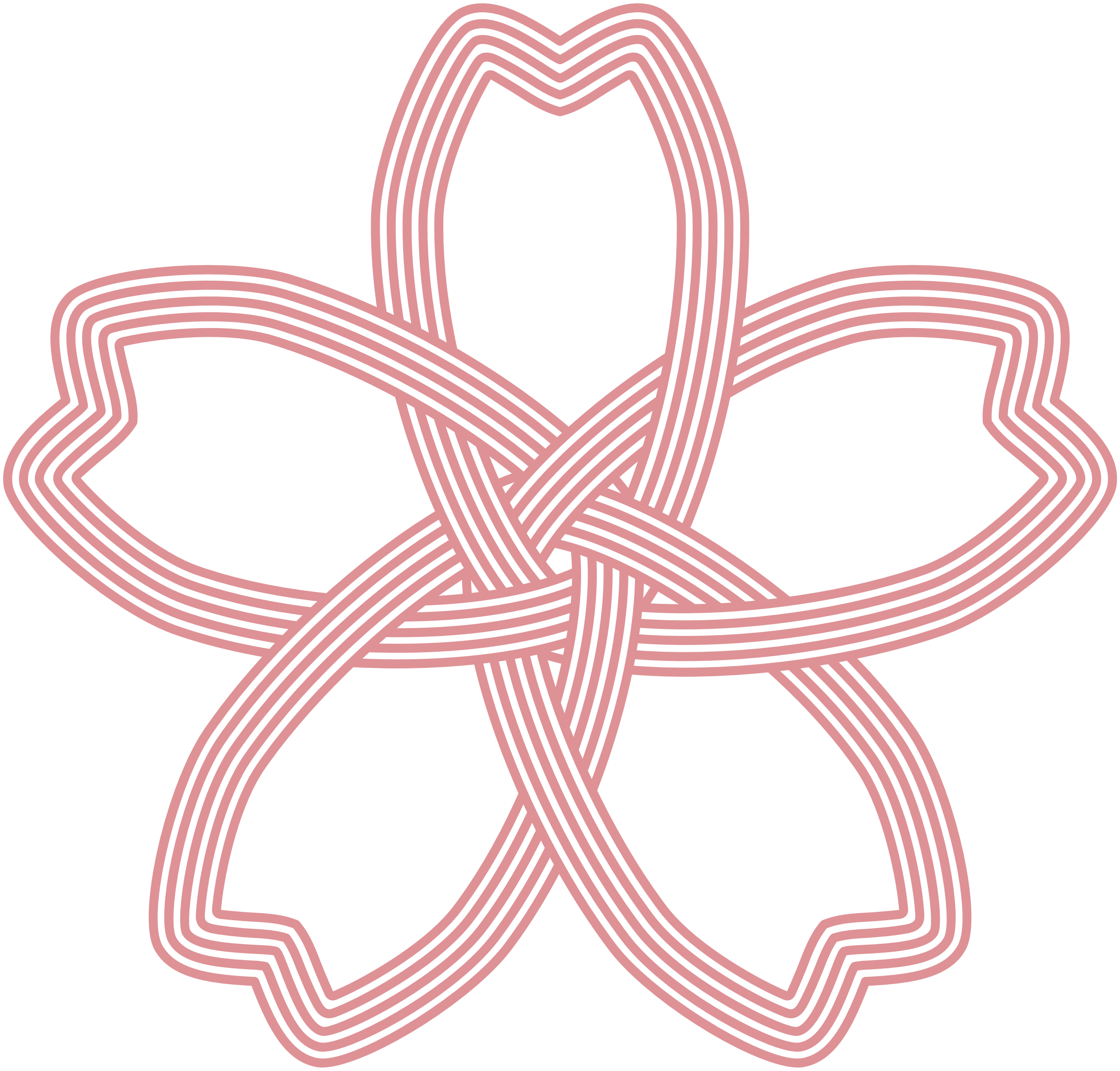
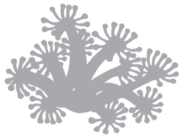


IBO2020 YEARBOOK

The 31st International Biology Olympiad 2020 Nagasaki, JAPAN
IBO Challenge 2020 (A Substitute for the 31st IBO 2020 Nagasaki, JAPAN)



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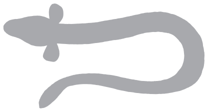

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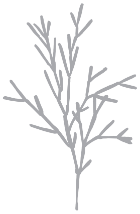
Branchiostoma japonicum



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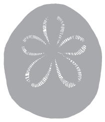

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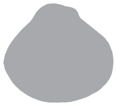
Conocephalum japonicum



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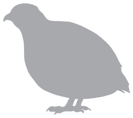

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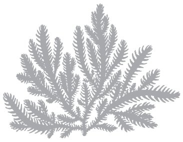
Cryptomeria japonica



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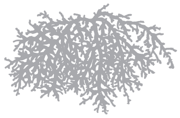
Dugesia japonica



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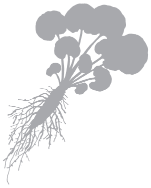

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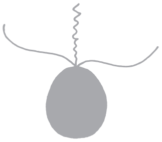

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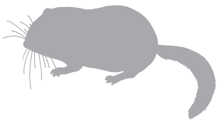

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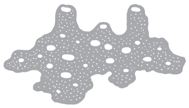

Halichondria japonica

Hydroglyphus japonicus

Hyla japonica

Hyleoglomeris japonica

IBO2020 YEARBOOK

This book is dedicated to the participants and supporters
of the IBO Challenge 2020, who together overcame the COVID-19 crisis.
We wish them all good health.
The IBO spirit never fades!

IBO2020 YEARBOOK

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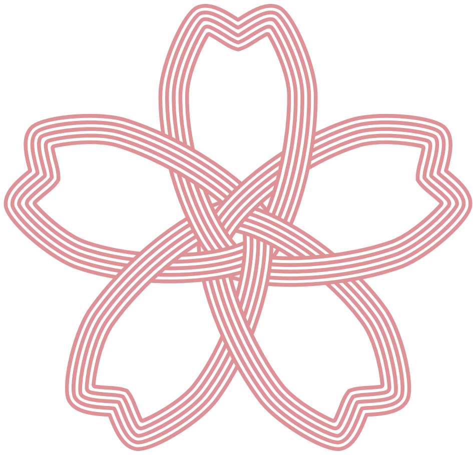

IBO2020 YEARBOOK

IBO2020 YEARBOOK

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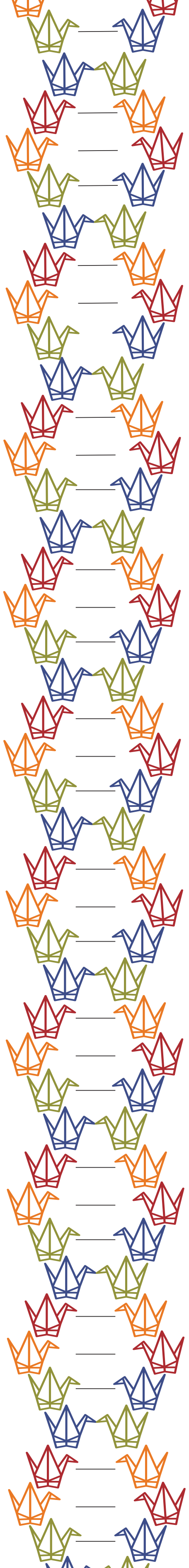


Introduction

IBO Challenge 2020

(A Substitute for the 31st IBO 2020 Nagasaki, JAPAN)

The 31st

IBO2020 Nagasaki, JAPAN

(Cancelled)

Enhancing the Wisdom of Youth Around the World Together

President of the IBO2020 Organizing Committee

Dr. Makoto Asashima

Emeritus Professor at the University of Tokyo
Special Research Professor at Teikyo University
Honorary Fellow of National Institute of Advanced Industrial Science and Technology (AIST)
Academic Adviser of Japan Society for the Promotion of Science (JSPS)
Foreign Academic Member of Lithuanian Academy of Science

First, I would like to express my sincere gratitude to all the people involved in the IBO Challenge 2020, who made this extremely international event possible and successful during the spread of COVID-19 worldwide. Although it was the first time for an IBO host country to organize a remote competition, we managed to successfully complete this event. I am truly appreciative for the cooperation and support of the students, jury members, and all of the other stakeholders. Despite our initial concerns, 53 countries and regions participated in the competition, far exceeding our original expectations for an event like this. We were also pleased that the students attended from various parts of the world.

We were able to successfully finish the practical exams on August 11th and the theoretical exam on August 12th without any major issues. On the 24th of the same month, we announced the names of the gold, silver and bronze medalists and their countries on our website without any specific rankings. After the event, we received emails and videos from many countries around the world about this remotely held international contest. Each of them was filled with joy and compliments about the event, which made us extremely happy.

Although the remote nature of the event limited us in most ways, it also pushed us to bring a new and unique activity to the IBO community: the International Group Project. Consisting of 4 students from different countries and a project facilitator, each group selected a topic from: (1) infectious diseases, (2) biodiversity and oceans, (3) genome editing, and (4) evolution. They then discussed their topic for more than two months and created their deliverables. This time, we asked IBO alumni and former volunteers, an extremely experienced, knowledgeable, and passionate group of youth, to be project facilitators for this event.

Within the Organizing Committee, the International Group Project was always considered to be as important as the examinations – that was how much this project meant to us. We could present a new opportunity for young people across the world to imagine, discuss, and propose something on a global scale. I believe that we succeeded in preparing students to be the next generation of world leaders who will lead the world with a new set of social, scientific, and biological issues. All the project posters that were submitted were wonderful despite various challenges that included the COVID-19 global pandemic, time zone differences, and poor internet connections.

In addition, I'd like to thank all of the organizing members who worked together from preparation to realization for nearly five years until the contest concluded, particularly the Secretariat Office and Ms. Mitsuko Kudo and Mr. Taiga Araki. I would also like to express my sincere gratitude to all of the companies and individuals who donated funds or sponsored us, as well as many other stakeholders such as the Japan Science and Technology Agency (JST) and the Japan Science Foundation (JSF).

Although our remote event was a success, I'm sure it made all of us miss the on-site IBO competition at the same time. It's a pity that the students and the international jury members didn't get to see the wonderful culture and nature of Japan this year, but I would like them to still come to Japan in the future. Thank you very much again to all of the people who are related to this IBO Challenge 2020. See you all in Portugal next year in 2021!

浅島 誠

Message from the Governor of Nagasaki

I would like to extend my sincerest congratulations on the holding of the 31st International Biology Olympiad in Nagasaki, Japan (IBO Challenge) with 202 participating students from 53 different countries and regions. It is unfortunate that the competition could not be held in Nagasaki and had to be conducted remotely due to the COVID-19 global pandemic. I believe, however, that the primary objectives of the competition have been fulfilled, as high school students from all around the world have been furnished with an opportunity to showcase their wits and compete in a test of biological knowledge on an international stage.

Although we did not have the chance to welcome you to Nagasaki this time, Nagasaki prefecture is blessed with extraordinary biodiversity. Nagasaki has many diverse ecosystems fostered by complex geography such as its remote islands, the Saikai National Park in Sasebo which possesses the Kujukushima or the “Ninety-Nine Islands,” and Mount Fugen on Shimabara Peninsula. Nagasaki also has many organisms that are born into rich, natural environments. I look forward to welcoming you all to Nagasaki in the near future so that you can explore its abundant charms.

I have high expectations that the competitors will play active roles as world leaders based on the experience they gained in this contest competing against their global counterparts in the field of biology. I pray for the continued good health and success of all those who have made this event possible.

Houdou Nakamura

Message from the Mayor of Sasebo

Sasebo City, Nagasaki Prefecture, located at the westernmost part of mainland Japan, is a port town with the beautiful scenery of the Saikai National Park "Kujukushima" archipelago. Kujukushima and its exceptional beauty are recognized amongst other internationally distinguished sceneries by the association of "The Most Beautiful Bays in the World" and has been deeply cherished and treasured by citizens. Its rich ecosystem has been formed corresponding with its complex topography, and its sea area is a cradle of life that contains various rare species. You can also enjoy huge bird migrations in the season. Sasebo City also contains various heritage sites such as the Hidden Christian site, which is designated as a World Cultural Heritage site, and the important military ruins of the Sasebo Naval District that is deeply involved with history of Sasebo.

The outbreak of COVID-19 has had a big impact all over the world and it was very unfortunate that the International Biology Olympiad 2020 Nagasaki had to be cancelled. However, I hope this extraordinary experience was a chance to be thankful for the "ordinary days" and to reaffirm your determination towards biology.

I hope you will work and learn hard, and that it will lead you to big success in your life. I also hope that IBO2020 will be an opportunity to lead you to Sasebo one day.

Norio Tomonaga

Message from the President of Nagasaki International University

To our regret, the 31st International Biology Olympiad in Nagasaki, Japan could not be held because of the worldwide COVID-19 infection. We prepared to welcome many students with hospitality who had a mind for biological research from all over world for more than 1 year. Fortunately, we are very glad to hear that the modified 'IBO in Nagasaki' was held on August 11 and 12 remotely, and that the results were announced on August 25. With drastic weather and environmental changes worldwide, the wave of students interested in biology should increase. We appreciate the help and various activities of the Organizing Committee and the participants.

Yukio Ando M.D. Ph.D.

Nagasaki International University — Event Venue

Located in Sasebo City, Nagasaki Prefecture, Nagasaki International University (NIU) is a private university founded in 2000. Since its foundation, the university has grown into a comprehensive university with four undergraduate departments (International Tourism, Social Work, Health and Nutrition, and Pharmacy) as well as three graduate schools. To this day, NIU cherishes its founding principle of "respect for human beings" and its motto of "always with humanity, always from the heart." The university is also known for its extensive incorporation of cultural education into its curriculum, such as the traditional tea ceremony.

IBO2020 Committee Members at NIU

Dr. Yoshinori Ohsumi

Dr. Yoshinori Ohsumi was born in Fukuoka in 1945. In 1963, he entered the University of Tokyo and chose to study molecular biology due to the influence of Prof. K. Imahori. As a graduate student, he enrolled in Rockefeller University to study under Dr. G. M. Edelman in 1974. He returned to the University of Tokyo as an assistant professor under Prof. Y. Anraku at the end of 1977. In 1988, he opened up his own small lab and started to work on the lytic function of the vacuole, and then found yeast autophagy by light and electron microscopy. After leaving the University of Tokyo he continued his research at the National Institute for Basic Biology at Okazaki. Then he moved to the Tokyo Institute of Technology in 2009 and received the Nobel Prize in Physiology or Medicine in 2016 for elucidating the mechanisms for autophagy.

Message from Yoshinori Ohsumi to the next generation of biologists

It's very unfortunate that the International Biology Olympiad must be held online this year, preventing us from discussing biology in person.

I'd like to send a message to the young generation of biologists.

Developments in our understanding of biology are truly striking. The establishment of foundational principles in molecular biology last century are the bedrock of this progress. In addition, rapid advances in the technologies available to researchers have also spurred on research. Contact between the fields of biology and medicine have become productive, with the fruits of biological research allowing us to overcome many diseases and improve human health in a direct and tangible way. These are without a doubt wonderful achievements. However, I believe that focusing on the practical application of biological knowledge alone is dangerous to the healthy development of biological research.

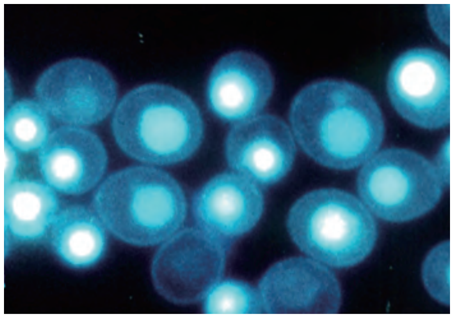
It is now clear that the activities of humans are having a strong impact on the natural environment and ecosystems. The ongoing COVID-19 pandemic is one example. Global warming is growing increasingly serious, and every year there are more and more natural disasters. Limits in the earth's resources are becoming ever more apparent.

Introduction

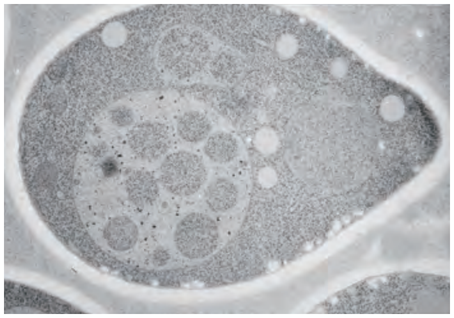
Message from Nobel Prize Winner

Meanwhile, great technological progress has ushered in an information age, and we are now faced with an overwhelming supply of information. While the COVID-19 pandemic continues to bring major changes to people's lives, we must now address problems never before faced by humanity. As future scientists, I believe that how you challenge these problems will have a direct effect on the future of our species.

I'd like to talk briefly about my research career. I began with an interest in a yeast vacuole. At the time, the vacuole was thought of as the place where cellular rubbish was collected, but I showed that the vacuole is able to transport amino acids and ions and has a V-ATPase, indicating it plays much more intricate roles in the cell. When I first started my own lab at the age of 43, I assumed that the vacuole is involved in intracellular degradation and set out to study the process of degradation. I discovered that yeast cells undergo massive self-degradation during starvation and showed that this was the same as previously observed autophagy. Then my group identified the genes required for autophagy and used these to unravel the molecular mechanism of this process. When I launched into this problem, the word 'autophagy' was virtually unheard of among biologists. Now that autophagy is such a major field of research, I feel like we are in a completely different age. As we discovered that the autophagy genes we identified are broadly conserved in animals and plants, autophagy research was revolutionized. The role of autophagy in a range of species, tissues, organs and individual organisms became clear, and we came to understand the amazing diversity of autophagy functions. When one considers that life is, essentially, a balance between synthesis and degradation, it is perhaps obvious to think that degradation is involved in every biological phenomenon, but this was overlooked for many years. Nowadays, many researchers are focusing on the involvement of autophagy in cancer and neurodegenerative diseases, as well as its higher-order functions such as longevity.



Fluorescence image of yeast vacuoles



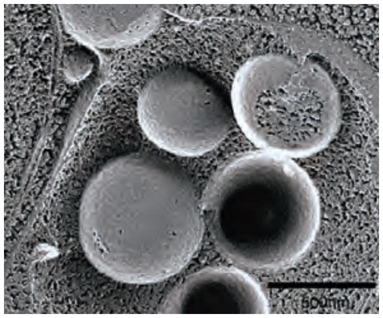
Electron microscopic image of a vacuolar proteinase-deficient cell under nitrogen-starvation conditions. The vacuole shows several autophagic bodies which contain a portion of cytoplasm.

I didn't set out on my scientific journey expecting that my research could be used to treat diseases. Most basic research begins instead with curiosity. This year's Nobel Prize in Chemistry for the development of gene editing technologies also recognized achievements at the forefront of microbial immunity. I chose to study the vacuole due to my interest in turnover, but also because it was overlooked by other researchers. Rather than taking part in fashionable areas of research that have already attracted a lot of attention, I think it's easier to make important progress without the distraction of competition by focusing on your own unique research topic.

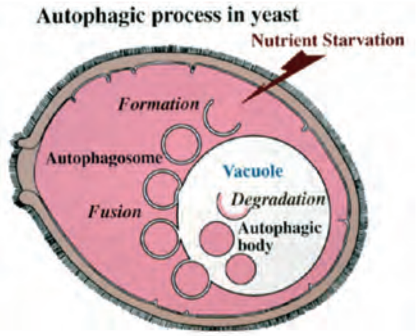
The most important thing in science is not simply the speed at which you are able to answer a question that is put to you. Today's answers can be rewritten by tomorrow's research. What is more important is identifying new questions that require answers. My hope is that young researchers can begin with scientific questions to which they can return throughout their career, rather than problems with immediate solutions. Paradigm shifts do not begin with questions that have direct and foreseeable answers.

While research is conducted by individuals, it is not a process that you should conduct alone. Big discoveries do not occur suddenly in isolation. Scientific research is ultimately a social activity, and it is important to work collaboratively with others. It's particularly important to discuss your research with people with a range of perspectives and who use different approaches to solve problems and even members of the public.

Finally, as future scientists, you should ensure that you experience the natural world as much as possible, and that you never forget that humans are only one form of life on earth. Draw inspiration from nature, discover new questions and allow yourself to be moved by the joy of discovery.



The very moment that an autophagosome fuses with the vacuole. Several autophagic bodies are also observed by freeze-fracture electron microscopic imaging.



Upon nutrient starvation, a membrane sac appears, expands and forms a double membrane bound structure, autophagosome, which contains a portion of the cytoplasm.

The outer membrane of the autophagosome fuses with the vacuolar membrane, releasing an inner membrane vesicle into the vacuolar lumen. The autophagic bodies are immediately degraded in wild type cells.

IBO Challenge 2020 (A Substitute for the 31st IBO 2020 Nagasaki, JAPAN)

From an On-site to Online Event

As we started hearing more and more news about the worldwide COVID-19 outbreak in February, we found ourselves in an increasingly difficult situation.

At first, we started evaluating multiple possibilities as precautionary measures, such as shortening the event's duration, postponement, and hosting it remotely. Many people from around the world, from country coordinators to selected competitors, started inquiring with us about the status of the event, which made us realize how high the stakes were. Whichever possibility we leaned toward, our biggest concern was that it seemed likely that we would have to alter or take away the once-in-a-lifetime opportunity that IBO provides its students, from testing their abilities in biology to interacting with like-minded youth from around the world.

As the pandemic grew bigger around the world, Japan halted all school activities in March. The nationwide state of emergency was issued in Japan in the following month. Even though we could no longer meet in person, the IBO2020 Organizing Committee kept the discussion going. Although we first assessed the possibility of hosting an on-site event with limited capacity, this was rejected as a result of the growing severity of the pandemic.

In the end, we concluded that changing the event to a remote competition was the best bet for us. Though not ideal, we thought it was a way to provide the IBO experience to as many students as possible in the world of lockdown and travel restrictions. Some raised concerns about the fairness of the examinations. We tackled this issue by awarding medals without releasing participant rankings, in addition to issuing exam operational guidelines against cheating. At the same time, we tried to give as much flexibility in exam operations as possible in order to accommodate all participating countries with varying situations. For example, each country could set their own exam timetables and exam venue (i.e., competitors gathered in one place or from their home). The International Group Project was also planned so that we could provide competitors with an opportunity to interact with each other online.

Although we received some criticism, almost all countries kindly and actively supported this new attempt. Thanks to the cooperation of the IBO community, we managed to host a successful event with 53 participating countries and regions.

Overview

Schedule

Phase	Event/Task	Date
Registration	Country/Personal Registration	31 July, 2020
	Exam Info Registration	5 August
Part 1 Examination	Practical Exams	11 August
	Theoretical Exam	12 August
	Results Released	24 August
Part 2 International Group Project	Group Project	3 August – 31 October
	Results Released	20 December

General Data

Part 1: Exams	
Participating Countries / Regions	47 + 3 Observers
Competitors	186
Jury Members	202
Exam Supervisors* *Non-jury people who oversaw the exams	45

- All questions were approved at the online jury meeting.
- All participating countries/regions conducted exams based on their own exam timetables. Announcements from the organizers were issued according to their local time zones.
- Competitors were permitted to take the exam from their home, as long as their countries/regions could set up a proper supervising environment. Out of the 47 participating countries/regions, seven conducted the exam completely online, nine incorporated some online examination, and 31 of them offered on-site supervision to all of their competitors.
- While optional, 31 countries/regions provided a link to their online supervision (Zoom, Skype, etc.).

Part 2: International Group Project	
Participating Countries / Regions	52
Competitors	202
Group Project Facilitators	37
Initial Number of Group Project Teams • Some competitors dropped out due to their academic responsibilities, vacations, time difference, connection issues, etc. • By the end of August, three teams were merged into other teams (46 remaining). 20 competitors had dropped out in that period.	49
Teams that successfully submitted the final deliverables by the deadline (November 5th)	39
Competitors who finished the group project	approx.130
Competitors who did not participate / quit halfway* *Due to illness, time difference, conflicted responsibilities, etc.	approx.70

Pre-Event Important Dates

2020 5 March	Announced that the event status of IBO2020 would be linked to the status of the Tokyo 2020 Olympic Games
24 March	Postponement of the Tokyo 2020 Olympic Games announced
25 March	Cancellation of IBO2020 Nagasaki (on-site event) and hosting of IBO Challenge 2020 (remote event) was approved by the IBO Steering Committee
26 March	Cancellation and the possibility of IBO Challenge 2020 announced on the website
30 March	IBO Challenge 2020 initial proposal submitted to the IBO Steering Committee
9 April	Discussion of the event details at an IBO Steering Committee meeting
20 April	General overview of the IBO Challenge 2020 finalized
27 April	Overview of the IBO Challenge 2020 announced to IBO member countries
7 May	Supplemental document released
1 June	Exam Operation Guidelines (version 1) released
10 June	International Group Project overview released
15 June	Practical Exam 2 (Bioinformatics) demo application released to all member countries
16 June	Participation poll started (deadline: June 21)
24 June	Country/Personal Registration started (deadline: July 31)
7 July	International Group Project facilitator registration started (deadline: July 20)
15 July	Exam Info Registration started (deadline: August 6)
16 July	Answer sheets distributed
30 July	Exam Operation Timetable and Instructions released
1 August	Subgroup Meeting
3 August	International Group Project grouping determined

IBO Challenge 2020

PART 1

Examination

Practical Exams

Theoretical Exam

Exam Timeline

Date	Event	
1-7 August	Online Subgroup Meeting	
7-11 August	Online Jury Meeting • 7 Aug: Exam questions revealed (English & Russian) • 8 Aug: Voting deadline to accept/reject each question • 10 Aug: English Official published & Translation deadline • 11 Aug: Certified questions distributed to all countries	Conducted based on each country's time zone (GMT +10 to GMT -6)
11-12 August	Exams • 11 Aug: Practical Exams Animal Physiology 3 hrs. & Bioinformatics 1.5 hrs. • 12 Aug: Theoretical Exam (3 hrs x 2 parts)	
13 August	Deadline to submit answer sheets / exam cover pages	
24 August	Results released on the website at 5 PM JST (Medal and special award recipients; no rankings)	

Thoughts Behind the Exam

Chief of the Practical Exams

Inspiring and Encouraging Young Talents

Hiroshi Wada (University of Tsukuba)

Photography by Tsuyoshi Asano

Usually in the IBO, students take four slots of practical exams of 90 minutes each. After the exams, students often say that they did not have enough time to complete the exams. It is a pity that most students do not even touch the prepared materials. After hearing about these experiences, one doubt hit me: Do our practical exams truly test the students for the abilities that are expected of good scientists? Sometimes it seems to me that the exams test for abilities found in good technicians, such as extracting DNA from cells as quickly as possible. Perhaps science is not an activity to compete in the speed of performance, but rather to compete, if we must compete, in originality or creativity. Thus, in the practical exam of IBO2020, I wanted to give students multiple chances to do trial-and-error. Yes, I wanted to give students the chance to make errors and to learn from those errors. As a trial case, we set up one practical exam to be twice as long as the usual exams (i.e., three hours).

I also like to refer back to the spirit of IBO that our activities are not for ranking students, but for inspiring and encouraging young talents in biology. Our practical exams should intend to show the new world of biology through the exams. In order to achieve this, I asked the scientists to include some messages in each exam. What do you want to show students through the exams? I hope students got the message and were inspired from the exams.

Chief of the Theoretical Exam

Finding Ways to Improve the World Through Biology

Tatsuhiko Noguchi (National Defense Medical College)

People who contributed to prepare the theoretical exams of the IBO Challenge 2020 are researchers who are currently active in many different fields of biology. I asked each author to freely create questions on biological topics that they considered most important. As a result, we were able to provide competitors with a diverse collection of problems covering a wide range of biological fields. We would be happy if they enjoyed answering our questions.

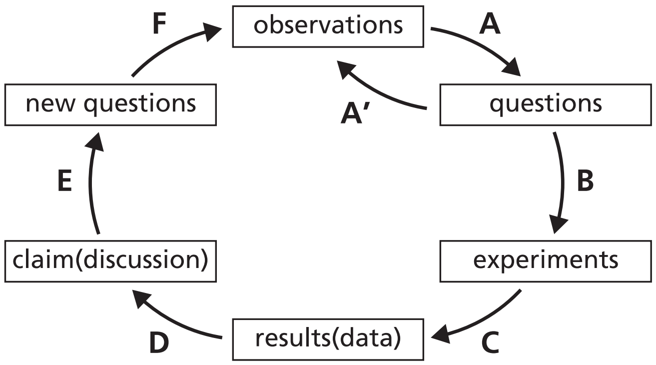
Here is a message from us to all of our competitors with great potential; in order for you to play an active role as social leaders, it is essential to acquire broad knowledge and wisdom from nature. This is true whether you become a scientific researcher or not. As nature and human society change faster than ever before, future leaders must have deeper understanding of various emerging challenges and choose the right solutions scientifically. By studying biology extensively, you will realize how wonderful the mechanisms of life are, and through the process, you should be able to find hints for solving many challenges humankind is or will be facing. So, please continue to study biology.

Practical Exam 1

Animal Physiology Team

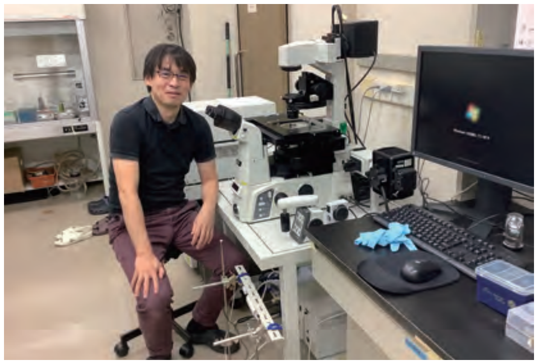
Kimiko Fukuda

For the practical exam in the original IBO2020, the Scientific Committee members decided to test the participants' abilities as future biologists.



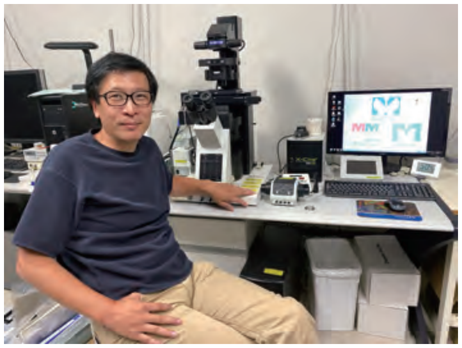
This diagram shows the cycle of scientific research. Researchers need to be able to do all of these seven processes, but typical practical exercises often test the participants' ability to perform mainly process C (as illustrated in the diagram). However, the IBO Challenge 2020 is remote, so it is impossible to conduct actual experiments. Therefore, we asked ourselves which competencies to test. The most important ability as a researcher is the process of deriving a question from observations. Researchers must be original. Originality is directly related to how deep the observation is and how interesting the questions are. Unfortunately, the thing that you don't train for during your high school education is how to observe deeply. In biological research, this "observation" includes not only observation with the eyes, but also many other means such as the modification of proteins, gene expression, exchange of substances, cell behavior, and the number of individuals. In this exam, we decided to have the participants observe the subject deeply and thoroughly with their naked eyes and express them. Instead of being unable to experiment, we also asked participants to design experiments to solve the question. At that time, you went back to the observation again (process A'). I would like to invite all of the participants to make deep observations of various subjects. It is a pleasure for researchers to solve the original questions from deep observations and produce their own results. I would like to meet the participants who are doing original research in the near future.

Iwao Koga
(Kamigoto High School)



Naoshi Takatori
(Tokyo Metropolitan University)

Kimiko Fukuda
(Tokyo Metropolitan University)



Guojun Sheng
(Kumamoto University)

Kyoko Fujimoto
(Nagasaki International University)

Practical Exam 2

Bioinformatics Team

Takeshi Kawashima

Sharing the Joy of Research
Through Simulating the Research Process

What we cared the most about when creating the bioinformatics exam for this event was to only use actual biological data. We didn't want to simplify the content by using artificial sequence data. We thought that the students' true biology talent would be best reflected in the way they analyze the real data. Because of this, we created questions recreating the actual research process of bioinformaticians.

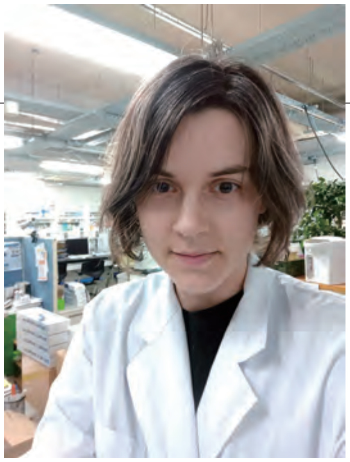
Bioinformatics is a field of science that has been most active in the use and analysis of databases of DNA and amino-acid sequences. In modern science, there is a massive effort to replace real-world observations with digital information. In other words, bioinformatics is the field that analyzes such digitally replaced biological data.

Therefore, considering the historical context of bioinformatics, we set the three essential abilities required for bioinformaticians as: 1) replacing observations with digital data (database), 2) approximating real life phenomena with equations (modeling), and 3) properly processing and analyzing the first two using a computer (computer science). Then, we planned to test these three abilities through our exam.

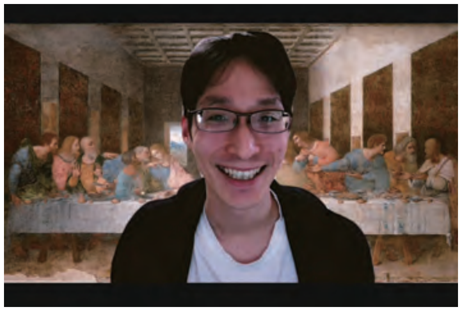
The most challenging part for us was making sure that there was one clear-cut answer for each question. The use of bubble sheets for answering greatly limited our flexibility, since we couldn't provide any open-ended questions to the competitors.

Another challenge for us was the IBO syllabus. In Campbell Biology, there is little mention of bioinformatics- the section about gene databases is about the only one. This forced us to begin every question with an explanation of the required background knowledge, which inevitably made the questions lengthy. We tried our best until the very last moment to create informative yet concise question sentences. Thanks to the generous cooperation of many people, we managed to make our questions into a web application that could be accessed from any device and implemented it on the internet.

In our exam, competitors started with some simple genome sequence data in hand. However, as they went through the questions, they could simulate the research process of gradually adding interpretations to the raw data using some computer programs. While anybody could reach the correct answers by simply trying all possible patterns, it required a series of educational guesses based on extensive biological knowledge to answer them correctly in a limited amount of time. We are greatly pleased if, through the exam, our competitors could experience the joy of biological research.

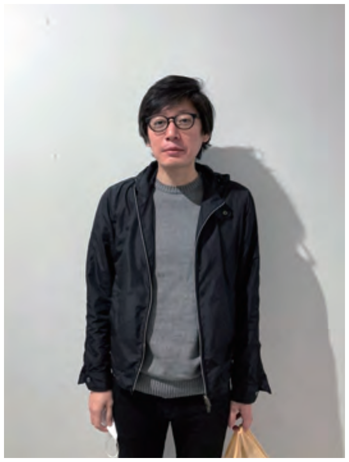


Josephine Galipon
(Keio University)



Haruka Ozaki
(University of Tsukuba)

Takeshi Kawashima
(National Institute of Genetics)



Tatsusada Yoshida
(Nagasaki International University)

Miho Totoki
(Sasebo Kita High School)

Practical Exam 3

Plant Physiology
Biochemistry
Plant Morphology Team

Koichiro Awai

If somebody asks you to name Japanese food you know, most of you would answer sushi first. Have you eaten sushi before? Unless you are completely new to sushi, you would know there are some green spicy paste between rice and raw fish; it's called Wasabi. Wasabi is an original spice of Japan and is produced by grinding the plant roots of *Eutrema japonicum*. This year, we planned to make the practical examination about Wasabi.

The exam included three topics: 1. Phylogenetic analysis of Wasabi by comparing the detailed structure of leaves among phylogenetically different kinds of plants, 2. Biochemical analysis of the enzyme responsible for producing spiciness of Wasabi by using fresh roots of *Eutrema japonicum*, and 3. Histochemical analysis of Wasabi to understand physiological role of the spiciness of Wasabi. It is widely believed that the purpose of Wasabi in sushi is to erase fishy smell of raw fish, but there is another and more important purpose: Wasabi can keep bacteria and fungi away from raw fish. Wasabi is, in fact, a traditional preservative.

Wasabi's spiciness is similar to the spiciness of radish or mustard, rather than red pepper. If you have a chance to eat sushi, please search for Wasabi and remember why it is good for sushi. Enjoy!

Koichiro Awai
(Shizuoka University)

Takuhiro Uto
(Nagasaki International University)

Shinichi Inoue
(Isahaya High School)

Kiyohiko Igarashi
(The University of Tokyo)

Shinichiro Sawa
(Kumamoto University)

Yoshihisa Kotake
(Saitama University)

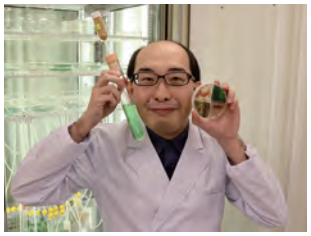
Theoretical Team 1

Biochemistry Team

Masahiko Ikeuchi

Science is a study of elucidating functions from mechanisms. In order to understand the functions of living organisms, it is important to learn underlying mechanisms that drive every biological function. The most fundamental part of biology is biochemistry, which deals with biological molecules and structures. If we could thoroughly understand their behaviors at the molecular level, we should be able to understand and predict cellular functions at higher levels, eventually all life phenomena, with further possible applications to medicine, industry, ecology, etc.

Masahiko Ikeuchi
(The University of Tokyo)



Kaisei Maeda
(Tokyo University of Agriculture)

Theoretical Team 2

Cell Biology Team

Shinji Kamimura and Tetsuro Mimura

Cells are the basic units that make up life forms. The same is true even for humans that are placed at the very tip of eukaryotic twigs in the evolutionary tree. In this respect, understanding the structures and functions of cells leads to the basic understanding of universal principles that are common to all other organisms, including bacteria, animals, plants and us, human beings. Considering such universality in the field of cell biology, we tried to prepare our questions to cover the whole field of cell biology and asked not only textbook knowledge but also your deeper understanding of biological principles based on physics and chemistry.

Shinji Kamimura
(Chuo University)

Tetsuro Mimura
(Kobe University)

Kimitsune Ishizaki
(Kobe University)

Yuta Otsuka
(The University of Tokyo)
IBO2011 Former Competitor

Hidehiro Fukaki
(Kobe University)

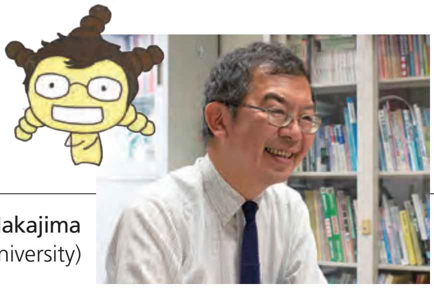
Thoughts Behind the Exam

Theoretical Team 3

Genetics Team

Harushi Nakajima

In the field of genetics, we created questions which required the ability to think logically and calculate accurately. Therefore, there are more problems based on fictitious experimental results and observations than problems based on actual academic papers. This type of question can be answered without any special knowledge as long as you read the question sentences carefully, but it is still difficult for examinees to solve because of the long sentences and heavy calculations. Despite some concerns, we believe that the competitors who participated in the IBO Challenge 2020 could compete well. We hope they enjoyed the problems.

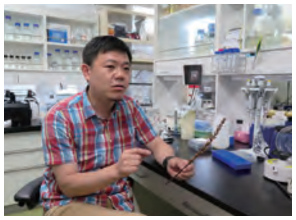


Harushi Nakajima
(Meiji University)

Takashi Osanai
(Meiji University)

Tomohiro Shimada
(Meiji University)

Masahiko Kato
(Meiji University)



Yoshiya Seto
(Meiji University)

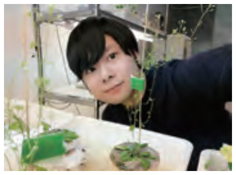
Theoretical Team 4

Plant Biology Team

Munetaka Sugiyama

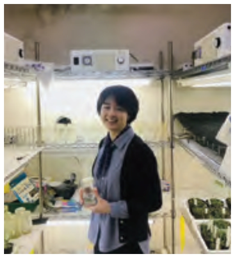
Plants represent a large group of multicellular eukaryotes of which structure, development, metabolisms, and physiological processes are quite different from those of animals in many aspects. By struggling with our questions of plant anatomy and physiology, we wanted the competitors to better understand the logics and mechanisms of underlying plant-specific forms and lifestyles, to get familiar with research in this field, and to become much more interested in the plant's world.

Left: Akihito Mamiya
(The University of Tokyo)
Right: Munetaka Sugiyama
(The University of Tokyo)



Kenji Nagata
(The University of Tokyo)

Yuta Otsuka
(The University of Tokyo)
IBO2011 Former Competitor



Hatsune Morinaka
(The University of Tokyo)

Takaaki Yonekura
(Nara Institute of Science and Technology)

Theoretical Team 5

Animal Biology Team

Tatsuo Michiue

There are more than one million species of animals living on Earth. Each animal has various shapes and characteristics, and lives in a variety of natural environments. There is no doubt that we, humans, are also animals. Once we think about our own bodies, we can easily understand that the body has so many function. We take food, move, breathe, support our body, supply oxygen throughout the body, and sometimes prepare for invasion from the outside. For thousands of years, human being has clarified how animals achieve these functions through observations and experiments. We prepared the exam questions with the hope that you would reconsider the splendor of animals through answering them.

Tatsuo Michiue
(The University of Tokyo)

Takayoshi Yamamoto
(The University of Tokyo)

Tatsuhiko Noguchi
(National Defense
Medical College)

Masafumi Inui
(Meiji University)

Shinji Kamimura
(Chuo University)

Manabu Yoshida
(The University of Tokyo)

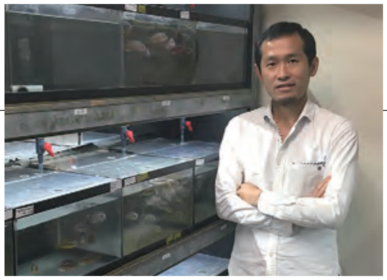
Hideki Abe
(Nagoya University)

Theoretical Team 6

Evolution Team

Masato Nikaido

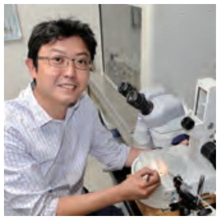
Knowing the evolution and the diversity of living things are directly linked to the idea of protecting the global environment. Please be aware that all living organisms are connected in the time of evolution; do not focus solely on the current research on mice and humans, but also have wider perspectives including the past, present, and future. Indeed, our questions covered a variety of organisms, including whales, mice, fish, insects and even algae. In addition, we prepared larger-scale questions that included extinct fossil animals as well as extant animals. We hope that solving our questions provided a good opportunity to consider the processes and mechanisms of biological diversification.



Masato Nikaido
(Tokyo Institute of Technology)

Takushi Kishida
(Museum of Natural and Environmental History, Shizuoka)

Masafumi Nozawa
(Tokyo Metropolitan University)



Koji Fujimura
(Niigata University)

Theoretical Team 7

Ecology Team

Tadashi Miyashita

Ecology is a field of science that deals with interactions between organisms and the environment, with an emphasis on exploring how ecosystems work. More specifically, ecology tries to identify the mechanisms of how the number of individuals and species richness are determined in nature, and how materials and energy flows in an ecosystem are controlled. Ecology also helps solve global issues, such as biodiversity conservation and sustainable use of ecosystem services. In recent years, reducing the risk of zoonotic diseases is becoming an urgent issue. Integration of ecology with other fields of biology as well as social sciences may be the key to achieve the long-term, harmonious coexistence of human society with nature.

Tadashi Miyashita
(The University of Tokyo)

Akira Mori
(Yokohama National University)

Yuya Fukano
(The University of Tokyo)

International Subgroup Meeting

Special Thanks to the Subgroup Members

It is since IBO2009 in Japan that the host country of IBO holds a subgroup meeting to shape up the exams prior to the jury meeting. For this IBO Challenge, we had two days of online subgroup meetings for both the practical and theoretical exams. Ten members of the subgroup devotedly spent four days reading the exams carefully. Thanks to their extremely constructive comments, our questions were brushed up to completion. We felt very happy when no question was rejected during the jury meeting.

Because we spent such an intense time together throughout the subgroup meeting, we felt like we already knew each other very closely, even though we just communicated over the comment function of Word files. I now feel it quite a pity that we couldn't see each other face-to-face. I hope we, including the scientists who made the questions, of course, can see each other in future IBOs.

My heartful thanks to all of the subgroup "family".

Chief, the IBO2020 Scientific Committee
Hiroshi Wada


Anindya Rana Sinha
(India)


Christiane Mühle
(Germany)


Gayane Ghukasyan
(Armenia)

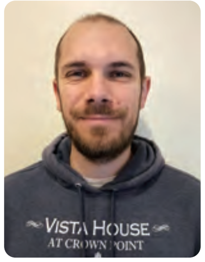

José Matos
(Portugal)


Joshua Hodgson
(UK)


Mary Oliver
(UK/Australia)


Poonpipope Kasemsap
(Thailand)


Saman Hosseinkhani
(Iran)


Vasili Pankratov
(Belarus)

Exam Operations

IBO Challenge 2020 was the first attempt for an IBO host country to coordinate examinations remotely. Due to the COVID-19 pandemic, the participating countries were in vastly different situations, which made our operations challenging. Although there were numerous challenges and limitations this year, we tried to build the best operational structure and methods for fair examinations. As a result, we managed to coordinate the exams with five staff members, only using existing services and platforms for nearly all operations.

1 Online Subgroup Meeting and Jury Meeting

- Exam questions for International Biology Olympiad, originally made by the host country, are first reviewed and edited by the international subgroup members appointed by the host. After that, the questions are released to the jury members of all participating countries. The jury members also review the questions and, if they find an objectionable part, a vote by all jury members is conducted to determine whether to keep, reject, or modify the part. Some jury members translate the questions during the meeting as well. After this whole process, the official IBO examinations are finally created.
- Just like the examinations, we had no choice but to organize subgroup and jury meetings completely online for the IBO Challenge 2020. Since it was logistically too difficult to host a real-time online meeting with around 50 countries that have different time zones, internet environments, and COVID-19 statuses, we utilized emails and cloud platforms like Google Drive for communication and an online form service (i.e., Cognito Forms) for voting.

2 Supporting Exam Operations Within Each Country

- Prior to the event, we published two essential documents for all participating countries: “Exam Operation Guidelines” for specific rules and recommendations to ensure fair examinations, and “Exam Operation Timetables and Instructions” for each country’s daily to-dos and step-by-step instructions for every task.
- During the event, the IBO2020 Secretariat Office sent a “daily reminder” email every day to remind them of the tasks and deadlines of that day.

3 Online Resources for the Practical Exams

- For the practical exams (animal physiology and bioinformatics), we required competitors to access online applications (i.e., webpages) to answer some of the questions. In order to avoid slow connections or server failures caused by access concentration, we prepared multiple servers (26 for the animal physiology exam and 33 for the bioinformatics exam) and placed them across the globe according to the geographical locations of the participating countries. We used Amazon Web Services for this operation. Although some minor issues were detected, we successfully managed the servers from the beginning of the jury meeting until the end of the practical exams. The online applications were developed for this event.

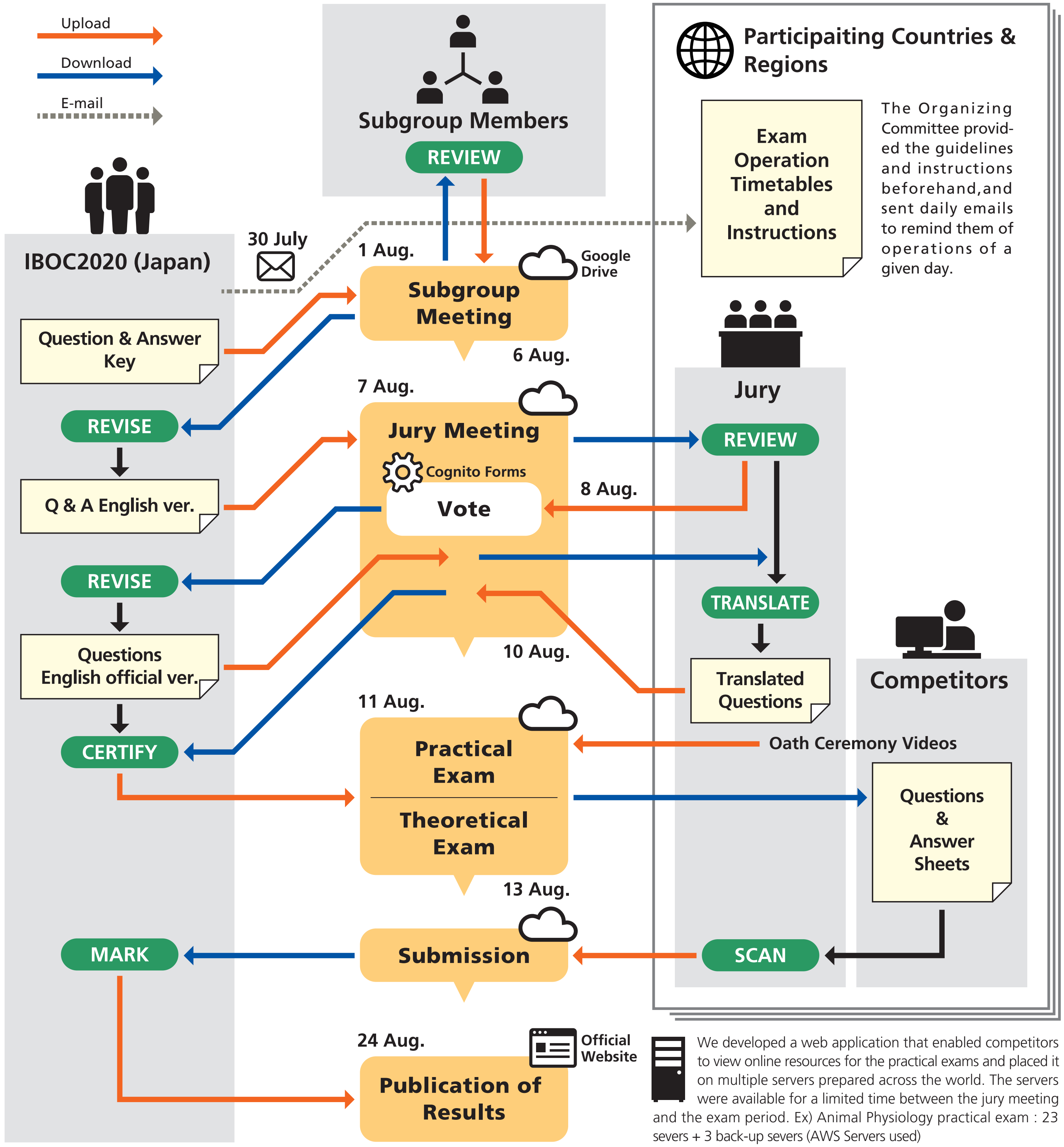
4 Cheating Prevention

- In order to minimize the risk of cheating, we announced various exam-related rules and recommended operational methods prior to the event. For instance, the participating countries had to film an oath ceremony before the exam (both competitors and jury) and upload it to a designated Google Drive folder. We also recommended countries to share an online supervision link (Zoom, Skype, etc.) with us so that we could randomly join their exam supervision for higher transparency.

5 Accommodating All Countries

- Throughout the event, from the beginning of the jury meeting until the end of the exams, we coordinated nearly everything based on each country’s local time zone instead of Japanese Standard Time. This was to ensure all participating countries had an equal amount of time to review and translate the questions, and to accommodate each country’s unique situations and challenges during the COVID-19 pandemic.

(Kentaroh Honda, IBO2007 Former Competitor)



We developed a web application that enabled competitors to view online resources for the practical exams and placed it on multiple servers prepared across the world. The servers were available for a limited time between the jury meeting and the exam period. Ex) Animal Physiology practical exam : 23 severs + 3 back-up severs (AWS Servers used)

Exam Results

Gold

Country/Region	Name
Thailand	Chaisrisawatsuk, Bhumpanya
Chinese Taipei	Chen, Chien-Yi
Vietnam	Duc, Ho Viet
South Korea	In, Heewon
Czech Republic	Janoušek, Jiří
Germany	Kessels, Fynn
Netherlands	Kooter, Berit
Russia	Kovalev, Maksim
USA	Lam, Judson
Singapore	Lin, Ziwei, Dewey
Azerbaijan	Muradli, Matin
Russia	Nagimov, Ruslan
Russia	Nikolaev, Nikolai
USA	Parsan, Nithin
China	Shao, Chengjun
Japan	Suematsu, Mahiro
Uzbekistan	Valijonova, Saida Atabekovna
China	Xu, Runtian
Chinese Taipei	Yang, Cheng-Chun
China	Yao, Qian
USA	Zhang, Albert

Silver

Country/Region	Name
Turkey	Ateş, Yiğit Can
Netherlands	Bosgoed, Dante
Hungary	Buzafalvi, Denes
Chinese Taipei	Chang, Hsiang-Chun
Latvia	Ekmanis, Toms
Azerbaijan	Gafarov, Sadiyar
UK	Grodzinski, Noah Joseph Baker
Germany	Groß, Damian
Azerbaijan	Gurbanov, Ranal
Vietnam	Ha, Dong Ngoc
Iran	Hassani, Arian
Chinese Taipei	Huang, Chi-Sheng
China	Jia, Hongzhe
Japan	Kanehisa, Ren
Uzbekistan	Karimov, Ravshanbek Mirgolib ugli
Japan	Kawamoto, Seita
South Korea	Kim, Geono
Russia	Kuzmenko, Oleg
Poland	Kwiatkowski, Jakub Krzysztof
USA	Liang, Derrick
Latvia	Lopatko, Rolands
Singapore	Lu, Kate
Indonesia	Marsetyo, Farrel Alfaza
Japan	Matsufusa, Manami
Lithuania	Melaika, Simonas
Iran	Moheimani, Nazgol
UK	Mulford, John
Indonesia	Nadia, Joan
Czech Republic	Pelánek, Ondřej
Turkey	Polat, Gencay Kaan
Singapore	Qiu, Xinzhi
Estonia	Rahe, Martin
Netherlands	Ruiten, Xanta van
Iran	Sabbaghi Lalimi, Mohammadamin
Singapore	Tan, Cheng Yat
Uzbekistan	Tojiboev, Sardor Eshqul ugli
Hong Kong, China	Tsang, Hoi Yeung
Hungary	Tusnady, Simon
Turkey	Yilmaz, Anil Deniz
South Korea	Yoon, Jae Won
Azerbaijan	Ziyaddinov, Asiman
Poland	Zurowski, Maciej Mateusz

Bronze

Country/Region	Name
Sri Lanka	Abbinanthan, Arulanantham
Latvia	Apsitis, Martins
Australia	Bahra, Priya Kaur
Latvia	Berzins, Mairis
Poland	Borak, Martyna Aneta
Poland	Buchalska, Barbara Anna
Australia	Bui, Nicholas Man Dac Vo
Hong Kong, China	Chan, Tsz Ching
Hungary	Czako, Balint Laszlo
Lithuania	Dapšys, Povilas
Sri Lanka	Dhanapala, Dhanapala Mudiyanselage Nilushi Navodya
Bulgaria	Dimitrov, Nikola Milenov
Lithuania	Duchovskytė, Marija
Luxembourg	Furlano, Jean-Marc Raffaello Matteo
Luxembourg	Gerhards, Frédéric
Bulgaria	Gilin, Viktor Dimitrov
Hungary	Gulacsi, Mate Mihaly
Denmark	Hansen, Tobias Spliid
Hong Kong, China	Ho, Ka Chun
Germany	Jaschinski, Ilka
South Korea	Kim, Dale
Armenia	Kurghinyan, Mher
Bulgaria	Kutrovski, Dimitar Marinov
Switzerland	Lanz, Kaspar Merlin
UK	Leung, William Henry Ty
Vietnam	Linh, Ha Vu Huyen
Sri Lanka	Malavige, Sauni Ruwanima
Luxembourg	Marth, Raffaël

Country/Region	Name
Finland	Marttinen, Harri Ensio
Indonesia	Maulana, Achmad Rizky
Czech Republic	Maxerová, Tereza
Slovenia	Mežnar, Anamarija
UK	Mousavi, Seyed Sepehr
Syria	Nasra, Majd
Philippines	Ng, Jeremy Ace Feliciano
Netherlands	Osenbruggen, Lucas van
Thailand	Piyanirun, Kantawich
Slovenia	Prelog, Ivo
Bangladesh	Raayan, Rafsan Rahman
Thailand	Rattanawannachai, Kittitach
Estonia	Remm, Mari
Switzerland	Salud, Anna
Nepal	Sapkota, Awahan
Germany	Sauer, David
Iran	Shahsavand Davoudi, Amirhossein
Bangladesh	Sharar, Raad
Thailand	Sima-Aree, Arthitaya
Kazakhstan	Taimanov, Adam
Estonia	Tamm, Johan
Indonesia	Tjandra, Nathanael
Bulgaria	Toshev, Kiril Teodorov
Belgium	Toussaint, Marie
Czech Republic	Tulis, Jan
Turkey	Tüney, Ali Berdan
Tajikistan	Vatanshoevich, Shams Davlyatbekov
Finland	Vuorela, Teemu Toivo Viljami

Certificate of Merit

Country/Region	Name
Armenia	Avanesyan, Gevorg
Kyrgyzstan	Azhybaev, Baktynur
Kazakhstan	Bissembayev, Arman
Turkmenistan	Hallayev, Hoshgeldi
Estonia	Haug, Sofia Marlene
Sri Lanka	Munasinghe, Samidhi Manthilani
Kazakhstan	Muratov, Yerassyl
Vietnam	Nga, Nguyen Thi Thu
Belgium	Van Roy, Mander
Hong Kong, China	Wong, Lok San
Australia	Zhou, Angie Jie

Special Awards

The 3D Reconstruction Award (Practical Exam 1: Animal Physiology)

Thailand	Chaisrisawatsuk, Bhumpanya

The Intron=Exon Boundary Award (Practical Exam 2: Bioinformatics)

Russia	Kovalev, Maksim
Russia	Nagimov, Ruslan
Indonesia	Nadia, Joan

The Champions of the Theoretical Exams (Theoretical Exam 1 & 2)

China	Shao, Chengjun
China	Yao, Qian

The Champions of Hard Questions (Theoretical Exam 1 & 2)

Chinese Taipei	Chang, Hsiang-Chun
Chinese Taipei	Huang, Chi-Sheng
Germany	Kessels, Fynn

About Medals

Gold	Kamuysaurus japonicus Read more on the next page
Silver	Nipponia nippon is an ibis of the order Pelecaniformes that can reach a length of 75 cm and a wingspan of 130 cm. They have white feathers with salmon-pink coloration under their wings and a bare, red face. The birds feed on amphibians, fish, and insects found in rice paddies. The crested ibis was once widespread in China, Japan, Korea, and eastern Russia. However, habitat destruction and pesticide use decimated ibis populations until they could only be found in Shaanxi Province, China. In 2008, joint Chinese-Japanese conservation efforts led to the reintroduction of crested ibises on Sado Island in Japan.
Bronze	Camellia japonica is a shrub or small tree species that grows to heights of 3-5 meters and is native to southern Japan and China. It is widely planted as an ornamental species around the world, with over 2,000 different cultivars in existence. This species is iconic for its beautiful white, pink, or red flowers that appear from late winter to early spring as well as its thick, glossy, and evergreen leaves. Its flowers produce a sweet nectar that is popular with several bird species and the monkeys of Yakushima island in southern Japan.

About Kamuysaurus

Dr. Yoshitsugu Kobayashi
Professor at the Hokkaido University Museum

Excavated skeleton of **Kamuysaurus japonicus**.

Kamuysaurus japonicus, newly named in 2019, is the best preserved and most complete large dinosaur skeleton from Japan. The genus name “Kamuy” refers to a mythological deity of the Ainu, the indigenous people of Hokkaido Island of Japan. “Saurus” and “japon” mean “reptile” and “Japan”, respectively, so *“Kamuysaurus japonicus”* has a meaning of “the god of Japanese dinosaurs”. The skeleton of *Kamuysaurus* was discovered from Cretaceous rock, dated as 72 million years ago, in Hobetsu area of Mukawa Town in Hokkaido Prefecture. This dinosaur was at least 12 years old at the time of death with a body length of approximately 8 meters and body mass of about 4.3 to 5 tons. *Kamuysaurus* is a duck-billed dinosaur, or hadrosaurid, which was common plant-eating dinosaurs during the Cretaceous. This discovery is not only significant for the people of Hokkaido and all of Japan, but it has global significance because this dinosaur shows us how the world has been connected through time. *Kamuysaurus* is closely related to dinosaurs from USA and Canada, *Edmontosaurus*, a duck-billed dinosaur found throughout much of western North America. Because these dinosaurs are so closely related, they provide evidence that long ago, Asia and North America were connected.

The initial discovery of the fossils came in April 2003 when a local resident unearthed 13 articulated vertebrae in the Upper Cretaceous Hakobuchi Formation in Hobetsu area of Mukawa Town. The vertebrae were initially considered to be a part of marine reptile plesiosaur, but were later identified as a partial tail of a dinosaur in 2011. Joint expeditions, held in the summers of 2013 and 2014, were launched to find the remaining parts of the dinosaur. In 2013, while exploring the same hill where the original

About Kamuysaurus

Left: Excavation of Kamuysaurus japonicus in Hobetsu area of Mukawa Town.
Above: Reconstruction of Kamuysaurus japonicus at the beach.

fossils were found, the paleontologists excavated multiple skeletal elements including isolated teeth. During the excavation in 2014, a large amount of rocks, containing dinosaur bones and parts of skull elements were excavated, and the dinosaur was considered to be a nearly complete skeleton. Preparation of the dinosaur took nearly 10 years with the help of many volunteers. The prepared fossils clearly demonstrate that this is a nearly complete skeleton including multiple cranial elements, nearly complete series of vertebrae, and nearly complete fore- and hind-limbs. It turns out that it was the biggest discovery of Japan ever.

The study of dinosaurs is not about studying monsters. It is about discovering the fascinating and incredible evolution of animals. Some dinosaurs evolved into strange figures. Good examples are long and large horns on a head like *Triceratops*, one-meter-long bony plates on a back like *Stegosaurus,* and a snorkel-like crest on the top of the head like *Parasaurolophus*. This diversity in shapes shows how successful dinosaurs were back in time. Other dinosaurs competed how big they could be. About 150 million years ago, a large meat-eating dinosaur, *Allosaurus*, conquered a niche in North America, where sympatric plant-eating dinosaurs, sauropods with body size of over 30 meters, became larger than predators for protection. Lastly, some dinosaurs challenged to fly and evolved into birds. This evolution was revolutionary because they had to adapt to a completely new habitat in the air. Innovative dinosaur research in recent years has revealed the evolutionary process from reptiles to birds, which gives us better understanding of how animals in ancient time evolved to animals in modern world and how all organisms are related and connected to each other.

I love what I do. I am always fascinated with dinosaurs. Dinosaurs tell us so many things we don't know. I wish you have a thing that you are interested in like I do. I hope you love what you do. I want you to be always curious, because it is a strong driving force and creates a bright future. If you're not interested in anything right now, dinosaurs might be the first step for you!

About IBOC2020 Goods

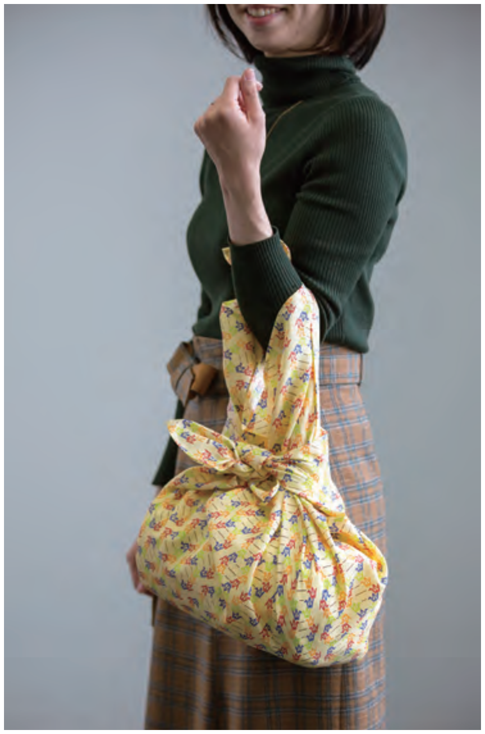
Official IBOC2020 T-Shirt

Traditional Round Fan (Uchiwa)

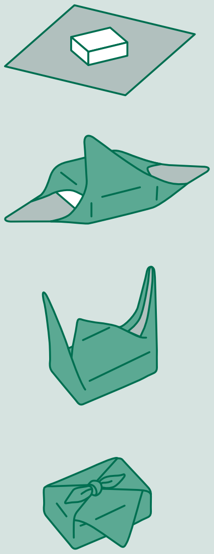
One of the two Japanese traditional gifts from IBO2020 is uchiwa, a round fan that is made from the wood of sustainably harvested Japanese cedar trees (*Cryptomeria japonica*). We hope you enjoy the scent of *Cryptomeria japonica*, a very distinctly Japanese aroma.

Traditional Cloth (Furoshiki)

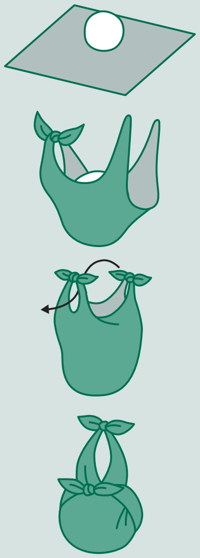
The other Japanese traditional gift is furoshiki, a traditional Japanese wrapping cloth. Depending on how you fold the cloth, it can transform from a bag or purse to a cover that secures important items. If you search for furoshiki on the internet, you can find many folding patterns for this cloth that you can try. Some examples are shown on the next page.



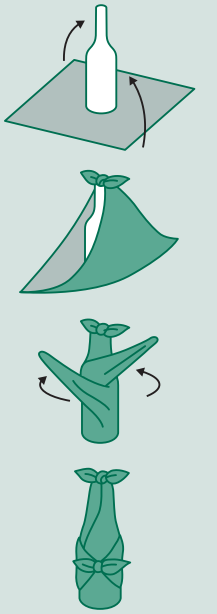
Otsukai Tsutsumi
(Basic Carry Wrap)



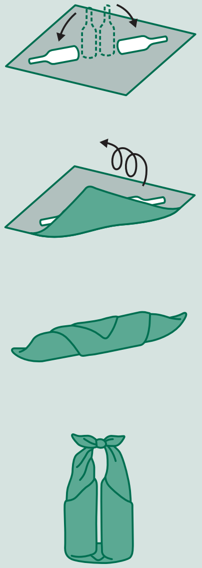
Suika Tsutsumi
(Watermelon Carry Wrap)



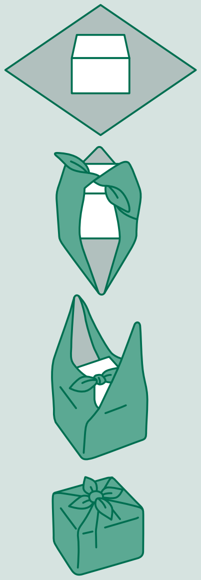
Bin Tsutsumi 1
(Bottle Carry Wrap 1)



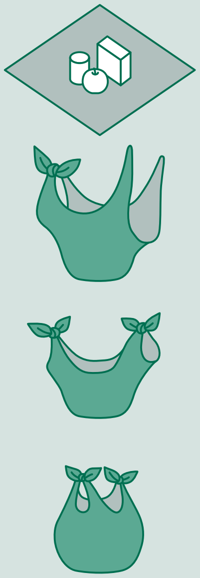
Bin Tsutsumi 2
(Bottle Carry Wrap 2)



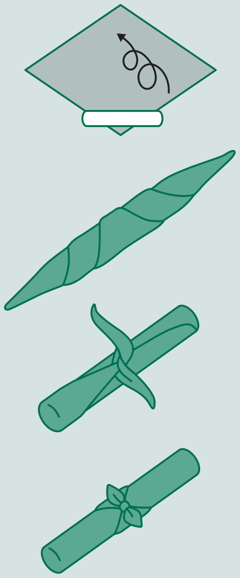
Yotsu Musubi
(4 Tie Wrap)



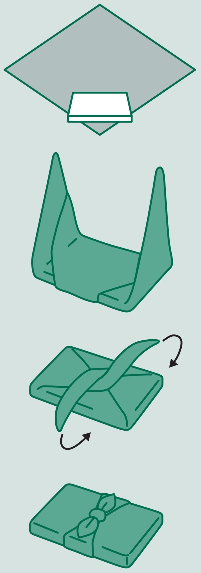
Tesage Bukuro
(Hand Carry Wrap)



Entou Tsutsumi
(Long Object Wrap)



Sao Tsutsumi
(Podding Carry Wrap)



Armenia

Competitors


Mher Kurghinyan


Ani Harutyunyan


Gevorg Avanesyan


Naeiri Sohrabian

Jury


Gayane Ghukasyan

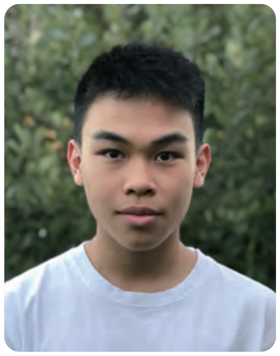

Arman Simonyan


Aren Petrosyan


Seda Marutyan

Australia

Competitors


Nicholas Man Dac Vo Bui


Angie Jie Zhou


Priya Kaur Bahra


Hamish Brodie Walker

Jury


Julie Cooke

Juliey Beckman

Supervisors

Nathan Bui / Nikki Mcdonald / Paul Mitchell

Azerbaijan

Competitors


Matin Muradli


Sadiyar Gafarov


Asiman Ziyaddinov


Ranal Gurbanov

Jury

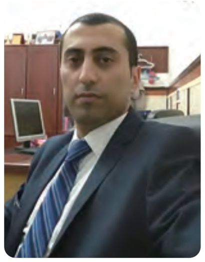

Rashad Salimov


Tural Javadzade


Hasan Hasanov


Elvin Abdullayev


Anar Gojayev


Elvin Huseynov


Ramil Khammadov


Nargiz Mammadli

Bangladesh

Competitors


Tasnim Binte Zulfiqar


Rafsan Rahman Raayan


Raad Sharar


Abrar Jamil

Jury


Md Samiul Alam Rajib


Saumitra Chakravarty


Rakha Hari Sarker


Md Habibur Rahman

Supervisors

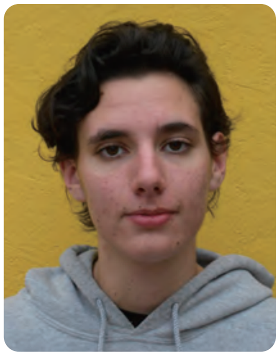
Muhammad Tarik Arafat / Saif Bin Salam Bondhon / Talukder Galib Shahriar Prince
Md. Minu Islam Khan / Samiha Sayeed / Md. Sahadat Hossain / Mahdi Hasan

Belgium

Competitors

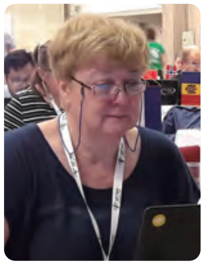

Milan Roelens


Mander Van Roy


Marie Toussaint


Sébastien Laurent

Jury


Marleen Caroline Van Strydonck


Hugo Paul Vandendries


Gérard Marie Cobut


Michaël Carmelo Terzo

Bulgaria

Competitors


Kiril Teodorov Toshev


Nikola Milenov Dimitrov


Dimitar Marinov Kutrovski


Viktor Dimitrov Gilin

Jury


Albena Georgieva Jordanova


Radoslav Aleksandrov Aleksandrov


Snezhanka Borisova Tomova-Gogova

China

Competitors


Qian Yao


Runtian Xu


Chengjun Shao


Hongzhe Jia

Jury


Yibo Hu

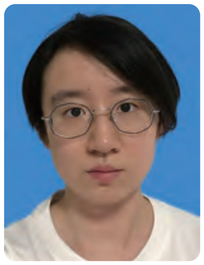

Xiangjun Tong


Dong Liu


Jinglan Wang


Yanyun Zhang


Xin Liang


Ruoting Tao

Supervisors

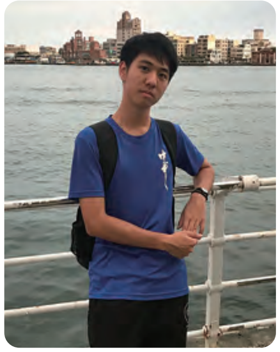
Fuwen Wei / Yongwen Zhang

Chinese Taipei / Republic of China

Competitors


Hsiang-Chun Chang


Chien-Yi Chen


Chi-Sheng Huang


Cheng-Chun Yang

Jury


Ying Wang


Chiu-Hsin Chu


Yu-Chung Chiang


Shu-Chuan Hsiao


Shen-Horn Yen

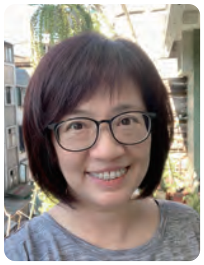

Jong-Kang Liu


Yi-Ling Yang


Jiin-Tsuey Cheng


Kuei-Shu Tung


Chih-Wei Shin


Fang-Lin Chu

Supervisors

Sheng-Pao Chen / Zi-I Song / Yu-Chi Chiu / Feng-Li Tsai

Czech Republic

Competitors


Ondřej Pelánek


Jan Tulis


Tereza Maxerová


JiříJanoušek

Jury


Lenka Libusová


Jan Černý


Antonín Reiter

Denmark

Competitors


Tobias Spliid Hansen


Josefine Møgelvang


Sofie Buur Beck


Jonatan Høhne

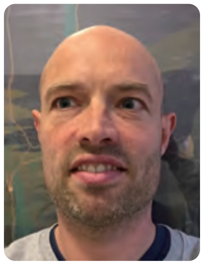
Jury


Kirsten Wøldike


Birthe Zimmermann


Vibeke Birkmann


Karen Helmig


Morten Eskildsen


Simon Albrechtsen

Estonia

Competitors


Sofia Marlene Haug


Mari Remm


Martin Rahe


Johan Tamm

Jury


Ando Vaan

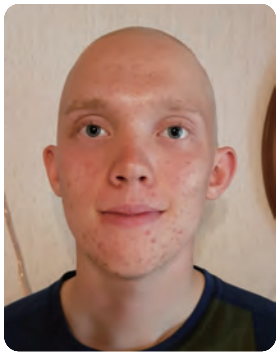

Karl Jürgenstein


Uku-Laur Tali


Sulev Kuuse

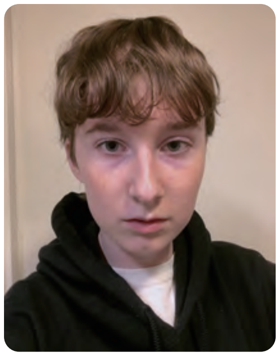
Finland

Competitors

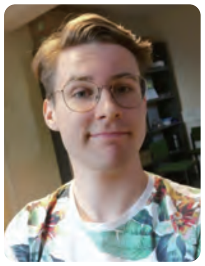

Harri Ensio Marttinen


Suvi Linnea Laitinen


Teemu Toivo Viljami Vuorela


Oona Elina Charlotta Kurola

Jury

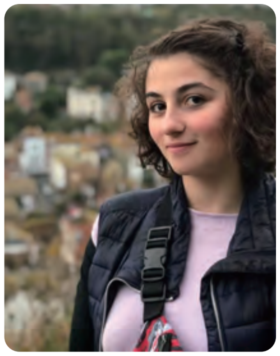

Niko Rainer Johansson

Tuomas Juha Eero Aivelo

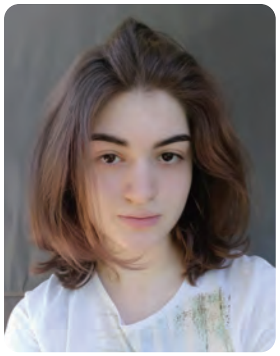

Jakke Sameli Neiro

Georgia

Competitors

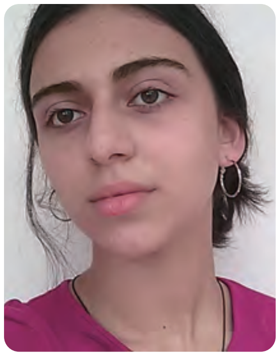


Elene Meskhi



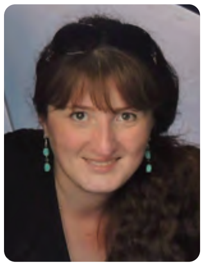
Khatia Nadiradze

Gega Karanadze



Tinatini Morchadze

Jury



Ekaterine Bakuradze

Irina Modebadze

Nana Barnaveli

Ekaterine Mitaishvili

Germany

Competitors


Damian Groß


Ilka Jaschinski


Fynn Kessels


David Sauer

Jury


Burkhard Schroeter


Dennis Kappei


Toni Gossmann


Christiane Mühle


Patricia Scholz


Cedric Cappel

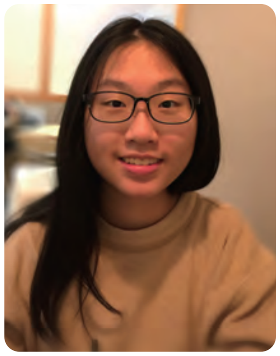

Jan Krieghoff

Hong Kong, China

Competitors


Lok San Wong


Ka Chun Ho


Hoi Yeung Tsang


Tsz Ching Chan

Jury


Ka Hoi Lau


Pui Yan Cecilia Yau

Hungary

Competitors

Balint Laszlo Czako

Denes Buzafalvi

Simon Tusnady

Mate Mihaly Gulacsi

Jury

Sandor Ban

Viktoria Gal

Zsolt Eros-Honti

Andrea Borbola

Adam Zoltan Seres

Supervisors

Eniko Gulyas / Anna Regina Krizsan

Iceland

Competitors

Katla Rut Robertsdottir Kluvers

Vitor Logi Þórisson

Kjartan Kristjánsson

María Guðjónsdóttir

Jury

Þórhallur Halldórsson

Ólafur Patrick Ólafsson

Arnór Bjarki Svarfdal

Indonesia

Competitors


Achmad Rizky Maulana


Farrel Alfaza Marsetyo


Joan Nadia


Nathanael Tjandra

Jury

Ahmad Faizal


Agus Dana Permana


Ida Bagus Made Artadana


Husna Nugrahapraja


Titis Setiyobudi


Syailendra Karuna Sugito

Iran

Competitors


Amirhossein Shahsavand Davoudi


Nazgol Moheimani


Mohammadamin Sabbaghi Lalimi

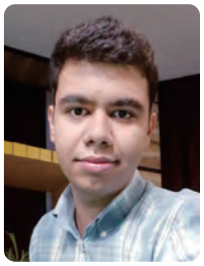

Arian Hassani

Jury


Alireza Majd


Amirhossein Zare Mohazabiyeh


Alireza Tanoori


Mohammad Ebrahim Katebi


Ali Yazdizadeh Kharrazi


Maryam Gholami Gharatappeh


Saman Hosseinkhani

Supervisor

Mohammad Karamudini

Japan

Competitors


Manami Matsufusa


Seita Kawamoto

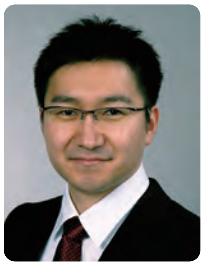

Mahiro Suematsu


Ren Kanehisa

Jury


Junichi Saito


Ryo Iwama


Daisuke Takahashi


Masayuki Hatta


Gaku Takimoto

Supervisors

Hiroko Hasegawa / Akari Soma / Jun Yatsu

Kazakhstan

Competitors

Arman Bissembayev

Dinmukhammed Urazbayev

Adam Taimanov

Yerassyl Muratov

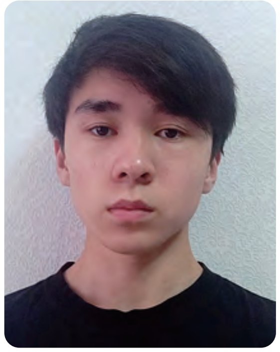
Jury

Adlet Sagintayev

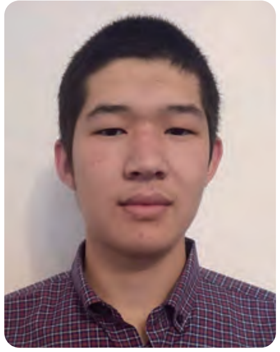
Ilyas Sakimov

Kyrgyzstan

Competitors


Azim Chyngozhoev


Aiatbek Kubanov


Baktynur Azhybaev


Aibek Medetbekov

Jury


Elbrus Tazhibaev

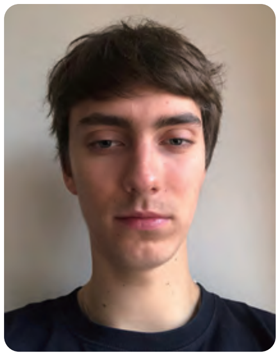
Supervisor

So Ho Kim

Latvia

Competitors

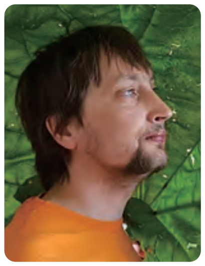

Toms Ekmanis


Martins Apsitis


Rolands Lopatko


Mairis Berzins

Jury


Janis Liepins

Agnese Kokina


Gunda Zvīgule Neidere


Katrina Daila Neiburga


Valdis Pirsko

Supervisors

Jana Kasaliete / Anitra Zile

Lithuania

Competitors


Povilas Dapšys


Marija Duchovskytė


Simonas Melaika


Edgaras Zaboras

Jury


Andrius Petrašiūnas


Rasa Sabaliauskaitė


Julius Juodakis


Dominykas Murza

Supervisor

Miglė Čiurinskaitė

Luxembourg

Competitors


Jean-Marc Raffaello Matteo Furlano


Raffaël Marth


Frédéric Gerhards

Jury


Thierry Marx


Alexandre Salsmann

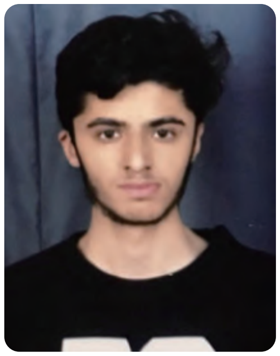

Sabrina Rodrigues Freitas

Nepal

Competitors


Garima Rokaya


Divya Prakash Yadav


Awahan Sapkota


Aadim Nepal

Jury


Surgeon BC


Dilip Bhattarai

Netherlands

Competitors


Berit Kooter


Dante Bosgoed


Lucas van Osenbruggen


Xanta van Ruiten

Jury


Ange Taminiau


Christine Moene


Leonie Cazemier


Nienke Nobel


Roel Baars

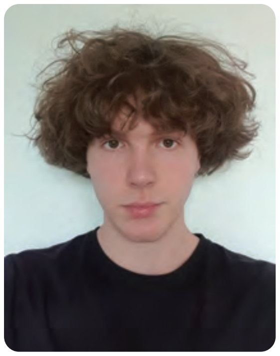
North Macedonia

Competitors


Stevan Bogdanov

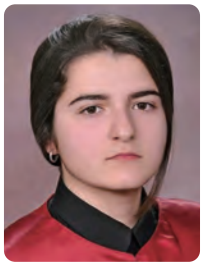

Jovana Stojcheska


Verica Gjeorgieva


Filip Petrovski

Jury


Lozenka Ivanova


Elena Rafailovska


Nikola Hadji Petrushev

Supervisors

Aleksandra Cvetkovska-Gjorgjievska / Biljana Miova / Cvetanka Cvetkoska / Maja Mladenova
Slavcho Hristovski / Oliver Tushevski / Sara Cvetanoska / Marija Trencheva

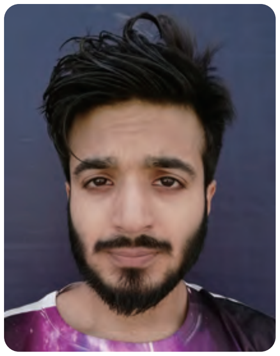
Pakistan

Competitors


Ahmed Kashif


Musa Salar


Umar Jamshad


Muneeb Waqas

Jury


Asma Imran


Asma Rehman

Philippines

Competitors


Sean Red Cruz Mendoza


Jeremy Ace Feliciano Ng


Elizabeth Rae Santiago Peralta

Jury


Ronald Allan Lopez Cruz

Poland

Competitors


Martyna Aneta Borak


Barbara Anna Buchalska


Jakub Krzysztof Kwiatkowski


Maciej Mateusz Zurowski

Jury


Takao Ishikawa


Lukasz Banasiak


Jakub Baczynski


Piotr Bernatowicz

Russia

Competitors

Oleg Kuzmenko

Maksim Kovalev

Ruslan Nagimov

Nikolai Nikolaev

Jury

Alexander M. Rubtsov

Calina Beliakova

Evgenii Shilov

Viktoriia Lavrenova

Saudi Arabia

Competitors

Zainab Al-Alawi

Maryam Ghalib Alhashim

Basil Habiballah

Omar Banjar

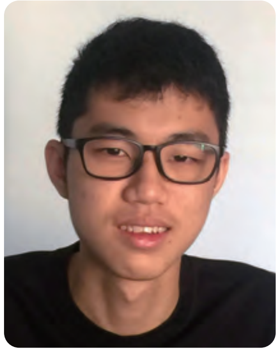
Jury

Yousef M. Al-Shahrani

Singapore

Competitors

Kate Lu



Cheng Yat Tan

Ziwei, Dewey Lin

Xinzhi Qiu

Jury

Dominic Jian Chien Heng

Beverly Pi Lee Goh

Ngan Kee Ng

Izavel Shu Yih Lee

Supervisors

Keene Lee / Renata Triani

Slovenia

Competitors

Ivo Prelog

Matic Smolič

Anamarija Mežnar

Dominik Primožič

Jury

Katja Ota

Mojca Ota

South Korea

Competitors


Dale Kim


Geono Kim


Jae Won Yoon


Heewon In

Jury


Jae Geun Kim


Kyoung Sang Cho


Kwang Pum Lee


Yong-Hwan Moon

Sri Lanka

Competitors


Sauni Ruwanima Malavige


Dhanapala Mudiyanselage Nilushi Navodya Dhanapala


Samidhi Manthilani Munasinghe


Arulanantham Abbinanthan

Jury


Hiran Samarasinghe Amarasekera


Nissanka Kolitha De Silva

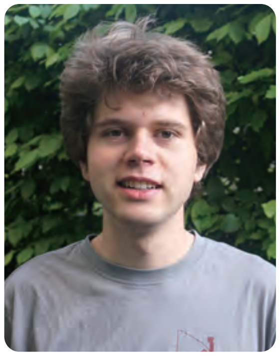

Jayantha Wijeyaratne

Switzerland

Competitors


Anna Salud


Orna Tabea Frohnert


Kaspar Merlin Lanz


Kalila Hörler

Jury


Thomas Schneeberger


Lorenz Widmer


Sarah Hilfiker


Linus Meier


Andrea Audétat


Nina Kathe


Alain Fauquex

Syria

Competitors


Majd Nasra


Aows Dayoub


Basel Alkanjo


Batoul Amraya

Jury


Abdul Qader Abbady


Nazir Khalil


Abdulsamie Hanano


Antonious Aldaoude


Mohamad Bashir Arnous


Chadi Soukkarieh

Supervisors

Razan Arour / Dania Kabbani

Tajikistan

Competitors

Munisa Pulatova Muminjonovna

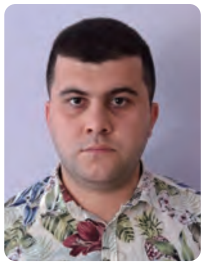
Shams Davlyatbekov Vatanshoevich

Zokirjon Mamadjonov Suhrobovich

Akramzoda Nazira Jamshed

Jury

Iskandar Ghayurov Sayvalievich

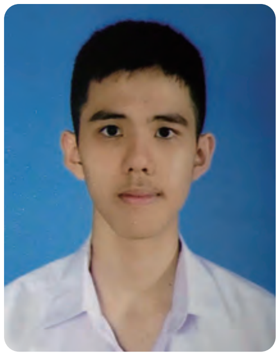


Dilovar Turaev Avrotovich

Thailand

Competitors


Arthitaya Sima-Aree


Bhumpanya Chaisrisawatsuk


Kantawich Piyanirun


Kittitach Rattanawannachai

Jury


Supachitra Chadchawan


Sittiporn Pattaradilokrat


Chatchawan Jantasuriyarat


Chatchawan Chaisuekul


Kittikhun Wangkanont


Panick Weingchai

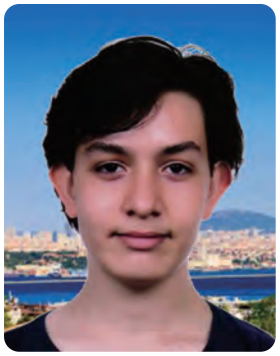
Supervisors

Charoensak Mueangkaew / Worachet Promruk

Turkey

Competitors


Yiğit Can Ateş


Anıl Deniz Yılmaz


Ali Berdan Tüney


Gencay Kaan Polat

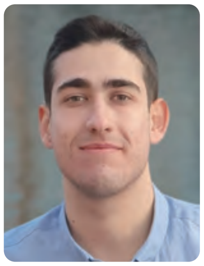
Jury


Leyla Açık


Yusuf Menemen


Batuhan Karakuş


Ismail Hakkı Dur


Atahan Durbaş

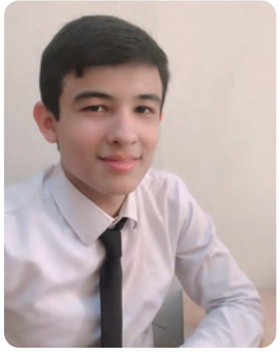

Sezgin Er

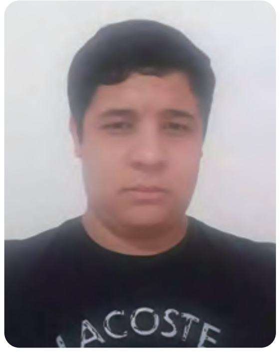

Ahmet Umur Topçu

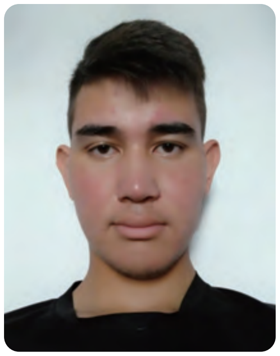
Turkmenistan

Competitors


Abdullah Deryayev


Azat Meredow


Rahym Rahymov


Hoshgeldi Hallayev

Jury


Atajan Rahmanov

Supervisor

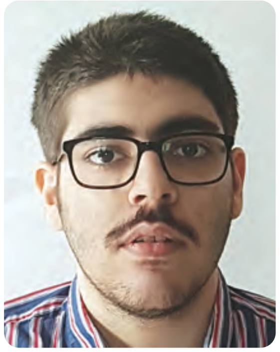
Berdimyrat Yazhanov

United Kingdom

Competitors


Noah Joseph Baker Grodzinski


John Mulford


Seyed Sepehr Mousavi


William Henry Ty Leung

Jury


Andrew Treharne


Matthew Johnston


Jiaqi Chen


Rebecca Peel


Katherine Lister

A | D
E | J
K | N
O | S
T | Z
Other Participants

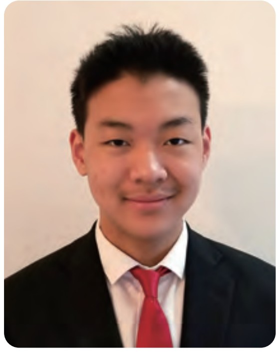
United States of America

Competitors


Nithin Parsan

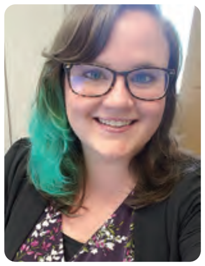

Derrick Liang


Albert Zhang


Judson Lam

Jury


Kathy Frame


Michelle King

Uzbekistan

Competitors


Barno Kuranboy Qizi Rustamova


Sardor Eshqul ugli Tojiboev


Saida Atabekovna Valijonova


Ravshanbek Mirgolib ugli Karimov

Jury


Davron Dilmurot ugli Tukhtaev


Nodirbek Islom ugli Kholikulov

Supervisor

Oybek Rustamboyevich Abdullaev

Vietnam

Competitors


Dong Ngoc Ha


Ha Vu Huyen Linh


Nguyen Thi Thu Nga


Ho Viet Duc

Jury


Mai Sy Tuan


Nguyen Quang Huy


Dinh Doan Long


Trieu Anh Trung


Le Thi Phuong Hoa


Nguyen Thi Hong Van


Le Ngoc Hoan


Vu Thi Thu


Le Hong Diep

Supervisors

Tran Duc Long / Bui Phuong Thao

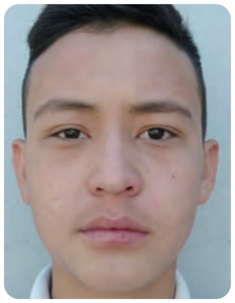
Afghanistan

Competitors


Tania Shams


Anara Hussaini


Rozina Haidary


Nizamuddin Mohibi

Jury


Qurban Ali Waezi

Canada

Competitors


Param Patel


Jia Ni Jenny Wu


Jessica Yu


Jiashen Jayson Tian

Jury


Sylvie Bardin

Croatia

Competitors


Ema Moskatelo


Olga Jerkovic Peric


Mihaela Simunic

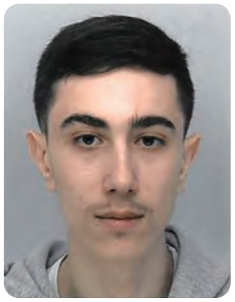
Jury


Andreja Lucic

France

Competitors


Rayan Pierquet


Eilone Nahon


Isaure Berard


Eve Rouquette

Jury


Jacques Chanteloup

Norway

Competitor


Heidi Berg

Jury


Malin Benum Roe

El Salvador / Ibero-American Countries

Jury


Huilhuinic Angel Orantes


Abizaí Clemente Chinchilla


Fátima Alejandra Hernández

IBO Challenge 2020

PART 2

International Group Project

International Group Project Timeline

Date	Event
25 July, 2020	Deadline to apply for Project Facilitators (Invitation sent to all accepted IBO2020 Nagasaki volunteers)
31 July	Competitors: deadline to submit their topic preferences
3 August	Grouping of competitors finalized (All competitors were assigned to a group of their first choice)
8-9 August	Grouping announced to Project Facilitators
13 August	The International Group Project begins • Slack used for communication between facilitators and organizers • Facilitators were asked to submit Progress Check Sheet every two weeks to be reviewed by Supporting Faculty Members
Early October	Project Facilitators recorded their meeting for archiving
31 October	Deadline to submit project deliverables
20 December	Results announced on the website

“Imagine, discuss, and propose the future of biology through current worldwide biological challenges.”

General Data

Participating Countries / Regions	52
Competitors	202
Group Project Facilitators	37
Initial Number of Group Project Teams • Some competitors dropped out due to their academic responsibilities, vacations, time difference, connection issues, etc. • By the end of August, three teams were merged into other teams (46 remaining). 20 competitors had dropped out in that period.	49
Teams that successfully submitted the final deliverables by the deadline (November 5th)	39
Competitors who finished the group project	approx.130
Competitors who did not participate / quit halfway*	approx.70

*Due to illness, time difference, conflicted responsibilities, etc.

IBO Challenge 2020

International Group Project

Human beings in the 21st century are facing various challenges that are more global and interdisciplinary than ever in history. We believe that biology is a key academic field in resolving these highly complicated issues and sustainably developing our society, as it deeply encompasses both knowing ourselves and knowing our surroundings.

Through the IBO Challenge 2020 International Group Project, we aimed to provide students with valuable opportunities to imagine, discuss, and propose the future of biology together with fellow young biologists across the world. Even during the COVID-19 pandemic, students had a chance to form life-long relationships with other IBO community members and obtain professional feedback on their deliverables.

Group Structure

Each group initially consisted of a maximum of five students, all representing different countries. Utilizing online communication tools (emails, messages, video calls, etc.), each group tackled a unique project that focused on a task and topic of their choice (see Tasks and Topics).

Project Facilitators, selected from accepted IBO2020 volunteers, oversaw the project in each group. They also worked as a bridge between the students and the IBO2020 Organizing Committee (organizer).

Tasks and Topics

Prior to the event, each student selected a) the task they would like to carry out and b) what topic area they would like to focus on. Based on these preferences, we matched students with others interested in the same tasks and topic areas.

Tasks (Choose One)

A. Plan and propose a creative experiment on a selected topic.
B. Discuss and propose how biology can address a selected topic.

Topics (Choose One)

1. Infectious Diseases
2. Biodiversity and Oceans
3. Genome Editing
4. Evolution

Project Deliverables

At the end of the project, all groups were asked to create and digitally submit their proposal in either of the following two forms. We purposefully didn't specify any layout or format requirements- the participants had the freedom to design their own work.

- PowerPoint: maximum 4 pages
- Poster (in PDF format): maximum 1 sheet

While groups could include photos or other static visual aids in their proposal, they were not allowed to include videos.

Awards

Top Groups:
After multiple evaluation sessions by professional scientists, we awarded 11 groups with top performances with some special prizes.

All Groups:
Submitted deliverables are available on our official website (password required). In addition, all groups received professional feedback on their works.

Co-host

The Ocean Policy Research Institute (OPRI),
The Sasakawa Peace Foundation

Covering 70% of the surface of the earth, the oceans are a treasure shared among all of humanity, and one on which we depend for our survival. The Ocean Policy Research Institute (OPRI) thus aspires to become a "think-and-do-tank," to address the many challenges to the ocean and thereby achieve the mission of the Sasakawa Peace Foundation to establish a new system of ocean governance. To do so, we will expand our research and advocacy activities, disseminate relevant information, facilitate necessary measures, and promote networking opportunities. Japan cannot solve all the ocean's problems on its own, however, which must be tackled from a global perspective through initiatives that are based on international discussion. We therefore sincerely wish to collaborate ever more closely with like-minded stakeholders who are also concerned about the oceans.

Feedback to the Participants

First, I would like to thank everybody who participated in the IBO Challenge 2020 International Group Project. Despite various difficulties, a lot of students worked very hard on their own project. As a head of this project, I'm very happy and honored to have been able to provide this opportunity of intercultural exchange through biology, which is one of the very important missions of the IBO.

As many of you know, our original plan was to host the group project at a beautiful beach in Sasebo city, Nagasaki. While we tried to provide students with the similar experiences through this remotely-hosted group project, it is not hard to imagine that a lot of students and country coordinators were confused by some big differences between the two. Since we hosted the project for a much longer period, some students were forced to drop out in order to prioritize their academic responsibilities. Reading the post-event questionnaires, we also found out that many groups had difficulties with time-zone difference, internet connection, or personal and nationwide issues. Even during this completely-remote event, we felt the negative effects of the COVID-19 pandemic everywhere. I expect that those valuable feedback will be carefully analyzed and utilized for the future IBO events.

Throughout the project, I was amazed by the students' enthusiasm and hard work on their topic, selected from (1) infectious diseases, (2) biodiversity and oceans, (3) genome editing, and (4) evolution. Even though it was not a smooth-sailing for most groups, I could easily see how hard they worked on their research and final deliverables. Some strongly reflected the students' cultural backgrounds. Some were at the level of a graduate school or even an academic conference. We enjoyed reading and evaluating every single one of them. If I need to say one thing, however, I believe that a lot of deliverables could have been more concise with less text. While I understand that they wanted to put everything they learned in a limited space, this is something to keep in mind in the future.

When we first planned this project, we were expecting some deliverables to be wild and eccentric while staying logically-sound, just like a thing we see in a good Sci-Fi movie. However, against our expectations, almost all deliverables were both realistic and feasible. Eccentric ideas, such as an idea that creates a huge breakthrough (Task A) or an idea that defines the perfect (but a bit unrealistic) state of the world (Task B), are always essential for the advancement of science. For example, genome editing techniques are products of these eccentric ideas. I believe it is young and talented students like the IBO participants who are expected to provide more "eccentricity" into the world.

Finally, I would like to thank all the project facilitators, mainly IBO alumni, who volunteered and supported the students during the project. After the event, a lot of students expressed that they could enjoy this difficult and demanding process because of the presence of the facilitators. I hope the competitors this year will also stay involved in the IBO just like them. (Akira Katoh)

Topic 1

Kazuhisa Ota

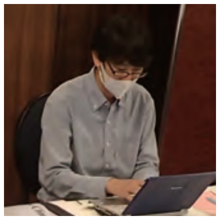
Topic 1 (infectious diseases) had 14 deliverable submissions; six from Task A and eight from Task B. Due to the current COVID-19 pandemic, six groups focused on the coronavirus as their target disease. On the other hand, their discussions were extremely diverse, from a disease model simulation to an effect of climate change. While the wide range of content made the evaluation quite challenging, we could enjoy the process from the beginning to the end because of that.

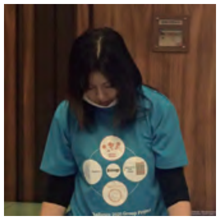
Picking a discussion theme for the Topic 1 might have been easy for most groups, as there are countless numbers of news related to infectious diseases and the demand of society is clear. On the contrary, I could tell that a lot of groups struggled to properly understand the current research or to find a creative solution, because the field is extremely competitive and there are almost too many articles you could base your discussion on. Nonetheless, I could see the students' hard work, passion, and talent in a lot of deliverables, which deserves our sincere applause.

Not only infectious diseases, but a lot of fields are now in need of decision-making and actions on a global scale. I expect all participants to take advantage of this experience and play an important role globally in the future.

Topic 2

Ko Tomikawa





Junko Toyoshima

For themes in Topic 2 (biodiversity and oceans), most groups chose problems that are recently becoming serious on a global scale, such as climate change, microplastics, and biodiversity. Each group explored solutions to the selected problem using their own original approaches along with some previous studies, and I'm proud to say that the quality of deliverables was very high. Additionally, all deliverables were well designed and composed, making their hard work attractive. The reviewers enjoyed reading them. While the feasibility of the proposed experiments and analyses were uncertain in part, we are excited about their work and looking forward to seeing their future improvement.

Feedback to the Participants

Topic 3

Akira Katoh

As you can see from this year's Nobel Prize in Chemistry on CRISPR-Cas9, genome editing (Topic 3) might have been the hottest and most familiar topic for the IBO participants. This topic attracted the largest number of participants, creating 19 groups to start with. Amongst them, 14 teams successfully submitted the final deliverables, which were all well-researched. Some had a great design, and others strongly reflected group members' diverse cultural background. We enjoyed evaluating them a lot.

For the Task A submissions, we primarily focused on the uniqueness in their viewpoint. While realistic and well-constructed, most of the deliverables were, unfortunately, not unique or creative enough to blowing our experts' mind. However, some groups took a step deeper and discussed the improvement of technique itself, which impressed the reviewers greatly.

For the Task B submissions, we valued their discussion section the most. All groups did a great job on analyzing the current issues, but not a lot of them used the analyses for creative discussions. Many groups could have had much higher evaluation if they had deeper and more creative discussions.

Even though genome editing is a relatively new tool for genetic engineering, a lot of research have already been done at this point. Society is waiting for the next techniques. I hope this event's participants will soon play a main role in discovering and developing the next research areas and techniques.

Topic 4

Shinichiro Sawa

Koichiro Awai

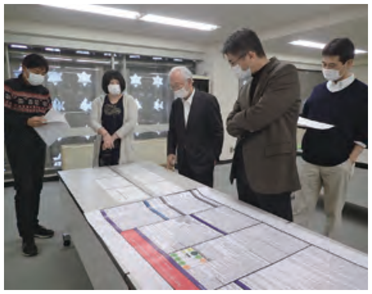
Topic 4 (evolution) had five deliverable submissions; two in the Task A, and three in the Task B. Focusing on animals like humans or birds, they discussed various topics such as problems related to behavioral ecology, effect of microwaves, and evolution of microbiota and diseases. All of them were well thought-out and discussed.

Since students had to either propose a creative experiment on evolution (Task A) or address an issue within evolution (Task B), we were concerned that the topic would be too difficult for students from the beginning to the end. We were expecting something simple because of those concerns, but they all betrayed us in a good way. All deliverables were well-constructed, and made us re-confirm where the field of evolution stands in the context of biology.

I believe the students will play an active and important role in society in the future. Even if that role is not related to biology, I wish that they will keep this evolutionary viewpoint somewhere in their mind.

IGP Results

The Awards of Excellence Given to groups with the best performance in each topic.	Topic1: 1A01	➔ page 106
	1B02+1A04	➔ page 120
	Topic2: 2A02	➔ page 136
	Topic3: 3A01	➔ page 146
	3A03	➔ page 150
	Topic4: 4B03	➔ page 180
The Ocean Policy Research Institute Award Given to one Topic 2 group with outstanding performance.	2B03	➔ page 142
The IBO2020 President Award Awarded by the president of the IBO2020 Organizing Committee, Dr. Makoto Asashima, to his choice of an outstanding group.	3B01	➔ page 162
The "Beyond Bio" Award Awarded for the group's creative solution beyond biology.	2B05	➔ page 144
The Uniqueness Award Awarded by Dr. Shinichiro Sawa, for the group's unique approach toward the project.	4A02	➔ page 174
The "Making Great Sense" Award Awarded by Dr. Akira Katoh, for the group's innovative idea related to the biosensor.	3A07+3B05	➔ page 156



About Participation Prizes

We have prepared participation prizes for everybody who took part in the International Group Project. After some discussion about what would be the best gift, we decided to give out handcraft resin-embedded specimens of two East Asian species.

Japanese goose barnacle

Scientific name:
Capitulum mitella (Arthropoda: Crustacea)
Distribution:
The western Pacific from the Japanese Archipelago to the Malay Archipelago.
Notes:
The Japanese goose barnacle, which looks like a shellfish, actually belongs to Crustacea. They have appendages which correspond to the legs of shrimps inside the hard shell. This species is also used for food in Japan. The specimen is designed to show both the external hard shell and the internal appendages.

Japanese maple

Scientific name:
Acer palmatum (Angiosperms: Sapindaceae)
Distribution:
East Asia from Japan to China.
Notes:
This species is the most common maple species in Japan and is representative of Japanese autumn leaves. In autumn, the leaves of the Japanese maple trees turn Japanese mountains red. This species has winged seeds, which are dispersed by wind. The specimen is designed to show the leaf and the seeds.

Questionnaire Summary

We asked both competitors and project facilitators to submit a post-event questionnaire within two weeks after the conclusion of the project. We collected responses from all but one of the facilitators and 76 out of 202 competitors.

According to the facilitator's responses, approximately 130 students successfully finished the project. The rest of the competitors, about 70, didn't participate in the project from the beginning or dropped out halfway. We started the project in mid-August with 49 groups, but had to restructure some groups and reduce the total count to 46 in September because of the increasing number of competitors dropping out from those groups. At the end, 39 groups submitted the final deliverables.

Even though the advancement of technology enabled us to hold this type of event virtually, most groups faced some major difficulties, such as time zone differences and internet connection issues, especially when they tried to schedule online meetings. Moreover, many groups also struggled with competitors' conflicting academic responsibilities or maintaining their motivation after the IBO exams. These factors must be carefully considered for future events.

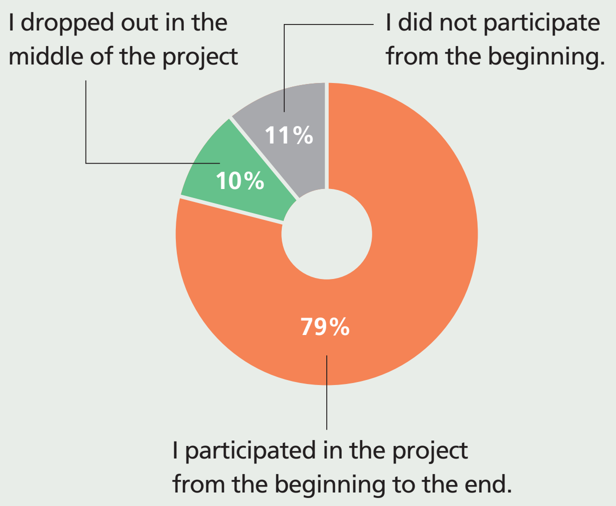
Nonetheless, a lot of competitors and facilitators commented that this project was a valuable opportunity for them, where they could interact and form friendships with like-minded people from around the world in the midst of lockdowns and travel restrictions.

We hope that this project could provide passionate and talented youth from around the world with a platform to express their creativity and to form meaningful friendships.

(Ryoko Utsumi)

Competitors

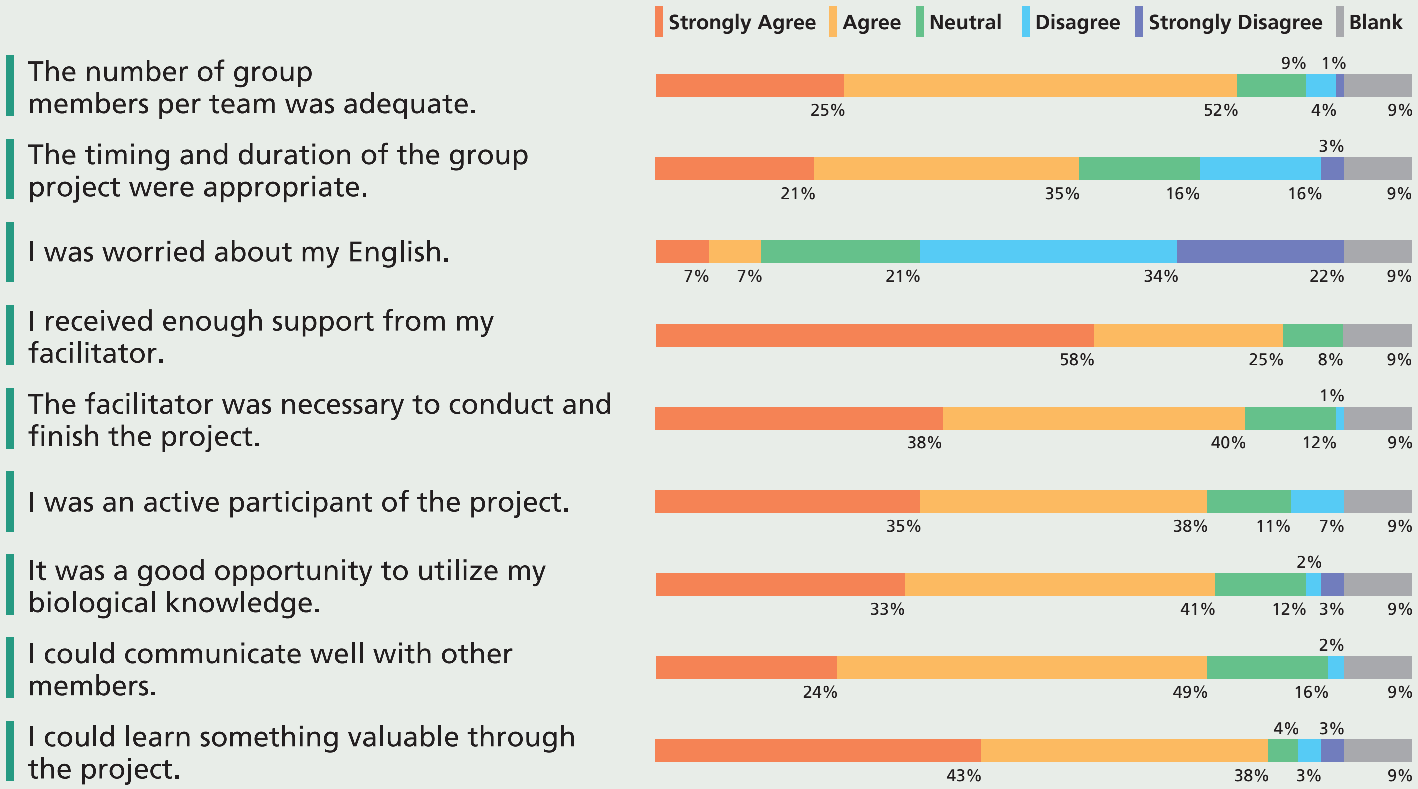
Your Participation Status



Please tell us the reason(s) why you did not participate / dropped out.

Questionnaire Summary

Competitors



Which factor(s) influenced your participation level?

Which factor(s) influenced your communication level with other members?

Comments

It was a wonderful experience to learn from other team members while staying safe during this pandemic.

It was an amazing opportunity and I really learned so much from this experience. I specifically enjoyed being able to talk to students who were ambitious and passionate about biology all over the world, connect, and make a project together. Thank you so much for organizing such an event amidst the COVID-19 crisis.

I believe the internet connection did influence the communication level negatively with one of our team members, as the team member did not have access to a stable internet, which made it impossible to hear the team member. However, we solved this by using the chat function on Zoom. The time zone differences were no problem at all.

All in all, I really liked and enjoyed this concept of an International Group Project. I hope it is a project that will continue within the Biology Olympiads the coming years as it is valuable both academically and socially. Thank you for organizing it this year.

I am beyond grateful that I got to be a part of this project. It was a really interesting task and a fantastic opportunity to make friends with like-minded individuals in different countries in the world. Thank you, IBO Challenge 2020 for making that happen!

I would like to thank very much for the opportunity to participate in the project as it was really great to make connection with participants from all over the world. It was for the first time in my life when I worked together with people from different countries on the same project and it was an amazing experience. If the next-year IBO will be held virtually, I'd like to suggest organizing of similar activity as was the International Group Project 2020.

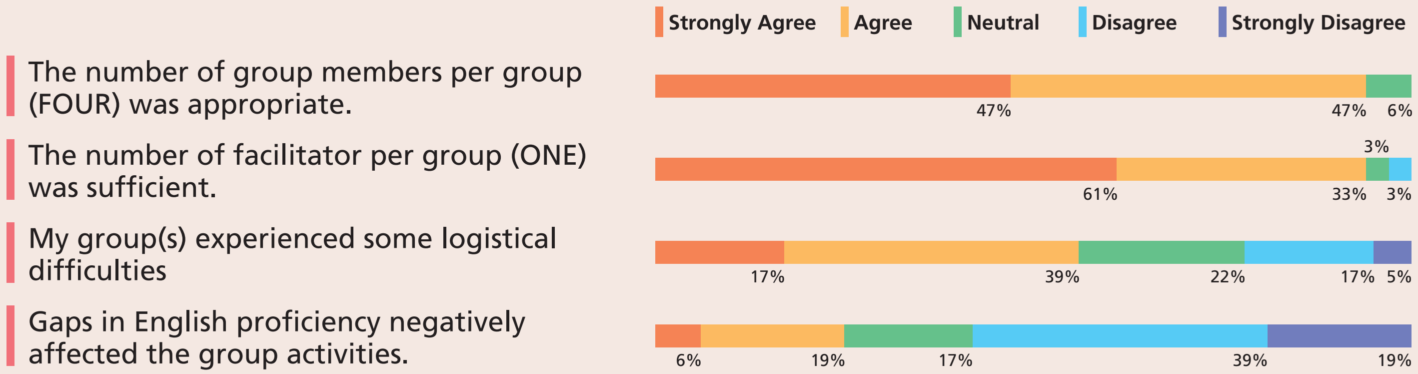
I'd rather have the group project being worked intensively one or two week after the IBO-C Test than having it span over 3 months after the test. For me it gives extra anxiety. Nevertheless, i love the idea and i want to communicate more with other competitors in other opportunities.

I would like to sincerely thank the people responsible for the IBO2020 international group project. For as this project has helped me to develop and learn crucial skills. And this project has also let me meet wonderful people from different countries which lead to exchange of knowledge, tradition and much more. This project has been a great experience and I hope the best for all.

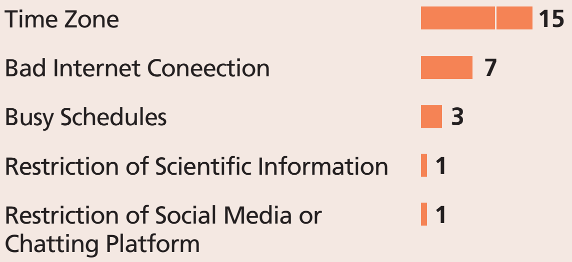
It was a wonderful experience to learn from other team members while staying safe during this pandemic.

Questionnaire Summary

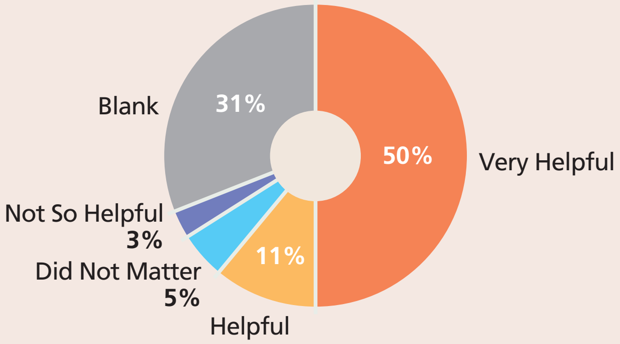
Facilitators



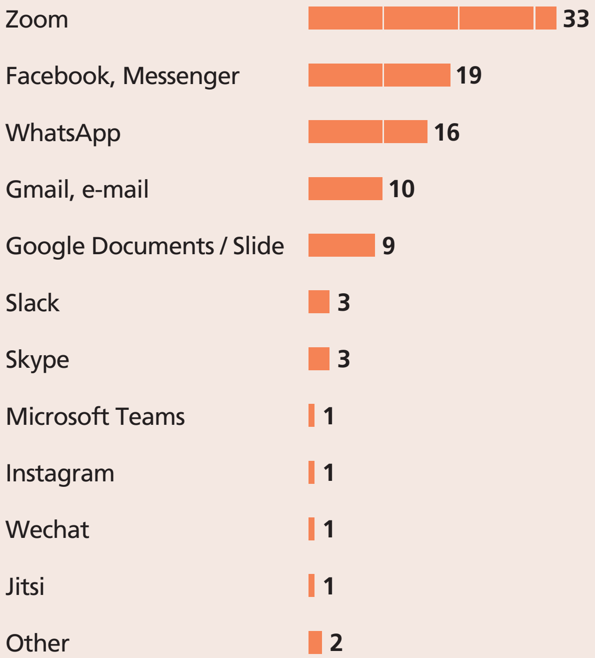
What kind of logistical difficulties did you experience?



If you acquired a Zoom account from us: how helpful was it?



Please list all the communication tools your group(s) used.

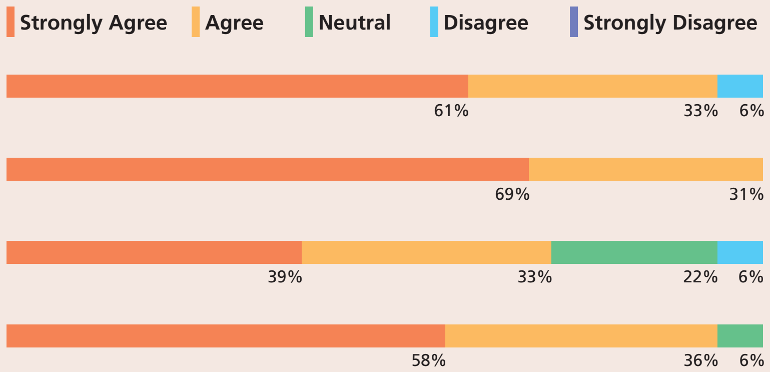


This experience as a facilitator was meaningful to myself.

Support from the organizers (via Slack, email, etc.) was helpful.

I would work as a facilitator even without the payment.

I'd like to sign up to be a facilitator again in the future.



Comments

This IBOC group project is definitely worth doing for participants. Working internationally on scientific theme (for such a long term) with strongly passionate peers is very rare and valuable opportunity for high school or university students.

The previous physical IBOs didn't have group projects, but this online version did. As such, I'm really impressed by the creativity and initiative some people took, which is far more interesting to see than the ability to just answer question papers.

Students actually got to experience SCIENCE and its ambiguity, facing the complexity they have to deal with. It is different from following written protocols and reading textbooks, because you actually have to start from scratch and dive into the scientific enterprise. This could be a nice complementary activity to IBO.

In this event, students of different nationalities and cultures have to put their differences aside and work together as a team. Although, I believe it would have been better to be there physically, I think this element is something fresh, exciting and also quite challenging.

I like how this project brings together a small group of people from different countries and allows them to bond together over a sustained project.

I would like to thank from the bottom of my heart all including IBO 2020 committee for their tremendous efforts to deal with today's situation and to provide such a wonderful opportunity for participants this year!

Overall, I am grateful to have been part of this project and I hope to participate in many more international events.

Project Facilitators

Consisting mostly of IBO alumni, project facilitators played a highly crucial, supportive role throughout the International Group Project. Assigned to one or two groups, each facilitator was tasked to handle various responsibilities for more than two months. Sometimes they acted as a "tour guide" for their group by asking insightful questions. Other times, they were mentors who introduced useful articles or shared their own research experiences. Most importantly, they were great supporters who made sure that the group activities proceeded as smoothly as possible.

During the project, group progress and documents were shared via cloud services, which all members from different time zones could access whenever most convenient. Meetings were held through online video conference tools.

The effort and contributions of facilitators were essential to the success of this completely online project. When a group member couldn't make it to an online meeting, most facilitators recorded and shared the meeting or created meeting notes for them. If a member struggled with communication in English, a lot of facilitators supported them by setting up a private meeting before or after a main meeting or sending separate emails in easier English to help them participate in the discussions more actively. Many operations and supportive methods were, in fact, taking advantage of the online nature of the event.

Here, we would like to thank all facilitators who generously offered their time and energy despite their multiple academic and non-academic responsibilities.

1A01

Jiwoo Nam
(South Korea)

I'm able to deliver my opinion in English fluently, but I can lose some of biological terms, so I'm studying them.

1A02 & 1B07

Lilian Demolin
(Belgium)

Med student and IBO competitor in UK 2017 and guide in Hungary. IBO community is the best in the world!!!

1A03

Valentino Sudaryo
(Indonesia)

Greetings from a fellow IBO 2014 competitor and IBO 2017 volunteer! Looking forward to a great time with all of you.

1A04 + 1B02

Akhila Imantha Nilaweera
(Sri Lanka)

As a past participant in IBO 2015,It's with great pleasure I'm joining this year's programme.

1A05 & 1B03

Birnur Sinem Karaoglan
(Turkey)

I'm quite excited about the dawning of new ideas and friendships which will be resulted from this IBO CHALLENGE!!!

1A06

Pavel Loginovic
(Lithuania)

1A07 & 1B01

Parmida Sadat Pezeshki
(Iran)

with fresh ideas, motivated minds, and big dreams, once we get started, we get ahead!

1B04

Nahida Harim
(Belgium)

IBO 2019 was the best moment of my life so I can't wait to join this new project and meet new people !

1B05

Jenna Tynninen
(Finland)

I'm a sporty golfer and future med student from Finland. As a former IBO competitor, I love the unique IBO community.

1B06

Ayaka Eguchi
(Japan)

I was a competitor of IBO2017. I'm sure this online project will be a very good experience for you. Have fun! :)

1B08

Anitra Zīle
(Latvia)

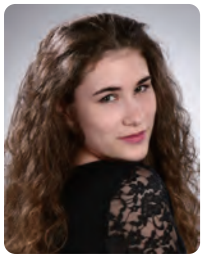
to be or to bio? - both

2A01

Diego Eduardo Kleiman
(Mexico)

My name is Diego Kleiman, from Argentina. I participated in IBO '14 and I am excited to volunteer for this online event!

2A02 & 2B04



Alexandra Nóra Piti
(Hungary)

Thank you to the IBO community for organizing this online event! I hope everyone will enjoy creating their proposals!

2A03

Rawand Fatah Abdalla Aziz
(Iraq)

2A04

Edwin Alejandro Chávez Esquivel
(Mexico)

Hi I'm Ed, from Mexico. I'm here because I want to support you in this edition of IBO, thank you for the opportunity c:

Project Facilitators

2B01

Anastasiya Valakhanovich
(Belarus)

Hi, I'm Anastasiya and I want to share my love for Biology with participants, volunteers, and the organization team!

2B02

Egemen Erbayat
(Turkey)

2B03

Mithun Diumantha Samaranayake
(Sri Lanka)

2B05

Maria Janine Juachon
(Philippines)

Hello everyone! Let's try to make the best out of our current situation! :)

3A01 & 3B09

Christopher Wang
(USA)

Hi! I'm Chris from the United States. I competed in the 2019 Hungary IBO and am a current neuroscience major in college.

3A02 & 3B04

Auddithio Nag
(Bangladesh)

Hi everyone! Auddithio here, although most friends just call me Audi. Looking forward to e-meeting all of you soon!

3A03 & 3B06

Diego Maldonado de la Torre
(Argentina)

Hi! My name is Diego, I am from Tijuana, Mexico. It is an honor to be a facilitator at IBO Challenge. Let's have fun!

3A04

Atahan Durbas
(Turkey)

I have been to IBO in 2016. It was an unfinished story for myself, that's why I am here to complete your story together!

3A05 & 3B01

Martyna Petrulyte
(UK)

3A06

Stuti Khandwala
(India)

Extremely excited to be a part of the first ever virtual IBO; looking forward to different but life-long relationships!

3A07 & 3B05

Anh Thi Minh Tran
(Vietnam)

Thank you to the IBO community for organizing this online event! I hope everyone will enjoy creating their proposals!

3A08 & 3B08

João Victor Silva Ribeiro
(Brazil)

Hi, guys.My name's Victor, 19 yo and I'm a medical student. I love doing cultural exchange and learning new languages :)

3A09 + 3B02

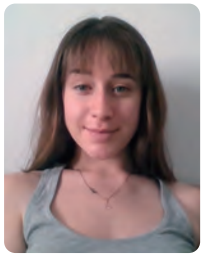
Györi Laszlo András
(Hungary)

Hey All! I'm Andris, and without IBO I doubt that I would have found my passion in biology. And I wasn't a competitor ;)

3A10

Shino Suda
(Japan)

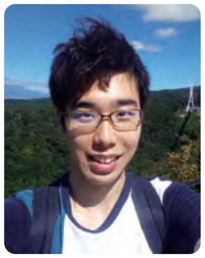
3B03



Danai Theou
(Greece)

I hope the students have as much of a great experience as I did when I was a competitor!

3B07



Eiichiro Kanatsu
(Japan)

4A01

Tomoyuki Wakashima
(Japan)

4A02

Vaidehi Devendra Rakholia
(India)

4A03

Uzuki Horo
(Japan)

4B02

Dominik Kopčak
(Slovakia)

4B03

Tymofii Sokolskyi
(Ukraine)

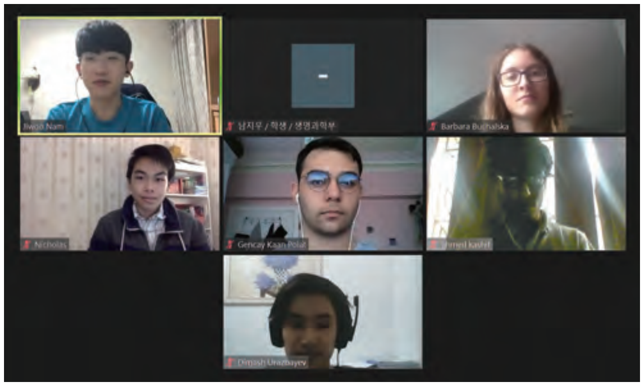
Hi! My name is Tym and I am a Ukrainian rising fourth-year student at Duke university.

4B04

Yasna Yeganeh
(Iran)

Hi this is Yasna!I am more than happy to help you and be at your service. let's make IBO2020 unique (^_^) がんばって！

1A01 Cross Immunity of Coronavirus



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Barbara Anna Buchalska (Poland)

Dinmukhammed Urazbayev (Kazakhstan)

Gencay Kaan Polat (Turkey)

Nicholas Man Dac Vo Bui (Australia)

The Awards of Excellence

Cross Immunity of C

Introduction

December 2019 marked the emergence of the Covid-19 pandemic, the deadliest and most infectious disease since the Spanish Flu. The deadliness of the Covid-19 comes from its protean complications and high infectivity through droplet spread and even possibly airborne. Scientists throughout the world have spent significant effort for nearly a year now, trying to understand the virus' pathogenicity and find a treatment for the condition. Recognising the urgency of the situation, many countries have simultaneously and parallelly taken up the challenge to produce a vaccine. Unfortunately, we still do not have a definitive front runner vaccine and a date of vaccine availability.

The key to developing a safe and effective vaccine is an excellent understanding of the virus' antigenicity and the subsequent body immunity against it. In the same light, there are questions regarding the possible cross-immunity between the Covid-19 and other viruses, and possible conferred protection against it.

Each virus contains a large array of macromolecules including proteins, polysaccharides and lipid molecules, some of which can form epitopes and potentially activate an immune response. Our body can also mount an immune response, not just against the epitopes, but also against the tertiary structure of the macromolecules. Contact with a complex entity such as a virus will elicit multiple immune responses from our body against the various epitopes and macromolecules presented on the virus' surface, but only a small number of these immune responses are effective or lethal against the pathogen. Because of these multiple, less-specific immunological reactions, some of the reactions will be cross-shared between different pathogens which have similar macromolecules or similar tertiary structures of the macromolecules. Individually, these cross immunological reactions may not be lethal to the pathogens, but they will weaken the pathogen to a various degree, reduce their infectivity and lethality. It is possible even that a combination of these cross immunological reactions may render the virus harmless. This approach presents as a possible weapon in our armamentarium in the treatment, containment, and vaccine development against the Coronavirus. Our paper aims to determine the feasibility of the cross-immunity approach against the Covid-19.

Purpose

There are two components t
identify the viruses with the
Covid-19. The second part a
viral species can be used in p

Methods

In our experiment we used s
compare SARS CoV-2 Spike
antigens.
At first in Immune Epitope
found epitopes that are simi
used *Blast – 70%* function to
similar in 70% to SARS Co
CoV-2 epitopes were obtaine
multi-epitope vaccine against
Then we compared in BLAS
sequence (NCBI Reference S
Epstein-Barr virus nuclear a
Protein Data Bank by 1VHI
has epitopes similar to SARS
in RCSB PDB Protein Comp
strain envelope protein E seq
Bank by 6IW4 code) with Sp
from Protein Data Bank by 6
algorithm.
Furthermore, we used Struct
to search for allergens that a
glycoprotein (NCBI Referen

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[1] Tamalika Kar, Utkarsh Nars
Filippo Castiglione, David M. M
candidate multi-epitope vaccine

Coronavirus

**Nicholas Bui, Ahmed Kashif, Barbara Buchalska,
Dinmukhammed Urazbayev, Gencay Polat**

onents to our proposal. The first is to
ith the highest antigenic similarity to the
d part aims to identify whether different
sed in producing a vaccine for Covid-19.

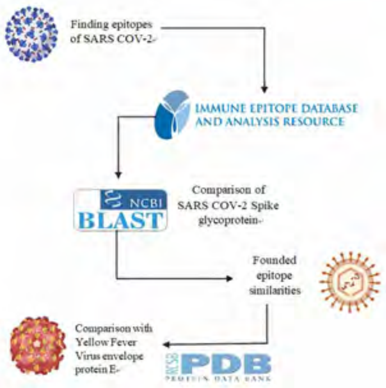
e used some bioinformatic programs to
-2 Spike glycoprotein sequence with other

Epitope Database and Analysis Resource we
are similar to those from SARS CoV-2. We
ction to locate linear epitopes which are
RS CoV-2 epitopes. Sequences of SARS
e obtained by us from an article *A candidate*
e against SARS-CoV-2 [1].
in BLAST program Spike glycoprotein
erence Sequence: YP_009724390.1) with
uclear antigen 1 sequence (obtained from
y 1VHI code), because Epstein-Barr virus
to SARS CoV-2 epitopes. We also compared
in Comparison Tool Yellow Fever Virus 17D
ein E sequence (obtained from Protein Data
with Spike glycoprotein sequence (obtained
ank by 6VXX code) using Smith-Waterman

d Structural Database of Allergenic Proteins
s that are similar to SARS CoV-2 Spike
Reference Sequence: YP_009724390.1).

arsh Narsaria, Srijita Basak, Debashrito Deb,
vid M. Mueller & Anurag P. Srivastava A
e vaccine against SARS-CoV-2

Methods



Results

Some studies have shown that there are some similarities between various antigens and Sars-CoV-2 epitopes. These similar epitopes are: Mycobacterium tuberculosis(MTB) epitopes , Penicillium chrysogenum epitopes, Leishmania infantia epitopes, Epstein-Barr virus epitopes and some epitopes of species causing severe diseases. There is some similarity between HBV nuclear protein and spike protein too.

Actually some previous studies have shown that BCG vaccine ,which is used for Mycobacterium tuberculosis, may decrease mortalitiy.

This highly similar epitope sequences give us an idea that we can use this vaccines to provide a cross-immunity. MTB can shown as an example to contribute this idea and there is strong evidences that HPV (Human Papilloma Virus) vaccines may have some possitive effects on decreasing the mortality. These strong evidences are contributing our idea that we can provide cross-immunity which is mediated by different vaccines that based on similar epitope sequences. HBV nuclear protein is strong candidate for being vaccine which can decrease the mortality rate of Sars-CoV-2.

Conclusion

As shown above, cross-reactivity between SARS CoV 2 and other viruses is a significant factor when dealing with immune responses to the virus as seen in many studies. Viral transmission can be reduced when pockets of susceptible communities show resistance to infection due to cross-immunity. This factor also needs to be considered in integrated efforts against viral spread. Populations which do not exhibit any cross-immunity are more vulnerable to SARS CoV 2. These populations can be vaccinated first and so mortality rates can be decreased.

Studies can be conducted to determine whether existing cross-immunity can contribute to herd immunity. This might reduce demands of vaccines in such populations. Dual immune responses to SARS CoV 2 which consist of cross-reactive immune cells and immune cell lines established by vaccines specific to SARS CoV 2 can counter the virus better than solely vaccine established immune responses. A more generalized immune response can be made because the cross reactive immune cell can target a different part of the virus than the vaccine established immune cell line. This can make it more difficult for the virus to mutate around the immune response. Thus complete viral annihilation can be achieved.

An indispensable part of preparing a sort of integrated management of the pandemic using existing vaccines with cross reactivity to SARS CoV 2 is to conduct detailed studies on the possibilities of immunopathology. Respiratory diseases have caused antibody dependent enhancement of disease (ADE) as observed in studies. Trial plans for the cross reactive vaccine and SARS CoV 2 vaccines could be performed concurrently to observe any damage to the respiratory epithelium/lung tissue. ADE in Covid19 could be ameliorated by using these cross reactive vaccines which are known not to cause ADE. Less trials might be needed to pass the cross reactive vaccine for use as trials have already been carried out and side effects of vaccines have already been explored.

Vaccines for yellow fever might be used as these vaccines require only one dose and provide lifelong protection. A cocktail of vaccines might also be used (BCG and yellow fever vaccine).

Inoculation should be done through normal routes used for yellow fever and other vaccines. However, the animals which most closely mirror human responses to SARS CoV 2 (syrian hamsters) can be used. Successful destruction of the virus will support the practical utility of a cross reactive mechanism to counter SARS CoV 2.

1A02 Quantification of structural response to prion disease of recombinant G127V-mutated and silenced PRPc in primary hippocampal mice cells using CRISPR-Cas9 and siRNAs

Facilitator

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Competitors

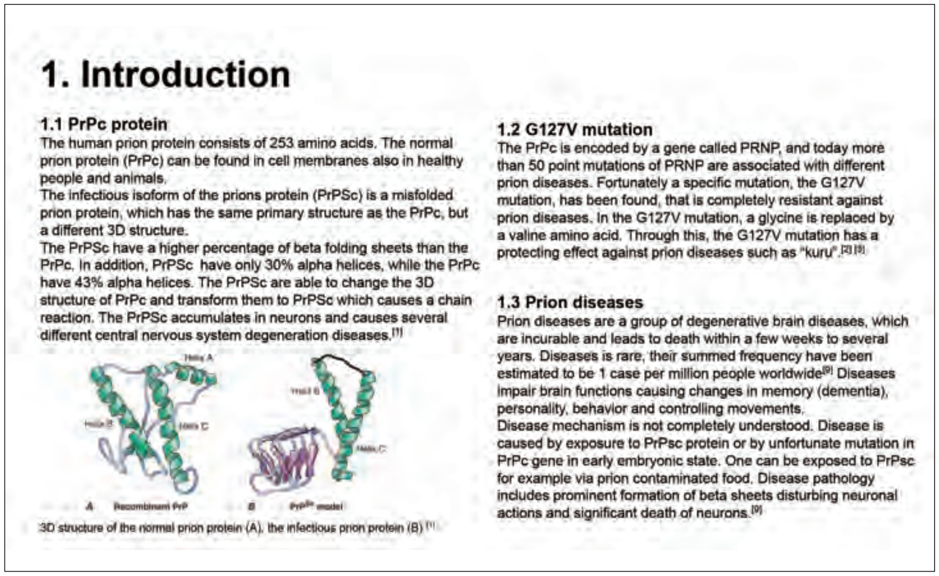
Anna Salud (Switzerland)
Sean Red Cruz Mendoza (Philippines)
Suvi Linnea Laitinen (Finland)

International Biology Olympiad Nagasaki 2020

Quantification of structural response to prion disease of recombinant G127V-mutated and silenced PRPc in primary hippocampal mice cells using CRISPR-Cas9 and siRNAs

International Biology Olympiad Challenge 2020
International Group Project

Laitinen, Suvi (Finland)
Mendoza, Sean Red (Philippines)
Salud, Anna (Switzerland)



1. Introduction

1.1 PrPc protein

The human prion protein consists of 253 amino acids. The normal prion protein (PrPc) can be found in cell membranes also in healthy people and animals.
The infectious isoform of the prions protein (PrPSc) is a misfolded prion protein, which has the same primary structure as the PrPc, but a different 3D structure.
The PrPSc have a higher percentage of beta folding sheets than the PrPc. In addition, PrPSc have only 30% alpha helices, while the PrPc have 43% alpha helices. The PrPSc are able to change the 3D structure of PrPc and transform them to PrPSc which causes a chain reaction. The PrPSc accumulates in neurons and causes several different central nervous system degeneration diseases.[1]

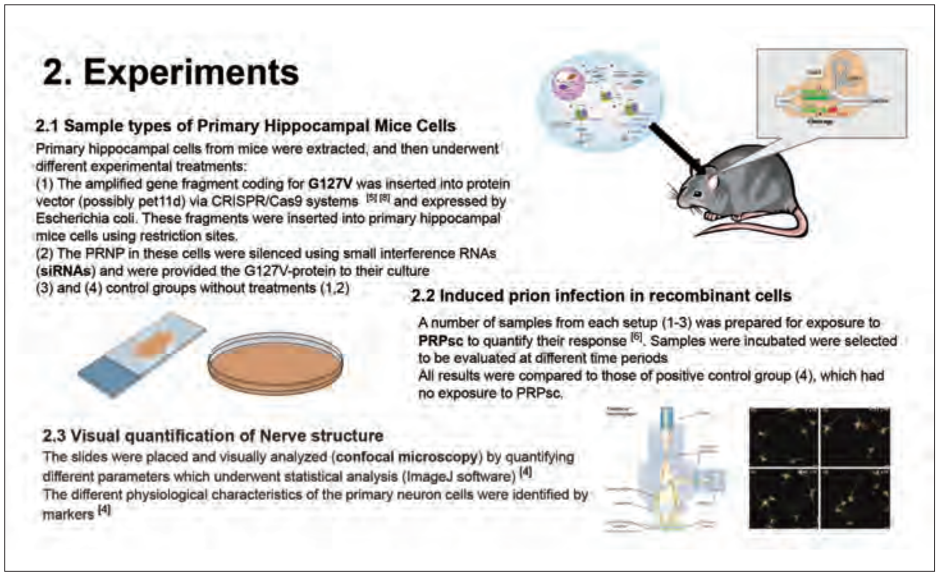
3D structure of the normal prion protein (A), the infectious prion protein (B) [1]

1.2 G127V mutation

The PrPc is encoded by a gene called PRNP, and today more than 50 point mutations of PRNP are associated with different prion diseases. Fortunately a specific mutation, the G127V mutation, has been found, that is completely resistant against prion diseases. In the G127V mutation, a glycine is replaced by a valine amino acid. Through this, the G127V mutation has a protecting effect against prion diseases such as "kuru".[2] [3]

1.3 Prion diseases

Prion diseases are a group of degenerative brain diseases, which are incurable and leads to death within a few weeks to several years. Diseases is rare, their summed frequency have been estimated to be 1 case per million people worldwide[9] Diseases impair brain functions causing changes in memory (dementia), personality, behavior and controlling movements.
Disease mechanism is not completely understood. Disease is caused by exposure to PrPsc protein or by unfortunate mutation in PrPc gene in early embryonic state. One can be exposed to PrPsc for example via prion contaminated food. Disease pathology includes prominent formation of beta sheets disturbing neuronal actions and significant death of neurons.[9]



2. Experiments

2.1 Sample types of Primary Hippocampal Mice Cells

Primary hippocampal cells from mice were extracted, and then underwent different experimental treatments:
(1) The amplified gene fragment coding for **G127V** was inserted into protein vector (possibly pet11d) via CRISPR/Cas9 systems [5] [8] and expressed by Escherichia coli. These fragments were inserted into primary hippocampal mice cells using restriction sites.
(2) The PRNP in these cells were silenced using small interference RNAs (**siRNAs**) and were provided the G127V-protein to their culture
(3) and (4) control groups without treatments (1,2)

2.2 Induced prion infection in recombinant cells

A number of samples from each setup (1-3) was prepared for exposure to **PRPsc** to quantify their response [6]. Samples were incubated were selected to be evaluated at different time periods
All results were compared to those of positive control group (4), which had no exposure to PRPsc.

2.3 Visual quantification of Nerve structure

The slides were placed and visually analyzed (**confocal microscopy**) by quantifying different parameters which underwent statistical analysis (ImageJ software) [4]
The different physiological characteristics of the primary neuron cells were identified by markers [4]

3. Methods and variables

3.1 Variables

The performance of each experimental treatment in preventing or limiting the propagation of PRPsc infection is compared using the below parameters

According to protein simulations, the presence of the G127V mutation in human PRP seems to prevent the formation of stable beta sheets and dimers in the host. This, in conjunction with other parameters indicating neurogenesis such as: development of synaptic-like contacts; neuronal polarity and axonal elongation; and life span of each cell, was used as a strategy in determining the efficacy of treatment 1 and 2

3.2 Hypothèses

Both gene editing with Crispr-Cas9, and silencing with siRNA/miRNA when compensatory protein is provided, would extend the lifespan of an individual neuron due to the significantly reduced formation of beta sheets. We hypothesize that in group 1 formation of beta sheets would not exist and it would be remarkably decreased in group 2. In addition, both groups (1,2) would have almost as high axonal elongation, formation rate of synaptic-like-connections and neuronal polarity than the positive control group (4)

3.3 Comparison of methods

Gene editing with Crispr-Cas9

- more modern technology with a lot of focus and investment
- wide array of mechanisms and methods to tackle prion infection: protein PcPr is important in neuron cell functions
- may not prevent neurodegenerative diseases as mechanisms of prion infection are not yet fully understood
- potential side effects to other structures and functions

Gene silencing

- siRNA / miRNA
- may not completely eliminate the presence of PrPc proteins that can still be infected

Development of synaptic-like contacts

Neuronal polarity and axonal elongation

Formation of stable beta sheets

Life span of each cell

4. Discussion

4.1 Discussion about experiments

In this study, only statistical information should be analyzed for several reasons. Firstly, the Crispr-Cas9 is a method which might cause other mutations in the genome of neurons and therefore resulting inaccuracy in single cells behavior. Secondly, cells would be extracted from a scatter of mice in maximum and therefore they may have even a major divergence between them.

4.2 Limitations of results

As our experiments would be performed *in vitro*, interactions between extracellular components and other cell types in brain would not have been taken into account. In addition, our inquiry is based on disease model in mice and therefore deductions cannot be straight generalized into humans. Hence, all results from these experiments would be rather suggestive than determinative.

4.3 Consideration about additional experiments

Extensive additional experiments would have to be performed to confirm the results. To inquire the effect of actual cellular environment to these methods, some experiments should be carried out *in vivo*. Moreover, with other variables more suitable to mature neurons, should these experiments be implemented to human cell samples from real patients.

4.4 Proposed methods to reduce PRPsc as possible therapies

Comprehensive further studies (considered briefly in 4.4) could lead to novel therapies for prion disease.

4.4.1 RNA-silencing

If RNA-silencing decreased significantly the progress of prion disease in extensive further examination, this method could possibly be developed to a therapy. It would be more affordable and it is rather treatment than prevention, which enables to be applied only to the patients. Nevertheless, RNA-silencing therapy would have significant disadvantages as well. Identifying patients as early as possible is crucial to this approach since there aren't any mechanism to cure damage already happen, rather to inhibit further losses. The therapy would lean to a lifelong medication and the delivery method would need to be deeply invasive such as brain pumps since both the silencing RNA and compensatory protein (G127V) has to be provided for the patient on a daily basis. However, patients are likely to accept troublesome treatment forasmuch as the disease is lethal.

4.4.2 Crispr-Cas9

Even though, we hypothesize that the Crispr-Cas9-treatment would have more significance in cellular experiments and perhaps in in vitro as well, the method scarcely could be applied as a future therapeutics. Indeed, the therapy would be exceedingly expensive; it would have to be applied in embryonic state and to everyone since there are very little or no possibility to identify future patients in forehand. In addition, gene-editing for embryos includes grave ethical concerns especially when the Crispr-Cas9 has induced unwanted changes in genome. [10] Even if those mutations are rare, in this application they would certainly cause problems due the massive number of patients.

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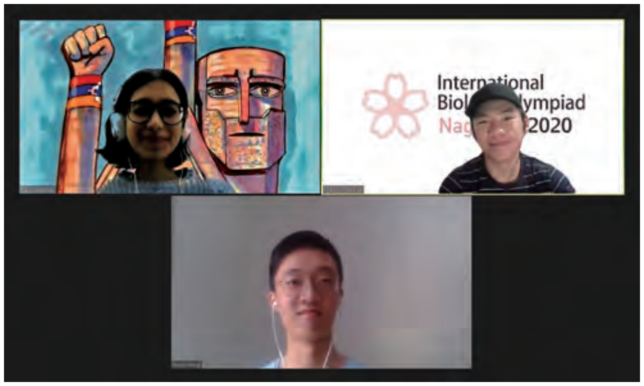
1A03 New Antibody-drug Conjugate strategy to Treat Novel SARS-CoV-2 virus



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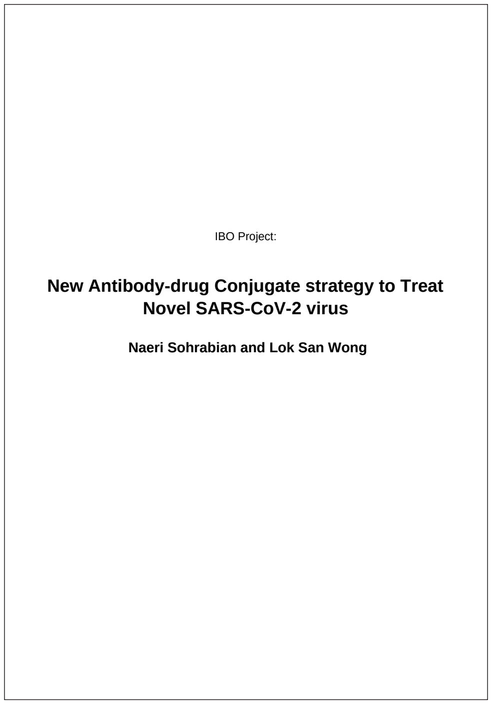
Naeiri Sohrabian (Armenia)

1

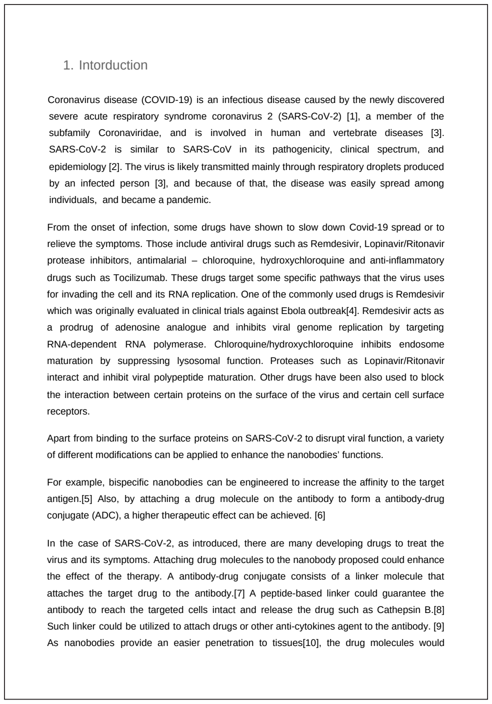


IBO Project:

New Antibody-drug Conjugate strategy to Treat Novel SARS-CoV-2 virus

Naeri Sohrabian and Lok San Wong

2



1. Intorduction

Coronavirus disease (COVID-19) is an infectious disease caused by the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], a member of the subfamily Coronaviridae, and is involved in human and vertebrate diseases [3]. SARS-CoV-2 is similar to SARS-CoV in its pathogenicity, clinical spectrum, and epidemiology [2]. The virus is likely transmitted mainly through respiratory droplets produced by an infected person [3], and because of that, the disease was easily spread among individuals, and became a pandemic.

From the onset of infection, some drugs have shown to slow down Covid-19 spread or to relieve the symptoms. Those include antiviral drugs such as Remdesivir, Lopinavir/Ritonavir protease inhibitors, antimalarial – chloroquine, hydroxychloroquine and anti-inflammatory drugs such as Tocilizumab. These drugs target some specific pathways that the virus uses for invading the cell and its RNA replication. One of the commonly used drugs is Remdesivir which was originally evaluated in clinical trials against Ebola outbreak[4]. Remdesivir acts as a prodrug of adenosine analogue and inhibits viral genome replication by targeting RNA-dependent RNA polymerase. Chloroquine/hydroxychloroquine inhibits endosome maturation by suppressing lysosomal function. Proteases such as Lopinavir/Ritonavir interact and inhibit viral polypeptide maturation. Other drugs have been also used to block the interaction between certain proteins on the surface of the virus and certain cell surface receptors.

Apart from binding to the surface proteins on SARS-CoV-2 to disrupt viral function, a variety of different modifications can be applied to enhance the nanobodies' functions.

For example, bispecific nanobodies can be engineered to increase the affinity to the target antigen.[5] Also, by attaching a drug molecule on the antibody to form a antibody-drug conjugate (ADC), a higher therapeutic effect can be achieved. [6]

In the case of SARS-CoV-2, as introduced, there are many developing drugs to treat the virus and its symptoms. Attaching drug molecules to the nanobody proposed could enhance the effect of the therapy. A antibody-drug conjugate consists of a linker molecule that attaches the target drug to the antibody.[7] A peptide-based linker could guarantee the antibody to reach the targeted cells intact and release the drug such as Cathepsin B.[8] Such linker could be utilized to attach drugs or other anti-cytokines agent to the antibody. [9] As nanobodies provide an easier penetration to tissues[10], the drug molecules would

3

detach and are thought to have better therapeutic results. Such modification on antibody and nanobody treatment is proposed to enhance the efficacy to reduce infection by SARS-CoV-2.

2. Methods and Results

To produce an effective antibody-drug conjugate against SARS-CoV-2, an existing nanobody being developed to target the RBD on the virus could be used. A specific immunomodulatory drug that inhibits cytokine level in patients could be used to alleviate the symptoms brought by cytokine storms in COVID patients. Therefore, in this research proposal, anti-IL6 drug Tocilizumab, is used.[11,12] Nanobodies named H11-D4 targeting RBD domain on virus spike protein are used specifically.[13]

1. Attachment of drug to antibody

To effectively bind the drug to the antibody, dithiothreitol(DTT) in PBS is incubated with the designated antibody in order to reduce the disulfide bond in the antibody. A drug linker molecule is used after reduction to create a drug-linker-ligand conjugate on the nanobody. A peptide linker called Cathepsin B is to be used. The drug can thus be released via hydrolysis of the peptide linker on the ADC.[14] The peptide linker is incubated with the nanobodies in ice and purified over a desalting column. It is purified through a size exclusion chromatography on G25 column containing DTPA. The larger size linked antibody could therefore be purified from other reacting agents. [15]

2. Confirmation Of the antibody-drug linkage

Proper linkage of the drug to the nanobody could be tested through an non-reducing SDS-PAGE analysis. SDS-PAGE measures the shift rate of proteins through the SDS gel, the larger ADC would move more slowly and therefore has a shorter shift rate than unreacted ones. By loading the modified nanobodies and control nanobodies into the gel separately, appropriately bonded ADC are expected to have a higher molecular weight than that of the control nanobodies. The expected result are shown below:

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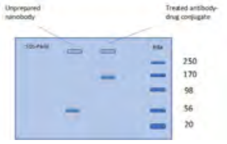


In order to measure the effectiveness of a potential drug, next generation *in vivo* tests have been conducted [16,17]. These tests are usually done on isolated tissues, organs, cells or on animals like rabbits, rats and canines.

Receptor-binding domain of the viral spike protein is a highly specific target of antibodies in SARS-CoV-2 patients. Thus many antibody tests are based on the receptor-binding domain (RBD) of the spike protein [20].

A new drug has to be tested in multiple steps, before making it into the market. Three important things have to be measured. Those include the efficacy of the drug (in our case: how well our antibody binds and neutralizes spike proteins), toxicity (harmfulness and side effects) and the dosage [21].

3. *In vivo* testing in animal model

A number of studies have investigated non-human primates as models for human infection. Rhesus macaques, Grivets, and common marmosets can become infected SARS-CoV-2 and become sick in laboratory settings [20, 22, 24]. Using animals such as those listed above in vivo tests are preferable (e.g. Rhesus macaques).

For this procedure we could divide Rhesus macaques into three groups.

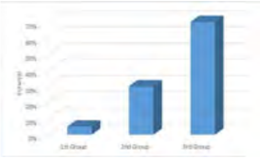
1st group: Test Subjects will be given only the pseudo virus, which have SARS-CoV-2 Spike Proteins expressed on the surface.

2nd Group: Test Subjects will be given both the pseudo virus and the unmodified, control antibody.

3rd Group: Test Subjects will be given the pseudo virus and the antibody with the selected ADC.

5

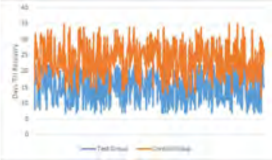
During 6 weeks survival percentage should be calculated from each group [23]. If the results are shown as below, then we predict that these conjugated drugs decrease mortality and are recommended as treatments. Additionally, the immune repertoire (immune cells and cytokine levels) of each primates could be characterized through flow cytometry and ELISA.



4. Clinical trials

If the conjugated drugs showed promising results on non-human primates, then final tests have to be done on humans. For example, patients could be randomly assigned into two Groups (Control Group and Test Group). Patients belonging to the Control Group should be given Placebo drugs, in contrast to patients of the Test Group, which will be given the active form of drug.

If the statistical results of the trail are shown as in Figure 2, then the drug has passed the tests and could be added on the market.



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3.Conclusion and final remarks

At present, COVID-19 is spreading very rapidly and there is yet no specific treatment. Some drugs have shown to slow down Covid-19 spread and to relieve the symptoms. Patients are given conjugate drugs to increase both the effectiveness of the treatment and to decrease recovery time. Finding and selecting the right drugs is a multistep complicated task. First compatible antibodies have to be selected from different animals or be made artificially. Then *in vitro* and *in vivo* experiments should be done to test which conjugated drugs are easing the symptoms, decreasing the recovery and the mortality rate.

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1A05 Modelling the Spread of Covid-19

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Modelling the Spread of Covid-19

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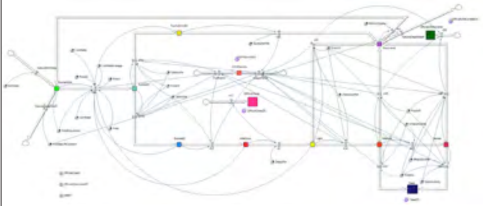
Priya Kaur Bahra , Australia
Aadim Nepal , Nepal

Hypothesis and Methodology

Previous research has investigated how COVID-19 spreads and how human intervention affects this spread. However, very little research extends this to predict how SARS-CoV-2 will consequently evolve to maximise its infectivity and minimise its mortality. We hypothesise that by modelling the spread of COVID-19, we can use the results to predict the future spread e.g. second waves, hence we can better prepare for them. Also, the model will demonstrate how our precautions against the spread of COVID-19 may affect the evolution of SARS-CoV-2.



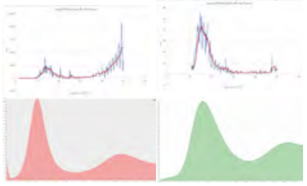
We created detailed flux charts in Anylogic to model the COVID-19 outbreak in Moscow, Paris and Tokyo. These models allowed for different parameters to be adjusted to study their effect on the spread of COVID-19. The parameters we chose to investigate were:

- if people with COVID-19 symptoms are isolated
- if quarantine orders were disregarded

Preliminary results

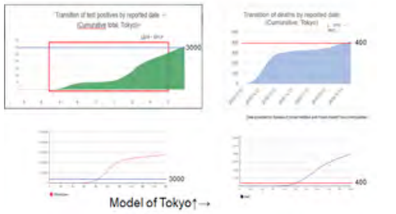
The results for Paris did predict the resurgence of infections after approximately 175 days. However, the scale is different. **Death counts support the hypothesis of underreporting of case numbers early on in the pandemic.**

Official cases/death counts (Paris):



Our Model for Paris

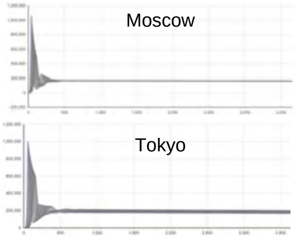
We could not make the model of Tokyo close to the actual numbers in time.
Although its curves look a little similar with real ones, the model shows about ten times the actual numbers.
Some of Tokyo's parameters seem to be far more different from others than we expected.



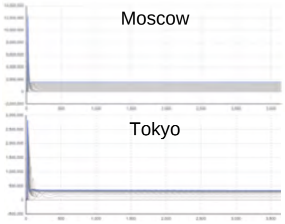
Preliminary Results - Moscow, Tokyo

How isolating people with COVID-19 symptoms affects its spread:

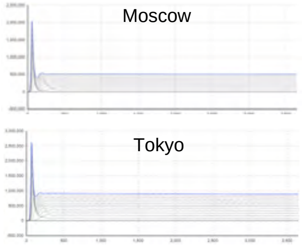
People with medium/severe infections are isolated in hospitals

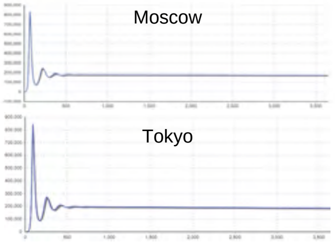


above: if people with light symptoms are isolated, increasing the duration of light infections does not affect COVID-19 infections significantly at equilibrium.



above: SARS-CoV-2 may evolve to increase the duration of asymptomatic infections. This significantly increases infections because asymptomatic people are not isolated.

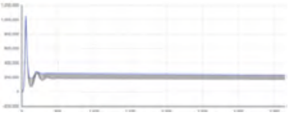




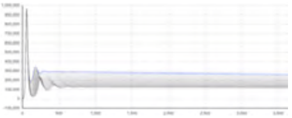
left: increased asymptomatic spread vs right: increased symptomatic spread (e.g. coughing). Thus COVID-19 may evolve to infect more effectively in an asymptomatic way e.g. persisting on surfaces.

How ignoring quarantine affects the spread (Moscow):

above: increased symptomatic infection (as people are not isolated) increases cases at equilibrium.



above: SARS-CoV-2 may evolve to persist for a longer time in the body. This increases the number of infections at equilibrium significantly. This may lead to more severe infections.



above: SARS-CoV-2 may evolve to minimise the immunity loss period as this increases case numbers.

Conclusion

Our model, while not perfectly refined, was able to predict some characteristics of an outbreak.

Furthermore, executing this experiment with a greater database and more detailed parameters would further increase the accuracy of the model and make it possible to reveal additional properties of SARS-Cov-2, such as giving better estimates about unreported case numbers.

Finally, it enables predictions about outbreaks in case the virus should mutate and its epidemic characteristics were changed. From our model, we found that SARS-CoV-2 will have three major directions of evolution if we isolate people who display symptoms:

1) Being asymptomatic rather than symptomatic
2) Evolving to spread effectively in an asymptomatic manner e.g. surviving longer on surfaces
3) Evolving to have less notable antigens, so that our immune system is more likely to lose memory about it, and immune loss will happen at a faster pace

If we disregard quarantines, symptomatic carriers will have more contact with susceptible people. Thus, SARS-CoV-2 will evolve to become more symptomatic and evolve to spread better through coughing and sneezing (i.e. symptomatic transmission).

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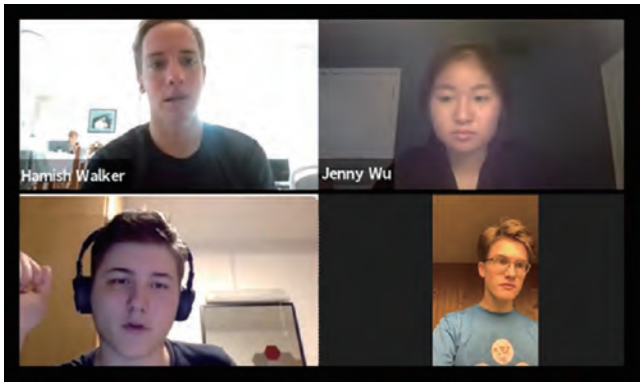
1A06 Dependence of prion uptake and colocalization on SDC3



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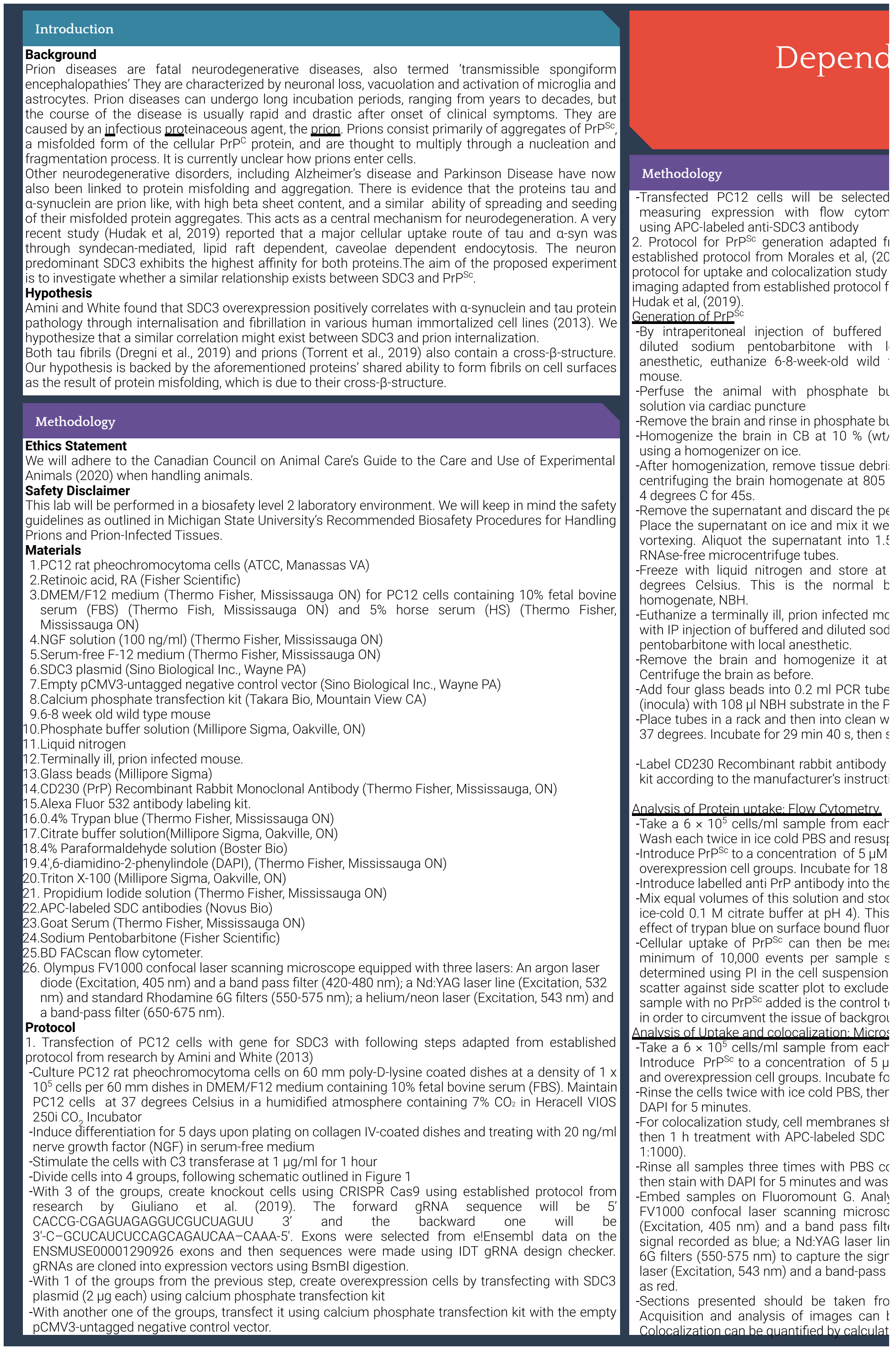

Introduction

Background

Prion diseases are fatal neurodegenerative diseases, also termed ‘transmissible spongiform encephalopathies’ They are characterized by neuronal loss, vacuolation and activation of microglia and astrocytes. Prion diseases can undergo long incubation periods, ranging from years to decades, but the course of the disease is usually rapid and drastic after onset of clinical symptoms. They are caused by an infectious proteinaceous agent, the prion. Prions consist primarily of aggregates of PrP^{Sc}, a misfolded form of the cellular PrP^{C} protein, and are thought to multiply through a nucleation and fragmentation process. It is currently unclear how prions enter cells.

Other neurodegenerative disorders, including Alzheimer’s disease and Parkinson Disease have now also been linked to protein misfolding and aggregation. There is evidence that the proteins tau and α-synuclein are prion like, with high beta sheet content, and a similar ability of spreading and seeding of their misfolded protein aggregates. This acts as a central mechanism for neurodegeneration. A very recent study (Hudak et al, 2019) reported that a major cellular uptake route of tau and α-syn was through syndecan-mediated, lipid raft dependent, caveolae dependent endocytosis. The neuron predominant SDC3 exhibits the highest affinity for both proteins.The aim of the proposed experiment is to investigate whether a similar relationship exists between SDC3 and PrP^{Sc}.

Hypothesis

Amini and White found that SDC3 overexpression positively correlates with α-synuclein and tau protein pathology through internalisation and fibrillation in various human immortalized cell lines (2013). We hypothesize that a similar correlation might exist between SDC3 and prion internalization.

Both tau fibrils (Dregni et al., 2019) and prions (Torrent et al., 2019) also contain a cross-β-structure. Our hypothesis is backed by the aforementioned proteins’ shared ability to form fibrils on cell surfaces as the result of protein misfolding, which is due to their cross-β-structure.

Methodology

Ethics Statement

We will adhere to the Canadian Council on Animal Care’s Guide to the Care and Use of Experimental Animals (2020) when handling animals.

Safety Disclaimer

This lab will be performed in a biosafety level 2 laboratory environment. We will keep in mind the safety guidelines as outlined in Michigan State University’s Recommended Biosafety Procedures for Handling Prions and Prion-Infected Tissues.

Materials

1. PC12 rat pheochromocytoma cells (ATCC, Manassas VA)
2. Retinoic acid, RA (Fisher Scientific)
3. DMEM/F12 medium (Thermo Fisher, Mississauga ON) for PC12 cells containing 10% fetal bovine serum (FBS) (Thermo Fish, Mississauga ON) and 5% horse serum (HS) (Thermo Fisher, Mississauga ON)
4. NGF solution (100 ng/ml) (Thermo Fisher, Mississauga ON)
5. Serum-free F-12 medium (Thermo Fisher, Mississauga ON)
6. SDC3 plasmid (Sino Biological Inc., Wayne PA)
7. Empty pCMV3-untagged negative control vector (Sino Biological Inc., Wayne PA)
8. Calcium phosphate transfection kit (Takara Bio, Mountain View CA)
9. 6-8 week old wild type mouse
10. Phosphate buffer solution (Millipore Sigma, Oakville, ON)
11. Liquid nitrogen
12. Terminally ill, prion infected mouse.
13. Glass beads (Millipore Sigma)
14. CD230 (PrP) Recombinant Rabbit Monoclonal Antibody (Thermo Fisher, Mississauga, ON)
15. Alexa Fluor 532 antibody labeling kit.
16. 0.4% Trypan blue (Thermo Fisher, Mississauga ON)
17. Citrate buffer solution(Millipore Sigma, Oakville, ON)
18. 4% Paraformaldehyde solution (Boster Bio)
19. 4',6-diamidino-2-phenylindole (DAPI), (Thermo Fisher, Mississauga ON)
20. Triton X-100 (Millipore Sigma, Oakville, ON)
21. Propidium Iodide solution (Thermo Fisher, Mississauga ON)
22. APC-labeled SDC antibodies (Novus Bio)
23. Goat Serum (Thermo Fisher, Mississauga ON)
24. Sodium Pentobarbitone (Fisher Scientific)
25. BD FACscan flow cytometer.
26. Olympus FV1000 confocal laser scanning microscope equipped with three lasers: An argon laser diode (Excitation, 405 nm) and a band pass filter (420-480 nm); a Nd:YAG laser line (Excitation, 532 nm) and standard Rhodamine 6G filters (550-575 nm); a helium/neon laser (Excitation, 543 nm) and a band-pass filter (650-675 nm).

Protocol

1. Transfection of PC12 cells with gene for SDC3 with following steps adapted from established protocol from research by Amini and White (2013)

-Culture PC12 rat pheochromocytoma cells on 60 mm poly-D-lysine coated dishes at a density of 1 x 10^5 cells per 60 mm dishes in DMEM/F12 medium containing 10% fetal bovine serum (FBS). Maintain PC12 cells at 37 degrees Celsius in a humidified atmosphere containing 7% CO_2 in Heracell VIOS 250i CO_2 Incubator

-Induce differentiation for 5 days upon plating on collagen IV-coated dishes and treating with 20 ng/ml nerve growth factor (NGF) in serum-free medium

-Stimulate the cells with C3 transferase at 1 μg/ml for 1 hour

-Divide cells into 4 groups, following schematic outlined in Figure 1

-With 3 of the groups, create knockout cells using CRISPR Cas9 using established protocol from research by Giuliano et al. (2019). The forward gRNA sequence will be 5’ CACCG-CGAGUAGAGGUCGUCUAGUU 3’ and the backward one will be 3'-C–GCUCAUCUCCAGCAGAUCAA–CAAA-5'. Exons were selected from e!Ensembl data on the ENSMUSE00001290926 exons and then sequences were made using IDT gRNA design checker. gRNAs are cloned into expression vectors using BsmBI digestion.

-With 1 of the groups from the previous step, create overexpression cells by transfecting with SDC3 plasmid (2 μg each) using calcium phosphate transfection kit

-With another one of the groups, transfect it using calcium phosphate transfection kit with the empty pCMV3-untagged negative control vector.

Depend

Methodology

-Transfected PC12 cells will be selected
measuring expression with flow cytom
using APC-labeled anti-SDC3 antibody

2. Protocol for PrP^{Sc} generation adapted f
established protocol from Morales et al, (20
protocol for uptake and colocalization study
imaging adapted from established protocol f
Hudak et al, (2019).

Generation of PrP^{Sc}

-By intraperitoneal injection of buffered
diluted sodium pentobarbitone with l
anesthetic, euthanize 6-8-week-old wild
mouse.

-Perfuse the animal with phosphate bu
solution via cardiac puncture

-Remove the brain and rinse in phosphate bu

-Homogenize the brain in CB at 10 % (wt/
using a homogenizer on ice.

-After homogenization, remove tissue debri
centrifuging the brain homogenate at 805
4 degrees C for 45s.

-Remove the supernatant and discard the pe
Place the supernatant on ice and mix it we
vortexing. Aliquot the supernatant into 1.5
RNAse-free microcentrifuge tubes.

-Freeze with liquid nitrogen and store at
degrees Celsius. This is the normal b
homogenate, NBH.

-Euthanize a terminally ill, prion infected mo
with IP injection of buffered and diluted sod
pentobarbitone with local anesthetic.

-Remove the brain and homogenize it at
Centrifuge the brain as before.

-Add four glass beads into 0.2 ml PCR tube
(inocula) with 108 μl NBH substrate in the P
-Place tubes in a rack and then into clean w
37 degrees. Incubate for 29 min 40 s, then s

-Label CD230 Recombinant rabbit antibody
kit according to the manufacturer’s instructi

Analysis of Protein uptake: Flow Cytometry.

-Take a 6 × 10^5 cells/ml sample from each
Wash each twice in ice cold PBS and resus

-Introduce PrP^{Sc} to a concentration of 5 μM
overexpression cell groups. Incubate for 18

-Introduce labelled anti PrP antibody into the

-Mix equal volumes of this solution and sto
ice-cold 0.1 M citrate buffer at pH 4). This
effect of trypan blue on surface bound fluor

-Cellular uptake of PrP^{Sc} can then be mea
minimum of 10,000 events per sample s
determined using PI in the cell suspension
scatter against side scatter plot to exclude
sample with no PrP^{Sc} added is the control t
in order to circumvent the issue of backgro

Analysis of Uptake and colocalization: Micros

-Take a 6 × 10^5 cells/ml sample from each
Introduce PrP^{Sc} to a concentration of 5 μ
and overexpression cell groups. Incubate fo

-Rinse the cells twice with ice cold PBS, the
DAPI for 5 minutes.

-For colocalization study, cell membranes s
then 1 h treatment with APC-labeled SDC
1:1000).

-Rinse all samples three times with PBS c
then stain with DAPI for 5 minutes and was

-Embed samples on Fluoromount G. Anal
FV1000 confocal laser scanning micros
(Excitation, 405 nm) and a band pass filt
signal recorded as blue; a Nd:YAG laser lin
6G filters (550-575 nm) to capture the sig
laser (Excitation, 543 nm) and a band-pass
as red.

-Sections presented should be taken fro
Acquisition and analysis of images can
Colocalization can be quantified by calcula

pendence of prion uptake and colocalization on SDC3

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be selected by
flow cytometry
ıtibody
ı adapted from
es et al, (2012);
:ation study and
ed protocol from

f buffered and
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ıosphate buffer

ıhosphate buffer.
ıt 10 % (wt/vol)

tissue debris by
ıate at 805 g at

scard the pellet.
ıd mix it well by
tant into 1.5 ml
ɔes.
ıd store at -80
ɜ normal brain

infected mouse
ɟ diluted sodium
:hetic.
ɡenize it at 10% in PBS containing Propidium iodide (PI).

ml PCR tubes. Mix 12 μl brain homogenate containing PrP^{Sc}
:rate in the PCR tubes. Mix well by pipetting.
into clean water filled sonication horn, inside dry incubator at
ı 40 s, then sonicate for 20s. Repeat cycle for 72 hours.

ɔit antibody (anti PrP) with Alexa Fluor 532 antibody labelling
ɛr's instructions.

Cytometry
e from each cell group, and an additional wild type sample.
S and resuspend in 0.5 ml physiological saline.
ion of 5 μM into the SDC3 knockout, one of the wild type and
ubate for 18 h at 37°C.
ɔody into the cell culture. Incubate for 1 hr.
:ion and stock solution of trypan blue (500 μg/ml dissolved in
pH 4). This lowers sample pH to 4.0, optimizing quenching
bound fluorescent proteins.
hen be measured via flow cytometry using a FACScan. A
ɜr sample should be analysed. Viability of cells should be
suspension (10 μg/ml) and appropriate gating in a forwards
t to exclude dead cells, debris and aggregates. The wild type
the control to which other cell groups should be compared to,
: of background noise from antibody binding to native PrP^{C}.
ation: Microscopic visualization
e from each cell group, and an additional wild type sample.
ation of 5 μM into the SDC3 knockout, one of the wild type
. Incubate for 18 h at 37°C.
ɔld PBS, then fix in 4% paraformaldehyde and stain nuclei with

ɜmbranes should be permeabilized with 1% Triton X-100, and
abeled SDC antibodies and labeled anti-PrP antibodies (both

with PBS containing 1% goat serum and 0.1% Triton X-100,
tes and wash three times with PBS.
unt G. Analyze distribution of fluorescence on an Olympus
ing microscope equipped with three lasers. A laser diode
nd pass filter (420-480 nm) should be used to capture the
ʼAG laser line (Excitation, 532 nm) and standard Rhodamine
:ure the signal recorded as yellow; and finally, a helium/neon
ı band-pass filter (650-675 nm) to capture the signal recorded

ɜ taken from approximately the mid-height of the cells.
ıages can be done using the Olympus Fluoview software.
ɟ by calculation of Manders' colocalization coefficient.

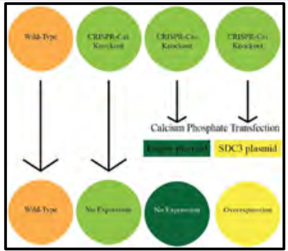


Figure 1. Generation of 4 experimental groups: After inducing differentiation, cells are separated into 4 groups. After following the steps described in the protocol above, there will be 1 wild-type group (orange), 2 groups with no SDC expression (light and dark green)—1 of which undergoes calcium phosphate transfection without a plasmid—and 1 group overexpressing SDC3 (yellow).

Methodology

-The wild type sample with no PrP^{Sc} added is the control to which other cell groups should be compared to, in order to circumvent the issue of background noise from antibody binding to native PrP^{C}.

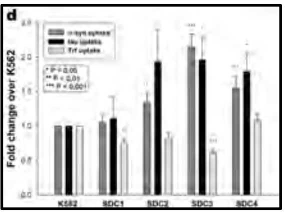
3. Qualitative and quantitative analyses with following steps adapted from research by Hudak et al. (2019).

Qualitative analysis

- Colours, shapes, textures, and patterns, will be noted for each of the 4 experimental groups. Nuclei will show up as blue, prions as yellow-green, and SDC3 as red.
- Use flow cytometry to measure fluorescence in the range of 550-625 nm to evaluate the expression of SDC3 for each experimental group separately.
- Combine this data in one graph with a line representing each experimental group's fluorescence levels

Quantitative analysis

- Using the Olympus Fluoview software, determine the fluorescence intensities of all experimental groups at the emission spectra for each dye.
- Generate all computer images and compare
- Normalize each experimental group's fluorescence intensity as percent deviation from the value of fluorescence intensity determined for the wild-type group. Calculate this as [(X-Y)/X]*100% where X is the fluorescence intensity calculated for the wild-type group without prion infection and Y is the fluorescence intensity calculated for the other experimental groups.
- Perform a one-way ANOVA test between the fluorescence intensity of each experimental group and the fluorescence intensity of the wild-type group with for prion protein. Determine a p_1 value
- Graph this data as a bar graph. Indicate error bars representing 1S.D.
- Digitally overlap images containing green-yellow (prions) and red (SDC) layers.
- Calculate Manders Colocalization Coefficient (MCC) for each experimental group. This reflects the extent of colocalization of prion protein and SDC3.
- Normalize each experimental group's MCCs as percent deviation from the value of MCCs determined for the wild-type group. Calculate this as [(X-Y)/X]*100% where X is the MCC calculated for the wild-type group and Y is the MCC calculated for any of the other experimental groups. Determine a p_2 value
- Perform a one-way ANOVA test between the MCCs of each experimental group and the MCCs of the wild-type group.
- Graph this average MCC data as a bar graph. Include error bars representing 1 S.D. Place an asterisk by bars that have $p<0.05$ from the one-way ANOVA test.



Example of bar graph where data is shown as compared to the values in the control group (in this case, K562 values) (Hudak *et al*. 2019)

Results & Discussion

This experiment could yield 3 cases with regards to the relationship between SDC3 expression and prion uptake. Within each case, there would be 3 subcases with regards to the relationship between SDC3 expression and colocalization with PrP^{Sc}.

Case 1: There is no correlation ($p_1 > 0.05$) between SDC3 expression and prion uptake by neuronal cells. There may not be a direct relationship between SDC3 and prion uptake. Results will be similar in all cell categories; that is, the results from amalgamated flow cytometry data should be very similar, but all have predominantly low intensity emissions.

a) No correlation ($p_2 > 0.05$) between SDC3 expression and colocalization. Correspondingly, the MCC results would need to demonstrate that there is little colocalization between SDC3 and PrP^{Sc} in all of the cell groups, and that all would have similar values for the MCC, in order to conclude this.

b) There is a positive correlation ($p_2 < 0.05$) between SDC3 expression and colocalization. SDC3 is likely a good binding site on the membrane compared to other potential binding sites. SDC3 is likely not directly involved in prion uptake. We could conclude this if the MCC value for the overexpression cell group was significantly higher than the wild type (prion infected) group, and the MCC value for the wild type (prion infected) group was significantly higher than the knockout group.

c) There is a negative correlation ($p_2 > 0.05$) between SDC3 expression and colocalization. SDC3 is most likely a poor binding site compared to other potential sites on the membrane. Proportionally,in cells with greater quantities of SDC3, less area of the membrane has greater binding affinity. We could conclude this if the MCC value for the overexpression cell group was significantly lower than the wild type (prion infected) group, and the MCC value for the wild type (prion infected) group was significantly lower than the knockout group.

Case 2: positive correlation ($p_1 < 0.05$) between SDC3 and prion uptake by neuronal cells. Prion uptake is likely promoted by SDC3. For this scenario, flow cytometry histograms of the emission spectra of the prion fluorescent antibodies may indicate that the greatest signal was seen for the overexpressed SDC3 group, next greatest for wild-type, and the lowest for the SDC3 knockout group.

a) Negative correlation ($p_2 < 0.05$) between SDC3 expression and colocalization: SDC3 is likely to promote PrP^{Sc} uptake, however, the mechanism for PrP^{Sc} uptake doesn't involve direct PrP^{Sc} to SDC3 binding. We would conclude this if the bar for the overexpressing experimental group has much lower MCC than the wild-type group with prion infection, and if the wild-type group with prion infection had a significantly lower MCC than the knockout cells.

Results & Discussion

b) No correlation ($p_2 > 0.05$) between SDC3 expression and colocalization: SDC3 is likely to promote PrP^{Sc} uptake, however, the role of SDC3 can not be determined. We would conclude this if the bars for any pair of wild-type, knockout, or overexpressing groups were not significantly different.

c) Positive correlation ($p_2 < 0.05$) between SDC3 expression and colocalization: SDC3 is likely to directly promote PrP^{Sc} uptake by binding to it. We would conclude this if the bar for the SDC3 overexpressing experimental group had a significantly higher MCC than the wild-type group with prion infection, and if the wild-type group with prion infection had a significantly higher MCC than the knockout cells.

Case 3: negative correlation ($p_1 < 0.05$) between SDC3 expression and prion uptake by neuron-like cells. Prion uptake is likely inhibited by SDC3. For this scenario, flow cytometry histograms of the emission spectra of the prion fluorescent antibodies may indicate that the greatest signal was seen for the knockout groups, next greatest for the wild-type group, and the lowest for the SDC3 overexpressing group.

a) Negative correlation ($p_2 < 0.05$) between SDC3 expression and colocalization: SDC3 is likely a poor binding spot compared to other potential binding sites on the cell membrane. Because on cell membranes with high SDC3, it covers a higher proportion of the membrane, then there are fewer areas where prions actually have a greater binding affinity. We would conclude this if the bar for the overexpressing experimental group has much lower MCC than the wild-type group with prion infection, and if the wild-type group with prion infection had a significantly lower MCC than the knockout cells.

b) No correlation ($p_2 > 0.05$) between SDC3 expression and colocalization: SDC3 is likely not a significantly better or worse binding spot compared to other potential binding sites on the cell membrane. Thus, if there is a cell-surface protein that contributes to the intake of prion protein, it is likely not SDC3, and further research is required. We would conclude this if the bars for any pair of wild-type, knockout, or overexpressing groups were not significantly different.

c) Positive correlation ($p_2 < 0.05$) between SDC3 expression and colocalization: SDC3 is likely a good binding spot compared to other potential binding sites on the cell membrane. However, the prion pathogenic activity would be limited to the membrane level and SDC3 effectively anchors the prions there. We would conclude this if the bar for the overexpressing experimental group has much higher MCC than the wild-type group with prion infection, and if the wild-type group with prion infection had a significantly higher MCC than the knockout cells.

Conclusion

Case 1: SDC3 is likely not involved directly in the process of prion internalization into neuronal cell culture. It is possible that an alternative endocytic pathway may be involved in the process. New hypotheses must be posed to investigate these alternate mechanisms.

Case 2: PrP^{Sc} like α-syn and tau, is very effectively internalized into neuronal cell culture as a result of SDC3 expression. Depending on the level of colocalization, the mechanism for internalization could involve PrP^{Sc} binding to SDC3 or another mechanism which does not involve direct PrP^{Sc} to SDC3 binding. Further research must be conducted to determine the exact mechanism of PrP^{Sc} internalization.

Case 3: PrP^{Sc}, unlike α-syn and tau, is not very effectively internalized into neuronal cell culture as a result of SDC3 expression. This may be due to a number of structural and chemical reasons. Efforts should be made to identify alternative proteins or other cell membrane components that could contribute to the internalization of PrP^{Sc}.

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All group members contributed equally and have approved the final proposal.

1A07 How Climate Change affects Infectious Diseases

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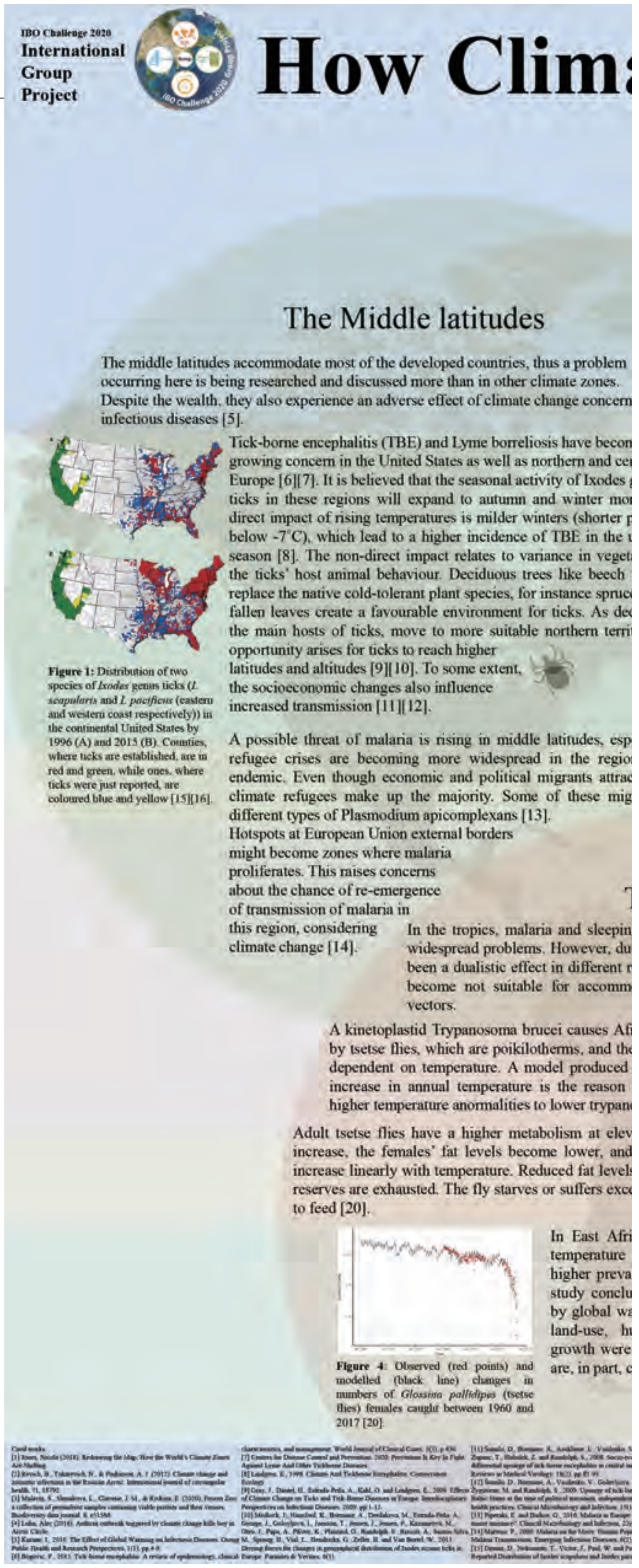

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International
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Project

How Clim

The Middle latitudes

The middle latitudes accommodate most of the developed countries, thus a problem
occurring here is being researched and discussed more than in other climate zones.
Despite the wealth, they also experience an adverse effect of climate change concern
infectious diseases [5].

Tick-borne encephalitis (TBE) and Lyme borreliosis have becon
growing concern in the United States as well as northern and cer
Europe [6][7]. It is believed that the seasonal activity of Ixodes
ticks in these regions will expand to autumn and winter mo
direct impact of rising temperatures is milder winters (shorter
below -7°C), which lead to a higher incidence of TBE in the
season [8]. The non-direct impact relates to variance in veget
the ticks' host animal behaviour. Deciduous trees like beech
replace the native cold-tolerant plant species, for instance spruc
fallen leaves create a favourable environment for ticks. As de
the main hosts of ticks, move to more suitable northern terri
opportunity arises for ticks to reach higher
latitudes and altitudes [9][10]. To some extent,
the socioeconomic changes also influence
increased transmission [11][12].

Figure 1: Distribution of two species of *Ixodes* genus ticks (*I. scapularis* and *I. pacificus* (eastern and western coast respectively)) in the continental United States by 1996 (A) and 2015 (B). Counties, where ticks are established, are in red and green, while ones, where ticks were just reported, are coloured blue and yellow [15][16].

A possible threat of malaria is rising in middle latitudes, esp
refugee crises are becoming more widespread in the regio
endemic. Even though economic and political migrants attrac
climate refugees make up the majority. Some of these mig
different types of Plasmodium apicomplexans [13].
Hotspots at European Union external borders
might become zones where malaria
proliferates. This raises concerns
about the chance of re-emergence
of transmission of malaria in
this region, considering
climate change [14].

T

In the tropics, malaria and sleepin
widespread problems. However, du
been a dualistic effect in different r
become not suitable for accomm
vectors.

A kinetoplastid Trypanosoma brucei causes Af
by tsetse flies, which are poikilotherms, and the
dependent on temperature. A model produced
increase in annual temperature is the reason
higher temperature anormalities to lower trypan

Adult tsetse flies have a higher metabolism at elev
increase, the females' fat levels become lower, and
increase linearly with temperature. Reduced fat level
reserves are exhausted. The fly starves or suffers exc
to feed [20].

Figure 4: Observed (red points) and modelled (black line) changes in numbers of *Glossina pallidipes* (tsetse flies) females caught between 1960 and 2017 [20].

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mate Change affects Infectious Diseases

Project by: Josefine Møgelvang (Denmark)
Edgaras Zaboras (Lithuania)

Facilitator: Parmida Pezeshki (Iran)

symbolizes tsetse fly
symbolizes Anthrax
symbolizes tick-borne encephalitis
symbolizes malaria

The Antarctic and Subantarctic

Due to the anthropogenic climate change, different environments are changing. One of them is the Arctic with its permafrost [1]. It is prognosed that 20-25 years from now, the permafrost will be reduced by 10-12% [2]. Its depth can be up to one kilometer and it makes up about 25% of the hard surface on our planet. As the permafrost constantly has subzero temperatures, it holds a sample of stored microorganisms. Therefore, the thawing of the permafrost poses a threat towards animals and humans, as diseases may (re-)emerge from the permafrost [3]. In the year of 2020, the world knows the threat of a new pathogen and therefore awareness of the thawing of the permafrost should be a priority.

Already, the thawing permafrost has claimed victims, such as the 12-year-old Russian boy, who in 2016 died due to an Anthrax exposure [4]. A concern is that grazing livestock consumes the endospores from Anthrax and thus exposes humans to Anthrax [2].

Furthermore, Revich, Boris et al. have uttered their concern for a prolonged activity of insect vectors, such as ticks, due to a prolonged amount of summer days. The warmer temperatures could increase the insect vector's chance of survival. In fact, there has been an increase in cases of borreliosis and encephalitis in the northern European Arctic [2].

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these migrants are carriers of

Covid-19

Beginning at the end of 2019, the world has faced a new threat: Covid-19. Easily spreading across the globe due to modern transportation such as airplanes, the virus has struck different countries with different severity. Climate change helps lay the groundworks for pandemics, such as Covid-19.

When a catastrophe hits, as seen in India and in the USA (Michigan) in May, a big amount of people needs to be relocated in a short amount of time. Upholding precautions in regard of Covid-19 is difficult during an emergency, thus leading to a greater spread of the disease [23], which is illustrated by the flow chart (figure 6). Furthermore, air pollution is an often-seen consequence due to our industrialization. Research from Harvard sheds light on the link between a greater mortality of Covid-19 cases and air pollution [24].

So far, no one has found a direct link between climate change and Covid-19. However, there are many factors resulting from climate change that affect human health and/or potential pathogens. With increasing temperatures or deforestation migration of animal species comes, thus resulting in new contact possibilities between humans, animals, and pathogens. Another factor is large livestock farms where spillover of infections can happen [25].

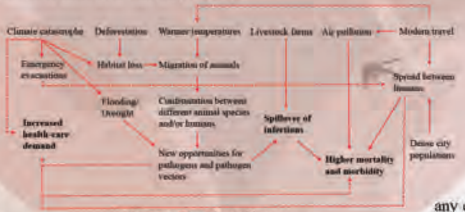

Figure 6: Flow-chart of factors contributing to epidemics

The Subtropics

The subtropics are known for its diversity in climate types. In northern Africa it is very arid, whereas southern Asia has its seasonal rainfalls, the monsoons. For example, Pakistan, experiences severe and frequent floods – one of the climate conditions worsened by climate change. Consequently, the country faces epidemics of diseases that are vector and water borne, such as diarrhea, leptospirosis, and malaria. Significant, however, is the morbidity and mortality of cases with respiratory infections after floods [17].

In a study by Chen, Mu-Jean et al. a link between extreme precipitation and outbreaks of water- and vector borne diseases, such as cholera and malaria, is investigated. An issue is the occurrence of stagnant water, which is an ideal environment for disease-carrying vectors. A figure from their study (figure 2) illustrates the occurrence of bacillary dysentery and the precipitation in different areas after the typhoon Nari in Taiwan in 2001 [18].

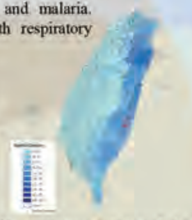

Figure 2: Mean precipitation is shown in shades of blue (se legend) and is measured in mm. The red dots represent cases of bacillary dysentery [18].

A review article by Metcalf C. et al from 2017 shows that the R_0 of an infectious disease plays a role in the incidence rate and the impact of precipitation on the occurrence of the disease. Figure 3 shows that an infectious disease with a low R_0 shows a greater correlation with the precipitation than an infectious disease with a high R_0 [19].

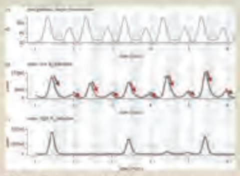

Figure 3: Low precipitation is shown periodically in a shade of grey. a) 6 years of simulated precipitation. b) Simulated cases of an infection with a low R_0. c) Identical infection as in b) but with a higher R_0 [19].

The Tropics

nd sleeping sickness have always been
owever, due to climate change, there has
different regions, as some habitats have
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causes African trypanosomiasis. It is transmitted
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sm at elevated temperatures and must feed more frequently. As temperatures
lower, and smaller pupae are produced. Rates of pupal fat consumption also
d fat levels in young adults decrease the chances to find its first meal before fat
suffers excess mortality as a consequence of taking additional risks in attempting

East African highlands, a consistent rise in
mperature over several decades led to a
gher prevalence of malaria [21]. Although the
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global warming are the key reasons, shifts in
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owth were also at play. However, these factors
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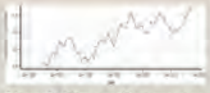

Figure 5: Mean monthly temperature (°C) anomalies in relation to a specific 1960–1990 period. [20]

Conclusion

Possible Solutions and Scientific Experiments

Researches outline three key factors that are needed to conclude that climate change causes variance in vector-borne infectious disease prevalence: 1. Evidence of biological sensitivity of the vector or parasite to climate; 2. Meteorological proof of climate change; 3. Possible evidence of any entomological and epidemiological changes that are connected with climate change [26]. If the correlation is proven, scientists try to forecast future climatic influences on infectious diseases by creating various models.

Statistical models look at the current geographic distribution of the disease and the specific climatic conditions in the location. With human intervention taken into account, the influence of climate change is assessed. Statistical equations that describe possible climate scenarios allow estimating the distribution in the future [27]. Landscape-based models join the climate-based models and spatial analytical methods to study the effects of climatic and other environmental factors. For instance, the influence of different vegetation types is investigated. Among other, it was used in several studies to estimate how future climate-induced changes at the ground level and the surface of water bodies in Africa would affect mosquitoes and tsetse flies and, in turn, malaria and sleeping sickness abundance [27]. Process-based or mathematical models use equations that reflect the relationship between climatic variables and biological parameters, for example, survival, biting rates, vector breeding, and incubation length. It allows assessing how climate change affects vector and parasite biology and, thus, disease transmission. This model has shown that small temperature increases raise the potential of malaria. If the global temperature rose by 2-3˚C, the number of people at risk of malaria would increase by around 3-5% (about several hundred million new cases), and the seasonal duration of malaria surges would increase in endemic areas [28].

Considering recent and old events and the facts presented, this project suggests among others a greater international cooperation to assess the climate change's effect on infectious diseases. More research is needed, using the above-mentioned key factors, to unravel the potential links between climate and infectious diseases, such as malaria, TBE, anthrax, and Covid-19. In regards of anthrax, one should take samples of soil in northern regions to ensure that no anthrax is found (e.g. by searching for eDNA). Also, one could make the TBE-vaccine more available to the population and continue developing a vaccine against borreliosis.

Furthermore, this project concretely proposes to also focus on 1) a better weather surveillance worldwide, 2) less deforestation, thus securing animal's natural habitats, 3) less use of fossil fuels and better filter systems on factories and vehicles to raise air quality, 4) smaller livestock farms to minimize spillover of infections and 5) more international focus on the overall health care system and its capacity and response in regard of natural disasters as well as epidemics/pandemics in each country, as infectious diseases in our modern world know no national borders.

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Figures & pictures:
Figure 1 is from cited work [15] and [16]. Background is removed.
Figure 2 is from cited work [18]. Background is partially removed.
Figure 3 is from cited work [19]
Figure 4 is from cited work [22]
Figure 5 is from cited work [20]
Figure 6 is a self-made flow-chart, partially based on a flow-chart from cited work [23]
The background world map is from https://www.pikpng.com/[illegible]
The mosquito picture is the second picture from https://www.clipartkey.com/search/mosquito/. Figure is colored black.
The tick picture is from https://www.goodfreephotos.com/vector-images/tick-crawling-drawing-vector-clipart.png.php
The biohazard warning sign is from https://freesvg.org/biohazard-warning-vector-sign
The tsetse fly picture is from https://freesvg.org/tsetse-fly. Background is removed.

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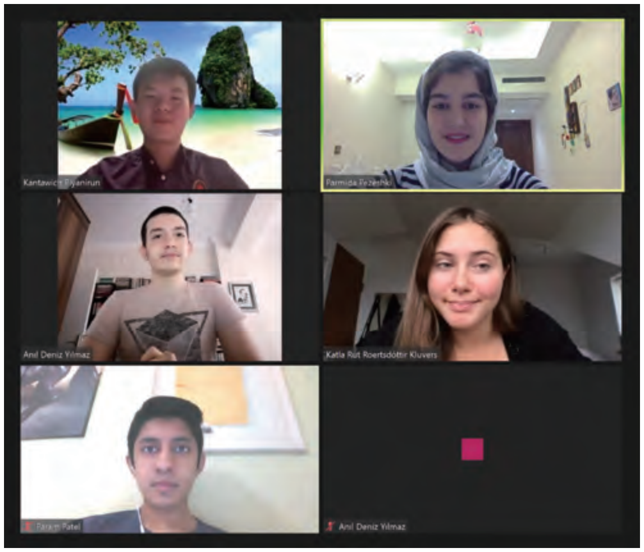
1B01 Host genetics of COVID-19



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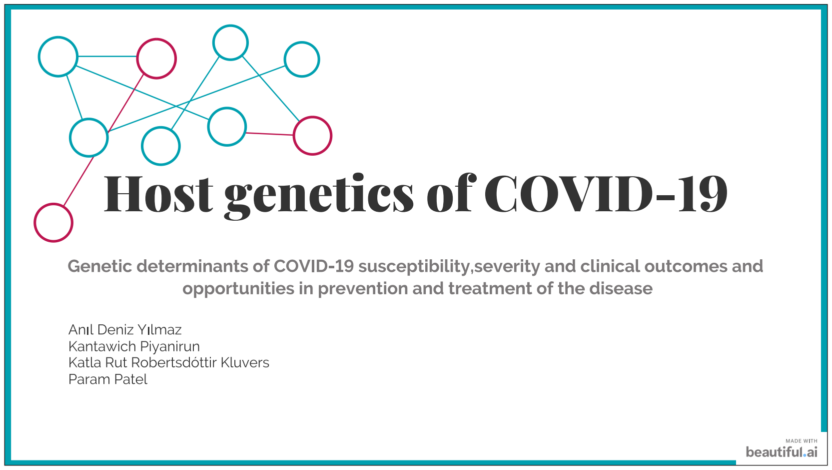
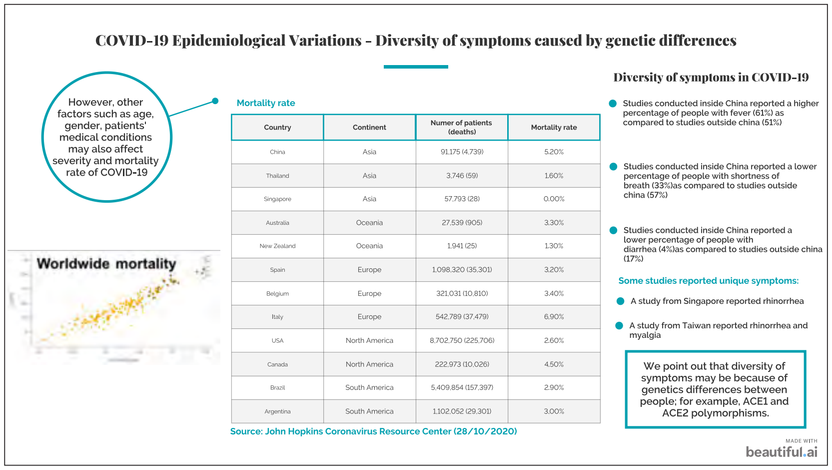


Host genetics of COVID-19

Genetic determinants of COVID-19 susceptibility,severity and clinical outcomes and opportunities in prevention and treatment of the disease

Anıl Deniz Yılmaz
Kantawich Piyanirun
Katla Rut Robertsdóttir Kluvers
Param Patel



COVID-19 Epidemiological Variations - Diversity of symptoms caused by genetic differences

However, other factors such as age, gender, patients' medical conditions may also affect severity and mortality rate of COVID-19

Worldwide mortality

Mortality rate

Country	Continent	Numer of patients (deaths)	Mortality rate
China	Asia	91,175 (4,739)	5.20%
Thailand	Asia	3,746 (59)	1.60%
Singapore	Asia	57,793 (28)	0.00%
Australia	Oceania	27,539 (905)	3.30%
New Zealand	Oceania	1,941 (25)	1.30%
Spain	Europe	1,098,320 (35,301)	3.20%
Belgium	Europe	321,031 (10,810)	3.40%
Italy	Europe	542,789 (37,479)	6.90%
USA	North America	8,702,750 (225,706)	2.60%
Canada	North America	222,973 (10,026)	4.50%
Brazil	South America	5,409,854 (157,397)	2.90%
Argentina	South America	1,102,052 (29,301)	3.00%

Source: John Hopkins Coronavirus Resource Center (28/10/2020)

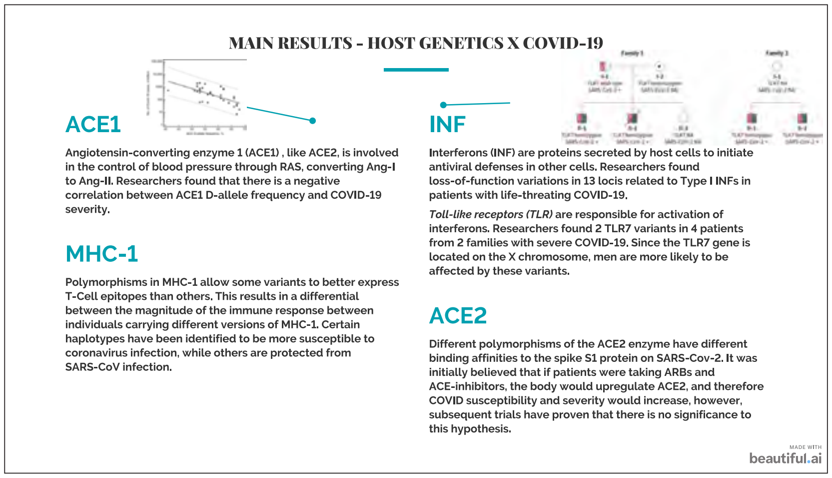
Diversity of symptoms in COVID-19

- Studies conducted inside China reported a higher percentage of people with fever (61%) as compared to studies outside china (51%)
- Studies conducted inside China reported a lower percentage of people with shortness of breath (33%)as compared to studies outside china (57%)
- Studies conducted inside China reported a lower percentage of people with diarrhea (4%)as compared to studies outside china (17%)

Some studies reported unique symptoms:

- A study from Singapore reported rhinorrhea
- A study from Taiwan reported rhinorrhea and myalgia

We point out that diversity of symptoms may be because of genetics differences between people; for example, ACE1 and ACE2 polymorphisms.



MAIN RESULTS - HOST GENETICS X COVID-19

ACE1

Angiotensin-converting enzyme 1 (ACE1) , like ACE2, is involved in the control of blood pressure through RAS, converting Ang-I to Ang-II. Researchers found that there is a negative correlation between ACE1 D-allele frequency and COVID-19 severity.

MHC-1

Polymorphisms in MHC-1 allow some variants to better express T-Cell epitopes than others. This results in a differential between the magnitude of the immune response between individuals carrying different versions of MHC-1. Certain haplotypes have been identified to be more susceptible to coronavirus infection, while others are protected from SARS-CoV infection.

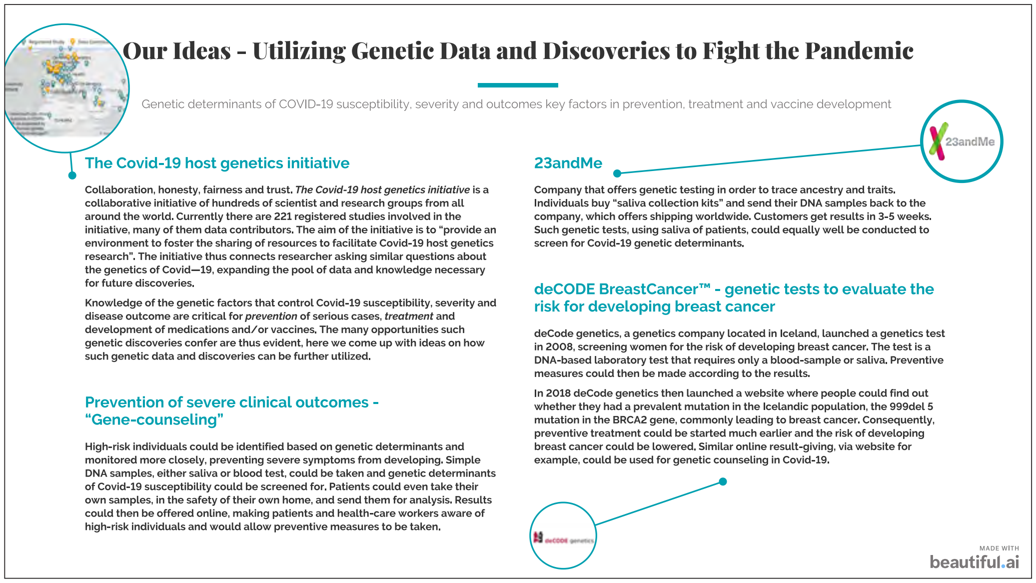
INF

Interferons (INF) are proteins secreted by host cells to initiate antiviral defenses in other cells. Researchers found loss-of-function variations in 13 locis related to Type I INFs in patients with life-threating COVID-19.

Toll-like receptors (TLR) are responsible for activation of interferons. Researchers found 2 TLR7 variants in 4 patients from 2 families with severe COVID-19. Since the TLR7 gene is located on the X chromosome, men are more likely to be affected by these variants.

ACE2

Different polymorphisms of the ACE2 enzyme have different binding affinities to the spike S1 protein on SARS-Cov-2. It was initially believed that if patients were taking ARBs and ACE-inhibitors, the body would upregulate ACE2, and therefore COVID susceptibility and severity would increase, however, subsequent trials have proven that there is no significance to this hypothesis.



Our Ideas - Utilizing Genetic Data and Discoveries to Fight the Pandemic

Genetic determinants of COVID-19 susceptibility, severity and outcomes key factors in prevention, treatment and vaccine development

The Covid-19 host genetics initiative

Collaboration, honesty, fairness and trust. ***The Covid-19 host genetics initiative*** **is a collaborative initiative of hundreds of scientist and research groups from all around the world. Currently there are 221 registered studies involved in the initiative, many of them data contributors. The aim of the initiative is to "provide an environment to foster the sharing of resources to facilitate Covid-19 host genetics research". The initiative thus connects researcher asking similar questions about the genetics of Covid—19, expanding the pool of data and knowledge necessary for future discoveries.**

Knowledge of the genetic factors that control Covid-19 susceptibility, severity and disease outcome are critical for *prevention* of serious cases, *treatment* and development of medications and/or vaccines. The many opportunities such genetic discoveries confer are thus evident, here we come up with ideas on how such genetic data and discoveries can be further utilized.

Prevention of severe clinical outcomes - "Gene-counseling"

High-risk individuals could be identified based on genetic determinants and monitored more closely, preventing severe symptoms from developing. Simple DNA samples, either saliva or blood test, could be taken and genetic determinants of Covid-19 susceptibility could be screened for. Patients could even take their own samples, in the safety of their own home, and send them for analysis. Results could then be offered online, making patients and health-care workers aware of high-risk individuals and would allow preventive measures to be taken.

23andMe

Company that offers genetic testing in order to trace ancestry and traits. Individuals buy "saliva collection kits" and send their DNA samples back to the company, which offers shipping worldwide. Customers get results in 3-5 weeks. Such genetic tests, using saliva of patients, could equally well be conducted to screen for Covid-19 genetic determinants.

deCODE BreastCancer™ - genetic tests to evaluate the risk for developing breast cancer

deCode genetics, a genetics company located in Iceland, launched a genetics test in 2008, screening women for the risk of developing breast cancer. The test is a DNA-based laboratory test that requires only a blood-sample or saliva. Preventive measures could then be made according to the results.

In 2018 deCode genetics then launched a website where people could find out whether they had a prevalent mutation in the Icelandic population, the 999del 5 mutation in the BRCA2 gene, commonly leading to breast cancer. Consequently, preventive treatment could be started much earlier and the risk of developing breast cancer could be lowered. Similar online result-giving, via website for example, could be used for genetic counseling in Covid-19.

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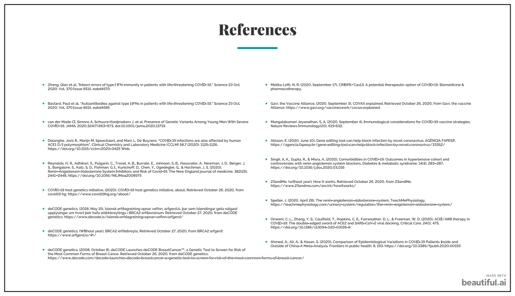

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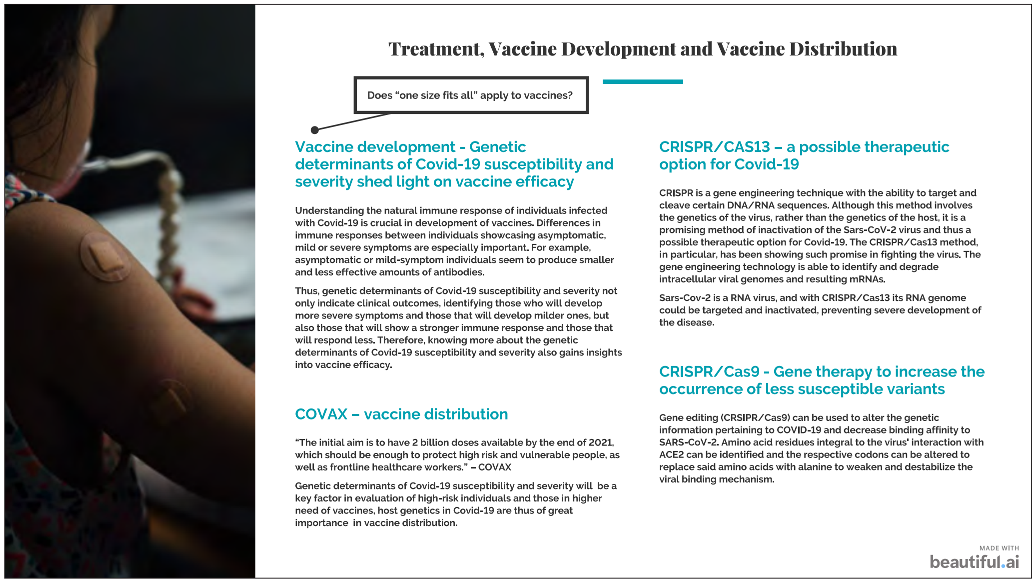

Treatment, Vaccine Development and Vaccine Distribution

Does "one size fits all" apply to vaccines?

Vaccine development - Genetic determinants of Covid-19 susceptibility and severity shed light on vaccine efficacy

Understanding the natural immune response of individuals infected with Covid-19 is crucial in development of vaccines. Differences in immune responses between individuals showcasing asymptomatic, mild or severe symptoms are especially important. For example, asymptomatic or mild-symptom individuals seem to produce smaller and less effective amounts of antibodies.

Thus, genetic determinants of Covid-19 susceptibility and severity not only indicate clinical outcomes, identifying those who will develop more severe symptoms and those that will develop milder ones, but also those that will show a stronger immune response and those that will respond less. Therefore, knowing more about the genetic determinants of Covid-19 susceptibility and severity also gains insights into vaccine efficacy.

COVAX – vaccine distribution

"The initial aim is to have 2 billion doses available by the end of 2021, which should be enough to protect high risk and vulnerable people, as well as frontline healthcare workers." – COVAX

Genetic determinants of Covid-19 susceptibility and severity will be a key factor in evaluation of high-risk individuals and those in higher need of vaccines, host genetics in Covid-19 are thus of great importance in vaccine distribution.

CRISPR/CAS13 – a possible therapeutic option for Covid-19

CRISPR is a gene engineering technique with the ability to target and cleave certain DNA/RNA sequences. Although this method involves the genetics of the virus, rather than the genetics of the host, it is a promising method of inactivation of the Sars-CoV-2 virus and thus a possible therapeutic option for Covid-19. The CRISPR/Cas13 method, in particular, has been showing such promise in fighting the virus. The gene engineering technology is able to identify and degrade intracellular viral genomes and resulting mRNAs.

Sars-Cov-2 is a RNA virus, and with CRISPR/Cas13 its RNA genome could be targeted and inactivated, preventing severe development of the disease.

CRISPR/Cas9 - Gene therapy to increase the occurrence of less susceptible variants

Gene editing (CRSIPR/Cas9) can be used to alter the genetic information pertaining to COVID-19 and decrease binding affinity to SARS-CoV-2. Amino acid residues integral to the virus' interaction with ACE2 can be identified and the respective codons can be altered to replace said amino acids with alanine to weaken and destabilize the viral binding mechanism.

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1B02+1A04 NEW PERSPECTIVES IN THE TREATMENT OF HUMAN RETROVIRUSES

Facilitator

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Tereza Maxerová (Czech Republic)
Yerassyl Muratov (Kazakhstan)
Ziwei, Dewey Lin (Singapore)

The Awards of Excellence

NEW PE

Retroviruses a
for 690,000 de
human effort t
These days, a c
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What is a retrovirus?

Retroviruses are viruses so named for their ability to perform reverse transcription. They are able to infect humans and cause a number of diseases. The most famous retrovirus is perhaps HIV, two species of lentiviruses which infect mostly CD4⁺ T helper cells[3]. Infection with HIV thus leads to a low CD4⁺ T helper cell count, and as T helper cells are vital to an effective immune response, HIV infection leads to a weakening of the immune system known as AIDS, making the body vulnerable to opportunistic infections[4]. Another example of a retrovirus is the Human T-lymphotropic virus type 1 (HTLV-1), responsible for neoplastic diseases such as adult T-cell leukaemia/lymphoma, inflammatory syndromes such as HTLV-1-associated myelopathy and uveitis and opportunistic infections such as *Strongyloides stercoralis* hyperinfection[5]. HTLV-1, in contrast to HIV, induces an immunostimulatory response in CCR4⁺ T cells[6].

The retroviral reproductive cycle

1. A viral glycoprotein on the surface of the retrovirus
2. The viral envelope fuses with the host cell membran
   the enzymes and RNA contained within.
3. Reverse transcriptase produces complementary DN
   degraded. The two cDNA strands form a week bond
4. cDNA is integrated into the host cell's genome, facili
   known as the provirus, and can stay dormant for a p
5. The provirus is transcribed to produce mRNA and vi
6. mRNA is translated to produce viral proteins using c
   are transported to and embedded in the host cell me
   remain in the cytoplasm.
7. Viral capsid proteins polymerize and encase viral ge
   new viral nucleocapsids.
8. Viral nucleocapsids bud off from the host cell, surrou
   in the process, which has now become the viral enve
9. New viruses are activated through the cleavage of vi
   glycoproteins and host receptors are cleaved, releasi
   ready to infect new host cells.

Untreated HIV infection is a life-threatening health problem and many different therapies for HIV/AIDS focusing mainl
the host viral load below a detectable and transferrable limit and do not ensure complete eradication of the virus from
immune system and anti-retroviral drugs[17]. As a result, patients must take antiretroviral drugs continually as the viru
the possibility of the emergence of viral resistance to drugs. Therefore, there has been interest in investigating possible
provirus in a latent phase. As a contrast to Block-and-Lock strategy, the goal of Shock-and-Kill strategy is to trigger the
system and combination of antiretroviral drugs[18][19][20].

BLOCK AND LOCK

Several epigenetic strategies that can permanently lock the virus in a latent phase have been investigated:

A drug potentially useful in the Block-and-Lock strategy treatments for HIV is didehydrocortistatin A (dCA), a specific and potent inhibitor of Tat, which can recruit other transcription factors to induce sustained transcriptional elongation from the viral promoter LTR[18]. As Tat has no cellular homolog, usage of dCA should not have significant cross-functional effects as is the case with many strategies that target gene regulation.

Other alternative methods include targeting agents that act in reactivation of the provirus, such as histone demethylases (HDMs). Their role has been shown in human cytomegalovirus (HCMV), a widespread pathogen that can remain in its latent phase for extended periods of time and reactivate in immunocompromised patients. The potential usage of various HDM inhibitors have been described and further experiments are being carried out[18].

As this epigenetic Block-and-Lock strategy is more sustainable than having to constantly deal with reactivated viruses emerging from reservoirs, scientists are searching for future alternatives. Of interest are natural mechanisms, such as X chromosome inactivation, where specific genomic modifications are applied, which can be identified and potentially utilized. One method of identification is by turning our attention to bacteria. Bacteria, such as *Listeria monocytogenes, Mycobacterium tuberculosis,* or *Helicobacter pylori* can utilize the recruitment of histone deacetylases (HDACs) to repress gene expression by deacetylating specific amino acids on the histones of specific genes[21]. If we will study these mechanisms of chromatin modifying thoroughly, we can use this knowledge to create new epigenetic strategies - we can for example direct some of these chromatin-modifying enzymes to histones that are associated with the provirus.

Another strategy for the permanent silencing of retroviral proviruses could be DNA methylation. There are known instruments, such as Zinc finger nucleases, TALENs or CRISPR-Cas9 platforms that can direct specific cytosine methylations[22]. However, their lack of specificity can result in off-site effects, so further research is needed to refine methods that involve these enzymes. A genetic tool dCas9–DNMT3ACD has recently shown lower levels of this effect in some research, though not in others[22]. Alternatively, we propose the usage *in vitro* synthesized modified histones. As the histones with different modifications can already be synthesized in vitro[23], working out methods for introducing them into living cells and directing them to specific genes could help in investigating the effects of possible histone modifications on infected cells.

The Block-and-Lock strategy is a promising area of research, though there is much work to be done before it can be implemented as a treatment option. Currently, the greatest hurdle we have yet to overcome is the lack of specificity and risk of off-site effects, which can have unpredictable and potentially severe consequences for the patient. Thus, more research has to be done before we are able to use this strategy as a tool in our ongoing fight against retroviruses.

SHOCK AND KILL

The most common approach i
infected cells. There are severa

One class of LRAs are inhibito
(HMT)[19]. These enzymes usu
increase the expression of tha
cells isolated from patients on
effective in other studies[25]. H
chromatin modifier inhibitors
cells tested *ex vivo*[27].

The next important class of LR
affecting the protein kinase C
Protein kinase C plays a role i
transcription factors at the lo
of latent provirus. Activation
of NFκB which causes increase
Promising PKC agonists are b
occurring[28] and synthetic ing
prostratin and phorbol-13-ste
NF-κB by IKK-dependent pho
degradation of IκBα[30]. In ano
myristate 13-acetate (PMA) o
in NF-κB activation[31].

This figure was inspired by the article: Kurdistani, S. K. and Gr
acetylation and deacetylation in yeast," *Nature reviews. Molec*

This figure was inspired by the article: Deeks, S. G. *et al.* (198
(Clinical research ed.), 296(6620), p. 514.

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Figures created with thanks to BioRender.com

All figures in this poster were created by 6 members of the IBO2020 Challenge Group project team "1B02-1A04" using tools from BioRender.com.

We would like to thank the BioRender.com development team for providing such a wonderful service, allowing biologists around the world to easily create vivid illustrations for their works.

Re

N PERSPECTIVES IN THE TREATMENT OF HUMAN RETROVIRUSES

Dong Ngoc Ha, Dewey Lin, Gega Karanadze,
Tereza Maxerová, Simonas Melaika and Yerassyl Muratov

INTERNATIONAL BIOLOGY OLYMPIAD e.V. IBO

International Biology Olympiad Nagasaki 2020

Retroviruses are responsible for many infections and deaths worldwide, with HIV/AIDS alone responsible for 690,000 deaths in 2019 due to the disease itself or the resulting complications[1]. Over many years of human effort to cure retroviral infections, researchers developed many approaches to tackling the virus. These days, a combination of antiretroviral drugs called combinatorial antiretroviral therapy (cART) is being frequently used to effectively suppress viral replication, reduce rates of transmission, and improve patients' life expectancy by prolonging the onset of AIDS[2]. However, because of the existence of retroviral reservoirs in bodies of patients, HIV-infected people on cART must continue to take the medications for the entirety of their lives, which can lead to great personal expenses, the development of viral resistance and the onset of toxic side effects. Because of these limitations, it is imperative that we continue to develop new alternative or additional therapeutic strategies to cART to fight retroviruses. A preliminary approach is to target the mechanism of viral entry into a cell. After all, the virus cannot infect a cell it cannot enter. Alternative approaches include the targeting of nucleic acids involved in the replication cycle of retroviruses, both RNA and DNA. Two of these possible approaches, known as the "Block and Lock" and "Shock and Kill" approaches, involve alteration of viral transcription frequency of the provirus in the host genome. While the Block-and-Lock approach attempts to silence the provirus within the host cell's genome, hopefully indefinitely, the Shock-and-Kill approach, on the other hand, is centred around the controlled reactivation of the provirus in infected cells in order to eradicate latent viral reservoirs. Finally, introduction of genetic modifications to a patient's own cells can help to tackle the retrovirus.

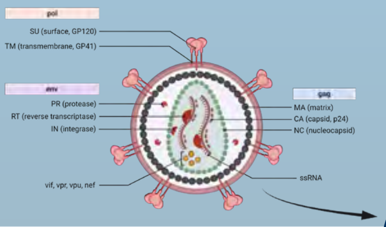
the retrovirus binds to a host receptor protein.
cell membrane. The viral nucleocapsid degrades, releasing
in.
lementary DNA (cDNA) from the viral RNA. Viral RNA is
a week bond and enter the cell's nucleus.
genome, facilitated by integrase. The integrated cDNA is
ormant for a period of time without being transcribed.
e mRNA and viral genomic RNA.
roteins using cellular apparatus. Viral envelope proteins
he host cell membrane. Viral capsid proteins and enzymes

encase viral genomic RNA as well as viral enzymes to form

ost cell, surrounding themselves with host cell membrane
the viral envelope. This forms the new viruses.
cleavage of viral glycoproteins. Bonds between viral
cleaved, releasing the viruses into the extracellular matrix,

Structure of a retrovirus

Retroviruses have an RNA genome which is coated with nucleocapsid proteins and packaged into the viral nucleocapsid along with RNA-dependent DNA polymerase (reverse transcriptase), DNA-dependent DNA polymerase, ribonuclease H, integrase and protease. The capsid is surrounded by the phospholipid viral envelope. Embedded in the viral envelope are viral glycoproteins. Viral glycoproteins have a multitude of functions, such as regulating host cell entry and viral assembly during replication[7].



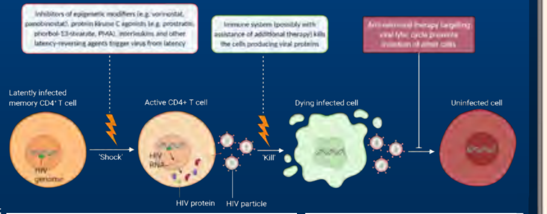
focusing mainly on the inhibition of retroviral proteins have been developed since discovery of HIV. However, these treatments only decrease
the virus from the body. This is because retroviruses can stay in the latent form in infected cells for several years, hidden both from the
ally as the virus can reemerge from reservoirs in infected cells after removal of the therapy. Moreover, prolonged treatment could increase
gating possible epigenetic strategies that could evade these drawbacks. One strategy, called Block-and-Lock, aims to permanently lock the
to trigger the exit of viruses from latency into their active form so that the infection can be completely eradicated from the body by immune

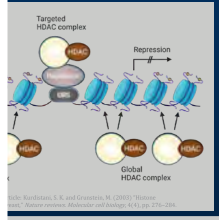
KILL

on approach in Shock-and-Kill strategy is introduction of latency-reversing agents (LRAs) into
here are several classes of LRAs that have been investigated:

As are inhibitors of epigenetic modifiers, such as histone deacetylase (HDAC), DNA methyltransferase (DNMT) or histone methyltransferase
e enzymes usually modify histones or DNA in a way which leads to lower expression of the modified segment, their inhibition should thus
pression of that segment. A HDAC inhibitor called vorinostat can successfully increase production of viral RNA of HIV-1 in memory $CD4^+$ T
om patients on antiretroviral therapy[24]. Some other HDAC inhibitors such as panobinostat in particular show themselves even more
r studies[25]. However, the fact that viral DNA can be incorporated at many different places in the host genome often hinders the effect of
ifier inhibitors[26]. Also, *in vitro* experiments using different cultured cell lines often fail to predict the effect of the LRA in infected patient
ivo[27].

tant class of LRAs are compounds
otein kinase C (PKC) signaling pathway.
C plays a role in the assembly of
ctors at the long terminal repeat (LTR)
us. Activation of PKC leads to activation
causes increased transcription of LTR.
agonists are both naturally
d synthetic ingenols[29]. In one study,
phorbol-13-stearate cause activation of
ependent phosphorylation and
IκBα[30]. In another study, phorbol 12-
etate (PMA) overperformed prostratin
tion[31].





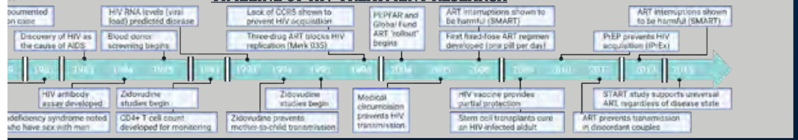
rticle: Kurdistani, S. K. and Grunstein, M. (2003) "Histone
east," *Nature reviews. Molecular cell biology*, 4(4), pp. 276–284.

Another class of LRAs are interleukins. When patients were treated with highly active antiretroviral therapy together with interleukin-2, the number of resting CD4+ T cells containing HIV in the blood was measured to be significantly lower than in patients who were receiving antiretroviral therapy alone[32].

Toll-like receptor agonists may also as LRAs. The activation of TLR-7 by GS-9620 agonist both stimulated induction of latent HIV and enhanced antiviral adaptive immunity response as a response to production of type I interferons[33][34].

A lot of other molecules have been demonstrated to act as effective LRAs[19]. However, there are still problems to be overcome to create an effective Shock-and-Kill strategy. *In vitro* experiments using cultured cell lines often fail to predict the effect of the LRA on infected patient cells tested *ex vivo*[27]. There are many different cell types which can serve as latent HIV reservoirs[19]. Signaling agonists on their own, such as PMA, interleukins and TLR-7 agonists in general induce a significantly lower transcription level compared to T cell receptor stimulation[19]. A combination of several LRAs will probably be needed to create a successful Shock-and-Kill therapy.

TIMELINE OF HIV TREATMENT RESEARCH



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PREVENTING VIRAL ENTRY

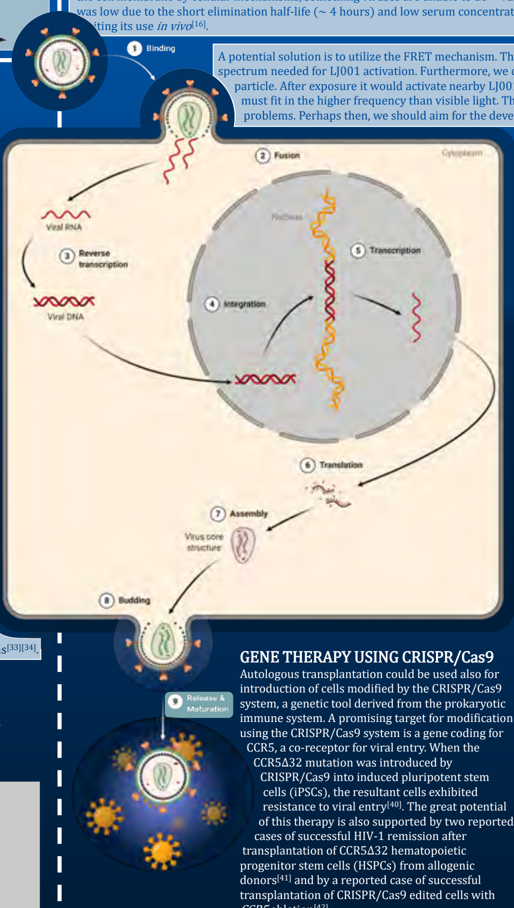
During antiretroviral therapy, a strategy utilizing a combination of both penetration and replication targeting drugs allows the usage of small doses of different drugs to treat the infection. A low concentration of cell-targeted suppressing molecules will not cause critical harm to the host, but can have a potent effect in reducing viral load. Although this may not completely stop the infection, it can give the patient's immune system an opportunity to establish control[8]. By using host-targeting as well as virus-targeting drugs in tandem, excess damage to host cells can thus be prevented.

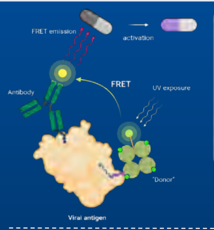
Oftentimes, viruses first utilize nonspecific electrostatic interactions with negatively charged sugars on the cell surface membrane for attachment before binding to a specific receptor that can facilitate viral entry. For example, many viruses, including HIV, first bind to sugars on heparan sulfate proteoglycans (HSPGs)[9][10][11]. Hence, it is possible to inhibit these interactions by utilizing trap particles that mimic these molecules. One strategy utilized by Cagno et al. involved utilizing nanoparticles carrying long flexible linkers that mimic HSPG to bind to multiple viral sites, thus irreversibly changing the structure of the virus[12]. This strategy has been noted to lose effectiveness *in vivo*, due to low bioavailability[13][14].

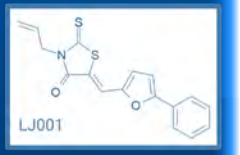
A potential solution is to localize the antiviral action by improving drug delivery through the application of nanotubes. This would also minimize the contact of cytotoxic elements with blood and allow us to achieve pinpoint localization. A caveat is that we must be certain of the location of viral accumulation, e.g. in lymphoid tissues where HIV proliferation mainly occurs.

LJ001 is a small molecule that oxidizes unsaturated phospholipids of both cellular and viral membranes[15]. This can affect the shape and fluidity of the membrane, preventing fusion. When both cellular and viral membranes are exposed to LJ001, only the latter are significantly affected, likely damaged lipids are quickly replaced in the cell membrane by cellular mechanisms, something viruses are unable to do[16]. LJ001 is thus effective *in vitro* as an antiviral agent. Unfortunately, *in vivo* efficacy was low due to the short elimination half-life (~ 4 hours) and low serum concentration of the compound. LJ001 also requires the presence of light to be activated, iting its use *in vivo*[16].

A potential solution is to utilize the FRET mechanism. The carrier of the drug can contain a fluorescent molecule with the emission spectrum needed for LJ001 activation. Furthermore, we can produce the "donor" antibody molecule, which sticks to the target viral particle. After exposure it would activate nearby LJ001 or its companion molecule. Considering Stoke's shift, the exposure radiation must fit in the higher frequency than visible light. The safest option is UV radiation, but even this precedes potential oncological problems. Perhaps then, we should aim for the development of analogues with improved stability and pharmacokinetics.







RNA INTERFERENCE

RNA interference (RNAi) is a mechanism commonly used in cells to regulate gene expression via controlled degradation of certain mRNAs[35]. In RNAi therapy, siRNAs (small interfering RNAs) or shRNA (short hairpin RNAs) can be used to degrade either viral RNA or host RNA which serves as a template for production of proteins associated with retroviral reproductive cycle. RNAi has already achieved notable success in treatment of some acute viral infections[36] and it is now on its way to being applied in the fight against long-term retroviral infections.

One approach in antiretroviral RNAi therapy is to induce the degradation of retroviral RNA genome via the introduction of antiretroviral siRNAs[38]. Ideal target viral sequences are those that are highly conserved and crucial for viral reproduction to prevent the emergence of drug resistance by mutation. These sequences should also be present at all stages of viral RNA splicing to make the therapy as effective as possible. The effectiveness of RNAi therapy can be also increased by targeting multiple viral sites at once[37]. Another approach is to target mRNAs of host proteins which take part in the retroviral reproductive cycle. These target proteins are for example CCR5 and CXCR4 coreceptors utilized for viral entry, LEDGF/p75, Importin-7 and Chaperonin which serve as cofactors in integration of viral DNA into host's genome, cellular proteins which interact with viral Tat protein and other proteins[38]. Targeting host structures reduces the likelihood of the emergence of retroviral drug resistance, but also carries the risk of negative side effects to uninfected host cells due to collateral exposure.

GENE THERAPY USING CRISPR/Cas9

Autologous transplantation could be used also for introduction of cells modified by the CRISPR/Cas9 system, a genetic tool derived from the prokaryotic immune system. A promising target for modification using the CRISPR/Cas9 system is a gene coding for CCR5, a co-receptor for viral entry. When the CCR5Δ32 mutation was introduced by CRISPR/Cas9 into induced pluripotent stem cells (iPSCs), the resultant cells exhibited resistance to viral entry[40]. The great potential of this therapy is also supported by two reported cases of successful HIV-1 remission after transplantation of CCR5Δ32 hematopoietic progenitor stem cells (HSPCs) from allogenic donors[41] and by a reported case of successful transplantation of CRISPR/Cas9 edited cells with *CCR5* ablation[42].

An important step in the process of RNAi and gene therapy is an effective means of drug delivery. In case of RNAi therapy, several specific *in vivo* delivery approaches have been proposed, such as the use of tissue specific serotypes of adeno-associated virus (AAV), antibodies and nucleic acid aptamers which undergo receptor-mediated endocytosis, and targeted lipid nanoparticles. However, each of these approaches has several limitations. Thus, probably the most effective approach is to perform modifications on cells *ex vivo* and then transplant these modified cells into patients[38]. In case of RNAi therapy, the most common way to introduce genes coding small RNAs into cell lines is by viral transduction. For example in one study, the lentiviral vector coding anti-CCR5 shRNA was transduced into CD34+ HPCs and CD4+ T-lymphocytes derived from a patient, and then transplanted back into the HIV-infected patient[39].

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Group project

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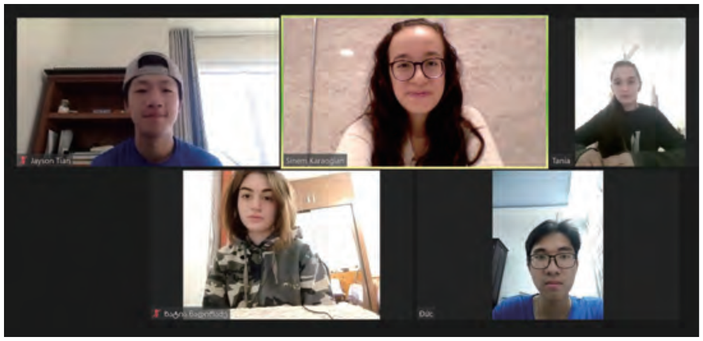
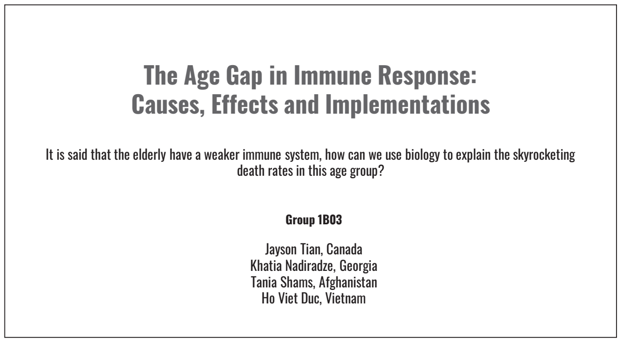
1B03 The Age Gap in Immune Response : Causes, Effects and Implementations





The Age Gap in Immune Response: Causes, Effects and Implementations

It is said that the elderly have a weaker immune system, how can we use biology to explain the skyrocketing death rates in this age group?

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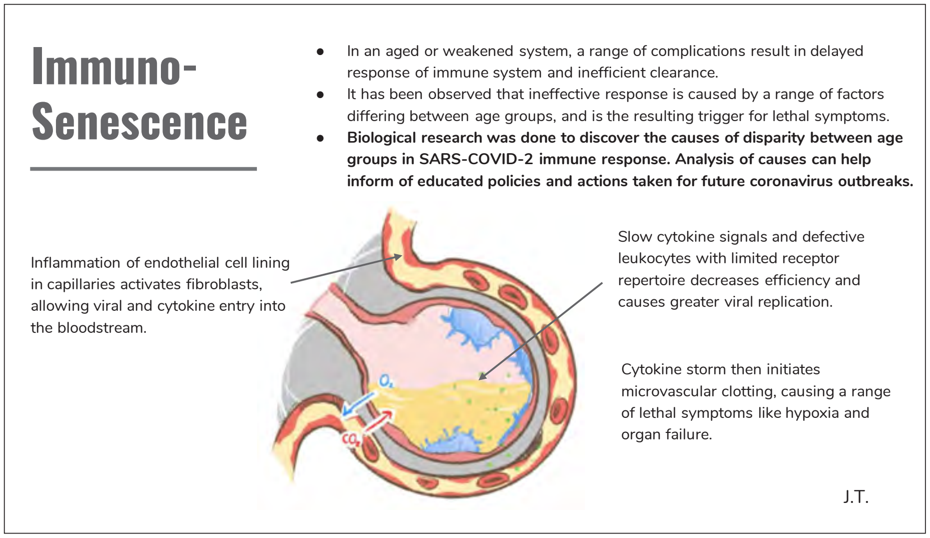

Immuno-Senescence

- In an aged or weakened system, a range of complications result in delayed response of immune system and inefficient clearance.
- It has been observed that ineffective response is caused by a range of factors differing between age groups, and is the resulting trigger for lethal symptoms.
- **Biological research was done to discover the causes of disparity between age groups in SARS-COVID-2 immune response. Analysis of causes can help inform of educated policies and actions taken for future coronavirus outbreaks.**

Inflammation of endothelial cell lining in capillaries activates fibroblasts, allowing viral and cytokine entry into the bloodstream.

Slow cytokine signals and defective leukocytes with limited receptor repertoire decreases efficiency and causes greater viral replication.

Cytokine storm then initiates microvascular clotting, causing a range of lethal symptoms like hypoxia and organ failure.

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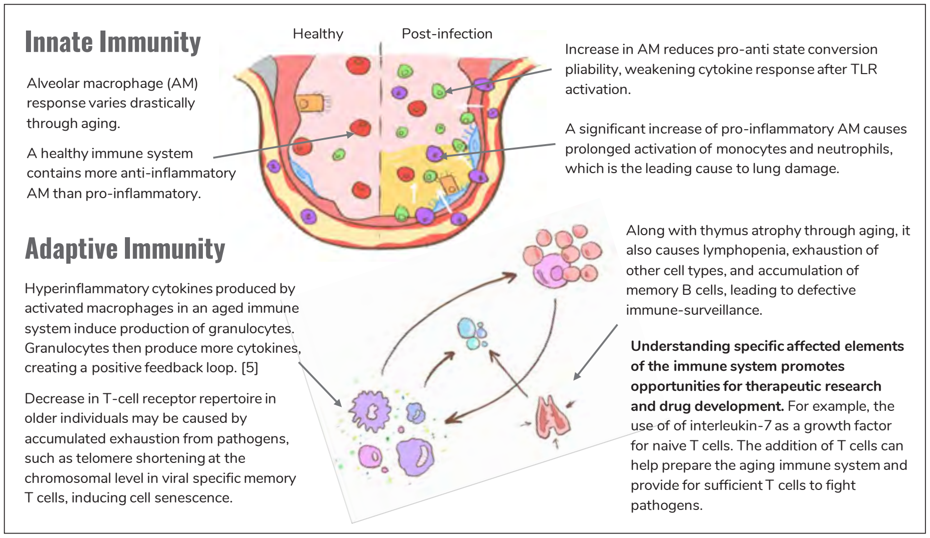


Innate Immunity

Alveolar macrophage (AM) response varies drastically through aging.

A healthy immune system contains more anti-inflammatory AM than pro-inflammatory.

Increase in AM reduces pro-anti state conversion pliability, weakening cytokine response after TLR activation.

A significant increase of pro-inflammatory AM causes prolonged activation of monocytes and neutrophils, which is the leading cause to lung damage.

Adaptive Immunity

Hyperinflammatory cytokines produced by activated macrophages in an aged immune system induce production of granulocytes. Granulocytes then produce more cytokines, creating a positive feedback loop. [5]

Decrease in T-cell receptor repertoire in older individuals may be caused by accumulated exhaustion from pathogens, such as telomere shortening at the chromosomal level in viral specific memory T cells, inducing cell senescence.

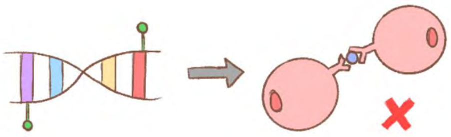
Along with thymus atrophy through aging, it also causes lymphopenia, exhaustion of other cell types, and accumulation of memory B cells, leading to defective immune-surveillance.

Understanding specific affected elements of the immune system promotes opportunities for therapeutic research and drug development. For example, the use of of interleukin-7 as a growth factor for naive T cells. The addition of T cells can help prepare the aging immune system and provide for sufficient T cells to fight pathogens.

Epigenetic Factors

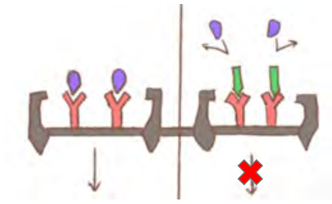
Chromatin relocation, modification over a lifetime can have negative impacts on the immune system. There is abundant evidence showing how changes to the epigenome by pathogens can weaken immune memory and function.

- For example, MERS-CoV uses DNA methylation to silence genes encoding for MHCs. This destructs the antigen presentation process of host immune cells.
- In a similar manner, SARS-CoV-1 methylates histones and long non-coding RNAs through activation of interferon-response genes.



Inflammaging

It was observed that elderly patients rapidly descend into systemic hyperactivation and hypercoagulation of vascular tissue. Secondary hemophagocytic lymphohistocytosis (SHL) is caused by specific interleukin molecules like IL-6. It secretes fibrin which produces D-dimer, a major inducer of vascular inflammation. Such inflammation increases in direct proportion As D-dimers increase with age.



Signal Transduction Cytokine Storm

No Signal Transduction

This information provides specific information on how scientists can target particular molecules that hampers normal immune system functions. In the field of pathogenic diseases, such insight can aid in the development of drugs. For example, Tocilizumad (Actemra) is a drug used to block IL-6 receptor activity, prohibiting signal transduction. This decreases the risks of cytokine storm and death within the elderly.

Discussion and Implementation

Thus, from using the biological knowledge of genetics, scientists can deduce changes in the epigenome caused by certain viruses.

For example, a study can be done to measure DNA methylation age of immune cells and other cell types throughout the infection process of SARS-CoV-2 - this way, we can find out of the epigenome in older patients impacts disease severity. This is also an understudied topic that may provide valuable evidence for development in drug trials.

Researchers can implement these aspects of biological knowledge in prediction of symptoms and pharmaceutical development. We are even using other viruses as research material for SARS-CoV-2 treatment methods.[2]

For example, dexamethasone is a type of corticosteroid medication which was thought would help with influenza[3] and now it is officially prescribed to COVID-19 patients. Another good example is remdesivir, medication which was created for HCV and RSV treatment and it is now used for SARS-CoV-2 patients.[4]

Such age disparities researched in this project demonstrated the need for different therapeutic and prevention strategies for individuals of different age groups. Biological research provides for a plethora of advantages, for example we can arrange **individual treatment methods for each age group** because of their immunological response differences, We can predict symptoms and outcomes of treating methods as well as create new treatment methods more efficiently. Of course the most relevant thing for us is medication and vaccine creating. **The history showed us use of the past research so we can assume right now we are doing the great job for future pandemics. Faster vaccine creation, more improved modeling systems for pandemics. Overall our research showed the need of implementing this knowledge in future epidemics. The biological overview of this problem can help them to be more efficient.**

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- All the illustrations are created by Jayson Tian.
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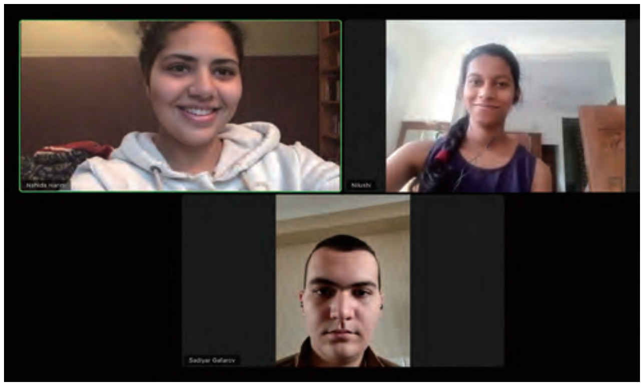
1B04 Using Biology to propose methods to prevent spread of Dengue



Using Biology to propose methods to prevent spread of Dengue

-IBO Challenge 2020 International Group Project-

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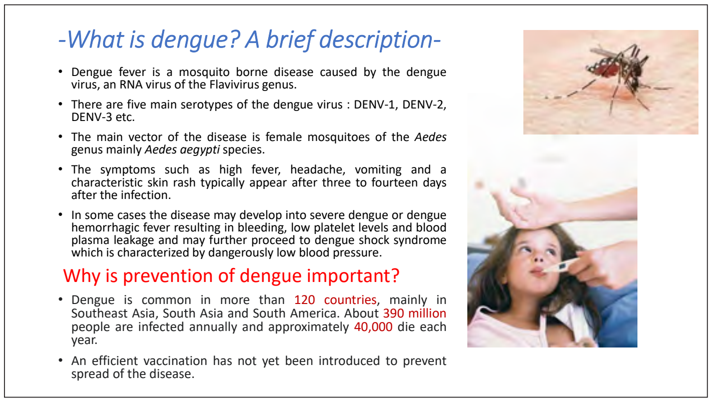
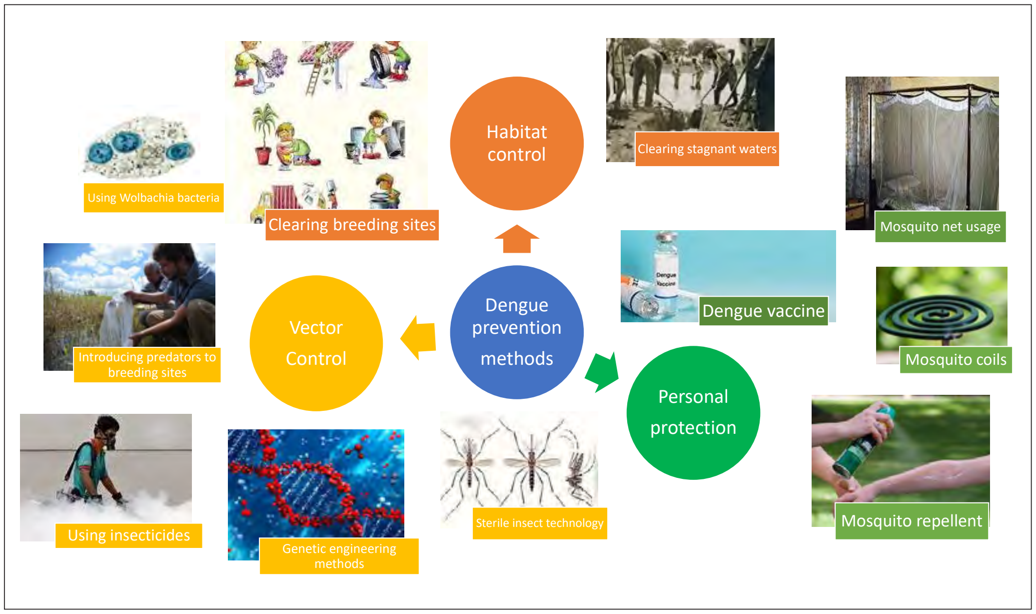


-What is dengue? A brief description-

- Dengue fever is a mosquito borne disease caused by the dengue virus, an RNA virus of the Flavivirus genus.
- There are five main serotypes of the dengue virus : DENV-1, DENV-2, DENV-3 etc.
- The main vector of the disease is female mosquitoes of the *Aedes* genus mainly *Aedes aegypti* species.
- The symptoms such as high fever, headache, vomiting and a characteristic skin rash typically appear after three to fourteen days after the infection.
- In some cases the disease may develop into severe dengue or dengue hemorrhagic fever resulting in bleeding, low platelet levels and blood plasma leakage and may further proceed to dengue shock syndrome which is characterized by dangerously low blood pressure.

Why is prevention of dengue important?

- Dengue is common in more than 120 countries, mainly in Southeast Asia, South Asia and South America. About 390 million people are infected annually and approximately 40,000 die each year.
- An efficient vaccination has not yet been introduced to prevent spread of the disease.

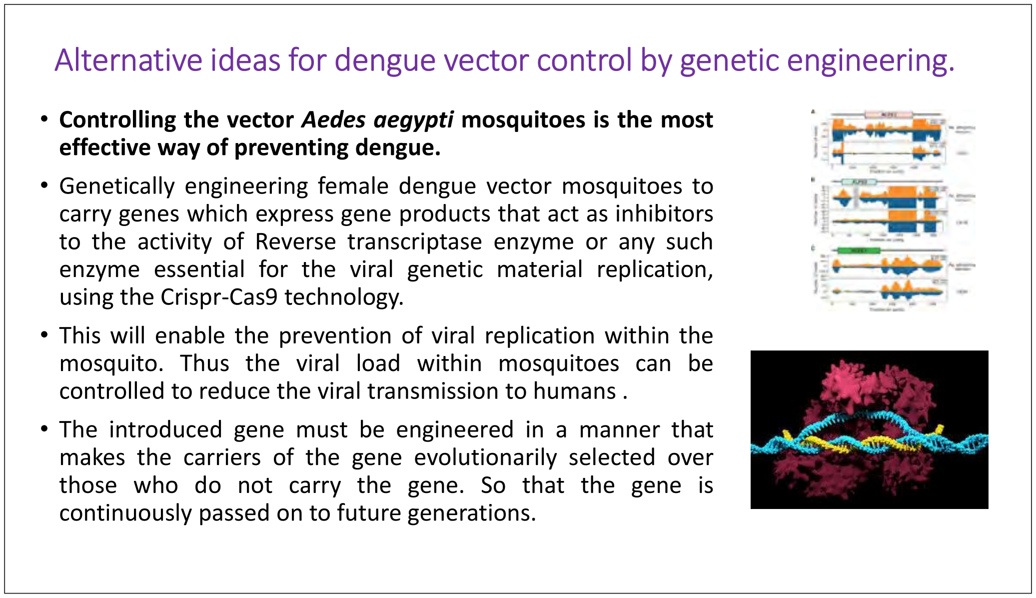


Dengue prevention methods

Habitat control
Clearing breeding sites
Clearing stagnant waters

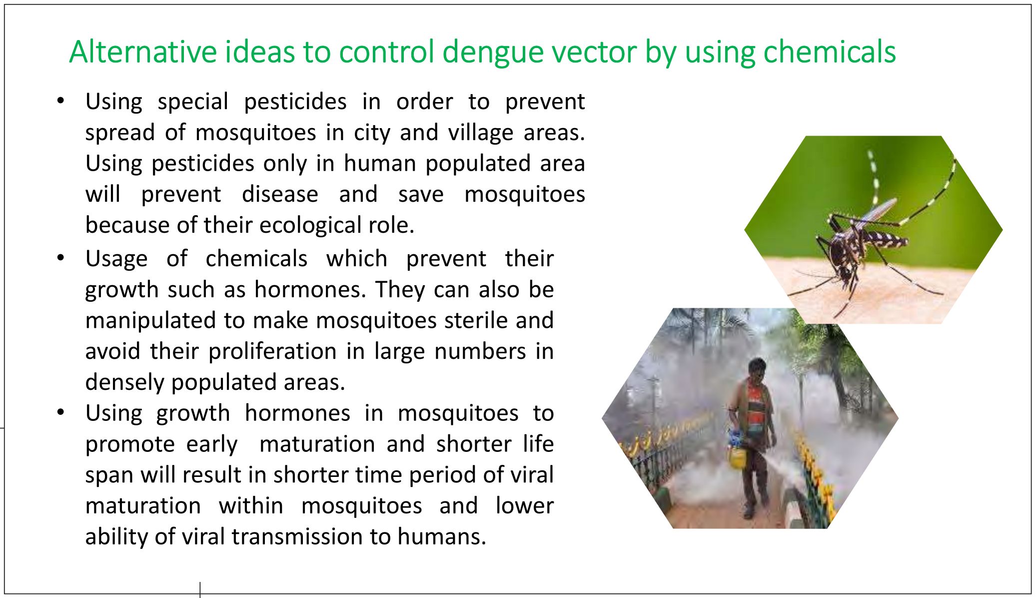
Vector Control
Using Wolbachia bacteria
Introducing predators to breeding sites
Using insecticides
Genetic engineering methods
Sterile insect technology

Personal protection
Mosquito net usage
Dengue vaccine
Mosquito coils
Mosquito repellent



Alternative ideas for dengue vector control by genetic engineering.

- **Controlling the vector *Aedes aegypti* mosquitoes is the most effective way of preventing dengue.**
- Genetically engineering female dengue vector mosquitoes to carry genes which express gene products that act as inhibitors to the activity of Reverse transcriptase enzyme or any such enzyme essential for the viral genetic material replication, using the Crispr-Cas9 technology.
- This will enable the prevention of viral replication within the mosquito. Thus the viral load within mosquitoes can be controlled to reduce the viral transmission to humans .
- The introduced gene must be engineered in a manner that makes the carriers of the gene evolutionarily selected over those who do not carry the gene. So that the gene is continuously passed on to future generations.



Alternative ideas to control dengue vector by using chemicals

- Using special pesticides in order to prevent spread of mosquitoes in city and village areas. Using pesticides only in human populated area will prevent disease and save mosquitoes because of their ecological role.
- Usage of chemicals which prevent their growth such as hormones. They can also be manipulated to make mosquitoes sterile and avoid their proliferation in large numbers in densely populated areas.
- Using growth hormones in mosquitoes to promote early maturation and shorter life span will result in shorter time period of viral maturation within mosquitoes and lower ability of viral transmission to humans.

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  J Devillers, C Lagneau, A Lattes, J.C Garrigues, M.M Clémenté and A. Yébakima

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1B05 MATHEMATICAL DISEASE MODELLING

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MATHEMATICAL DISEASE MODELLING

Project by: Arian Hasani, Elizabeth Rae Peralta, Kjartan Kristjansson and Кирил Тошев

What is mathematical disease modeling?

Mathematical disease modelling is a means through which epidemiologists can quantitatively forecast how an infectious disease will progress. These models can mathematically process collected data to return values which can help make informed decisions on how best to intervene. These interventions include, but are not limited to, mass vaccinations, enforcing quarantines and investment in necessary equipment. Disease models have been used with success to counter the spread of diseases such as HIV, Hepatitis and Tuberculosis.

Today, however, disease modelling has seen far more exposure to the public eye than ever before for its use during the Covid-19 pandemic due to the spread of the disease and greater access to information in the internet era.

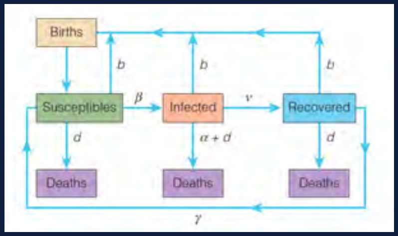

Image 1

What constitutes a good disease model?

A disease model consists of variables. These variables can be objective or based on assumptions. Most "primitive" models consist exclusively of objective variables. For example the SIR model is composed of three data-driven variables representing, respectively, people who are susceptible, infectious and those who have recovered. Another example of an objective variable is the basic reproduction number (R0), the value of which describes how many others an infected person will on average spread the disease to. Assumed variables on the other hand are based on assumptions rather than concrete information.

A good model can use assumed variables to account for various factors. For example, elderly people being more susceptible, certain communities being more likely to spread the disease to others within that community and people who live a certain lifestyle, such as farming, being more or less likely to contract the disease. Modern models also use techniques such as stochastic estimation in which the variables are assigned a degree of randomness so that a wider array of predictions can be made.

The compartmental model

One of the most common and convenient mathematical models for explaining how diseases transmit and behave is the Compartment model. This model divides the host's population into different parts, shown by boxes in illustrations of this model. Each box contains one of the subpopulations of the host. This is why we call this model the compartment model.

Mostly the population is divided into three parts: (this kind of compartment model is called SIR because of these three groups).

Susceptible:

they don't have the disease but they are not immune to it.

Infected:

They have got the disease and their mortality rate is increased.

Recovered:

They got the disease and just recovered and now have temporary immunity against the pathogen.

A host from each subpopulation can move to another one in some rates, shown by some constants. The birth rate of the population is shown by b. The death rate of all the population is shown by d, but the infected people die at the rate of $(d + \alpha)$.

ß is the rate of infection for those who are susceptible and infected people can be recovered in rate of v and the recovered people can lose their immunity and become susceptible again at the rate of y.

This is further illustrated in image 1.

Image 1

Human-animal transmission modeling

Many of the most deadly diseases in human history were converged tthrough animals. Some examples being the black death, swine flu, rabies and now most notoriously, COVID-19.
These diseases have all been projected with compartmental models closely related to the aforementioned SIR model.
An example is the anthrax model. This disease is caused by the bacteria Bacillus anthracis and can infect both humans and animals the model uses the dynamics of the SIR model however, it accounts for both susceptible animals and humans transmitting the disease.
Therefore, the rate of infection in humans or animals is (Ih+ Iv)ß with other constants and variables being mostly the same.
The model contains 7 compartments which are further illustrated in image 2:

Susceptible vector (Sv)
Infected vector (Iv)
Vaccinated vector (Vv)
Recovered vector (Rv)
Susceptible human (Sh)
Infected human (Ih)
Recovered human (Rh)

Factors that affect disease transmission models

There are different factors that affect disease transmission models. One of these factors is demographics.
Respiratory diseases, according to past studies, generally show a similar trend wherein younger populations generally show reduced susceptibility to the disease due to the fact that children are generally less exposed. Another contributing factor as to why children are less susceptible is because of the production of non-specific antibodies from other respiratory diseases. Although different diseases show different trends with respect to younger age groups, older age groups have similar findings. Older age groups typically show increased susceptibility to diseases because of their weakened immune system, among others. Aside from age, social status, population distribution, and population growth all contribute to disease transmission, with the latter currently used by Centers of Disease Control in different countries for early detection of outbreaks. The environment is also a major factor in disease transmission modelling. Climate conditions as well as the mobility of the population determine how fast a virus moves within a population or an area. The evolution of the disease also plays a part in disease modelling because any major evolutions to a pathogen's genetic code will set back any developments made against the pathogen.

Limitations of Disease Transmission Modelling

Disease transmission models help with the mitigation and surveillance of different diseases all around the world, but it does have its limitations.
Although disease evolution can be predicted, the nature of the mutations is random, making it hard to create accurate models. The pathogen is not the only that is constantly evolving. The world population is also constantly changing. It is also heterogenous, meaning that studies conducted on a particular population may not have the same conclusions as that of another population. With this, there also needs to be multiple sources of information for models to be as accurate as possible.
Different disease models may also differ in findings, where status-based models have different results as compared to history-based or individual-based models.
Lastly, one of the biggest problems of those in the field of public health is that real time scenarios are often different from scenarios predicted by the models, meaning the process in creating these models should be further developed.

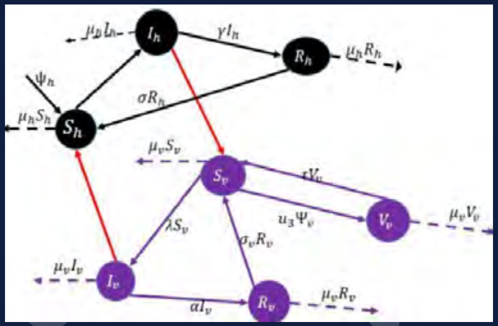

Image 2

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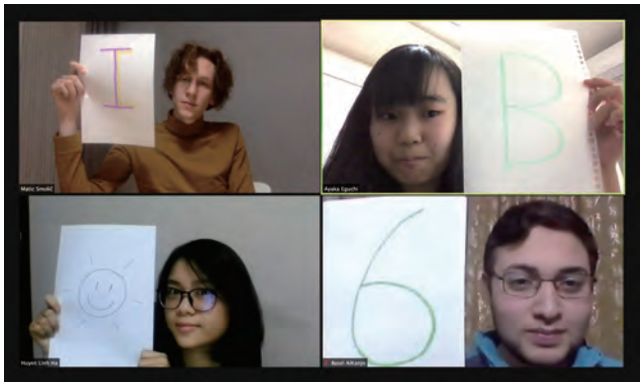
1B06 The Roles of Nanobiotechnology in Combatting Infectious Diseases



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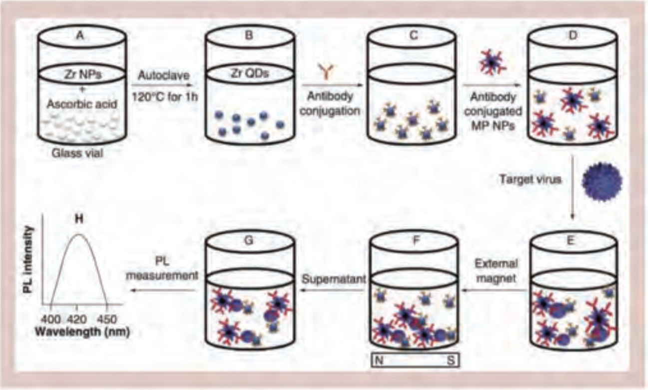

Figure 2 : One Type of Detection Procedures (1)

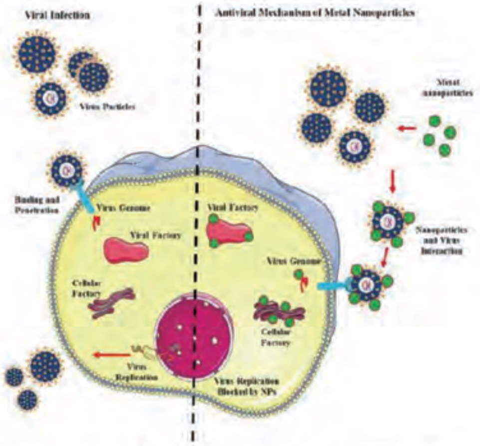

Figure 3 : Antiviral Mechanism of Nanoparticles (2)

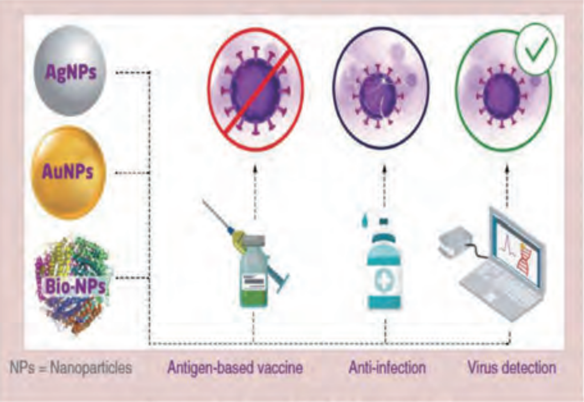

Figure 1 : Graphical Abstract (1)

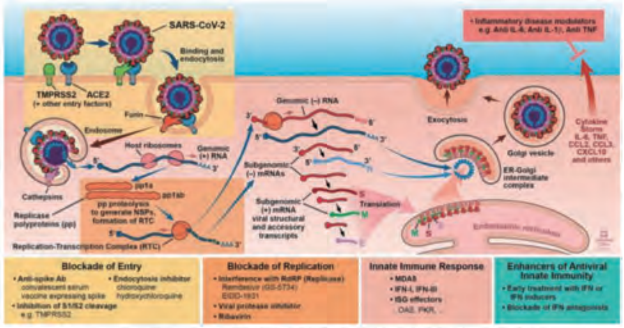

Figure 4 : SARS-COV-2 Replication Cycle (3)

The Roles of Nanobiotechnology in Combatting Infectious Diseases

Authors: (alphabetically ordered)

Basel AlKanjo (Syria), **Harri Martinnen** (Finland), **Huyen Linh Ha** (Vietnam), **Matic Smolič** (Slovenia)

Abstract :

Infectious diseases present public health challenges worldwide. The current COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a global issue nowadays. With the alarming COVID-19 outbreak, there is an urgent need to develop efficient methods for the treatment, detection, and possibly prevention of spreading of this virus. In this regard, researchers shed light on the applications of a new field of science called nanobiotechnology that could offer promising solutions for many challenges concerning infectious diseases. This paper focuses on the reliability of nano-applications as new approaches for confronting the global Covid-19 pandemic. Thus, nanobiological intervention is discussed in terms of designing effective nanoparticles to counter the conventional limitations of common antiviral and biological procedures.

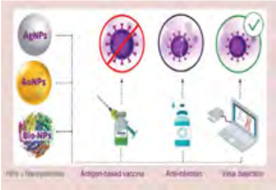

Figure 1 : Graphical Abstract (1)

Detection of Infectious Viruses :

Nowadays, Global pandemic of COVID-19 is an immerse problem. This arouses many research groups to work on the diagnosis of viral particles. It is now admitted that the RT-qPCR is the most accurate test for diagnosis the COVID-19, however, the usage of this method is based on the detection of the genetic material of virus, which could be in some cases undetectable due to its degradation or limitation. So, in order to avoid false either positive or negative results that can occur by using such standard detection methods for COVID-19, the usage of nanotechnology in viral diagnosis, based on the detection of native viral particle, has shown to be a promising approach.

Firstly, gold nanoparticles can be used for COVID-19 detection since they possess specific optical and electrical features making them suitable as detector probes against virus, and these Au NPs are furthermore known for a special phenomenon called surface plasmon resonance (SPR). Due to the interaction between Au NPs with the guest particles the effects of SPR are changed and can be therefore used as a signal for biosensor applications. For example, detection method based on colorimetric assays can enable COV detection using Au associated with various entities such as double stranded DNA that specifically binds to COV or by using Ag NPs attached to acpcPNA, which remain dispersed in the presence of complementary COV derived DNA, giving rise to a detectable color change.

Secondly, as mentioned before, silver nanoparticles could be alternatively applied in detection techniques, since their optical properties are quite similar to those of Au NPs. Ag NPs are now used in different metal nano-arrays, which improve the plasmatic activity with the assistance of Raman labelling with active components.

Thirdly, attaching specific anti-viral antibodies to magnetic beads and then separating the target from a sample by applying a magnetic field (magnetic bead-based immunoassay) could be also used for detection of COVID-19. In the past, MnFe2O4 magnetic beads have already been conjugated with anti-influenza antibodies to detect viruses. The target complex was then conducted visually based on fluorescence intensity. Likewise, another method proposes immunoassay to detect the IgG antibodies against HBV antigens and similar approaches could be potentially used for detection of COVID-19. Additionally, fluorescent Zr QDs and magnetic nanoparticles are in the process conjugated with antibodies that specifically bind to COV. As a result, in the presence of COV, a particular fluorescent complex is consequently formed and furthermore isolated magnetically and discerned by fluorescence measurements .

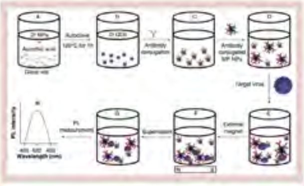

Figure 2 : One Type of Detection Procedures (1)

Fourthly, an alternative type of COV diagnosis can also rely on the usage of nano-traps, which capture and concentrate corona viruses, leading to improvement of their stability and furthermore facilitating their detection over a long period of time. Moreover, some references also report the possibility of COV detection with use of biosensor made out of carbon electrodes that contain gold nanoparticles (Au NPs) associated with viral spike proteins. Comparably, other approach proposes the use of field-effect transistors (FET) coated with graphene sheets attached to antibodies that are able to recognize COVID-19 spike proteins thereby allowing the detection of these proteins in different medias — phosphate-buffered saline, culture medium, clinical samples.

Lastly, nanotechnology could be used to further improve already existing widespread methods and could be therefore easily implemented. The PCR technique is currently the most broadly-used method for COVID-19 detection. The efficiency of PCR, which is based on the synthesis of cDNA from genomic RNA and is followed by amplification, could be improved by using NPs. In general, reverse transcription PCR would be carried out in the presence nanoparticles, improving the efficacy of the polymerase chain reaction. Consequently, this would result in a better sensitivity, and help avoiding cross-contamination with other viruses.

All in all, it seems pertinent to say that using nano-materials can change for the better our existing COVID-19 -detection methods by either allowing specific COVID-19 binding at nanoparticle's surface or improving PCR efficacy, generally leading to better sensitivity compared with other detection methods. Nevertheless, it is important to note that costs, durability, and environmental effects of materials used should be considered before their general application.

Treatment of Infectious Diseases :

A nanomedicine strategy is a powerful equipment to evolve new medical applications against infectious diseases and improving COVID-19 therapeutic management .

One of the most exceptional properties that NPs acquire is their high surface to volume ratio due to their tiny size, which accommodates for their special physical , chemical, and biological properties. Recently, nanomediated combination therapy , which uses NPs as carriers for the antiviral drugs , have shown immense promise in nanomedicine activities against viral diseases. Nanocarrier based therapeutics offer several opportunities to overcome the limitations and difficulties of current antiviral therapy applications. Many fundamental challenges can be solved by nanocarrier based antiviral drugs delivery. NPs have been shown to be efficient for the delivery of therapeutic moieties such as drugs, vaccines, siRNAs and certain peptides. They are widely used to deliver hydrophobic compounds which alone exhibit poor solubility in the blood. Connecting the right therapeutic candidate to the right nanocarrier is crucial and essential for the commercial success of nanomedicine applications against the SARS-CoV-2. Using NPs assisted therapy that can act as a delivery system has many benefits. Firstly, it can readily cross biological membranes in which most drug agents cannot cross alone, including cell membranes. This remarkable property gives NPs the capability of fighting against intracellular pathogens.

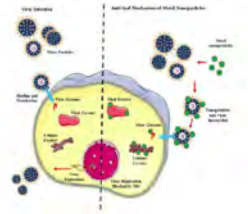

Figure 3 : Antiviral Mechanism of Nanoparticles (2)

Some NPs contributes in promoting body immune responses to detect viral antigens and fight against the viral infection. Nanoparticles have shown their ability to target both adaptive (T cells, B cells) and innate immune systems (macrophages, monocytes, neutrophils) at the cellular level. Modulating APCs using nanoparticles could be very important, particularly for COVID-19 vaccine strategies.

The great effectiveness of NPs reveals up in homeotherapy; Targeted nanoparticles provide an improved rate of endocytosis which better ensures delivery of a therapeutic nanoparticle dose into the target cell, as a result ,it can lower the required dosage for the treatment of patients , being cost effective , and also reducing side effect risks by restricting the entry and distribution of the drug reagents into only the target cells.

Nanomaterials have been regularly applied in as antiviral agents , including :NPs which can act as receptor antagonists ; efforts are being made to find certain NPs which can serve as antagonists for the ACE2 receptor that coronaviruses utilize to enter into host cells. Silver NPs and certain kinds of nanopolymers exhibit inhibitory effects for many essential steps in the viral replication cycle, such as reverse transcription, negative and positive RNA strand synthesis, virion budding, etc.. It is further possible to target a specific cellular and intracellular sites involved in the pathophysiology of SARS-CoV-2 using nanomediated medicine applications.

Prevention of Infectious Diseases Spreading :

Scientists had been actively inventing protective equipments that can limit the spreading of infectious viruses .

Firstly, protective customs against infectious viruses include eye protections, face masks, lab coats, gloves, and boots that are made from metallic nanoparticles and silver NPs. These products have the ability to remove virus-size particles resulting in their antiviral and antimicrobial functions as they could minimize air filter pressure and enhance the filtration process. These features allow today's technology to produce reusable masks which preserve intact nanomembranes, exhibit filtration efficiency, and are water resistance after washing many times.

In addition to that , these products also minimize financial pressure in production since they use cellulose nanofiber made from waste plant materials such as sugar cane bagasse and other agricultural products, they are also able to produce large quantities just in little time.

Secondly, Nanomaterials-based coatings are currently used for several applications and different products are now available. Various nanomaterials, such as silver, bismuth, or titanium nanoparticles, have been developed for coating surfaces. They support the prevention applications in terms of reducing the attachment of pathogens and disrupting the structure of pathogens.

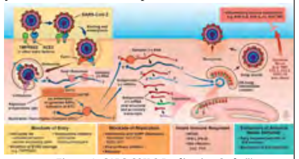

Figure 4 : SARS-COV-2 Replication Cycle (3)

Thirdly, sanitizers made from NPs, for example silver salts, are safe for sanitizing purposes and have the capability to inactivate the viruses on surfaces and reduce the presence of SAR-CoV2. Other facilities, like air filter and wound dressing, which are made from NPs ,are also able to reduce or remove viral particles.

Adjuvants which are made from NPs play an important role in stimulating immune responses in order to enhance the vaccine's effects. For example, gold NPs stimulate IgG response using nanovaccines made from spike S of SARS-CoV2, Nanocarriers -based delivery systems such as metal oxide NPs, liposomes, and dendrimers protect nanovaccines from premature degradation, increasing stability of nanovaccines' structure.

Nanovaccine candidates include: Lipid NPs which encapsulate mRNA-based vaccine that encodes for a full-length stabilized spike protein of SARS-CoV2 or a DNA plasmid encoding the spike protein.

Conclusion and Future Directions :

The nano-based technology system currently presents novel approaches to overcome conventional challenges and has thus attained significant attention in confronting infection pandemics. However, there are still some limitations involved, especially in biosafety and nanotoxicity. Despite many advantages, some nanomaterials exhibit poisonous effects on the cells which might interfere with normal metabolism, cause the improvement of some bacteria's fitness, and alter bacterial metapopulation of the human body. In addition, some NPs are toxic at a particular level of its concentration, therefore, suitable doses of these compounds must be in careful consideration.

Although there are still many challenges and barriers to achieve full potential and effectiveness of nanomedicine, the field seems to be promising against Covid-19 pandemic. Consequently, future directions should explore the possibilities of improving conventional applications by using advanced research in nanobiotechnology.

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and for the **IBO Committee 2020**.

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1B07 Infectious Diseases Finding the permanent cure for HIV?

1B07

Infectious Diseases

Finding the permanent cure for HIV?

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Kim Dale, Republic of Korea

Munasinghe Samidhi Manthilani, Sri Lanka

Prelog Ivo, Slovenia

The Two Lucky Humans Who Have Escaped From HIV

Berlin Patient: Timothy Ray Brown

Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

London Patient: Adam Castillejo

The New York Times
Rare Treatment Is Reported to Cure AIDS Patient

CCR5-Δ32 deletion shows promise

Science News
Second patient has been cured of HIV, study suggests
Long-term follow-up of the London patient suggests no detectable active HIV virus remains in the patient

Timothy Ray Brown: the accidental AIDS icon
The 'Berlin patient' didn't ask to be cured of HIV; now he wants a cure for all

Medical News Today
2nd person cured of HIV thanks to stem cell transplant

The New York Times
H.I.V. Is Reported Cured in a Second Patient, a Milestone in the Global AIDS Epidemic

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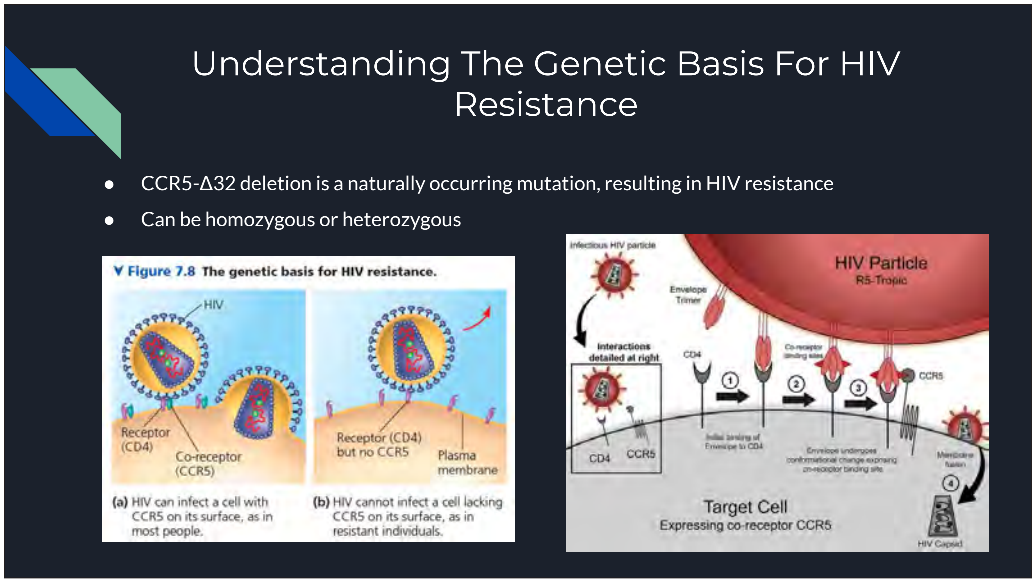


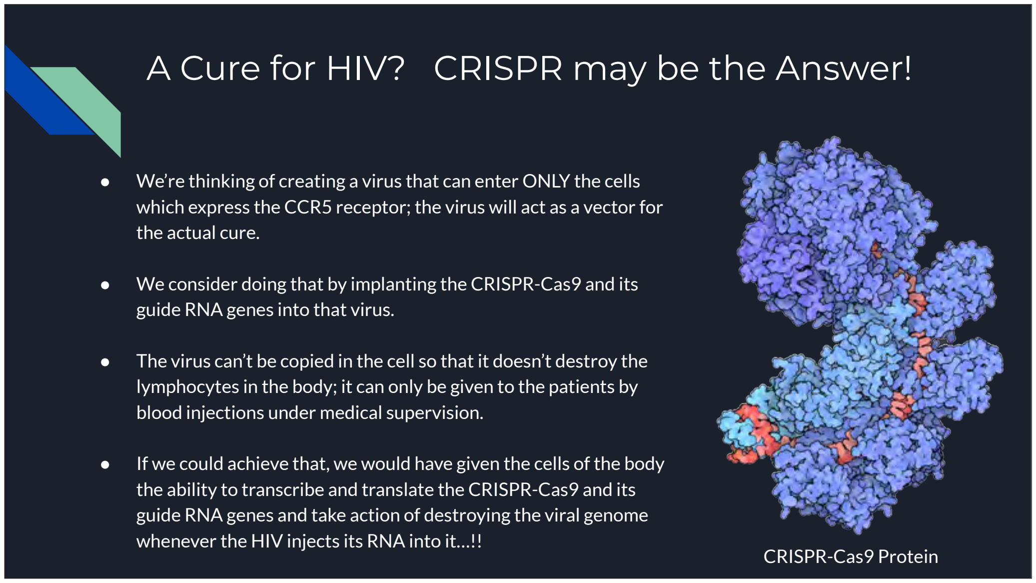
Understanding The Genetic Basis For HIV Resistance

- CCR5-Δ32 deletion is a naturally occurring mutation, resulting in HIV resistance
- Can be homozygous or heterozygous

Figure 7.8 The genetic basis for HIV resistance.

(a) HIV can infect a cell with CCR5 on its surface, as in most people.

(b) HIV cannot infect a cell lacking CCR5 on its surface, as in resistant individuals.



A Cure for HIV? CRISPR may be the Answer!

- We're thinking of creating a virus that can enter ONLY the cells which express the CCR5 receptor; the virus will act as a vector for the actual cure.
- We consider doing that by implanting the CRISPR-Cas9 and its guide RNA genes into that virus.
- The virus can't be copied in the cell so that it doesn't destroy the lymphocytes in the body; it can only be given to the patients by blood injections under medical supervision.
- If we could achieve that, we would have given the cells of the body the ability to transcribe and translate the CRISPR-Cas9 and its guide RNA genes and take action of destroying the viral genome whenever the HIV injects its RNA into it…!!

CRISPR-Cas9 Protein

Gene Edited Twins and Ethical Concerns

- November 2018: First gene edited babies (twins) were born in China
- CCR5 gene was disabled (not in the same way as naturally) → effects cannot be predicted
- Global backlash
- DNA changes are passed down the generations
- CRISPR is not perfect → can alter important genes

Literature and Sources

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1B08 CAN PHAGE THERAPY SOLVE ANTIBIOTIC RESISTANCE?

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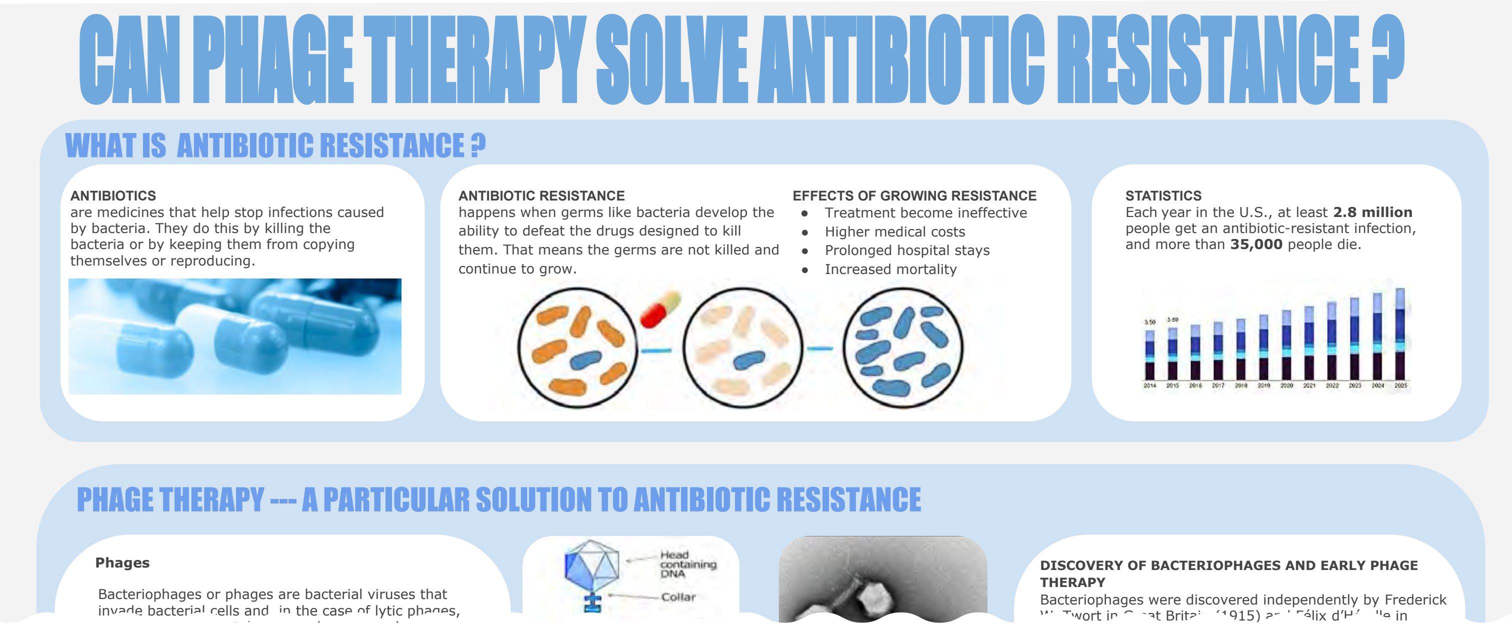


CAN PHAGE THERAPY SOLVE ANTIBIOTIC RESISTANCE ?

WHAT IS ANTIBIOTIC RESISTANCE ?

ANTIBIOTICS
are medicines that help stop infections caused by bacteria. They do this by killing the bacteria or by keeping them from copying themselves or reproducing.

ANTIBIOTIC RESISTANCE
happens when germs like bacteria develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow.

EFFECTS OF GROWING RESISTANCE
- Treatment become ineffective
- Higher medical costs
- Prolonged hospital stays
- Increased mortality

STATISTICS
Each year in the U.S., at least **2.8 million** people get an antibiotic-resistant infection, and more than **35,000** people die.

PHAGE THERAPY --- A PARTICULAR SOLUTION TO ANTIBIOTIC RESISTANCE

Phages

Bacteriophages or phages are bacterial viruses that invade bacterial cells and in the case of lytic phages,

DISCOVERY OF BACTERIOPHAGES AND EARLY PHAGE THERAPY
Bacteriophages were discovered independently by Frederick W. Twort in Great Britain (1915) and Félix d'Hérelle in

Phages

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse.

Main characteristics

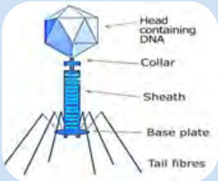
- NOT cells
- Have no cell structure
- Can't grow, move and feed outside of host cell
- Reproduce inside living cell
- Made up of a core of DNA or RNA surrounded by a protein coat (capsid).

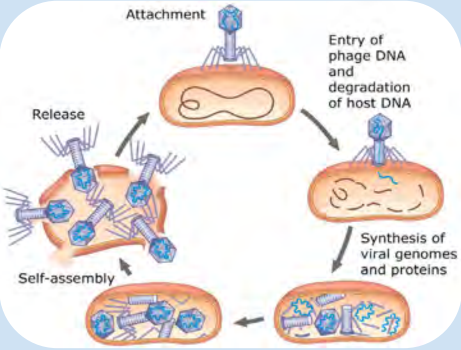
Virulent bacteriophages – Lytic cycle

Virulent bacteriophages happen to be those that play in our interest. This bacteriophage type uses the lytic cycle for replication.

Lysis or lytic cycle is a cytoplasmic viral replication process in which the bacteriophage injects its genetic material into a host cell, which allows this genetic material to replicate, producing many new phages. Once the host cell is filled with new bacteriophages, the host cell raptures from within, releasing the newly formed phages.

- It is important that the bacteriophages that are used for phage therapy are all virulent phages





DISCOVERY OF BACTERIOPHAGES AND EARLY PHAGE THERAPY

Bacteriophages were discovered independently by Frederick W. Twort in Great Britain (1915) and Félix d'Hérelle in France (1917).

The first reported application of phages to treat infectious diseases of humans came in 1921 from Richard Bruynoghe and Joseph Maisin who used bacteriophages to treat staphylococcal skin disease. The bacteriophages were injected into and around surgically opened lesions, and the authors reported regression of the infections within 24-48 h.

PROPHYLAXIS AND TREATMENT OF BACTERIAL INFECTIONS IN HUMANS

- Completed phase 2. Significant reduction of P.aeruginosa load from baseline in phage group of patients in UK
- Pyophage treatment was applied via nebulization, P.aeruginosa reduced in cystic fibrosis patient in UK
- Bacteriophages cocktail as a prebiotic treatment for gastrointestinal disorders was tested in USA
- Completed phase 2. Pathogenic bacteria decreased in patients with urological infections treated Pyo bacteriophage (S. aureus, E.coli, Streptococcus spp., P.aeruginosa) in Georgia
- Completed phase 1. No safety concerns with WPP-201-(S. aureus, E.coli, P.aeruginosa) treatment for venous leg ulcers were found in USA.

MAJOR ADVANTAGES

- **Reduction of bacterial resistance.**
  Because phages and bacteria are both living organisms they both evolve at the same time. So when a bacterium has developed a resistance against a phage it will also develop a mutation that will thwart the bacterium's resistance.

- **Auto "dosing".**
  When phages kill bacteria they can increase in number specifically where hosts are located. So phages themselves contribute establishing the phage dose.

- **Minimal disruption of flora.**
  On one hand, because of their host specificity phages only minimally impact health-protecting normal flora bacteria. Indeed they can infect from only few strains of a bacterial species to more than one relatively closely related bacterial genus. On the other hand, many chemical antibiotics are prone to induce superinfections since they eradicate a huge amount of bacteria (pathogene or not).

- **Bactericidal agents.**
  Bacteria that have been successfully infected by obligately lytic phages are unable to regain their viability. However, certain antibiotics are bacteriostatic (such as tetracycline) That means that bacteria may more readily permit bacterial evolution towards resistance.

PROBLEMS OF PHAGE THERAPY AND POSSIBLE SOLUTIONS

- **Phages have a limited range:** one type of phage can only target a few kinds of bacteria.
  1. **Use phage proteins:** It's effective against more types of bacteria, but loses most of the advantages of phages. Consider evolving phages against the resistant strains, then collecting proteins produced, to negate the effects of evolution of the bacteria against the protein.
  2. **Rapidly identify the type of bacteria causing the infection, then use the corresponding phage against it:** It enables effective targeting, but the techniques and equipment aren't widespread enough to be used in most clinical settings. Technological advancements may eventually lower the cost of the process, solving this problem.

- **Phages may induce allergic reactions in the body or be eliminated by antibody production of the body.**
  1. **Purify phages, and consider selecting for phages with a lower probability of causing antibody production and allergic reactions:** It has been shown by past research that it can be done. Antibody production should not be a major issue, because the time for antibody production by the body is long enough for the therapy to take effect.

- **Difficulties in finding active phages.**
  1. **Suitable phages can be found by screening through natural habitats:** Sewage water, pond water, etc. yields various types of phages, and can be isolated and cultured in the lab.
  2. **Consider genetically modifying existing phages:** It is prohibited by law to use genetically modified phages for human therapy, but an extensive understanding of the phage genome should enable us to genetically modify phages to reach desired effects.

- **Producing phages of a suitable quality for clinical use.**
  Phage preparations need rigorous purification before human usage, to prevent unwanted immune reactions. This means that production may be slowed by limited production sites that guarantee quality.

- **Phage therapy needs more research in order to prove its effect, and laws need change to enable extensive research and human trials of the phage therapy.**

CONCLUSION

To conclude, phages seem to be one of the best known alternative to antibiotics even if there are some issues that need to be solved. The biggest problem phage therapy is facing is probably the lack of knowledge, even if there were discovered before antibiotics. However all the advantages offered by phage therapy have led to pleading in favor of the establishment of a global phage production for clinical use.

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2A01 Can the physiological response to microplastic exposure be used to determine their impact on an ecosystem?

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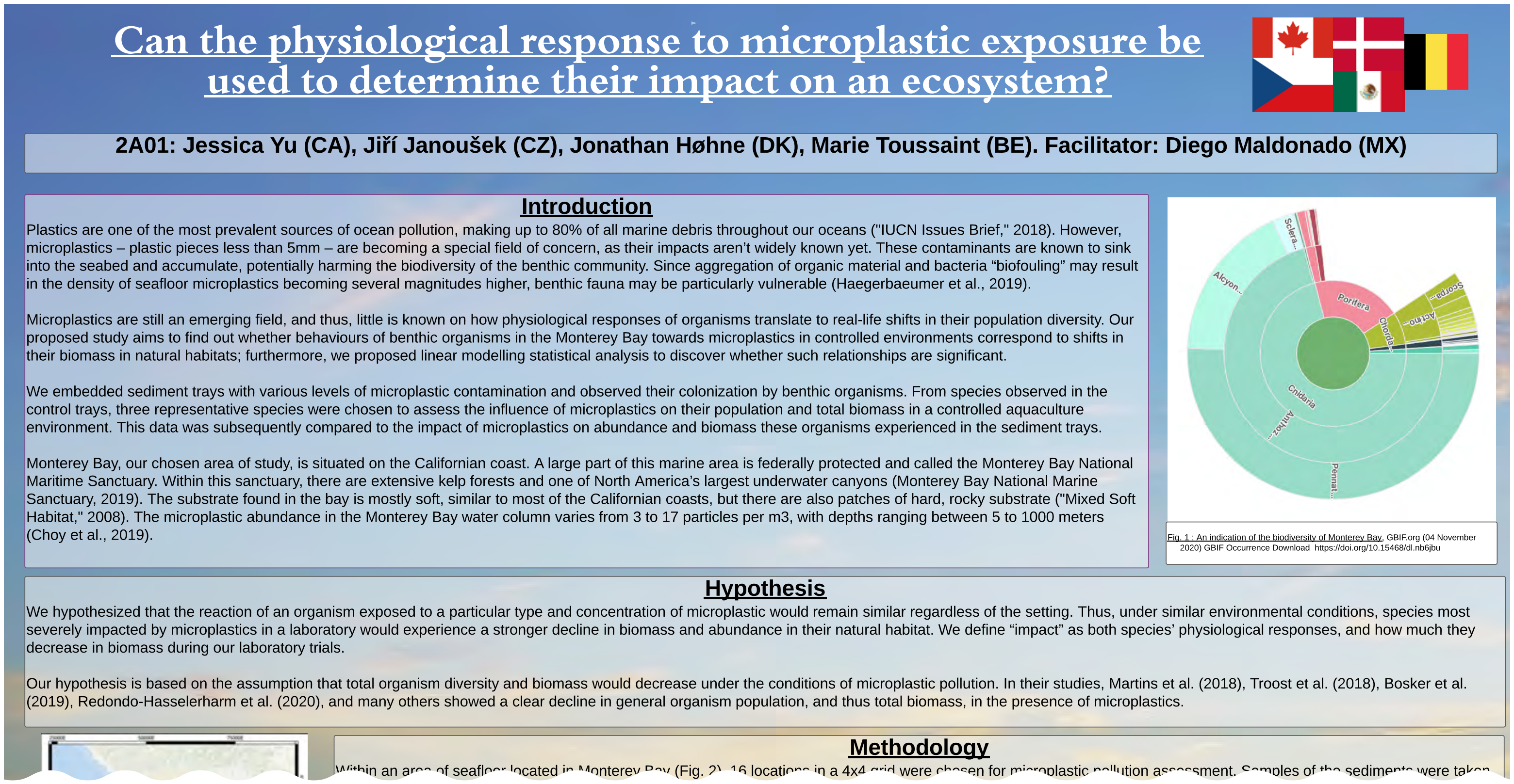

Can the physiological response to microplastic exposure be used to determine their impact on an ecosystem?

2A01: Jessica Yu (CA), Jiří Janoušek (CZ), Jonathan Høhne (DK), Marie Toussaint (BE). Facilitator: Diego Maldonado (MX)

Introduction

Plastics are one of the most prevalent sources of ocean pollution, making up to 80% of all marine debris throughout our oceans ("IUCN Issues Brief," 2018). However, microplastics – plastic pieces less than 5mm – are becoming a special field of concern, as their impacts aren't widely known yet. These contaminants are known to sink into the seabed and accumulate, potentially harming the biodiversity of the benthic community. Since aggregation of organic material and bacteria "biofouling" may result in the density of seafloor microplastics becoming several magnitudes higher, benthic fauna may be particularly vulnerable (Haegerbaeumer et al., 2019).

Microplastics are still an emerging field, and thus, little is known on how physiological responses of organisms translate to real-life shifts in their population diversity. Our proposed study aims to find out whether behaviours of benthic organisms in the Monterey Bay towards microplastics in controlled environments correspond to shifts in their biomass in natural habitats; furthermore, we proposed linear modelling statistical analysis to discover whether such relationships are significant.

We embedded sediment trays with various levels of microplastic contamination and observed their colonization by benthic organisms. From species observed in the control trays, three representative species were chosen to assess the influence of microplastics on their population and total biomass in a controlled aquaculture environment. This data was subsequently compared to the impact of microplastics on abundance and biomass these organisms experienced in the sediment trays.

Monterey Bay, our chosen area of study, is situated on the Californian coast. A large part of this marine area is federally protected and called the Monterey Bay National Maritime Sanctuary. Within this sanctuary, there are extensive kelp forests and one of North America's largest underwater canyons (Monterey Bay National Marine Sanctuary, 2019). The substrate found in the bay is mostly soft, similar to most of the Californian coasts, but there are also patches of hard, rocky substrate ("Mixed Soft Habitat," 2008). The microplastic abundance in the Monterey Bay water column varies from 3 to 17 particles per m3, with depths ranging between 5 to 1000 meters (Choy et al., 2019).

Fig. 1 : An indication of the biodiversity of Monterey Bay, GBIF.org (04 November 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.nb6jbu

Hypothesis

We hypothesized that the reaction of an organism exposed to a particular type and concentration of microplastic would remain similar regardless of the setting. Thus, under similar environmental conditions, species most severely impacted by microplastics in a laboratory would experience a stronger decline in biomass and abundance in their natural habitat. We define "impact" as both species' physiological responses, and how much they decrease in biomass during our laboratory trials.

Our hypothesis is based on the assumption that total organism diversity and biomass would decrease under the conditions of microplastic pollution. In their studies, Martins et al. (2018), Troost et al. (2018), Bosker et al. (2019), Redondo-Hasselerharm et al. (2020), and many others showed a clear decline in general organism population, and thus total biomass, in the presence of microplastics.

Methodology

Within an area of seafloor located in Monterey Bay (Fig. 2), 16 locations in a 4x4 grid were chosen for microplastic pollution assessment. Samples of the sediments were taken

Fig. 2 : Survey points at the Monterey Bay sea floor. The orange dots signify locations where sediment samples were taken for microplastic polution assesment. The inner points of the 4x4 grid indicate the placement of the sediment trays.

Methodology

Within an area of seafloor located in Monterey Bay (Fig. 2), 16 locations in a 4x4 grid were chosen for microplastic pollution assessment. Samples of the sediments were taken and analyzed for microplastic concentrations according to the methodology described in Courtene-Jones et al. (2020).

A total of 80 sediment trays were placed on the seafloor. The trays were prepared according to a method previously described in Redondo-Hasselerharm et al. (2020); they were divided into 4 groups (placed at the inner 4 points in the 4x4 grid), with 5 treatments at 4 technical replicates per treatment within each group. The microplastic contamination of the sediment was set to be 2 times, 4 times, 8 times and 16 times the background average of the local microplastic contamination, in addition to an unaltered control. After 24 months, the trays were extracted and the sediment was washed with water over a 0.5 mm sieve until all fine particles were removed. The leftover material was sorted and macroscopic organisms were determined. For each present species, the total biomass and number of individuals were measured. From the three most abundant phyla in the control sample (Annelida, Arthropoda and Mollusca), a representative species was chosen for laboratory cultivation.

The organisms of the representative species were cultivated in aquaculture according to Besseling et al. (2017). The experimental setup included 60 aquaria divided equally among 3 experimental species, 5 treatment groups treated using the same sediment as described above and 4 technical replicates. Afterwards the total number and biomass of the cultivated organisms in the cultures was measured.

An linerar modeling analysis was performed to determine the effect of microplastic contamination on the abundance and biomass of each species in the sediment trays and aquaculture, as well as the total organism biomass and biodiversity in the trays (the analysis was performed not with absolute biomass and abundance, but the relative value in comparison to the control). In the case that our hypothesis is true, we would expect the two effects of plastic concentration and environment to be fully additive. The presence of a significant non-additive effect (interaction of variables) would, on the other hand, disprove our hypothesis.

Results

We predict that our experiment may show a significant disparity between the effect of microplastics on certain organisms in aquaculture compared to the studied sea floor area. Particularly, we predict the decline in abundance and total biomass of annelid worms in response to increasing microplastic contamination of the sediment trays, with no such effect observed in the aquaculture setting at comparable microplastic particle concentration. This prediction is based on the results of previous studies of the impact of microplastics on benthic invertebrates, namely Hasselerharm et al. 2018 and 2020:

In the 28-day long experiment by Hasselerharm et al. (2018) (Fig. 3), no effects of the microplastics was observed on the organisms exposed to micro- and nanoplastics in a controlled environment except for a reduction in growth of Gammarus pulex. The other five species (notably including two members of the phylum Annelida) were not measurably affected in any way, despite the very high microplastic concentrations being used (reaching up to 40 % of the dry weight of the sediment).

However, Hasselerharm et al. (2020) showed significant differences in the effect of microplastic and nanoplastic contaminated sediment on benthic community composition in a natural setting (Fig. 4 & 5). Namely, annelid worms showed a notable decline, which contributed to an overall decrease in macroinvertebrate abundance due to micro and nanoplastic contamination. This effect was observed with both examined particle sizes, but in both cases only at the highest tested concentration of plastic particles, which was 5 % of sediment weight.

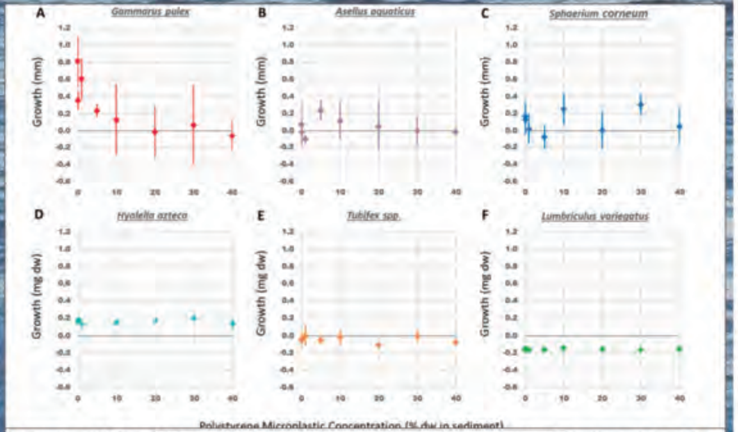

Fig. 3 (Hasselerharm et al. 2018) : effect of microplastic pollution on the growth of six species of inverebrates in a controlled environment. The graph represent the growth of six inverebrate species kept in aquaculture with microplastic contaminated sediment, with polystyrene plastic particle concentration ranging from 0 to 40 % dry sediment weight (axis X). Growth was measure in milimetres or in miligrams of dry weight. The vertical lines represent standart deviation.

Fig. 4 (Hasserlerharm et al. 2020) : abundance of diferent classes of invertebrates under different level of microplastic exposure The graph represents the number of individuals of different invertebrate classes (calculated as mean of 4 replicates) in microplastic contaminated trays with concentrations of polystyrene microplastic particles ranging from 0 to 5 % dry sediment weight (axis X) after 3 months (M3) and 15 months (M15).

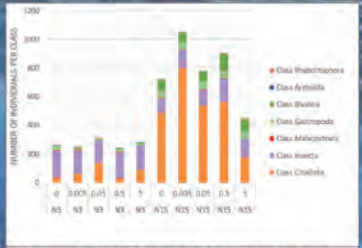

Fig. 5 (Hasserlerharm et al. 2020) : abundace of different classes of invertebrates under different level of nanoplastic exposure. The graph represents the number of individuals of different invertebrate classes (calculated as mean of 4 replicates) in nanoplastic contaminated trays with concentrations of polystyrene nanoplastic particles ranging from 0 to 5 % dry sediment weight (axis X) after 3 months (N3) and 15 months (N15).

Discussion

A vast quantity of research on the issue of microplastics is available (Guzzetti et al. 2018, Wright et al. 2013), however, there are many varying factors such as the types of plastics, particle sizes and shapes, observed physiological impacts, and general methodologies; therefore, directly comparing the resulting data is extremely difficult. Furthermore, the ecological relevance of these findings are diminished by the fact that the effects of microplastics on different organisms are often studied at unrealistically high particle concentrations (de Sá et al., 2018) and over short periods of time.

The comparison of two sets of data gathered in a field experiment (Fig. 4 and 5) and controlled environment experiment (Fig. 3) shows a notable discrepancy; while annelid worms were the most impacted in the field experiment, the controlled environment experiment showed no effect on the two tested annelid species, but rather a growth reduction of amphipod crustaceans.

These results indicate that present controlled environmental studies are likely not sufficient in determining the impact of microplastic pollution on real ecosystems. This is important, as we rely heavily on laboratory-based studies to learn about how microplastics affect species. Further studies in the future should be tailored to gather more representative data about microplastic pollution in an ecological context by using particle concentrations realistically encounterable in natural environments. Treatments and observations across longer time frames would likely be beneficial as well in encompassing acute and chronic effects of microplastic exposure.

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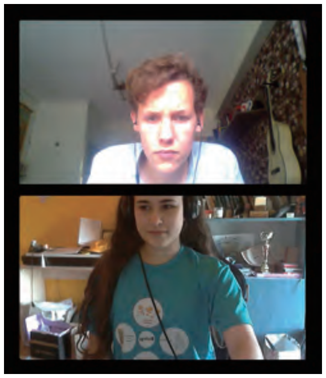
2A02 Research on Mapping the Biodiversity of Microorganisms Living on Garbage Patches



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The Awards of Excellence

Research on Mapping the Biodiversity of Microorganisms Living on Garbage Patches

By: Dante Bosgoed, The Netherlands, Yao Quian, China, Hoshgeldi Hallayev, Turkmenistan
Facilitator: Piti Alexandra Nóra

The last few years the problem of garbage patches, huge areas at sea covered with garbage such as plastics (see fig. 1), became more clear. These patches, created by ocean currents accumulating large amounts of garbage, have an influence on the ecosystems of oceans.

Not only can larger animals die from accidents with plastics, but micro-organisms could also play an enormous role on influencing the ecosystem [2]. Succession of these organisms could lead to new habitats for others. This can be disruptive (see fig. 2). F.e. could microplastics that are produced by degradation processes be accumulated in organisms of a higher trophic level [3]. Not much research is done about the biodiversity of microorganisms on those garbages patches and their effects.

Fig. 1: A picture of a part of a garbage patch in the Pacific ocean. (To clean up patches like these a Dutch student started a non profit organisation "Ocean Cleanup" to clean the oceans while making use of sea currents, floaters and special collecting stations.) [1]

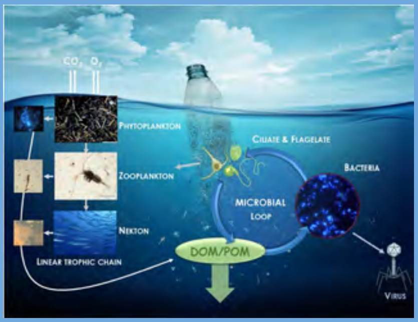

Fig. 2: picture of a potential influence microorganisms could have. [3]

Garbage Patches as a habitat

In our research, instead of researching the effects of this garbage patches on the ecosystems of oceans, we will consider those patches as a new niche and almost a new habitat. Evidently, we then can do research on the biodiversity of microorganisms on those patches. There are already some papers written about communities living on garbage patches. F.e. they discovered that those communities were net autotrophic and that there consisted of both eukaryotes and prokaryotes (see fig. 3) [4].

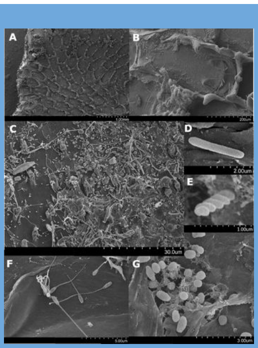

Fig. 3: SEM images of microorganisms living on pieces of microplastic. [4]

Research Question

To really understand the communities on those garbage patches we will need a map of distribution and diversity of microorganisms living there. The central question in this research will therefore be: "Which microorganisms live on the garbage patches and how are they distributed?" We will try to find an answer to this question by making a map of the abundance of protists and prokaryotes (microorganisms).

Hypothesis

Firstly, there is almost an unlimited amount of plastic in the garbage patches, hence we expect to find prokaryotic and eukaryotic organisms that are able to degrade plastics. Moreover, we expect microorganisms that can live in harsh conditions. This is because of the fact that those organisms are almost like pioneers in primary succession. On top of that, we expect the amount of microorganisms to differ from other areas in the ocean. To summarize, we expect to find an abundance of microorganisms that significantly differs from that of other areas in (the middle of) the ocean.

Difficulties

When trying to make such a map we will encounter a couple of difficulties. On the one hand, we could have difficulties collecting samples of microorganisms. On the other hand, we could have difficulties with the ever changing shape of garbage patches due to currents. Together with our method of determining which microorganisms we have found, we will try to explain our ideas of dealing with these difficulties.

Methods and Materials

To map the diversity we will have to start with dividing the patches into smaller area. These may not be to small, because the area of garbage patches is huge. The Great Pacific Garbage Patch f.e. has an area of more than 1,3 million km^2[5]. Therefore we will divide it in areas of 4 km^2. These areas will be projected on the garbage patch and a program needs to be written to correct the areas for the possible distortion of the patches. This will be done by providing photos of the garbage patch over time, by which the computer program then will correct the areas. We will also study three areas in more detail. Those three areas, one near the edge, one near the middle and one in between, are 4 km^2 areas that will divided into 100 m^2 areas.

To collect the samples we will use ships. Although this is sufficient for large areas, in smaller areas the ship can disturb the patches too much. Because of this and the fact that so many measurements have to be done we will use a drone to collect the samples of the areas studied in more detail.

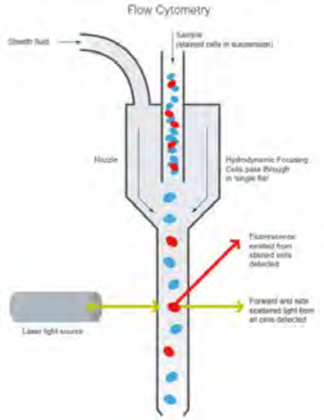

Fig. 5: A sample of stained cells will flow to a tube. Where the laser beam goes through the tube it is width enough for 1 cell at the time. A sensor will detect the differences in incoming signal per cell. [6]

When the samples are collected we have to do research on them. The conventional way to find out the different species in samples is by 16S rRNA sequencing. Although this is really accurate, it is also very expensive and time consuming. Because of the large amount of samples this method cannot be used.Instead of 16S rRNA sequencing or sequencing of any kind we decided to use flow cytometry to determine the diversity of microorganisms. Flow cytometry is a method which makes use of the phenotypic differences between microbial cells. The stained cells go in a flow through a laser beam. A sensor will read the disturbance of the light beam and, with a couple of statiscal methods, a fingerprint is made (see fig. 4). This can be done in less than 15 minutes per sample. Later, with already existing ecological data, these fingerprints can be coupled to a species and so diversity can be found. With this method we will look both at alpha-diversity, diversity in a sample, and beta-diversity, community turnover. We can calculate a Hill number to get a value of diversity. This we can map over the garbage patch and we can map the species and families over the patch. [7]

Conclusion

All in all, it is our proposal to map the biodiversity and abundance of microorganisms on garbage patches in the oceans. We will make use of modern technology in this research like the use of a drone to collect the samples of organisms from the patches.We also use the modern technology of flow cytometry to determine the kind of microorganisms we have. In the end we will have mapped out: the distribution of microorganisms, their abundance and their relative abundance.

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Relevance

Our research is of great relevance to the study of both ecosystems of oceans near garbage patches and to biotechnology. Firstly, because this study will provide use with a better understanding of the biodiversity on garbage patches. Hence we will better be able to do research on microorganisms that, by degradation of plastic, produce microplastics. Which is really important for understanding the rate and way and as a consequent the hazards of water pollution by microplastics. Secondly, because this study provide us with a better understanding of microorganisms that degrade plastics. Therefore this research could be used to find certain organisms that we can use in biotechnology to degrade plastic in a process like the one described in fig. 5. This could be a solution of our (plastic) waste problem. In this way our research will benefit both ecological and biotechnological research fields. This study will be of use for both the studying of the existing problem and possibly solving it.

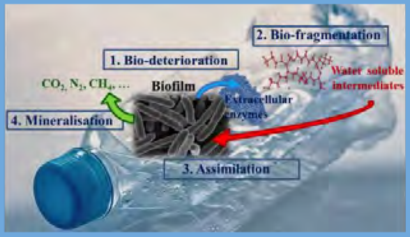
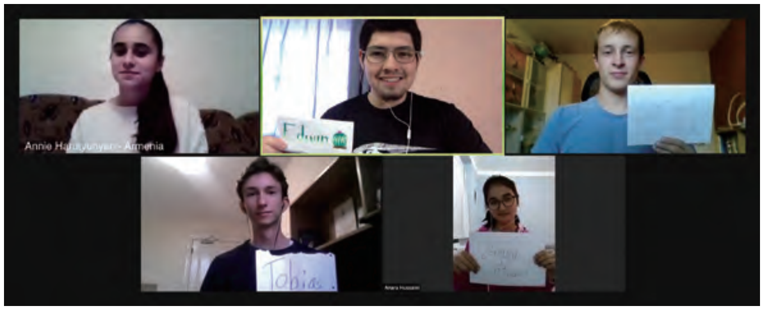


Fig. 5: Biodegradation of plastic by microorganisms [8]

2A04 Zooxanthellae Translocation



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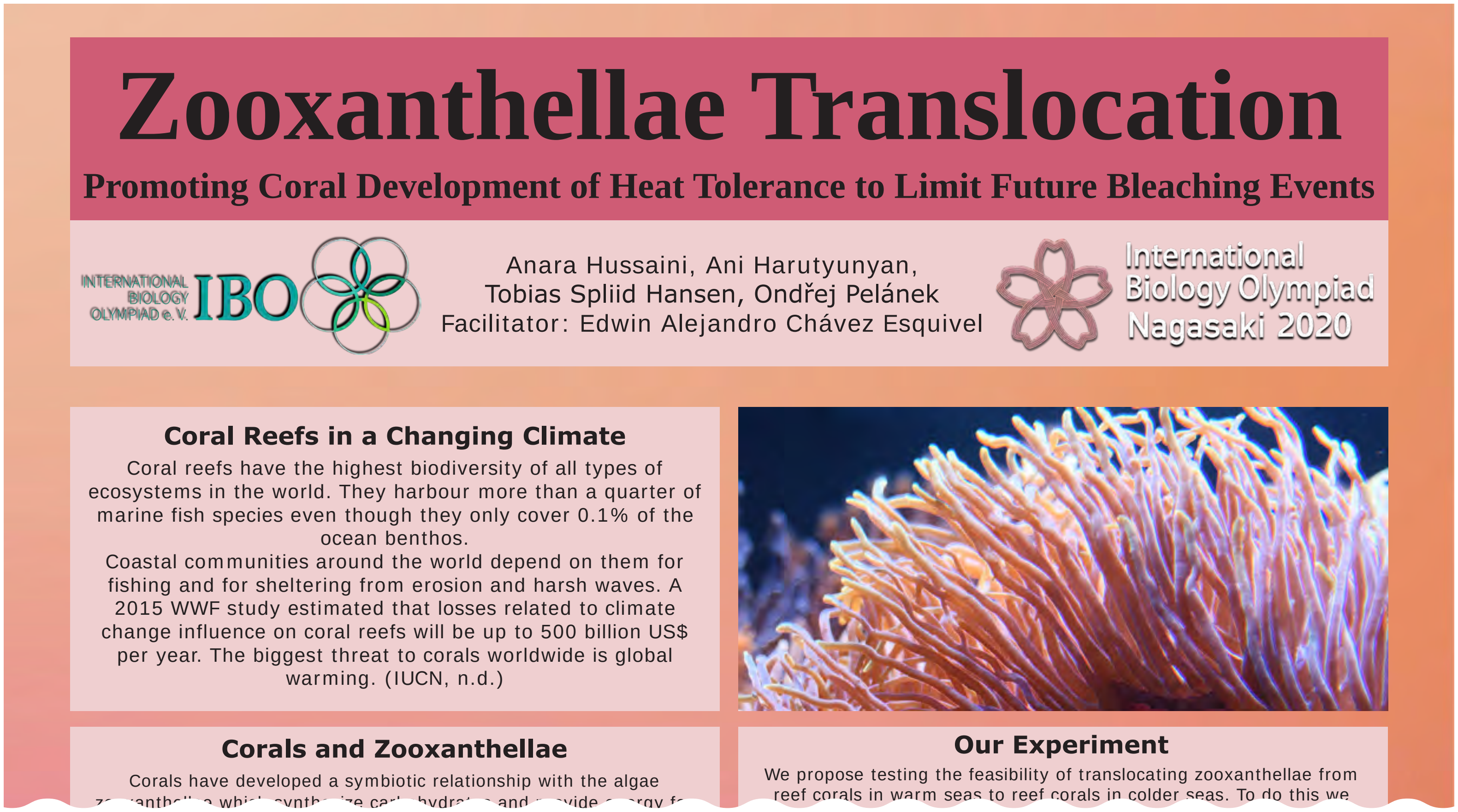


Zooxanthellae Translocation

Promoting Coral Development of Heat Tolerance to Limit Future Bleaching Events

Anara Hussaini, Ani Harutyunyan,
Tobias Spliid Hansen, Ondřej Pelánek
Facilitator: Edwin Alejandro Chávez Esquivel

Coral Reefs in a Changing Climate

Coral reefs have the highest biodiversity of all types of ecosystems in the world. They harbour more than a quarter of marine fish species even though they only cover 0.1% of the ocean benthos.

Coastal communities around the world depend on them for fishing and for sheltering from erosion and harsh waves. A 2015 WWF study estimated that losses related to climate change influence on coral reefs will be up to 500 billion US$ per year. The biggest threat to corals worldwide is global warming. (IUCN, n.d.)

Corals and Zooxanthellae

Corals have developed a symbiotic relationship with the algae

Our Experiment

We propose testing the feasibility of translocating zooxanthellae from reef corals in warm seas to reef corals in colder seas. To do this we

Corals and Zooxanthellae

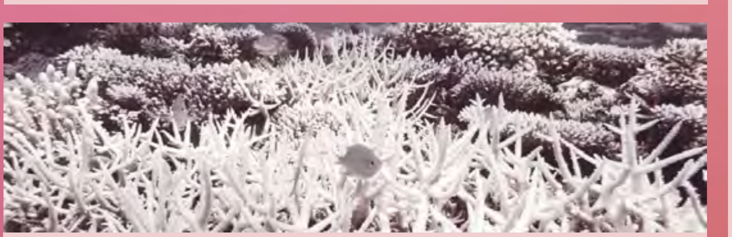
Corals have developed a symbiotic relationship with the algae zooxanthellae which synthesize carbohydrates and provide energy for the corals which in turn shelter them and provide them with nutrients.

When corals are exposed to high temperatures, they expel the zooxanthellae and "bleach". Most corals will not recover from bleaching and full recovery of the reef can take a decade by which time new extremes may have killed off some reefs completely (JCU, 2019). Some species of zooxanthellae allow corals to tolerate temperatures as high as 34-36ºC. One such species is *Symbiodinium thermophilum* (Hume et al., 2015).

Our proposed solution

It is already known that corals with different clades of zooxanthellae have different heat tolerance. There is also evidence that over time corals will swap to heat tolerant symbionts by themselves (Baker et al., 2004), however the current rate of climate change means that most coral reefs will not achieve this before it is too late.

We propose translocating heat tolerant zooxanthellae from reef corals in warm seas to reef corals in colder ones to promote the spread of these zooxanthellae and thereby heat tolerance in these corals.



High temperatures cause corals to expel their symbiotic partners and bleach. In nature, few corals are able to recover from this while the rest die. In the controlled laboratory conditions we can ensure that corals get the chance to repopulate with better fitted symbionts.
Picture source: Australian Institute of Marine Science. Scott reef 2016.

Prospects of the Investigation

Our experiment is designed to be proof of concept for our proposed solution to climate change effect on reef corals.

For our proposed solution to be feasible our experiment must at the very least show that the corals are successfully repopulated with the zooxanthellae. Ideally the repopulated corals will have a highly increased heat tolerance as well as having high growth at lower temperatures to be able to compete with naturally occurring corals and zooxanthellae and spread in the natural habitats.

If the experiment is successful, further research into the effectiveness of translocation of different zooxanthellae species in different corals, even non reef corals, would be highly relevant to preserve as many coral communities as possible.

Our Experiment

We propose testing the feasibility of translocating zooxanthellae from reef corals in warm seas to reef corals in colder seas. To do this we have designed an experiment testing the effectiveness of translocating the highly heat tolerant *Symbiodinium thermophilum* to a variety of corals of other seas.

- **Collecting zooxanthellae**
    - Collect water from benthos near corals known to be associated with *Symbiodinium thermophilum* in the Arabian Gulf.
    - Divide the collected water into many groups (just a few zooxanthellae in each) which are supplied with nutrients and light at a 30ºC to promote growth of heat tolerant zooxanthellae and inhibit that of non-heat tolerant zooxanthellae.
    - Sample the groups after some days and sequence them by qPCR to determine which have a mix of zooxanthellae and which contain only the desired species.
    - Mix the groups containing only the desired zooxanthellae to ensure genetic diversity in the new population. This will be the stock population.
- **Transferring the zooxanthellae to reef building corals**
    - Collect corals of different species from different sites around the world and acclimatize them in lab with their in situ temperature.
    - For every species of corals 3 groups will be made. One group will be the positive, unbleached control. Another group will be the negative, bleached control. And the final group will be bleached and exposed to *Symbiodinium thermophilum*.
    - All samples are treated with antibiotics.
    - Aquarium temperature is slowly raised by 5ºC over 14 days and then maintained at that temperature to achieve bleached corals. Except for the non-bleached control which is maintained at the in situ temperature.
    - Non-control corals are exposed to the stock population of zooxanthellae for 4 weeks at the corals' in situ temperature with good water circulation. Then follows five days where they are not exposed to any zooxanthellae.
    - Sequencing by qPCR is performed to detect the presence of any unwanted zooxanthellae.
- **Measuring performance of the corals**
    - We divide each of the three groups into two which will be exposed to two different temperatures. One temperature is the current average yearly in situ temperature of the corals. And the other is the highest average yearly temperature that scientific models predict the corals in situ within this century.
    - Measure the acquired heat resistance by measuring the growth (circumference of the base and the height of the coral) every 5 days for a month.

Predictions for our Experiment

We expect to find that some coral species are able to be repopulated with the zooxanthellae species and others which are not.

We expect that the newly introduced zooxanthellae will cause highly increased growth at the higher temperature but reduced growth at the lower temperature compared to the controls.

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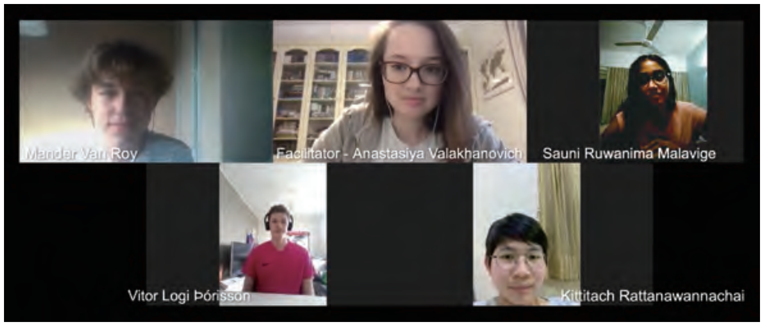
2B01 THE SMALL SAVIORS OF OUR EARTH?



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THE SMALL SAVIORS OF OUR EARTH?

About algae and climate change

By Sayuni Ruwanima Malavige
Kittitach Rattanawannachai
Viktor Logi Þórisson
Mander Van Roy
Facilitated by Anastasiya Valakhanovich

Climate change is one of the major issues, if not the major issue of the 21st century. We, as adolescents living in Sri Lanka, Belgium, Iceland and Thailand, already experience the implications of climate change:

The Indian Ocean has one of the largest seasonal phytoplankton blooms among tropical seas, due to the cycling of nutrients from the sub-surface to the surface, leading to high primary productivity. It has also experienced an increase of surface temperature by 1.2°C, over the last century compared to global surface warming of 0.8°C. Studies show that there has been a 20% decrease in phytoplankton over the last six decades.This finding has several economical implications, phytoplankton sustain marine food webs and account for half of the global net primary production. Loss of phytoplankton effects fisheries catches apart from disrupting biogeochemical cycles and climate processes.

In Belgium, 9 of the 10 warmest years ever measured, occured after the year 2000. Belgium has had more and more very dry summers over the last years too. This is indisputably no coincidence, and is linked to climate change.

As Iceland is an arctic country, it has a lot of glaciers which are all melting, some have even completely melted. Iceland is also susceptible to rising sea levels as most of the population is located by the sea.

Thailand's temperature trend fluctuated from 1951 to 2018. The average temperature at noon stays around 31-32 degrees fahrenheit. Nevertheless, the average temperature at night has been increasing since 1951. This trend is inevitably correlated to climate change as you know that climate change raises the atmospheric carbon dioxide concentration. As a result, in the night, heat cannot radiate from the ground and raises Thailand's night temperature trend.

The biodiversity of the ocean suffers under global warming too. Therefore, we think that we, as an international society, have to find solutions for this problem. We think that algae can have a major role in mitigating the problem. That is why we would like to discuss the potential of algae as a mitigating actor in climate change in this review.

On the verge of disaster?

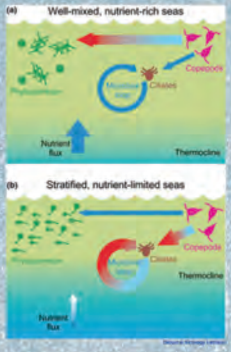
Several studies have shown that sea surface warming caused by global warming has led to a

Seaweed (f)arming against climate change

We believe that seaweed farming is a very promising actor in climate change reduction as

On the verge of disaster?

Several studies have shown that sea surface warming caused by global warming has led to a decline in phytoplankton biomass globally over the last century. This is a worrying trend because phytoplankton account for around half the global net primary production, form the basis of marine food webs and are involved in several biogeochemical cycles and climate processes.



Sea surface warming hinders nutrient cycling to the upper sea layers because it causes stratification, less dense warmer water remains on the top and this limits vertical cycling during which nutrient rich subsurface waters mix with the upper layers where the majority of photosynthesis occurs due to presence of sunlight.

Since phytoplankton are at the base of almost every marine food web, changes in phytoplankton dynamics or distribution affects marine biodiversity which can have several economic and ecological implications. For example due to sea surface warming, cold water plankton are moving further North, affecting species at different strata in local food chains.

In addition phytoplankton are also affected by ocean acidification, ocean pH is predicted to drop by another 0.3 to 0.4 units by the end of this century, increasing [H+] by around 100% to 150%; Thus affecting calcifying and non-calcifying phytoplankton, by changing ion concentrations in the ocean.

Increase in UV-B radiation due to the thinning of the ozone layer also negatively impacts photosynthetic phytoplankton. Increased UV exposure damages phytoplankton cells, causing damage to DNA or reducing photosynthetic pigments available. All these factors together combined can have detrimental effects on phytoplankton, indirectly damaging marine biodiversity, biogeochemical processes with ecological and economic implications.

...but there's still hope.

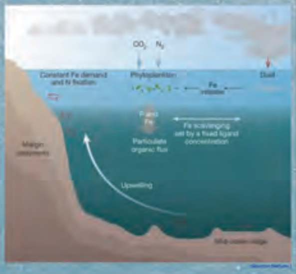
These negative impacts are worrying, so immediate action is required. We think that microalgae and plankton could play a major future role in mitigating climate change, and therefore have a positive impact on the ocean (biodiversity) in a couple of different ways.

Primarily, the phytoplankton takes up a lot of carbon dioxide and thus sequesters a lot of the (anthropogenic) greenhouse gasses. This is due to the fact that it is very efficient at photosynthesising and its potential to grow very fast, in comparison with a lot of land plants. Applying limiting factors (i.e. nutrients) in the ocean could make algae grow quicker, and therefore enhance the carbon sequestration potential of the population. We have to keep in mind that this artificial influx of nutrients can potentially provoke a HAB (Harmful Algae Bloom), which can cause a hypoxic dead zone. Further research on how the (pseudo?-)controlled addition of nutrients (such as iron) can be used, is needed.

Secondarily, the algae and plankton can play a role in weakening climate change via other pathways. One of these pathways is its emission of DMS (dimethyl sulfide). This substance can react with other particles in the air, forming condensation nuclei (little particles by which clouds can be formed). Clouds reflect all the incoming light - this is why they seem white to us. This contributes to the earth's albedo (the ability to reflect incoming light), and therefore can help to diminish the temperature rise.

Ocean 'Fe'rtilisation

OIF or Ocean Iron Fertilisation is one of the ways to counteract climate change, that could be started at short notice on relevant scales. It is based on the reasoning that adding trace amounts of iron to iron-limited phytoplankton of the Southern Ocean will lead to blooms, mass sinking of organic matter and ultimately sequestration of significant amounts of atmospheric carbon dioxide (CO2) in the deep sea and sediments. This hypothesis is tested by multiple mesoscale experiments that provided strong support for its suitable condition: stimulation of a diatom bloom accompanied by significant CO2 drawdown. However, the ratio of the iron added to the ocean does highly affect marine lives. In the worst case scenario, the pelagic ecosystems might be destroyed by the algae blooming.



Seaweed (f)arming against climate change

We believe that seaweed farming is a very promising actor in climate change reduction as well, and that it can be used for adaptation to the impacts of climate change. First of all, the farms contribute in a direct way to biodiversity in two ways.

Firstly, the algae can raise the pH of the farm areas significantly, because of the intake of carbon(ic acid) during the Calvin cycle, which is beneficial to calcifiers (who can't survive in low pH areas) and other animals.

Secondly, they can oxygenate areas. Due to the rising ocean temperature, a lot less oxygen can be dissolved in the ocean. The seaweed farms can locally oxygenate a region, which enhances biodiversity, because a lot of ocean organisms need oxygen in order to survive. (Because the algae are farmed, they aren't decomposited: a process that would require oxygen.)

Seaweed farming is becoming increasingly popular in Asia. Using current technology, extensively available sea areas may be cultivated to produce crops that require no freshwater or fertilizers, while providing a variety of valuable ecosystem services.

To grow seaweeds, farmers have to cut the seagrasses and remove the sea urchins before constructing the farm. Seedlings are then tied to monofilament lines and strung between mangrove stakes pounded into the substrate. Cultivation of seaweed in Asia is a relatively low-technology business with a high labor requirement. There have been many attempts in various countries to introduce high technology to cultivate detached plants growth in tanks on land in order to reduce labor, but they have yet to attain commercial viability. This project is believed to be highly successful in many regions in the world. It is proposed to decrease 22% of the total carbon dioxide in the atmosphere within 20 years.

Furthermore, seaweed farming can mitigate climate change and enhance biodiversity in more indirect ways. Due to its low setup price, the farms can offer a sustainable financial and nutritional substitute for (over)fishing to countries, especially to developing countries. This obviously leads to more biodiversity in the ocean. Besides, the algae (macro and micro) can be used as biofuell. We will tell more about it in the next alinea.

Healthy floating fuel in the ocean?

For phototrophic organisms like algae can transform electromagnetic energy into chemical energy, they can be converted into biofuels. The transformation of algae into biofuel could offer a sustainable way of acquiring fuels. In addition, algae have a couple of advantages over land biofuel crops.

Firstly, the algae consume less water than land crops, which can be an important factor to lands with an arid climate.

Secondly, a lot of nitrogen fertilizers are used in the cultivation of land biofuel crops. The use of these fertilizers generates a lot of nitrogen oxides, which are very potent greenhouse gases (and deplete the ozone layer). These emissions could be strongly reduced by using algae.

However, algae farming inevitably brings along some problems too. Due to its high water content, the algae need to be dried first before being processed. This is an endoenergetic process. On the other hand, microalgae farms for biofuel are relatively expensive and need extensive care.

Summary

As we have demonstrated in this review, climate change can have a devastating impact on microalgae and plankton in the sea and subsequently on the entire ocean wildlife. There are, however, numerous ways we can combat this, but we must be aware of the potential risks involved. We can enhance the growth of microalgae and plankton by applying limiting factors. But this can potentially cause a HAB. We can also increase seaweed farming, which increases the pH and oxygen in the area. It also offers nutrition to developing countries and is affordable. Algae farming can offer a sustainable way of acquiring biofuels but is expensive and needs constant care. The future is bright, it´s not going to be easy, it will take a unified global effort but together we can solve this climate change problem.

2B03 The Great Amazon Reef System

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The Ocean Policy Research Institute Award

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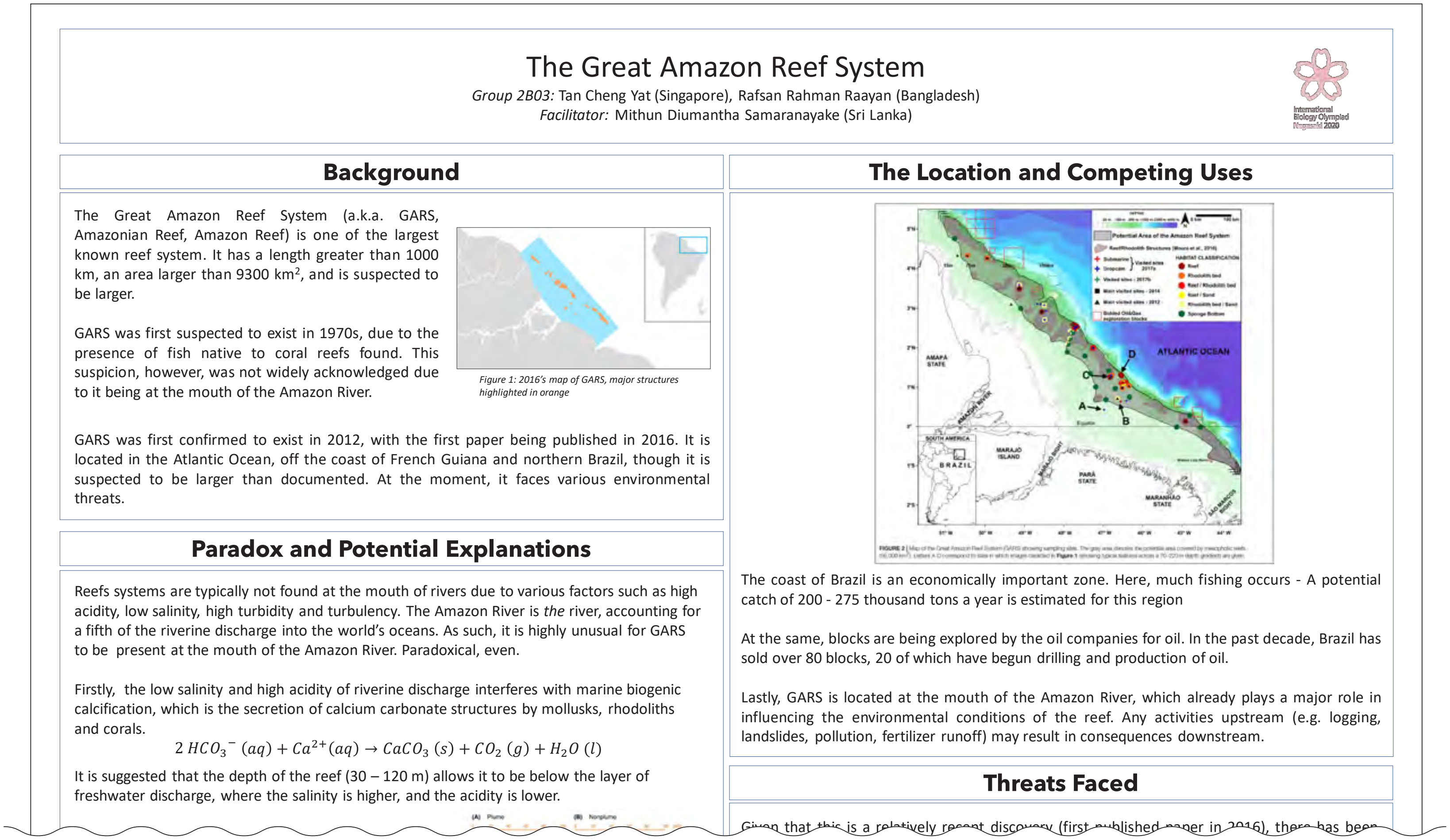


The Great Amazon Reef System

Group 2B03: Tan Cheng Yat (Singapore), Rafsan Rahman Raayan (Bangladesh)
Facilitator: Mithun Diumantha Samaranayake (Sri Lanka)

International Biology Olympiad Nagasaki 2020

Background

The Great Amazon Reef System (a.k.a. GARS, Amazonian Reef, Amazon Reef) is one of the largest known reef system. It has a length greater than 1000 km, an area larger than 9300 km^2, and is suspected to be larger.

GARS was first suspected to exist in 1970s, due to the presence of fish native to coral reefs found. This suspicion, however, was not widely acknowledged due to it being at the mouth of the Amazon River.

Figure 1: 2016's map of GARS, major structures highlighted in orange

GARS was first confirmed to exist in 2012, with the first paper being published in 2016. It is located in the Atlantic Ocean, off the coast of French Guiana and northern Brazil, though it is suspected to be larger than documented. At the moment, it faces various environmental threats.

Paradox and Potential Explanations

Reefs systems are typically not found at the mouth of rivers due to various factors such as high acidity, low salinity, high turbidity and turbulency. The Amazon River is *the* river, accounting for a fifth of the riverine discharge into the world's oceans. As such, it is highly unusual for GARS to be present at the mouth of the Amazon River. Paradoxical, even.

Firstly, the low salinity and high acidity of riverine discharge interferes with marine biogenic calcification, which is the secretion of calcium carbonate structures by mollusks, rhodoliths and corals.

$$2\,HCO_3^-\,(aq) + Ca^{2+}(aq) \rightarrow CaCO_3\,(s) + CO_2\,(g) + H_2O\,(l)$$

It is suggested that the depth of the reef (30 – 120 m) allows it to be below the layer of freshwater discharge, where the salinity is higher, and the acidity is lower.

The Location and Competing Uses

The coast of Brazil is an economically important zone. Here, much fishing occurs - A potential catch of 200 - 275 thousand tons a year is estimated for this region

At the same, blocks are being explored by the oil companies for oil. In the past decade, Brazil has sold over 80 blocks, 20 of which have begun drilling and production of oil.

Lastly, GARS is located at the mouth of the Amazon River, which already plays a major role in influencing the environmental conditions of the reef. Any activities upstream (e.g. logging, landslides, pollution, fertilizer runoff) may result in consequences downstream.

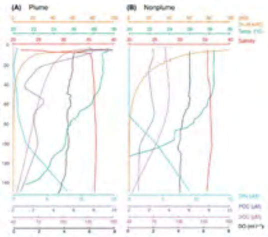
Threats Faced

Given that this is a relatively recent discovery (first published paper in 2016), there has been and corals.

$$2\,HCO_3^-\,(aq) + Ca^{2+}(aq) \rightarrow CaCO_3\,(s) + CO_2\,(g) + H_2O\,(l)$$

It is suggested that the depth of the reef (30 – 120 m) allows it to be below the layer of freshwater discharge, where the salinity is higher, and the acidity is lower.

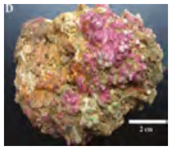
Depth alone, however, is unable to explain how GARS manages to exist despite turbid waters. Riverine discharge carries high amounts of sediments, which while can sustain detrivores, have the detrimental effect of causing sedimentation of the seabed, and low light penetration. Zooxanthellae and other photosynthetic microorganisms experience low photosynthetic rate, causing low oxygenation of waters.



Currently, there is no consensus as to an explanation, but a popular one is seasonal plumage - depending on location, there are periods in the year where there is sufficient light penetration for photosynthesis. This is supported by correlation of southern parts of the reef experiencing greater biodiversity. In fact, perhaps our answer lies in the composition of the reef.

Reef Composition

One interesting observation is that the reef is predominantly rhodoliths rather than corals. Rhodoliths are marine red algae that resemble coral. They secretes calcium carbonate structures but not attached to substrate. This is a potential explanation as to how the reef exists, due to rhodoliths being hardier. In fact, GARS has even been suggested to be a new class of biome.



Example of a rhodolith

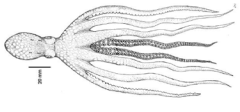
The sponges of GARS appear to be adapted to the environment they are in. It is common to find sponges with narrow atrium and high pumping, adaptions to contend with strong currents and high suspended sediments. At the same time, other growth forms adapted for light capture, steady current and sediment resistance have been observed, such as the large, erect and cup-like massive structures of <u>Geodia</u> <u>spp.</u> as pictured below.

The main food source of these sponges (and other organisms such as corals) are theorized to be very small picoplankton and mesozooplankton in riverine discharge.

The biodiversity of GARS has been called lacking, but a 2016 paper has documented 61 species of sponge, 73 species of fish and much more. This number has only risen across the years. Rare, unique or even *new* animals include:

<u>Pythonichthys</u> <u>sanguineus</u>: the rare Caribbean mud eel

<u>Lutjanus</u> <u>alexandrei</u>: a newly discovered snapper species



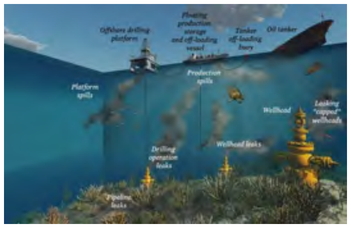
<u>Lepidoctopus</u> <u>joaquini</u>: a newly discovered octopus species

influencing the environmental conditions of the reef. Any activities upstream (e.g. logging, landslides, pollution, fertilizer runoff) may result in consequences downstream.

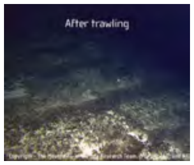
Threats Faced

Given that this is a relatively recent discovery (first published paper in 2016), there has been few steps taken to reduce the severity of threats to the reefs, due to a lack of understanding. However, there are three readily apparent threats:

<u>1. Oil Drilling</u>
Oil drilling is notorious for having severe ecological impacts, especially in spillage or accidental releases. Oil is opaque, it reduces light penetration into the reef below from already poor to poorer.



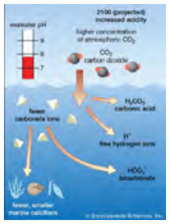
Greenpeace, amongst other activist groups, have been petitioning for the Brazilian government to hand out oil blocks more judiciously.



<u>2. Overfishing</u>
Fishing can directly or indirectly affect the biodiversity, by disrupting the ecological balances and food webs. This is especially true for unsustainable fishing methods, that can be hard to enforce against. An example would be trawling, a method of fishing where nets are dragged across seabeds. Trawling can indiscriminately kill organisms, dislodge corals or disrupt the reef

The Brazilian government have been implementing various instruments to control over-fishing. Examples include seasonal fishing bans to marine reserves.

<u>3. Climate Change</u>
Climate change is a particularly big issue to reefs systems, not just limited to GARS. It is a systemic issue with multiple issues that pose threats to reefs.



Firstly, increased carbon dioxide levels in end lead to ocean acidification, which reduces/diminishes marine biogenic calcification. This is especially an issue in GARS given the already acidic riverine discharge and the fact that the calcium carbonate structures formed by rhodoliths are not affixed to the seabed, relying on gravity and weight instead.

Secondly, rising sea temperatures lead to metabolic stress on the organisms. Photosynthetic rate is already poor due to river plume, much less with increased temperatures. Furthermore, ejection of zooxanthelle from corals may occur, leading to coral bleaching.

A comparative example of coral bleaching

So what's being done? Globally, there is an increased awareness of how our various practices contribute to climate change and steps are being taken to reduce our impact.

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2B05 Biological Thinking : To discuss ocean biodiversity in a 'serious' way

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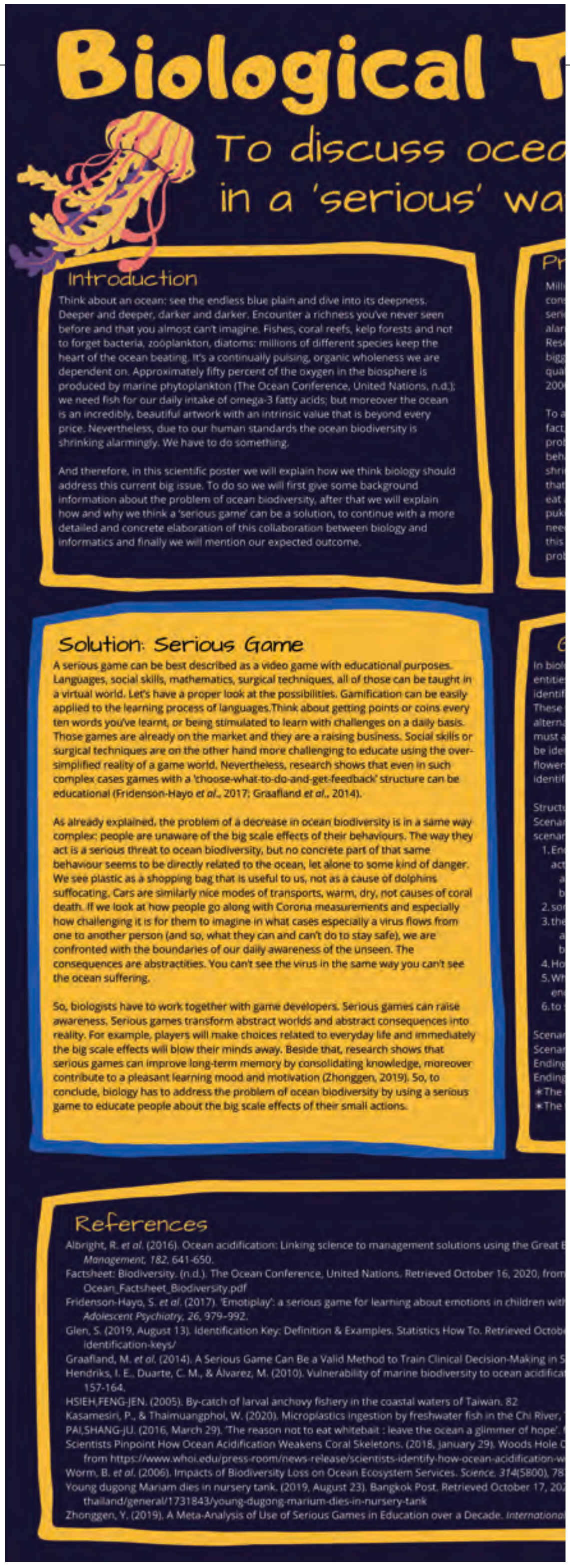
Xanta van Ruiten (Netherlands)

The "Beyond Bio" Award



Introduction

Think about an ocean: see the endless blue plain and dive into its deepness. Deeper and deeper, darker and darker. Encounter a richness you've never seen before and that you almost can't imagine. Fishes, coral reefs, kelp forests and not to forget bacteria, zooplankton, diatoms: millions of different species keep the heart of the ocean beating. It's a continually pulsing, organic wholeness we are dependent on. Approximately fifty percent of the oxygen in the biosphere is produced by marine phytoplankton (The Ocean Conference, United Nations, n.d.); we need fish for our daily intake of omega-3 fatty acids; but moreover the ocean is an incredibly, beautiful artwork with an intrinsic value that is beyond every price. Nevertheless, due to our human standards the ocean biodiversity is shrinking alarmingly. We have to do something.

And therefore, in this scientific poster we will explain how we think biology should address this current big issue. To do so we will first give some background information about the problem of ocean biodiversity, after that we will explain how and why we think a 'serious game' can be a solution, to continue with a more detailed and concrete elaboration of this collaboration between biology and informatics and finally we will mention our expected outcome.

Solution: Serious Game

A serious game can be best described as a video game with educational purposes. Languages, social skills, mathematics, surgical techniques, all of those can be taught in a virtual world. Let's have a proper look at the possibilities. Gamification can be easily applied to the learning process of languages.Think about getting points or coins every ten words you've learnt, or being stimulated to learn with challenges on a daily basis. Those games are already on the market and they are a raising business. Social skills or surgical techniques are on the other hand more challenging to educate using the over-simplified reality of a game world. Nevertheless, research shows that even in such complex cases games with a 'choose-what-to-do-and-get-feedback' structure can be educational (Fridenson-Hayo *et al.*, 2017; Graafland *et al.*, 2014).

As already explained, the problem of a decrease in ocean biodiversity is in a same way complex: people are unaware of the big scale effects of their behaviours. The way they act is a serious threat to ocean biodiversity, but no concrete part of that same behaviour seems to be directly related to the ocean, let alone to some kind of danger. We see plastic as a shopping bag that is useful to us, not as a cause of dolphins suffocating. Cars are similarly nice modes of transports, warm, dry, *not* causes of coral death. If we look at how people go along with Corona measurements and especially how challenging it is for them to imagine in what cases especially a virus flows from one to another person (and so, what they can and can't do to stay safe), we are confronted with the boundaries of our daily awareness of the unseen. The consequences are abstractities. You can't see the virus in the same way you can't see the ocean suffering.

So, biologists have to work together with game developers. Serious games can raise awareness. Serious games transform abstract worlds and abstract consequences into reality. For example, players will make choices related to everyday life and immediately the big scale effects will blow their minds away. Beside that, research shows that serious games can improve long-term memory by consolidating knowledge, moreover contribute to a pleasant learning mood and motivation (Zhonggen, 2019). So, to conclude, biology has to address the problem of ocean biodiversity by using a serious game to educate people about the big scale effects of their small actions.

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Thinking:
cean biodiversity
way

Problems of Ocean Biodiversity

Millions of different species populate the oceans. Together, they constitute an enormous, organic network of life. Nevertheless, they are seriously threatened. The species abundance in the world oceans is alarmingly shrinking and the consequences can't be overlooked. Research shows that the greater the loss in marine biodiversity, the bigger the impaired ability of an ocean is to provide food, maintain the quality of its waters and to recover from disturbances (Worm, B. *et al.*, 2006). So, we have to prevent this from happening. But how?

To answer this disturbing question we must know the cause. What, in fact, is the seed for such a loss in ocean biodiversity? The answer is probably hard to look at: we are it, we, human beings and our behaviours. Due to our lifestyle the species abundance in oceans is shrinking. Overfishing, plastics and acidification are just a few threats that can be called. And all of them have their roots in our behaviour: we eat a lot of fish, we use a lot of plastics, we need a lot of carbon dioxide puking factories to produce our stuff. But ocean biodiversity radically needs the opposite, and we need ocean biodiversity. So, how to turn this over? In this poster we'll discuss how biology should address this problem.

Game structure

In biology, an identification key is a device that aids the identification of biological entities, such as plants, animals, fossils,and microorganisms. Traditionally identification keys have most commonly taken the form of single-access keys. These work by offering a fixed sequence of identification steps, each with multiple alternatives, the choice of which determines the next step. At each step, the user must answer a question about one or more features (characters) of the entity to be identified. For example, a step in a botanical key may ask about the color of flowers, or the disposition of the leaves along the stems. A key for insect identification may ask about the number of bristles on the rear leg.

Structure:
Scenario 1:Offer some background knowledge and describe the"situation"of the scenario(by telling a short story).
1. Encounter the first question.The player can choose one from the following two actions.
   a. action a (the relatively better action)..................................to 2
   b. action b ....to 3
2. some explanations of why this is better..................................to 6(3)
3. the ramification that the action leads to (describing a condition or a problem)
   a. solution a (the relatively more effective one)..........................to 4
   b. solution b ....to 5
4. How can this solution help solve the problem................................to 6(1)
5. Why can't this solution help solve the problem ,or why this isn't effective enough. Introduce recommended solution ....to 6(0)
6. to scenario 2

Scenario 2:
Scenario 3:
Ending 1: 0~5 points
Ending 2: 6~9 points
∗The number in "6(_)" represents the point the player gets in the scenario.
∗The total point the player gets determines the ending of the story.(｡･ω･｡)

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Introduction to the Scenarios

Following are the scenarios of the serious game; they're a lot more story-like than only the formal structure mentioned before. Reading through them you will encounter three different threats to ocean biodiversity: overfishing, plastics and acidification. All are situated in the cultural context of a different country. Not only are these scenarios trying to raise awareness of the big scale effects of small deeds, but also to educate young humans about how biology can help solve these problems that are already actual. So, let's read these scenarios and don't forget to visualise the filmlike gameworld in your head!

The Ocean Game

Created by
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CHI SHENG HUANG - Chinese Taipei
Xanta van Ruiten - The Netherlands
Facilitator: Maria Janine Juachon

Beginning Story

One day afternoon, you are walking along the street by the coast on your way home. Suddenly, you hear someone calling you from the sea. Feeling strange, you stop to look at the sea and do a double take.There is a three-spined stickleback (From Theoretical Test) jumping out of the sea and saying "Is anyone there?" simultaneously. It's weird, but you are driven by curiosity and decide to walk closer to the fish.
The Fish : Hey you human! We've gotten some trouble in the sea. We need your biological knowledge to help solve the problems.
You: I didn't do well in today's biology test.(´・_・`)
The Fish : Never mind! As long as you are able to deal with the real problem, who cares about the score? Let's go!
the fish drags(by a fish?!) you into the sea
The Fish : (cast a spell on you) You are now able to breath and see clearly in the water. You have been familiar with the sea since you were young. Don't you find something different between the current sea and the one you remember? By the way, my name is Iboc.
You look around and discover that there are fewer fish in the sea and the coral reef is less colorful than before.
Iboc : Our habitat is ruined. The number of life species and individuals are decreasing year by year. And even the environmental pH value is becoming lower and lower these years.The situation is just getting worse and worse.
You : (noticing something floating) Is that a jellyfish?
Iboc : No, it's probably a deserted plastic bag.
You : Oops...So, how can I help?
Iboc : My purpose of bringing you here is to improve this situation .(cast another spell on you) From now on, what you learn and feel will be imprinted in all human beings' mind. You will need to pass the trial consisting of three stages with different scenarios based on the three countries' situation .Let's start with the first one in Taiwan : Overfishing.

Overfishing

Background knowledge : Whitebait is a collective term for the immature fry of fish (juvenile fish),such as herring, sardines, mackerel, bass and many others. The particular species that are marketed as "whitebait" varies in different parts of the world, and for Taiwan they are usually anchovies (*Engraulis japonica, Encrasicholina heteroloba, Encrasicholina punctifer*)(PAI,SHANG-JU, 2016).In Taiwan, whitebait are tender and can be regarded as a delicacy. Since the council of agriculture of Taiwan introduced "Bull Trawling" from Japan in 1977, the fishery yield has increased. This improved the economic situation of some fishing villages. In recent years, fishermen have found that the catch of some medium and large-sized fish decrease.
Situation : You are a fisherman and you are ready to do your work : go fishing ,especially whitebait, on the sea. In the surrounding sea area, there are porgy, mackerel, trevally, bonito, tuna, whitebait, etc. Since the catch has kept decreasing recently, you need to think of a method to catch more fish.
1. Now, choose which net you want to use in this voyage.
   a. fine net (mesh size:0.2cm*0.2cm)..............................to 2
   b. another net (mesh size:2cm*2cm)...............................to 4
2. The fine net helps you catch more fish, but the fish is in all sizes. This means that you catch many fry, juvenile and adult of several kinds of fish. Many of them are bycatch. Over the course of time, there will be fewer and fewer fish, such as trevally and porgy, in the sea, and this can cause those fish who prey on them to decrease in number, too. To solve this situation, you can choose your solution from the following two options.
   a. stop catching whitebait or establish closed fishing season..............to 3
   b. pick out the bycatch fish after every catching and release them back to the sea ....to 5
3. In this way, you reduce the catch of whitebait. This is the most straightforward solution since it leaves the fish undisturbed and waiting for them to grow back to their former populations...to 6(1)
4. The mesh size is so big that it can't catch any whitebait. Though this may make you be unsatisfied with the catch, this is actually the more environmentally friendly action. The decrease of some medium and large-sized fish is a warning and it has something to do with whitebait overfishing. In fact, due to bycatching, catching whitebait can make an impact on over two hundred fish species, not to mention whitebait overfishing. Therefore, it's a good way to catch other fish rather than whitebait by using nets with larger mesh size ....to 6(3)
5. Unfortunately, most of the fry and juvenile already die in the process, so it'll be useless to pick them out. It's better to take action before catching them up......to 6(0)
6. To the next scenario_Plastics

Plastics

Background knowledge: Mariam, a baby dugong which was found on Krabi Beach in Thailand, was rescued and nursed. She was on the watch from staff and locals, and was kept in her natural habitat near the community. Unfortunately, she mistook a plastic for seagrass she normally eats, and it could not be digested properly. Shortly after, she got a severe infection in her intestine and died (Bangkok Post, 2019).
Situation: You are going shopping with your family. You have some shopping bags in your house, but the department store usually offers plastic bags for free.
1. You have two choices:
   a. You choose to ask for the plastic bags..............................to 2
   b. You bring your own reusable shopping bags...........................to 3
2. Every time you go shopping, you always ask for the plastic bags at the store. Now you have lots of plastic bags kept in your house, so many that your house is starting to run out of space! What should you do now?
   a. Bring them to the store, reuse them as shopping bags...................to 4
   b. Dump them all in the trash..to 5
3. Great job! By using the reusable bags, you actually help reduce the plastic waste, thereby reducing the amount of plastic waste in the ocean. Even though it's only a small act, the result will become bigger and bigger as you keep doing it..........to 6(3)
4. Plastics can be bad for the environment if you use them wastefully. But for this case, you can reuse them! If they are still clean, you can use them as shopping bags a number of times. If they are not clean enough, you can use them as trash bags instead. However, avoid using them as much as you can. Good job on using the plastics effectively though!..to 6(1)
5. Oh no! The plastic bags you just dumped just got blown away by strong wind! Some of them ended up in the canal near your house, which were carried on to the river, and eventually reached the ocean. Now, the plastic can go from useful to harmful. Lives in both the river and the ocean may consume plastics (Kasamesiri & Thaimuangphol, 2020). If the plastics are too big, the animals that ate them mostly cannot survive, like the baby dugong Mariam's case. If the plastics were broken down into smaller pieces, some animals may still be alive, but they still cannot digest the plastics. What if they were caught and used for food? That means we have to eat the plastics we dumped, and I don't think that's good for our health. Next time you go shopping, use reusable shopping bags, or if you have to use plastic bags, just reuse them as shopping bags or trash bags!..to 6(0)
6. To the next scenario_Acidification

Acidification

Background knowledge: In the Dutch language, we have a lot of different words to mention a particular rainy weather type. They show a necessity: obviously it rains a lot in the Netherlands. Drizzle is one of the most common and one of the least favorite types, I can assure you. Despite these too frequent rain showers, our Dutch world is known for its cycling-love and incredibly good bicycle infrastructure: apparently we've got more bicycles than humans in our small country. These two cultural phenomena have led to the next scenario.
Situation: It's morning, time to go to school. But when you look outside, nothing but a greyness stares back at you. Small raindrops cover the windows and the bleak wind whistles. You shiver, even though a warm house embraces you. It's drizzling and cold, but you've to go to school. You know it's only about fifteen minutes bicycling and you know a rain suit will keep you dry and warm, nevertheless the world outside is so cold and stormy, that a little trip with the car can't hurt, right?
1. So, what are you gonna do? Will you ask your mum to bring you or will you in the end go by bicycle?
   a. The car would be nice..to 2
   b. Well, I would nevertheless prefer to go by bicycle....................to 3
2. *Iboc: let's see what the big scale effects of your small deed are.* And the viewpoint travels to smaller and smaller scales, we zoom in on the gases of the moving car. It's almost as if we were a part of that ugly, dirty smoke constantly coming out of that transport thing. And we see molecules dancing, bumping into each other. We give them names and are finally in real contact with these small things we had only read about. But after our first amazement we start worrying about the enormous amount of the molecules we call 'carbon dioxide' and we fly into the sky following their diffusion. What an increase of carbon dioxide concentration does this trip with the car cause! Let's follow one of those molecules, and mention how he slowly but surely approaches the big ocean. Suddenly, a quick dive makes him (and us) become one with that blue waterness. It's as if our carbon dioxide starts a relationship with a water molecule: carbonic is formed and a small, positively charged proton is rejected. *Iboc: this is what we call 'ocean acidification' – in the following we will find out why especially this is harmful for biodiversity.* What's our small proton doing? He diffuses to the areas of coral reefs where calcium carbonate is absolutely necessary for the continued existence of this organic richness: its skeletons are made out of aragonite, which is a form of calcium carbonate. Look at how our proton fights with that coral arm over one molecule of calcium carbonate: he wins, fuses with the carbonate, leaves the calcium alone. Suddenly the softened coral breaks, he can no longer stand the streams of the water. And after that it goes quickly; the whole coral world starts to break up, to dissolve in the water (Woods Hole Oceanographic Institution, 2018). Small fishes and zooplankton dependant on the existence of coral reefs, are dying too. We see how a loss of biodiversity is working its way through all the trophic levels, since all the animals are connected in the food cycle (Hendriks *et al.*, 2010). What we end with is a serious reducement of ocean biodiversity; the beauty of coral reefs is gone and with that some of our food and possible sources for medicines and so on and so forth. *Iboc: to prevent these big scale effects of carbon dioxide emission on ocean biodiversity, which of the following two solutions would be best?*
   a. Use assisted evolution to promote well-adapted genotypes (and especially well-adapted to those elevated carbon dioxide concentrations) in order to maintenance a large population size and genetic diversity in threatened ocean areas (Albright *et al.*, 2016)..................................to 4
   b. Remove the anthropogenic carbon dioxide from the atmosphere and the surface oceans with modified and quickly growing diatoms (Albright *et al.*, 2016)...to 5
3. [First: look at the same video as in 2, then, while rewinding:] *Iboc: but this didn't happen, due to your choice. A bicycle doesn't emit anything: gratulations!* And again we dive into the ocean, but now perceive a colourful and lively coral reef...to 6(3)
4. *Iboc: great choice! When the surroundings are changing, genetic diversity is essential for a ecosysteem to maintain its biodiversity. Imagine for example a family of fishes, some of them brighter coloured than others – this variety is called 'genetic diversity'. When a new predator is introduced in their territory, the brighter ones are visible and therefore eaten, in contrast to the others. Therefore, without this genetic diversity, all of them would have been bright coloured and all of them would have died: genetic diversity increases the adaptability of species to new circumstances, it prevents species from extinction. In the same way promoting genetic diversity and assisted evolution, will make the coral reef resilient to acidification: natural selection will just pick out the corals that love growing in more acid circumstances.* And so, we see an acid ocean, since a lot of protons are moving around. Nevertheless: it's still lively and colourful there: the coral reef is adapted to these new circumstances and can now easily gather its calcium carbonate in these much more acid surroundings....................to 6(1)
5. *Iboc: gambled wrong! It will take at least thousands of years to remove all anthropogenic carbon dioxide this way and we simply don't have that time. Probably modified diatoms could promote this process, but we have to wait until such genetic engineered microorganisms are proved to be creatable in a not that costly way. So, we still see that same process happening:* [look at the same video as in 2]..to 6(0)
6. Go to 'ending'

Ending Story

If the player got 0~5 points: Ending 1
Here, the actions that you have chosen to do weren't so effective. The ocean still needs a lot more attention in order to be a sustainable source of life for us. Now, there's a drastic decrease in ocean biodiversity. We won't be able to survive for long if technology doesn't support this sudden change.

If the player got 6~9 points: Ending 2
Congratulations! You have successfully complete the game with excellent choices of action. If you keep up with these good practices, the ocean can be a sustainable source for humanity and help keep the world's ecosystem in balance. Thank you for saving us!

Discussion

The game has finally come to an end. How was it? Did you enjoy it? And did you learn something new today? The method used in the game is an adaptation from what we call an "Identification key". The identification key is mainly used in taxonomy (Glen, 2019), a field of biology which focuses on identification, classification, and nomenclature of organisms. Here, the key is used to describe what you will face after deciding to do things. Each action has its own pathway and is rated by points given. The more the point you got, the more effective your decisions were! Not quite hard to understand, does it?

As you can see, what you do might seem small compared to the effects they have on our planet. Those effects not only cause changes to the ocean, but to other areas as well, including where we live. Our small deeds will eventually return to us, whether good or bad, sooner or later. Whether you did well or not in this game, you still have time to take the action now, in your daily life. Keep up with the good practices. Protect the ocean, the world, and humanity!

The issues we select are hard issues. This means they are very complicated and should be viewed by many different aspects. However, we simplify them in order to fit them into the game structure. Therefore, we highly recommend that players think more about these issues after the game. They are more than what they are in the serious game.

As we chose to educate people using a game, we expect that it will gain a lot of interest, especially from the children. By learning how the identification key works, and also using critical thinking, this game provides a better understanding of biology, the importance of ocean biodiversity, and how to help conserve it. Hopefully, the game will help bring attention to ocean biodiversity conservation, or even inspire children to learn more about science.

3A01 CRISPR CGBE1-based editing on CD34+ stem cells to grow Bombay blood group compatible red blood cells

Facilitator

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Competitors

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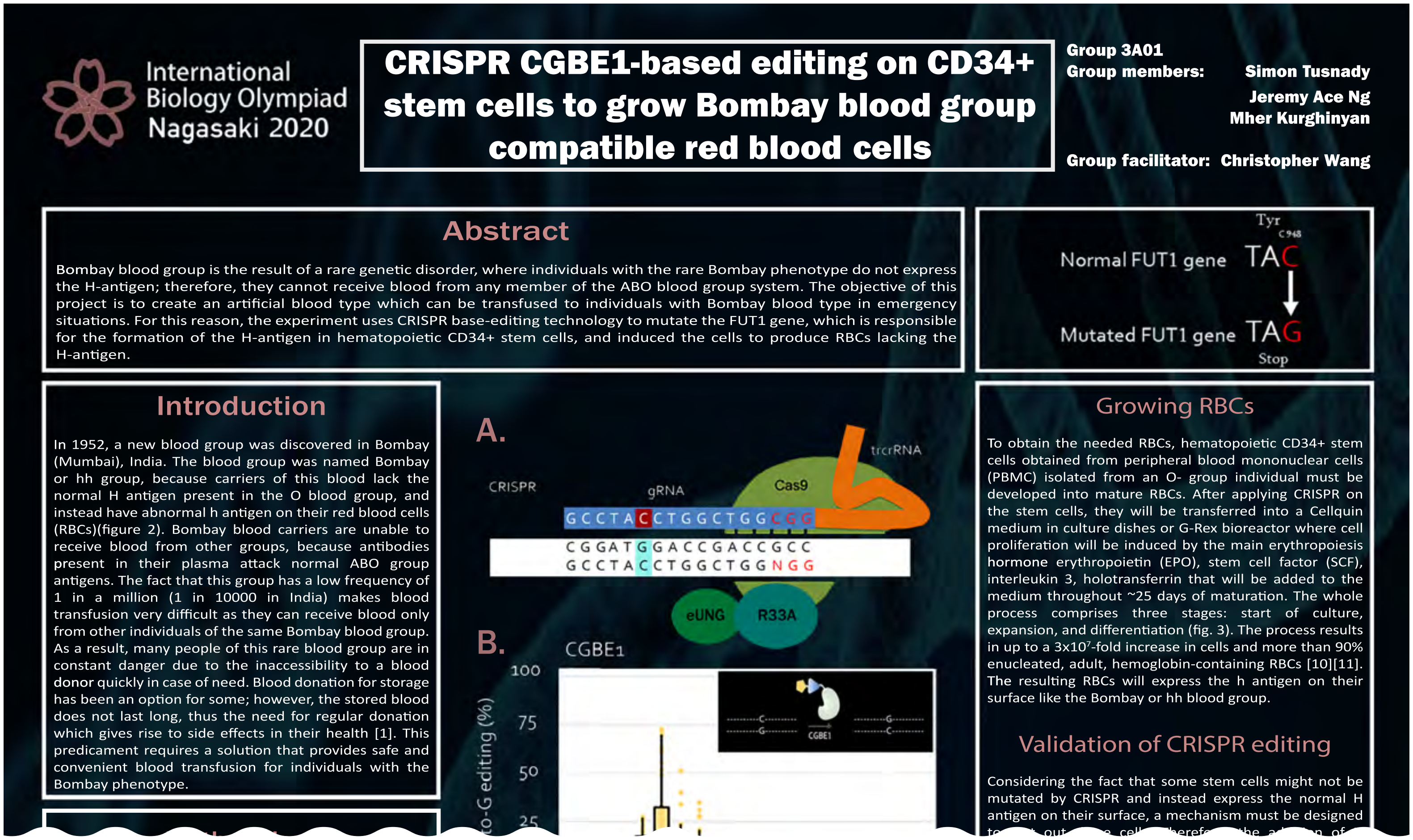

International
Biology Olympiad
Nagasaki 2020

CRISPR CGBE1-based editing on CD34+ stem cells to grow Bombay blood group compatible red blood cells

Group 3A01
Group members: Simon Tusnady
Jeremy Ace Ng
Mher Kurghinyan

Group facilitator: Christopher Wang

Abstract

Bombay blood group is the result of a rare genetic disorder, where individuals with the rare Bombay phenotype do not express the H-antigen; therefore, they cannot receive blood from any member of the ABO blood group system. The objective of this project is to create an artificial blood type which can be transfused to individuals with Bombay blood type in emergency situations. For this reason, the experiment uses CRISPR base-editing technology to mutate the FUT1 gene, which is responsible for the formation of the H-antigen in hematopoietic CD34+ stem cells, and induced the cells to produce RBCs lacking the H-antigen.

Introduction

In 1952, a new blood group was discovered in Bombay (Mumbai), India. The blood group was named Bombay or hh group, because carriers of this blood lack the normal H antigen present in the O blood group, and instead have abnormal h antigen on their red blood cells (RBCs)(figure 2). Bombay blood carriers are unable to receive blood from other groups, because antibodies present in their plasma attack normal ABO group antigens. The fact that this group has a low frequency of 1 in a million (1 in 10000 in India) makes blood transfusion very difficult as they can receive blood only from other individuals of the same Bombay blood group. As a result, many people of this rare blood group are in constant danger due to the inaccessibility to a blood donor quickly in case of need. Blood donation for storage has been an option for some; however, the stored blood does not last long, thus the need for regular donation which gives rise to side effects in their health [1]. This predicament requires a solution that provides safe and convenient blood transfusion for individuals with the Bombay phenotype.

Growing RBCs

To obtain the needed RBCs, hematopoietic CD34+ stem cells obtained from peripheral blood mononuclear cells (PBMC) isolated from an O- group individual must be developed into mature RBCs. After applying CRISPR on the stem cells, they will be transferred into a Cellquin medium in culture dishes or G-Rex bioreactor where cell proliferation will be induced by the main erythropoiesis hormone erythropoietin (EPO), stem cell factor (SCF), interleukin 3, holotransferrin that will be added to the medium throughout ~25 days of maturation. The whole process comprises three stages: start of culture, expansion, and differentiation (fig. 3). The process results in up to a $3x10^7$-fold increase in cells and more than 90% enucleated, adult, hemoglobin-containing RBCs [10][11]. The resulting RBCs will express the h antigen on their surface like the Bombay or hh blood group.

Validation of CRISPR editing

Considering the fact that some stem cells might not be mutated by CRISPR and instead express the normal H antigen on their surface, a mechanism must be designed donor quickly in case of need. Blood donation for storage has been an option for some; however, the stored blood does not last long, thus the need for regular donation which gives rise to side effects in their health [1]. This predicament requires a solution that provides safe and convenient blood transfusion for individuals with the Bombay phenotype.

Methodology

Design of gRNA and base editor constructs

A nonsense mutation Tyr-316–>Ter in the FUT1 gene through a C948–>G transversion is responsible for the inactivation of the allele in erythroid H-deficient Bombay individuals [2][3]. To induce the targeted C-to-G base transversion, CGBE1, a unique base editor consisting of an RNA-guided Cas9 nickase, an Escherichia coli–derived uracil DNA N-glycosylase (eUNG) and a rat APOBEC1 cytidine deaminase variant (R33A)(fig. 1), will be used to efficiently induce the base edits previously impossible in CBEs and ABEs with reduced indel mutations [4][5]. Cas9 nickase has been reported to efficiently mutate genes without detectable damage at known off-target sites [6], thus allowing for the precision and safety imperative in the experiment. The gRNA sequence was identified by locating the C948 base in the FUT1 gene and searching for the nearest PAM sequence (NGG). The CGBE1 base editor construct used in this study will be cloned into a mammalian expression plasmid backbone and fused with P2A-eGFP under the control of a CMV promoter for later enrichment, while the expression of the gRNA in a pUC19-based entry vector will be driven by a U6 promoter as described in the methods of Kurt et al. [5], in preparation for synthesis. Highly efficient C-to-G editing was most observed on base 6 of the protospacer [5], thus, the gRNA sequence was shifted to accommodate the position of intended mutation.

gRNA sequence: 5'-GCCTACCTGGCTGGCGG-3'

Delivery of base editor and gRNA

Gene modification through CRISPR/Cas9 has been previously shown to be successful in CD34+ hematopoietic stem cells [7]. To generate a sustainable and generative supply of H-antigen-free reticulocytes, cells derived from the CD34+ cell line will be subjected to electroporation-assisted transfection of either the synthesized control or base editor plasmid and the gRNA plasmid.

Enrichment by FACS and verification of on- and off-target mutations by whole genome sequencing

Fluorescence-activated cell sorting (FACS) will be used to enrich the transfected cells that successfully express GFP, a fluorescent protein marker. The top ten percent of GFP-expressing cells will be sorted and propagated for subsequent screening and analysis [8]. RNA will be removed using RNase to eliminate possible interference with the sequencing. Next generation sequencing will then be performed on the isolated genomic DNA from both control and transfected cells. Bioinformatic analyses will be performed to identify discrepancies between the sequences of the control and treated cells in order to determine possible off-target mutations [9], and to sort out effects not derived from the manipulation caused by transfection.

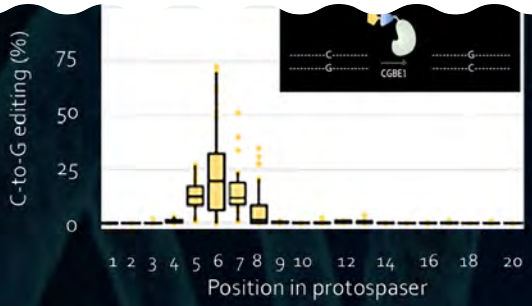

Fig 1. CGBE1 base editor (A) CGBE1 base editor with the designed gRNA sequence (B) Distribution of C-to-G frequencies per nucleotide across the protospacer from Kurt et al. (2020)

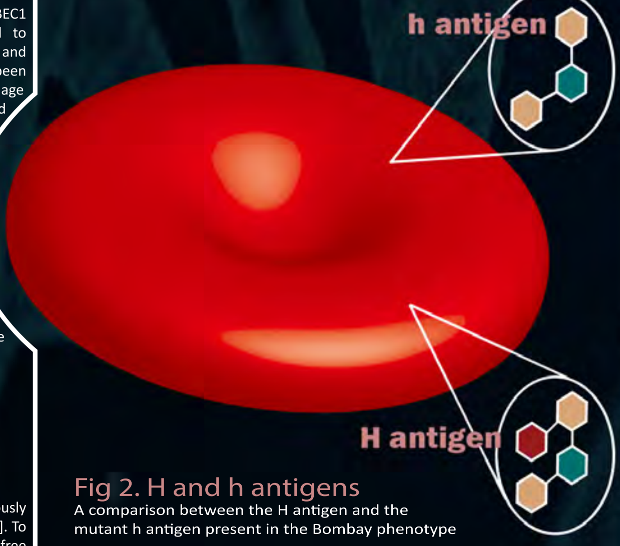

Fig 2. H and h antigens
A comparison between the H antigen and the mutant h antigen present in the Bombay phenotype

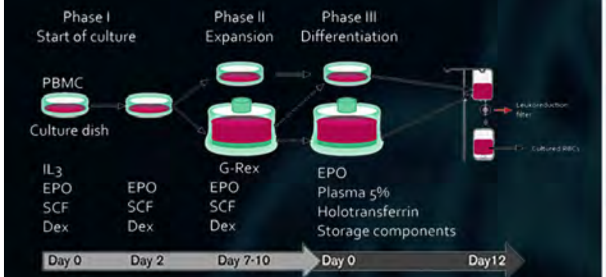

Fig 3. Growing and filtration of RBCs
The growing process comprises three stages: start of culture, expansion, and differentiation. This is then followed by the filtration of RBCs.

The resulting RBCs will express the h antigen on their surface like the Bombay or hh blood group.

Validation of CRISPR editing

Considering the fact that some stem cells might not be mutated by CRISPR and instead express the normal H antigen on their surface, a mechanism must be designed to sort out these cells. Therefore, the addition of H antibodies in the filtration process will result in agglutination of H-carrying RBCs and formation of bigger clusters. The cultured RBCs will pass through leukoreduction filters [10], and as a result, the clusters will be captured by the filter and h cells will remain.

Preparing for transfusion

Donor-to-donor variation is not the only problem of blood transfusion. The storage time influences the quality of red blood cells, as RBCs age in the storage bag and are exposed to storage lesion. According to some studies [12][13], storage lesion leads to post-transfusion enhanced clearance, reduced oxygen carrying capacity, release of potentially toxic substances, and immunomodulation with potential unwanted transfusion-related clinical outcomes, such as acute lung injury or higher mortality rate. However, there are several methods to prevent these unwanted effects, such as cryopreservation or anaerobic storage. These forms of storage could be an excellent solution to the clinical problems. In this experiment, the cryopreservation method will be utilized; storage of RBCs at ultra-low temperature stops cellular metabolism, preventing the progressive deterioration that is responsible for the RBC storage lesion, allowing conservation of red blood cells for long-term periods [12][13].

Discussion

In conclusion, the experiment is designed to create red blood cells that lack H-antigens on their surface, similar to the red blood cells of Bombay-mutant individuals. Using CRISPR base editing technology and methods for enrichment and validation, a large amount of H-antigen-deficient RBCs can be generated. With proper maintenance and storage of the packed RBCs, the packed cells can be sent to major hospitals for use in cases of emergency requiring blood transfusion for people with Bombay phenotype.

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3A02 Creating novel CRISPR-Cas variants with altered PAM sequences using a hybrid approach

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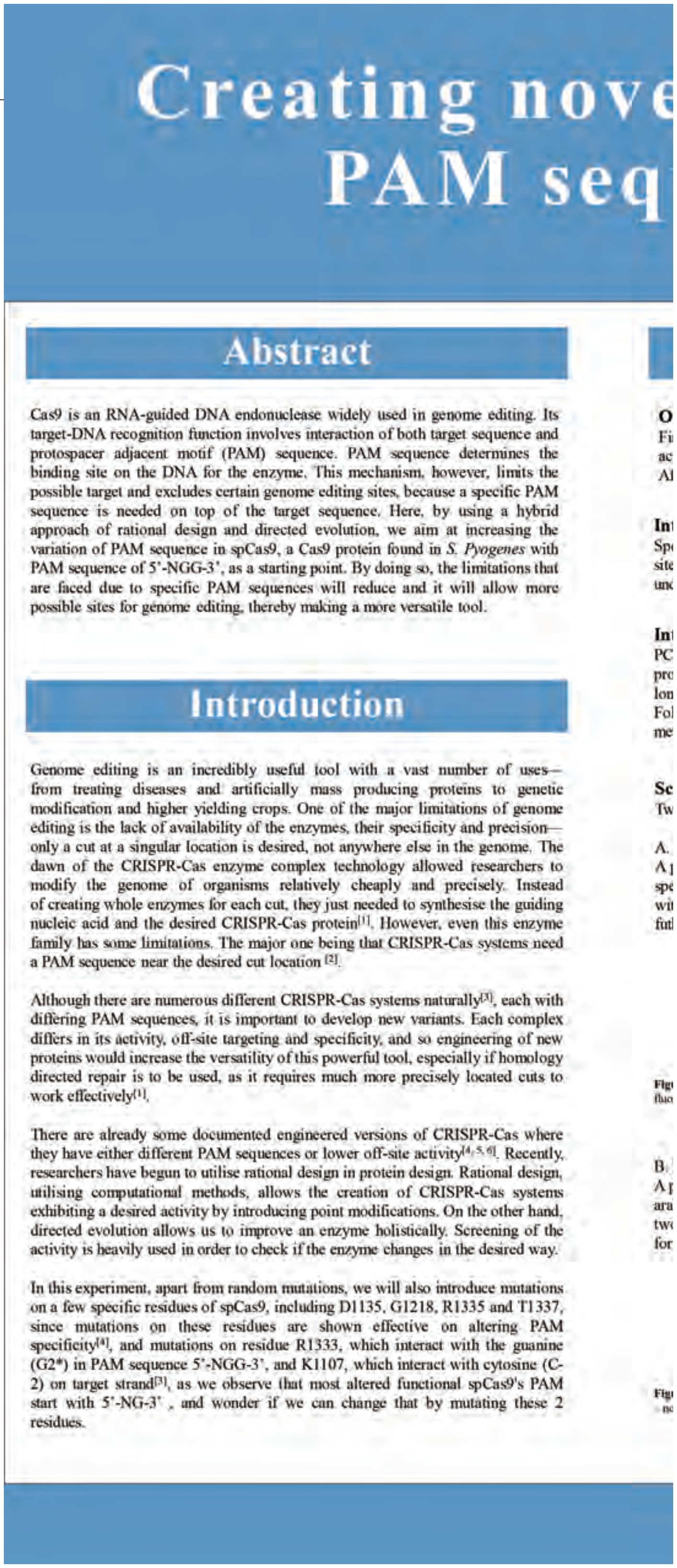


Creating nove
PAM seq

Abstract

Cas9 is an RNA-guided DNA endonuclease widely used in genome editing. Its target-DNA recognition function involves interaction of both target sequence and protospacer adjacent motif (PAM) sequence. PAM sequence determines the binding site on the DNA for the enzyme. This mechanism, however, limits the possible target and excludes certain genome editing sites, because a specific PAM sequence is needed on top of the target sequence. Here, by using a hybrid approach of rational design and directed evolution, we aim at increasing the variation of PAM sequence in spCas9, a Cas9 protein found in *S. Pyogenes* with PAM sequence of 5'-NGG-3', as a starting point. By doing so, the limitations that are faced due to specific PAM sequences will reduce and it will allow more possible sites for genome editing, thereby making a more versatile tool.

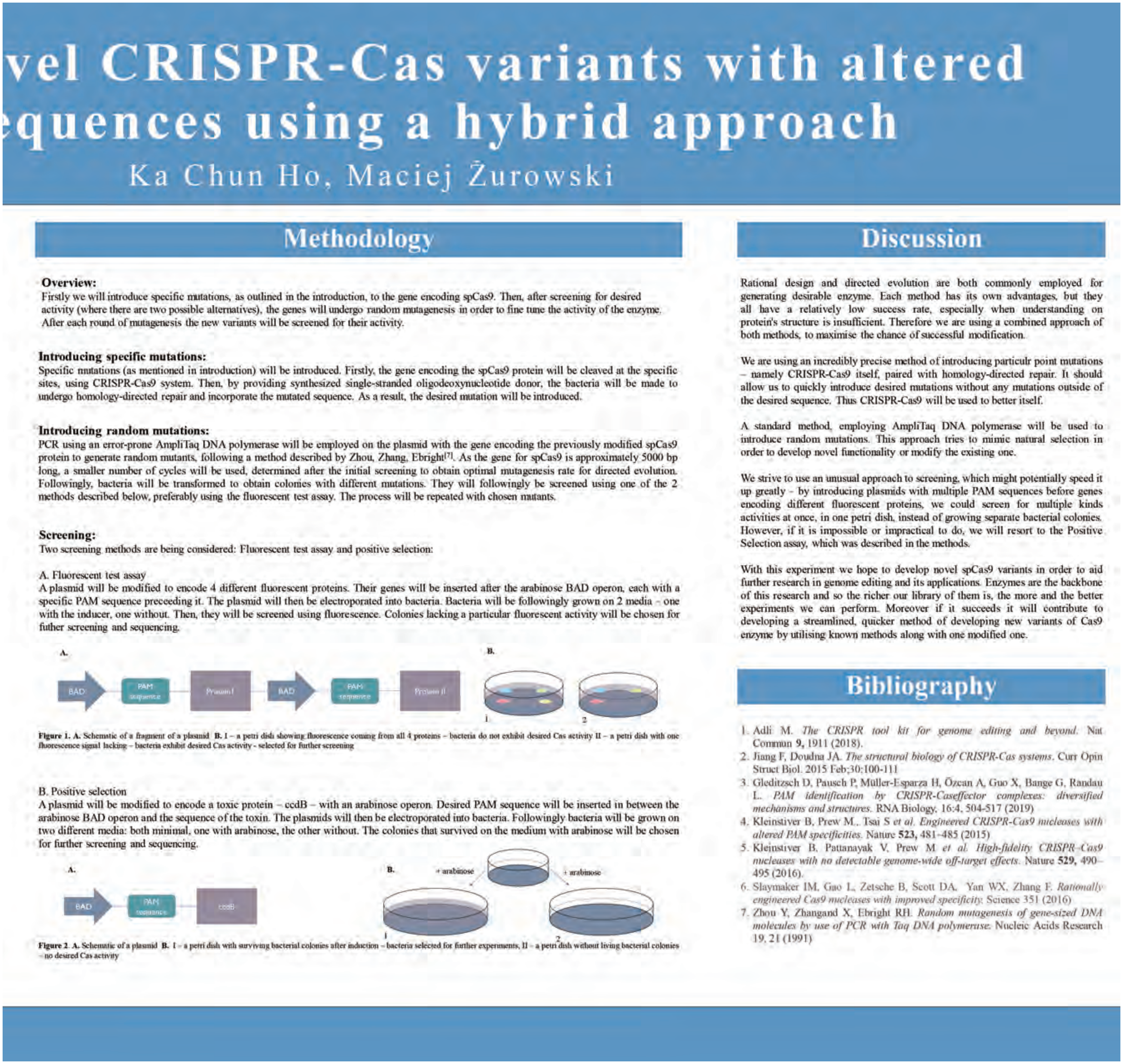
Introduction

Genome editing is an incredibly useful tool with a vast number of uses—from treating diseases and artificially mass producing proteins to genetic modification and higher yielding crops. One of the major limitations of genome editing is the lack of availability of the enzymes, their specificity and precision—only a cut at a singular location is desired, not anywhere else in the genome. The dawn of the CRISPR-Cas enzyme complex technology allowed researchers to modify the genome of organisms relatively cheaply and precisely. Instead of creating whole enzymes for each cut, they just needed to synthesise the guiding nucleic acid and the desired CRISPR-Cas protein[1]. However, even this enzyme family has some limitations. The major one being that CRISPR-Cas systems need a PAM sequence near the desired cut location [2].

Although there are numerous different CRISPR-Cas systems naturally[3], each with differing PAM sequences, it is important to develop new variants. Each complex differs in its activity, off-site targeting and specificity, and so engineering of new proteins would increase the versatility of this powerful tool, especially if homology directed repair is to be used, as it requires much more precisely located cuts to work effectively[1].

There are already some documented engineered versions of CRISPR-Cas where they have either different PAM sequences or lower off-site activity[4, 5, 6]. Recently, researchers have begun to utilise rational design in protein design. Rational design, utilising computational methods, allows the creation of CRISPR-Cas systems exhibiting a desired activity by introducing point modifications. On the other hand, directed evolution allows us to improve an enzyme holistically. Screening of the activity is heavily used in order to check if the enzyme changes in the desired way.

In this experiment, apart from random mutations, we will also introduce mutations on a few specific residues of spCas9, including D1135, G1218, R1335 and T1337, since mutations on these residues are shown effective on altering PAM specificity[4], and mutations on residue R1333, which interact with the guanine (G2*) in PAM sequence 5'-NGG-3', and K1107, which interact with cytosine (C-2) on target strand[3], as we observe that most altered functional spCas9's PAM start with 5'-NG-3' , and wonder if we can change that by mutating these 2 residues.



vel CRISPR-Cas variants with altered equences using a hybrid approach

Ka Chun Ho, Maciej Żurowski

Methodology

Overview:
Firstly we will introduce specific mutations, as outlined in the introduction, to the gene encoding spCas9. Then, after screening for desired activity (where there are two possible alternatives), the genes will undergo random mutagenesis in order to fine tune the activity of the enzyme. After each round of mutagenesis the new variants will be screened for their activity.

Introducing specific mutations:
Specific mutations (as mentioned in introduction) will be introduced. Firstly, the gene encoding the spCas9 protein will be cleaved at the specific sites, using CRISPR-Cas9 system. Then, by providing synthesized single-stranded oligodeoxynucleotide donor, the bacteria will be made to undergo homology-directed repair and incorporate the mutated sequence. As a result, the desired mutation will be introduced.

Introducing random mutations:
PCR using an error-prone AmpliTaq DNA polymerase will be employed on the plasmid with the gene encoding the previously modified spCas9 protein to generate random mutants, following a method described by Zhou, Zhang, Ebright[7]. As the gene for spCas9 is approximately 5000 bp long, a smaller number of cycles will be used, determined after the initial screening to obtain optimal mutagenesis rate for directed evolution. Followingly, bacteria will be transformed to obtain colonies with different mutations. They will followingly be screened using one of the 2 methods described below, preferably using the fluorescent test assay. The process will be repeated with chosen mutants.

Screening:
Two screening methods are being considered: Fluorescent test assay and positive selection:

A. Fluorescent test assay
A plasmid will be modified to encode 4 different fluorescent proteins. Their genes will be inserted after the arabinose BAD operon, each with a specific PAM sequence preceeding it. The plasmid will then be electroporated into bacteria. Bacteria will be followingly grown on 2 media - one with the inducer, one without. Then, they will be screened using fluorescence. Colonies lacking a particular fluorescent activity will be chosen for futher screening and sequencing.

Figure 1. A. Schematic of a fragment of a plasmid **B.** I – a petri dish showing fluorescence coming from all 4 proteins – bacteria do not exhibit desired Cas activity II – a petri dish with one fluorescence signal lacking – bacteria exhibit desired Cas activity - selected for further screening

B. Positive selection
A plasmid will be modified to encode a toxic protein – ccdB – with an arabinose operon. Desired PAM sequence will be inserted in between the arabinose BAD operon and the sequence of the toxin. The plasmids will then be electroporated into bacteria. Followingly bacteria will be grown on two different media: both minimal, one with arabinose, the other without. The colonies that survived on the medium with arabinose will be chosen for further screening and sequencing.

Figure 2. A. Schematic of a plasmid **B.** I – a petri dish with surviving bacterial colonies after induction – bacteria selected for further experiments, II – a petri dish without living bacterial colonies – no desired Cas activity

Discussion

Rational design and directed evolution are both commonly employed for generating desirable enzyme. Each method has its own advantages, but they all have a relatively low success rate, especially when understanding on protein's structure is insufficient. Therefore we are using a combined approach of both methods, to maximise the chance of successful modification.

We are using an incredibly precise method of introducing particulr point mutations – namely CRISPR-Cas9 itself, paired with homology-directed repair. It should allow us to quickly introduce desired mutations without any mutations outside of the desired sequence. Thus CRISPR-Cas9 will be used to better itself.

A standard method, employing AmpliTaq DNA polymerase will be used to introduce random mutations. This approach tries to mimic natural selection in order to develop novel functionality or modify the existing one.

We strive to use an unusual approach to screening, which might potentially speed it up greatly - by introducing plasmids with multiple PAM sequences before genes encoding different fluorescent proteins, we could screen for multiple kinds activities at once, in one petri dish, instead of growing separate bacterial colonies. However, if it is impossible or impractical to do, we will resort to the Positive Selection assay, which was described in the methods.

With this experiment we hope to develop novel spCas9 variants in order to aid further research in genome editing and its applications. Enzymes are the backbone of this research and so the richer our library of them is, the more and the better experiments we can perform. Moreover if it succeeds it will contribute to developing a streamlined, quicker method of developing new variants of Cas9 enzyme by utilising known methods along with one modified one.

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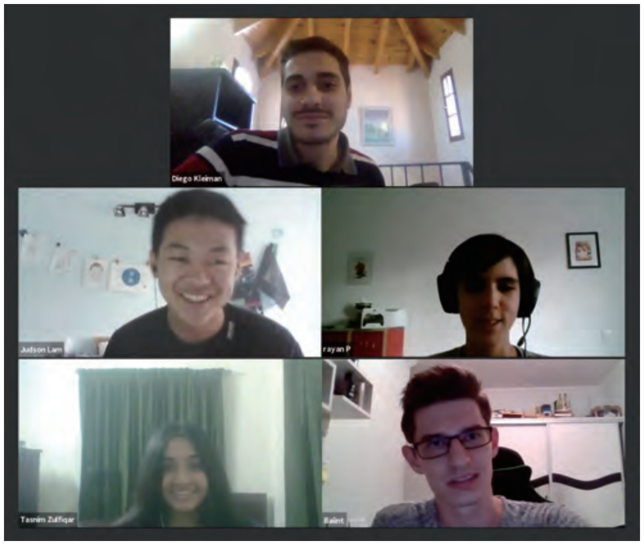
3A03 Applications of CRISPR to mtDNA using the MITO-porter



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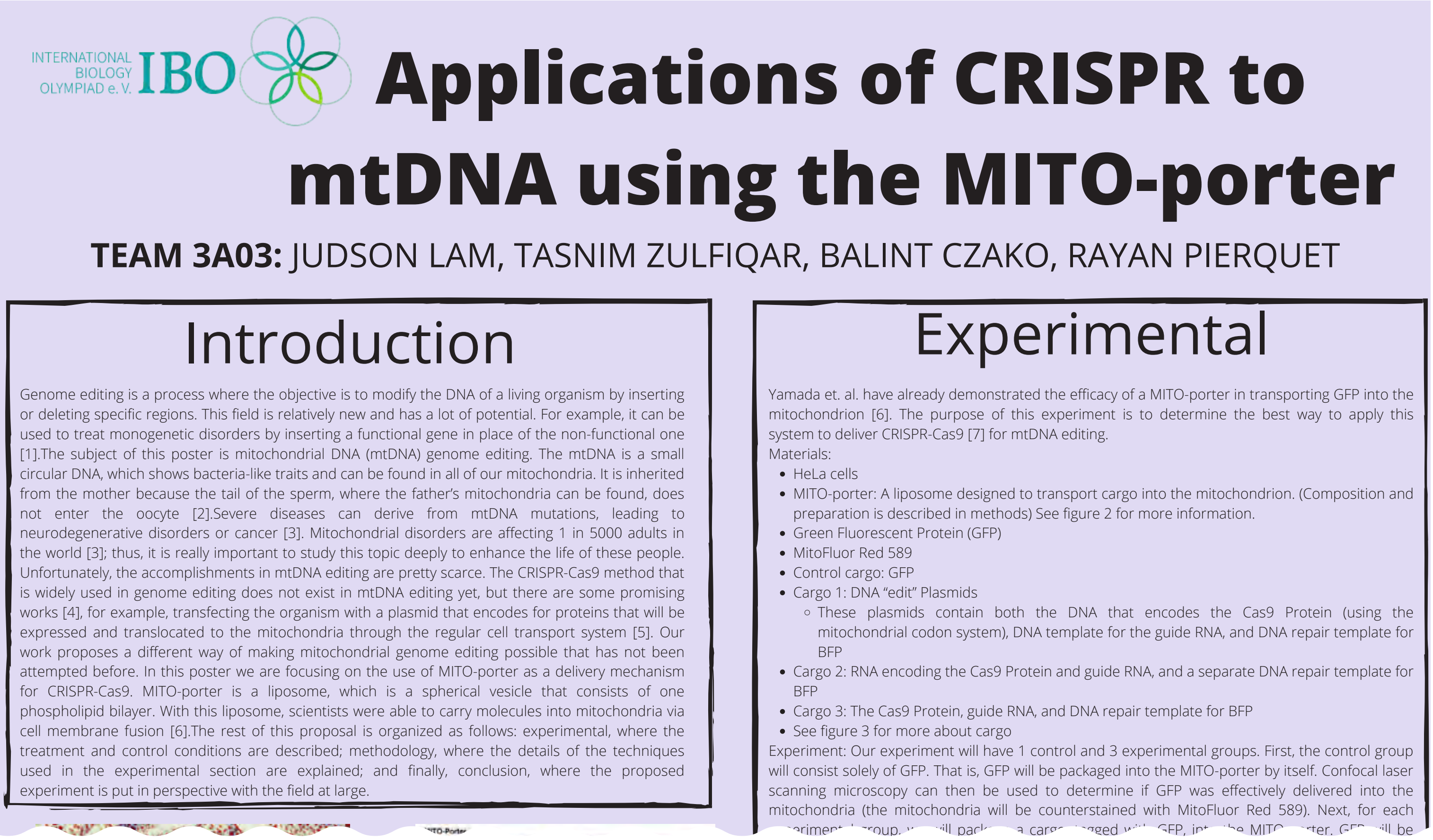

INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Applications of CRISPR to mtDNA using the MITO-porter

TEAM 3A03: JUDSON LAM, TASNIM ZULFIQAR, BALINT CZAKO, RAYAN PIERQUET

Introduction

Genome editing is a process where the objective is to modify the DNA of a living organism by inserting or deleting specific regions. This field is relatively new and has a lot of potential. For example, it can be used to treat monogenetic disorders by inserting a functional gene in place of the non-functional one [1].The subject of this poster is mitochondrial DNA (mtDNA) genome editing. The mtDNA is a small circular DNA, which shows bacteria-like traits and can be found in all of our mitochondria. It is inherited from the mother because the tail of the sperm, where the father's mitochondria can be found, does not enter the oocyte [2].Severe diseases can derive from mtDNA mutations, leading to neurodegenerative disorders or cancer [3]. Mitochondrial disorders are affecting 1 in 5000 adults in the world [3]; thus, it is really important to study this topic deeply to enhance the life of these people. Unfortunately, the accomplishments in mtDNA editing are pretty scarce. The CRISPR-Cas9 method that is widely used in genome editing does not exist in mtDNA editing yet, but there are some promising works [4], for example, transfecting the organism with a plasmid that encodes for proteins that will be expressed and translocated to the mitochondria through the regular cell transport system [5]. Our work proposes a different way of making mitochondrial genome editing possible that has not been attempted before. In this poster we are focusing on the use of MITO-porter as a delivery mechanism for CRISPR-Cas9. MITO-porter is a liposome, which is a spherical vesicle that consists of one phospholipid bilayer. With this liposome, scientists were able to carry molecules into mitochondria via cell membrane fusion [6].The rest of this proposal is organized as follows: experimental, where the treatment and control conditions are described; methodology, where the details of the techniques used in the experimental section are explained; and finally, conclusion, where the proposed experiment is put in perspective with the field at large.

Experimental

Yamada et. al. have already demonstrated the efficacy of a MITO-porter in transporting GFP into the mitochondrion [6]. The purpose of this experiment is to determine the best way to apply this system to deliver CRISPR-Cas9 [7] for mtDNA editing.

Materials:

- HeLa cells
- MITO-porter: A liposome designed to transport cargo into the mitochondrion. (Composition and preparation is described in methods) See figure 2 for more information.
- Green Fluorescent Protein (GFP)
- MitoFluor Red 589
- Control cargo: GFP
- Cargo 1: DNA "edit" Plasmids
  - These plasmids contain both the DNA that encodes the Cas9 Protein (using the mitochondrial codon system), DNA template for the guide RNA, and DNA repair template for BFP
- Cargo 2: RNA encoding the Cas9 Protein and guide RNA, and a separate DNA repair template for BFP
- Cargo 3: The Cas9 Protein, guide RNA, and DNA repair template for BFP
- See figure 3 for more about cargo

Experiment: Our experiment will have 1 control and 3 experimental groups. First, the control group will consist solely of GFP. That is, GFP will be packaged into the MITO-porter by itself. Confocal laser scanning microscopy can then be used to determine if GFP was effectively delivered into the mitochondria (the mitochondria will be counterstained with MitoFluor Red 589). Next, for each experimental group, we will package a cargo tagged with GFP, into the MITO-porter. GFP will be works [4], for example, transfecting the organism with a plasmid that encodes for proteins that will be expressed and translocated to the mitochondria through the regular cell transport system [5]. Our work proposes a different way of making mitochondrial genome editing possible that has not been attempted before. In this poster we are focusing on the use of MITO-porter as a delivery mechanism for CRISPR-Cas9. MITO-porter is a liposome, which is a spherical vesicle that consists of one phospholipid bilayer. With this liposome, scientists were able to carry molecules into mitochondria via cell membrane fusion [6].The rest of this proposal is organized as follows: experimental, where the treatment and control conditions are described; methodology, where the details of the techniques used in the experimental section are explained; and finally, conclusion, where the proposed experiment is put in perspective with the field at large.

Figure 1: Normal morphology of a mitochondria observed with transmission electron microscopy [10]

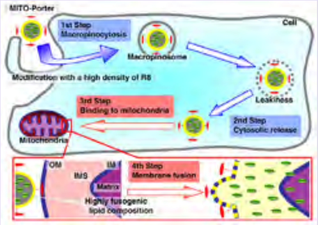

Figure 2: Brief description of how MITO porter reaches mitochondria [6]

Methods

MITO-porters (liposomes) are prepared with a lipid mixture (DOPE:PA:STR-R8 in a ratio of 9:2:1) by a hydration method [6]. This consists of hydrating a lipid film by evaporating an organic solvent in which lipids were dissolved. We obtain pieces of bilayer which we transform into liposomes by extrusion. The cargo is encapsulated in an aqueous phase. (see figure 4) To verify proper encapsulation, we separate the liposomes from the free GFP-labeled cargo by centrifugation and calculate the encapsulation efficiency by measuring the fluorescence intensity emitted by the liposomes vs. the free solution [6].The cell culture is incubated with MITO-porters which are uptaken via macropinocytosis. Then, the MITO-porters escape the macropinosome into the cytoplasm, fuse with the outer membrane of the mitochondria due to the fusogenic activity induced by PA, and finally the cargo diffuses from the intermembrane space to the mitochondrial matrix.Distribution of GFP and BFP will be measured through fluorescent microscopy. We will use these fluorescent tags to detect localization of our cargo in the cells.PCR and DNA sequencing will be used to analyze the resulting modifications on mtDNA.

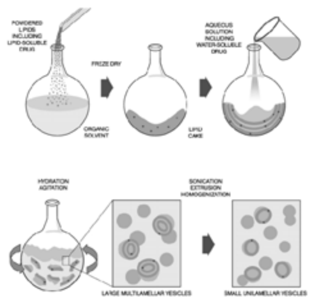

Figure 4: Liposome preparation using hydration method [11]

  - These plasmids contain both the DNA that encodes the Cas9 Protein (using the mitochondrial codon system), DNA template for the guide RNA, and DNA repair template for BFP
- Cargo 2: RNA encoding the Cas9 Protein and guide RNA, and a separate DNA repair template for BFP
- Cargo 3: The Cas9 Protein, guide RNA, and DNA repair template for BFP
- See figure 3 for more about cargo

Experiment: Our experiment will have 1 control and 3 experimental groups. First, the control group will consist solely of GFP. That is, GFP will be packaged into the MITO-porter by itself. Confocal laser scanning microscopy can then be used to determine if GFP was effectively delivered into the mitochondria (the mitochondria will be counterstained with MitoFluor Red 589). Next, for each experimental group, we will package a cargo, tagged with GFP, into the MITO-porter. GFP will be used to determine whether the cargo has been delivered into the mitochondrion properly (a yellow signal from MitoFluor Red + GFP should be observed). Afterwards, the cells will be incubated for a few generations, and we will look for the presence of BFP with a fluorescent microscope. A successful experiment will show yellow under a fluorescent microscope in the first generation of cells, and blue after a few generations if the CRISPR-Cas9 system correctly incorporated the donor DNA. If green is present, but blue is not, this indicates that the cargo was unsuccessful in incorporating a functional BFP gene in the mitochondrial genome. If neither green nor blue is present, the MITO-porter failed to deliver the cargo into the mitochondria. In any case, the mitochondrial DNA will be purified, PCR will be run to amplify the region of interest, and the DNA will be sequenced to look for the presence of the BFP gene (or forms of it).

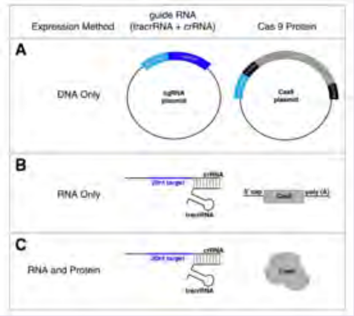

Figure 3: The three different cargo types not including the BFP DNA template [7]

Conclusion

This work proposes a way to test a delivery system that could be used to insert CRISPR RNPs to mitochondria to edit mtDNA. This tool is an advancement in genome editing because it will allow us to edit mtDNA using CRISPR, which was previously nearly impossible due to the inability to deliver it to the mitochondria. This work has implications in research [8] and clinical settings [9] because it provides an alternative method to prevent or treat mitochondrial disorders, and could be used to bring the CRISPR-Cas gene editing system to mtDNA where such methods to make such precise changes are still in their early stages. Although CRISPR-Cas9 has been used widely in research, there remains ethical and safety-related concerns regarding its application, especially on human subjects. Therefore, this novel tool should be under the same level of scrutiny as existing genome editing methods. Additionally, it is relevant to check the specificity and accuracy of this delivery system and its potential unintended effects on nuclear DNA. Future work should involve analyzing the aforementioned aspects, using the new technique on animal models to study the effectiveness of mtDNA editing and trying other delivery systems to introduce genome editing tools in the mitochondria.

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Abbreviations:
- mtDNA: mitochondrial DNA
- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats Repetitive
- Cas: CRISPR associated protein
- GFP: green fluorescent protein
- BFP: blue fluorescent protein
- DOPE: 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine
- PA: phosphatidic acid
- STR-R8: stearyl octaarginine

3A05

From Crocus Sativus to a super plant
- with the magic of genetical engineering

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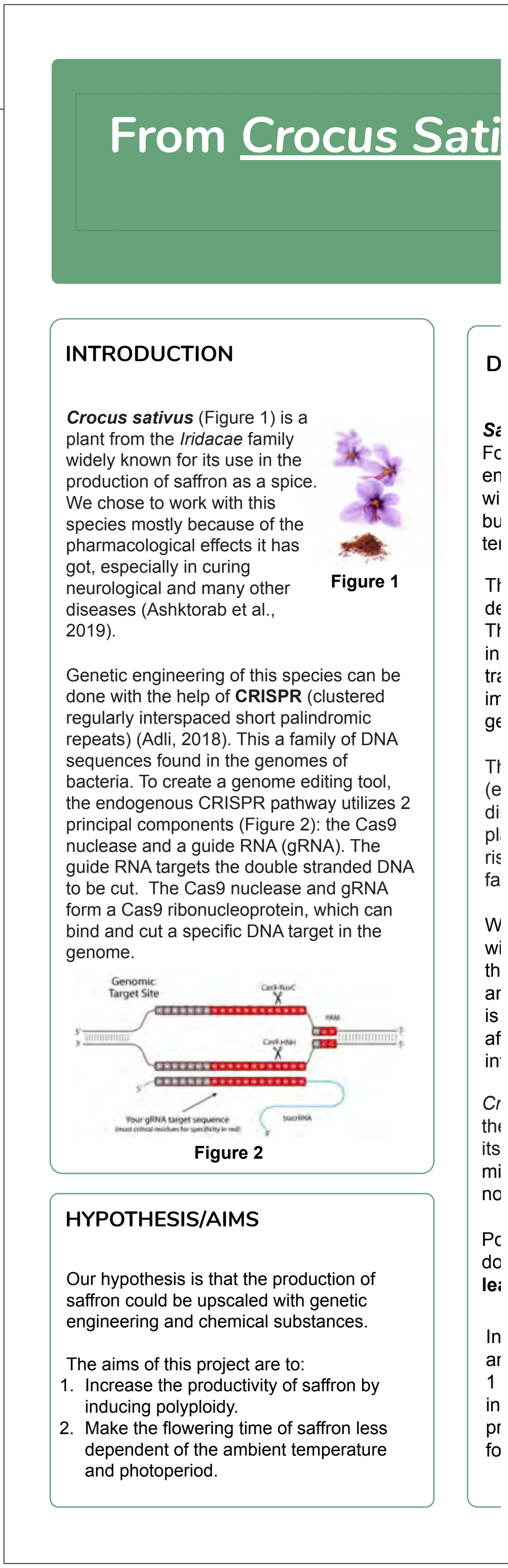

From Crocus Sati

INTRODUCTION

Crocus sativus (Figure 1) is a plant from the *Iridacae* family widely known for its use in the production of saffron as a spice. We chose to work with this species mostly because of the pharmacological effects it has got, especially in curing neurological and many other diseases (Ashktorab et al., 2019).

Figure 1

Genetic engineering of this species can be done with the help of **CRISPR** (clustered regularly interspaced short palindromic repeats) (Adli, 2018). This a family of DNA sequences found in the genomes of bacteria. To create a genome editing tool, the endogenous CRISPR pathway utilizes 2 principal components (Figure 2): the Cas9 nuclease and a guide RNA (gRNA). The guide RNA targets the double stranded DNA to be cut. The Cas9 nuclease and gRNA form a Cas9 ribonucleoprotein, which can bind and cut a specific DNA target in the genome.

Figure 2

HYPOTHESIS/AIMS

Our hypothesis is that the production of saffron could be upscaled with genetic engineering and chemical substances.

The aims of this project are to:
1. Increase the productivity of saffron by inducing polyploidy.
2. Make the flowering time of saffron less dependent of the ambient temperature and photoperiod.

Sativus to a super plant - with the magic of genetical engineering

Stevan Bogdanov

3A05 international Biology Olympiad 2020

DISCUSSION

Saffron crocus blooms only **once a year** and unlike most spring-blooming plants, saffron crocus does not blossom until autumn. For example, in China, the daughter corms begin to grow at the end of January and mature at the end of May and subsequently, enter a **dormant period** until mid-August. During the period, the corms are dug out from the soil when the leaves turn yellow and wilted and moved into the door to store. Experiencing the **high temperature** treatment in summer (ranged from 23 to 27 °C), the buds are broken up from dormancy in the middle of August and the floral primordia begin to initiate. When the average room temperature falls to **15–17 °C** in mid-autumn, most apical buds are in blossom.

The potential candidate gene, the enhancing of which would reduce the dependance of this plant to the external conditions is the **LFY gene**. This is a transcription factor that promotes early floral meristem identity in **synergy with APETALA1** which is required subsequently for the transition of an inflorescence meristem into a floral meristem, by an immediate upstream regulation of the **ABC classes** of floral homeotic genes, activating directly APETALA1.

The LFY gene can be enhanced by the technique of using CRISR (explained in the introduction), which is expected to promote flowering directly, without causing interfering in other genetic pathways of the plant. As seen on **Figure 3**, modifying other genes is potentially more risky, furthermore proving that the most reliable “candidate gene” is in fact the above mentioned LFY.

With the above mentioned methods, *Crocus sativus* can become a plant with little to no effect of the outside factors to its flowering. However, there is another problem which needs to be overcome as well. The amount of the active substance produced by this plant is too little, and it is possible to uprise it as well. Doing it with genetic engineering can affect the plant in many pathways, so the safer way to do it is introducing polyploidy with chemical substances, such as **colchicine**.

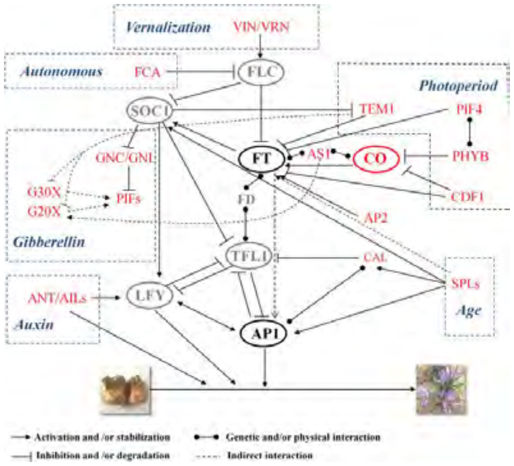

Figure 3

Crocus sativus is a sterile **triploid** (2n = 3x = 24) cultivated species, of unknown origin from other diploid and polyploid species in the genus Crocus (*Iridaceae*). By adding colchicine to this plant, we can potentially double its ploidity, resulting in producing more of its product. This has been done to other species successfully. The mechanism is the following: the colchicine prevents the microtubule formation during cell division, thus the chromosomes do not pull apart like they normally do. The end result is a cell that now has double the number of chromosomes that it would normally have.

Polyploid plants are generated in an effort to create new plants that have new characteristics. Even though there are some downsides (sometimes the polyploidy plants are sickly and not viable), in general the polyploid plants are expected to have l**arger leaves and flowers.**

In ordinary saffron crocuses, each flower produces only 3 stigmas, and tt takes an enormous amount of flowers (between 15,000 - 16,000 flowers) to produce 1 kilogram of saffron. However**.** using this chemical or others similar to it should increase the organs of *Crocus sativus* - including their stigmas, where saffron is produced and harvested from, thus cutting down the number of flowers needed for producing a kilogram of the product by half.

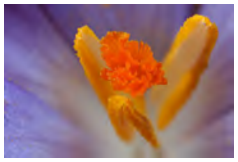

Figure 4

CONCLUSION

Combining genetic engineering with other methods - like using chemical substances is a way to:

1. Get more of the product of *Crocus sativus* and get it more frequently in places where it already grows (which could be implied in my region in the Republic of North Macedonia and the Hellenic Republic)
2. Make *Crocus sativus* grow and flower in places where it was never able to because of the local temperatures (one of the example being the territories of the northern hemisphere relatively close to the Pole).

The production of this plant is highly important as it is widely used in the field of medicine. To be more concrete, the extracts of its, mostly **safranal and crocin**, have been and still are used to treat a wide range of conditions, among which are: neurological, inflammatory and cardiovascular diseases.

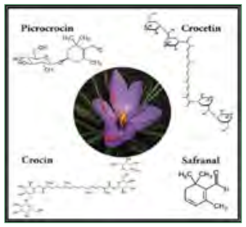

Figure 5

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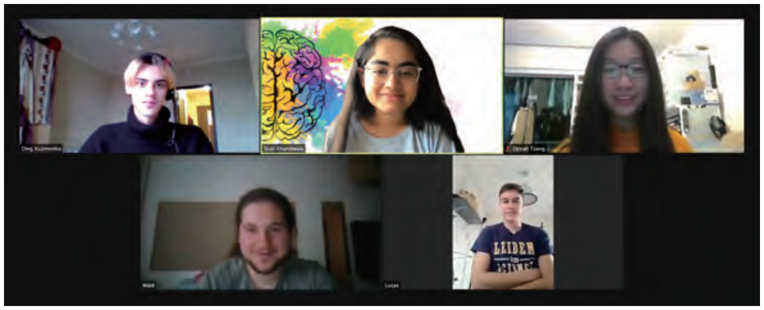
3A06 TelomExtend: A Genetic Approach to Reduce Rate of Aging by Extending Telomeres



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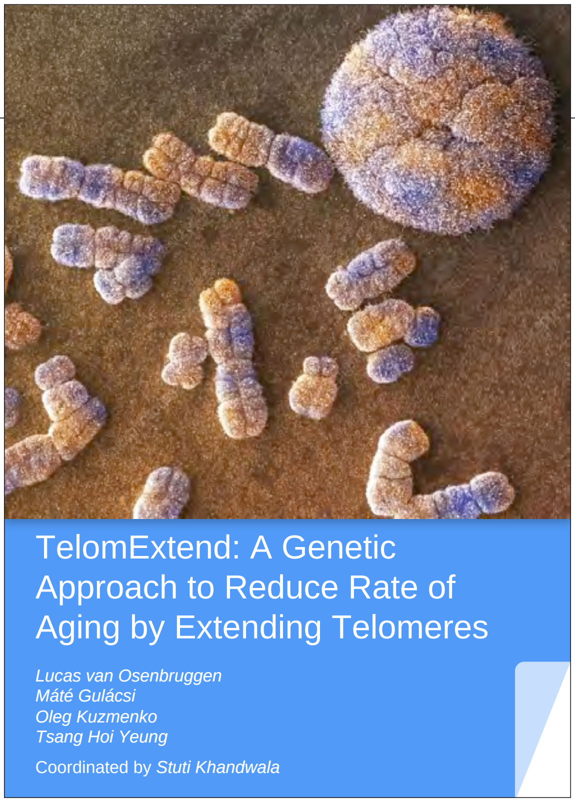

TelomExtend: A Genetic Approach to Reduce Rate of Aging by Extending Telomeres

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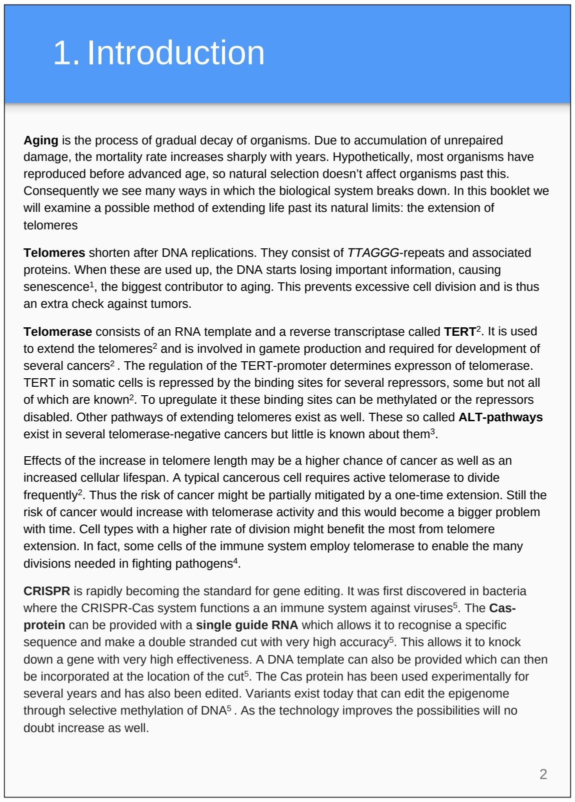

1. Introduction

Aging is the process of gradual decay of organisms. Due to accumulation of unrepaired damage, the mortality rate increases sharply with years. Hypothetically, most organisms have reproduced before advanced age, so natural selection doesn't affect organisms past this. Consequently we see many ways in which the biological system breaks down. In this booklet we will examine a possible method of extending life past its natural limits: the extension of telomeres

Telomeres shorten after DNA replications. They consist of *TTAGGG*-repeats and associated proteins. When these are used up, the DNA starts losing important information, causing senescence[1], the biggest contributor to aging. This prevents excessive cell division and is thus an extra check against tumors.

Telomerase consists of an RNA template and a reverse transcriptase called **TERT**[2]. It is used to extend the telomeres[2] and is involved in gamete production and required for development of several cancers[2] . The regulation of the TERT-promoter determines expresson of telomerase. TERT in somatic cells is repressed by the binding sites for several repressors, some but not all of which are known[2]. To upregulate it these binding sites can be methylated or the repressors disabled. Other pathways of extending telomeres exist as well. These so called **ALT-pathways** exist in several telomerase-negative cancers but little is known about them[3].

Effects of the increase in telomere length may be a higher chance of cancer as well as an increased cellular lifespan. A typical cancerous cell requires active telomerase to divide frequently[2]. Thus the risk of cancer might be partially mitigated by a one-time extension. Still the risk of cancer would increase with telomerase activity and this would become a bigger problem with time. Cell types with a higher rate of division might benefit the most from telomere extension. In fact, some cells of the immune system employ telomerase to enable the many divisions needed in fighting pathogens[4].

CRISPR is rapidly becoming the standard for gene editing. It was first discovered in bacteria where the CRISPR-Cas system functions a an immune system against viruses[5]. The **Cas-protein** can be provided with a **single guide RNA** which allows it to recognise a specific sequence and make a double stranded cut with very high accuracy[5]. This allows it to knock down a gene with very high effectiveness. A DNA template can also be provided which can then be incorporated at the location of the cut[5]. The Cas protein has been used experimentally for several years and has also been edited. Variants exist today that can edit the epigenome through selective methylation of DNA[5] . As the technology improves the possibilities will no doubt increase as well.

2

3

Cell cultures have become the prevalent technology providing basic information on cells' proliferation, differentiation or product formation under strictly controlled conditions. Cells, isolated from human tissues, can be expanded in vitro and experimented on. Often these cells are limited in the amount of divisions, making repeating results more difficult. A solution often used is cell lines of cancerous cells. Different conditions allow cultivating different types of human cells in cultures consisting of single type of cells, or even in highly organised agglomerates resembling real tissues or organs[6].

Organoids - three-dimensional complex tissue cultures, are self-organising miniaturized versions of a organ[7]. They are able to realize physiological processes similar to that in the human body[7]. Organoids also provide us with more information on how cells are interacting within a tissue or organ and how changes in cellular processes can affect the whole organism. Organoids are derived from the pool of stem cells that can give rise to the different cell lines of the organ[8]. Researchers can also influence the development by applying specific active molecules, such as proteins involved in determining the cell fate or just by cultivating precursor cells in different artificially created environments[9]. Organoids thus provide a much needed intermediate between cell cultures and living organisms.

2. Methods

To evaluate the effects of telomere extension on human physiological processes such as aging or possible cancerogenesis, manipulations are carried out on organoids. The reasons for this decision are the moderate turnover rate[10], relative ease of cultivation and the involvement in physiological aging processes. A kidney organoid is created consisting of genetically modified cells with upregulated telomerase. Normal age-related changes in the kidney include a decrease in the relative abundance of **mesangial** cells, which results in the impairing of glomerular filtration rate[11]. This is something that can be measured as a quantity of age in the organoid by staining for specific markers like *CD44*[12]. The presence of abnormal growth can be detected by simple microscopic comparison.

In order to cultivate such a organoid the *Takasato multi-step protocol* is used[13], since it allows to achieve the highest heterogeneity of cells within the organ. The human iPS cell line *CRL1502* will be genetically modified and then cultivated into a mature organoid[13].

The telomere length can be determined using *terminal restriction fragment analysis*. This technique uses a restriction enzyme that cuts often but not inside the repeated sequence of telomeres. This digested DNA is put into gel electrophoresis and a complementary nucleic acid probe is added for detection[14].

4

To lengthen telomeres, the repressors of the TERT gene should be downregulated. A *CRISPR-sp-dCas9-KRAB complex*[15] can be used to silence the genes coding for these repressors in a highly specific manner[16]. In this experiment *rAAV*, the standard vector for gene therapy, will be used to carry the complex[17,18]. Specifically, pAnc80L65 plasmid and AAV2 would be used to target mesangial cells due to its high specificity and efficiency[19]. As rAAV can only accommodate around 4.8 kb of DNA, the long sequence coding for the protein domains is to be splitted into 2 vectors[20]. This will be inserted in the cell using *hybrid AAV dual vector strategy*[21]: the two separated AAVs open at their inverted terminal repeat (ITR) and either undergo homologous recombination, or undergo concatemerization and subsequent transplicing where *ITRs* are removed[22]. The original sequence would be restored after both approaches. This doubles the opportunity of transgene regeneration. The mentioned plasmid and other helper plasmids will be inserted into *HEK293* cells, generating AAV2 carrying the crucial sequence.

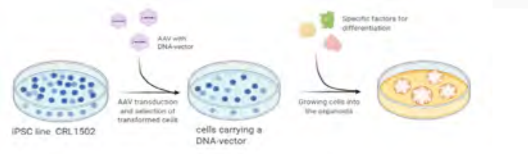

Figure 1. General scheme for experimental setup

Dual AAVs have a relatively low immunogenicity, so it would also be useful in gene therapy. With the aid of an *sgRNA,* the *CRISPR-sp-dCas9-KRAB* complex binds to the transcription start site of the TERT repressors and prevents them from being expressed, as the complex can halt RNA polymerase progression by *steric hindrance*[23]. As a result TERT is activated and telomeres will be extended. This effect will be temporary as the complex will eventually be broken down.

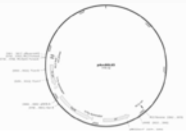

Figure 3. Plasmid used for carrying coding sequences[24]

Figure 2. Crucial sequences inserted in the rAAV variants

5

3. Hypothesis

H0: Increased expression of TERT doesn't affect the lifespan of cells. This could be explained by the complex protein network involved in this process which would downregulate telomerase via different pathways.

H1: Increased expression of TERT results in lengthening of telomeres in mesangial cells, which leads to a greater number of cell divisions and a longer lifespan for the organoid.

We will perform a statistical test comparing edited cells to the control group using $p < 0.05$ to rule out coincidence. All data will be published.

Expected negative effects are increased chances of malignant transformation and uncontrolled cell division causing disturbance of normal kidney structure or its physiological functions.

4. Future Developments

Living organisms: The next logical step if the results indicate increased cellular age would be to apply this treatment to a living model organism, such as *Mus musculus*.This would provide us with a better model of how telomere lengthening actually affects aging.

Negative feedback: The action of this experiment is genomically *scarless* and reversible**.** A negative feedback system using similar techniques might be developed that targets repressors and prevents the excessive extension of telomeres. This could help in cancer treatments.

Cyclical expression: A possible way to avoid excessive lengthening and transformation as a result of our treatment is adding a molecular switch, that would restrain it to express only in at certain times in the circadian rhythm or even more rarely by using annual molecular signals.

Clinical approach: Age-dependent impairment of glomerular filtration rate may be caused by decreased numbers of mesangial cells. Increasing their renewal rate would help in therapy of nephropathy. What's more, this technique can be extended to other cell types. This would be a powerful treatment for multiple diseases and could lead to a cure for aging itself.

6

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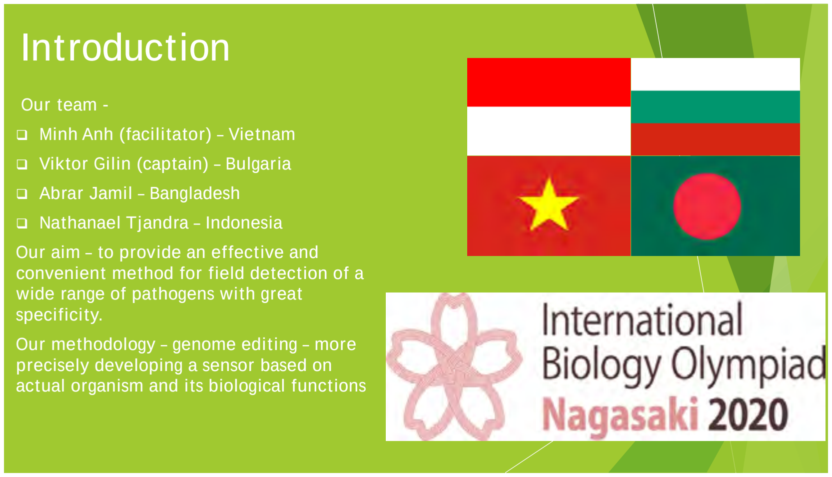
3A07+3B05 Yeast biosensor

IBO Group Project 3A07
– Yeast biosensor



Introduction

Our team -

- Minh Anh (facilitator) – Vietnam
- Viktor Gilin (captain) – Bulgaria
- Abrar Jamil – Bangladesh
- Nathanael Tjandra – Indonesia

Our aim – to provide an effective and convenient method for field detection of a wide range of pathogens with great specificity.

Our methodology – genome editing – more precisely developing a sensor based on actual organism and its biological functions

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Competitors

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Asiman Ziyaddunov (Azerbaijan)

Cheng-Chun Yang (Chinese Taipei)

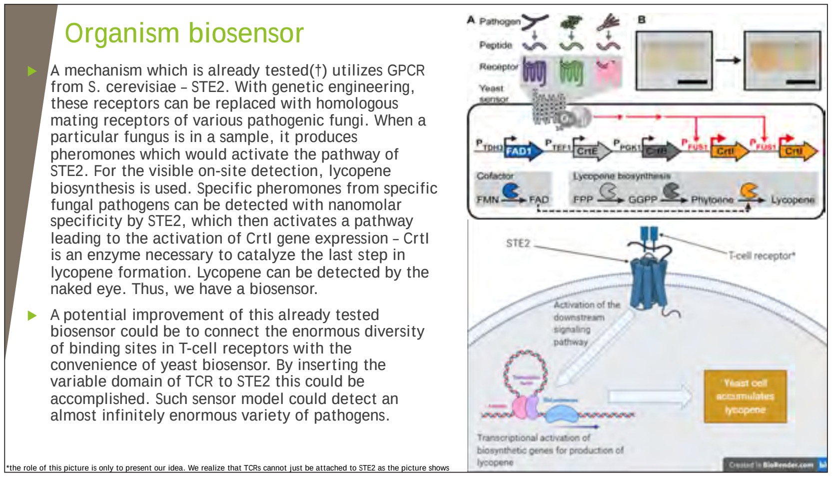
Heewon In (South Korea)

Munisa Pulatova Muminjonovna (Tajikistan)

Nathanael Tjandra (Indonesia)

Viktor Dimitrov Gilin (Bulgaria)

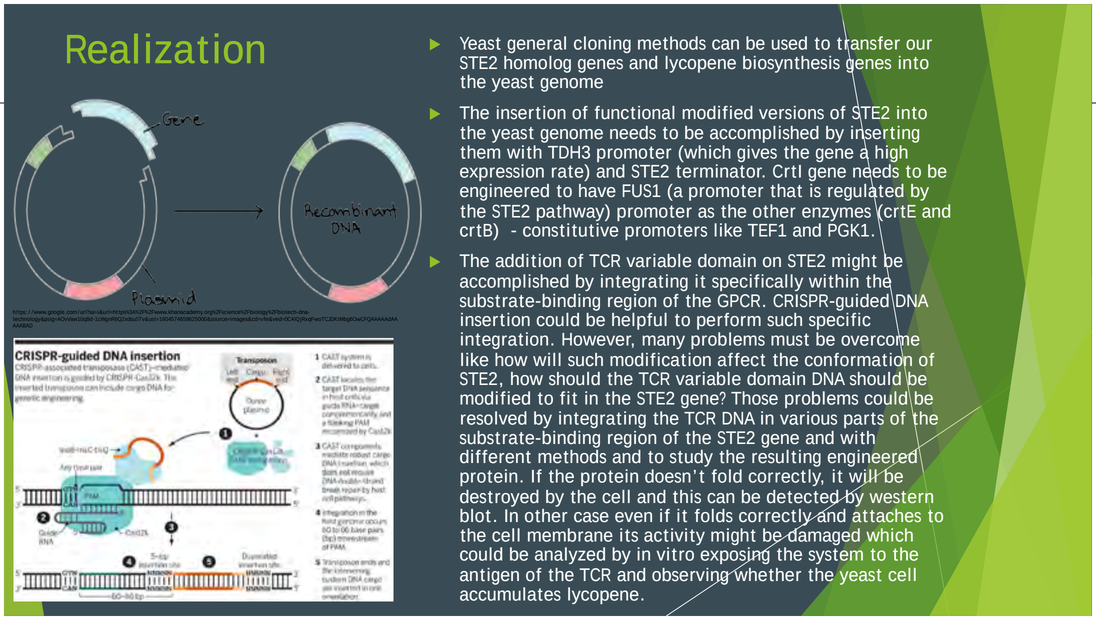
The “Making Great Sense” Award



Organism biosensor

- A mechanism which is already tested(†) utilizes GPCR from S. cerevisiae – STE2. With genetic engineering, these receptors can be replaced with homologous mating receptors of various pathogenic fungi. When a particular fungus is in a sample, it produces pheromones which would activate the pathway of STE2. For the visible on-site detection, lycopene biosynthesis is used. Specific pheromones from specific fungal pathogens can be detected with nanomolar specificity by STE2, which then activates a pathway leading to the activation of CrtI gene expression – CrtI is an enzyme necessary to catalyze the last step in lycopene formation. Lycopene can be detected by the naked eye. Thus, we have a biosensor.
- A potential improvement of this already tested biosensor could be to connect the enormous diversity of binding sites in T-cell receptors with the convenience of yeast biosensor. By inserting the variable domain of TCR to STE2 this could be accomplished. Such sensor model could detect an almost infinitely enormous variety of pathogens.

*the role of this picture is only to present our idea. We realize that TCRs cannot just be attached to STE2 as the picture shows



Realization

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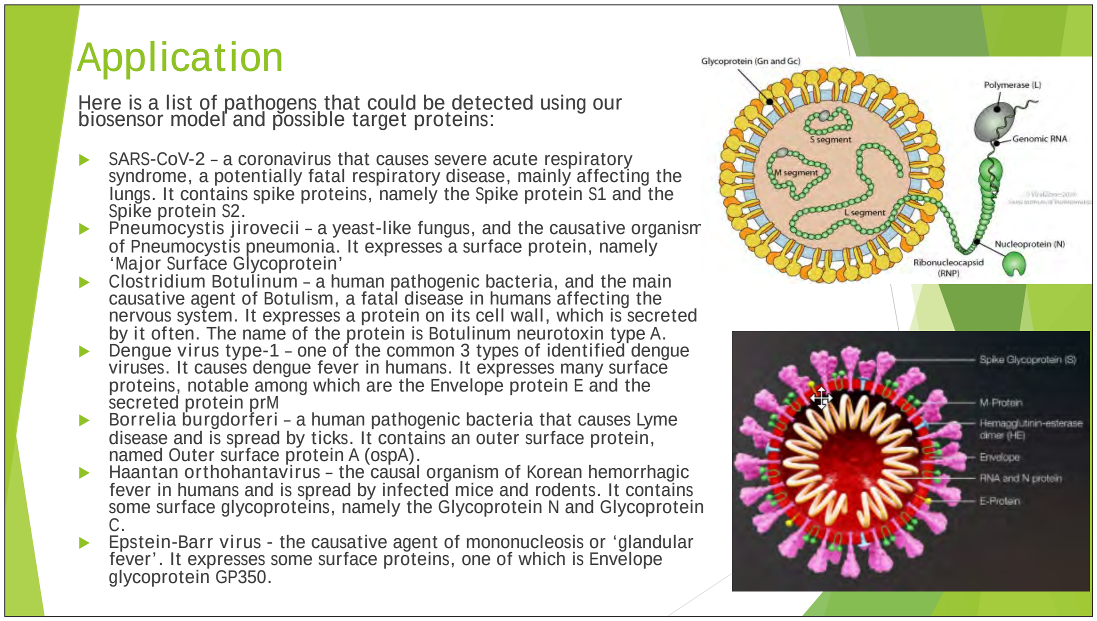
- Yeast general cloning methods can be used to transfer our STE2 homolog genes and lycopene biosynthesis genes into the yeast genome
- The insertion of functional modified versions of STE2 into the yeast genome needs to be accomplished by inserting them with TDH3 promoter (which gives the gene a high expression rate) and STE2 terminator. CrtI gene needs to be engineered to have FUS1 (a promoter that is regulated by the STE2 pathway) promoter as the other enzymes (crtE and crtB) - constitutive promoters like TEF1 and PGK1.
- The addition of TCR variable domain on STE2 might be accomplished by integrating it specifically within the substrate-binding region of the GPCR. CRISPR-guided DNA insertion could be helpful to perform such specific integration. However, many problems must be overcome like how will such modification affect the conformation of STE2, how should the TCR variable domain DNA should be modified to fit in the STE2 gene? Those problems could be resolved by integrating the TCR DNA in various parts of the substrate-binding region of the STE2 gene and with different methods and to study the resulting engineered protein. If the protein doesn't fold correctly, it will be destroyed by the cell and this can be detected by western blot. In other case even if it folds correctly and attaches to the cell membrane its activity might be damaged which could be analyzed by in vitro exposing the system to the antigen of the TCR and observing whether the yeast cell accumulates lycopene.



Application

Here is a list of pathogens that could be detected using our biosensor model and possible target proteins:

- SARS-CoV-2 – a coronavirus that causes severe acute respiratory syndrome, a potentially fatal respiratory disease, mainly affecting the lungs. It contains spike proteins, namely the Spike protein S1 and the Spike protein S2.
- Pneumocystis jirovecii – a yeast-like fungus, and the causative organism of Pneumocystis pneumonia. It expresses a surface protein, namely 'Major Surface Glycoprotein'
- Clostridium Botulinum – a human pathogenic bacteria, and the main causative agent of Botulism, a fatal disease in humans affecting the nervous system. It expresses a protein on its cell wall, which is secreted by it often. The name of the protein is Botulinum neurotoxin type A.
- Dengue virus type-1 – one of the common 3 types of identified dengue viruses. It causes dengue fever in humans. It expresses many surface proteins, notable among which are the Envelope protein E and the secreted protein prM
- Borrelia burgdorferi – a human pathogenic bacteria that causes Lyme disease and is spread by ticks. It contains an outer surface protein, named Outer surface protein A (ospA).
- Haantan orthohantavirus – the causal organism of Korean hemorrhagic fever in humans and is spread by infected mice and rodents. It contains some surface glycoproteins, namely the Glycoprotein N and Glycoprotein C.
- Epstein-Barr virus - the causative agent of mononucleosis or 'glandular fever'. It expresses some surface proteins, one of which is Envelope glycoprotein GP350.

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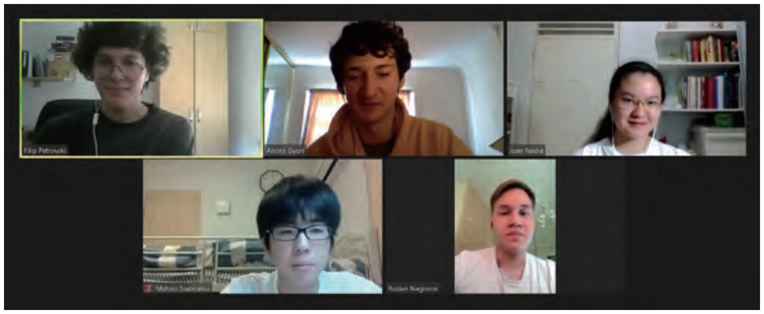
3A09+3B02 Enhancement of Triacylglycerol Content and Mass Cultivation of Dunaliella salina



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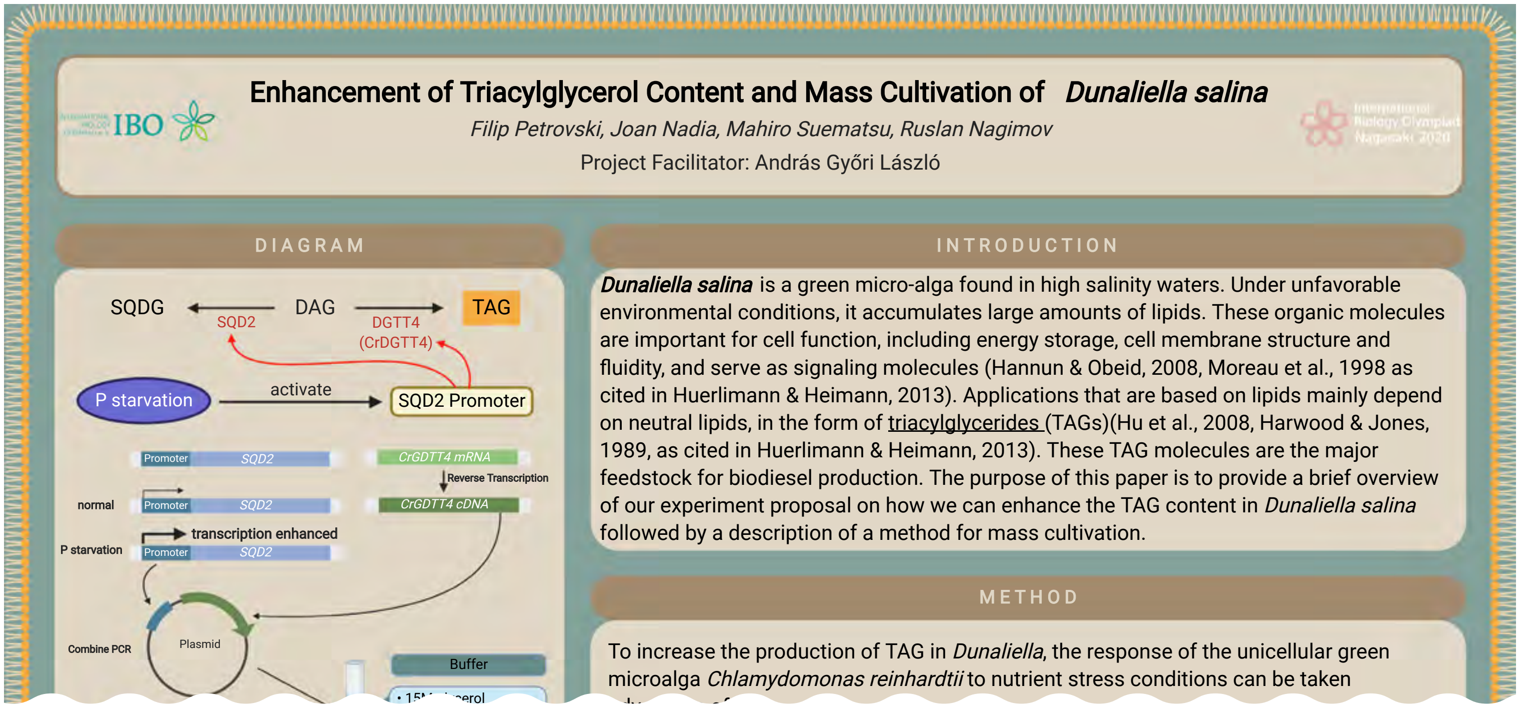


Enhancement of Triacylglycerol Content and Mass Cultivation of *Dunaliella salina*

Filip Petrovski, Joan Nadia, Mahiro Suematsu, Ruslan Nagimov

Project Facilitator: András Győri László

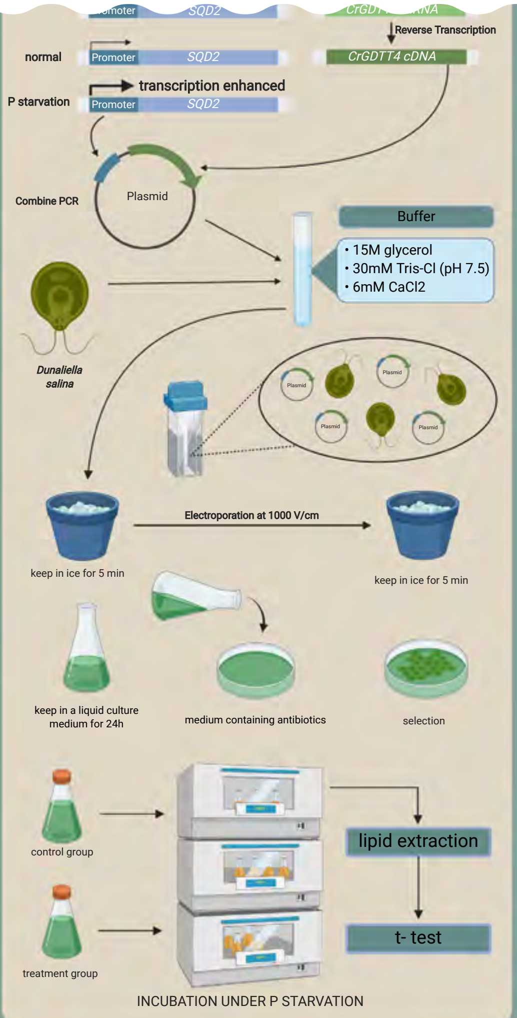
DIAGRAM

INTRODUCTION

Dunaliella salina is a green micro-alga found in high salinity waters. Under unfavorable environmental conditions, it accumulates large amounts of lipids. These organic molecules are important for cell function, including energy storage, cell membrane structure and fluidity, and serve as signaling molecules (Hannun & Obeid, 2008, Moreau et al., 1998 as cited in Huerlimann & Heimann, 2013). Applications that are based on lipids mainly depend on neutral lipids, in the form of triacylglycerides (TAGs)(Hu et al., 2008, Harwood & Jones, 1989, as cited in Huerlimann & Heimann, 2013). These TAG molecules are the major feedstock for biodiesel production. The purpose of this paper is to provide a brief overview of our experiment proposal on how we can enhance the TAG content in *Dunaliella salina* followed by a description of a method for mass cultivation.

METHOD

To increase the production of TAG in *Dunaliella*, the response of the unicellular green microalga *Chlamydomonas reinhardtii* to nutrient stress conditions can be taken



SOME FACTS

D. salina is capable of producing large amounts of carotenoids to protect against light. For this feature in 1966 the USSR began to actively cultivate it. The most active production of carotenoids occurs under conditions of high salinity. Due to the abundance of β-carotene, which is vitamin A precursor, *D. salina* is a popular pro-vitamin A food supplement and cosmetic additive.

The cells do not have a rigid cell wall, which makes the organism susceptible to osmotic pressure. To maintain osmotic balance, it produces a lot of glycerol.

feedstock for biodiesel production. The purpose of this paper is to provide a brief overview of our experiment proposal on how we can enhance the TAG content in *Dunaliella salina* followed by a description of a method for mass cultivation.

METHOD

To increase the production of TAG in *Dunaliella*, the response of the unicellular green microalga *Chlamydomonas reinhardtii* to nutrient stress conditions can be taken advantage of.

In *C. reinhardtii* (and all green alg*ae), TAG* is synthesized from diacylglycerol (DAG), catalyzed by diacylglycerol acyltransferase (DGAT). DAG is also used to create sulphoquinovosyl diacylglycerol (*SQDG),* photosynthetic membrane lipid. Sulphoquinovosyldiacylglycerol 2 (SQD2) catalyzes this reaction. During phosphate starvation, SQD2 promoters in *C. reinhardtii* are up-regulated and, what's important, enhance the overexpression of DGTT4 (a type of DGAT in *C. reinhardtii*), leading to the increased TAG accumulation (Goncalves et al., 2016; Iwai et al., 2014).

In order to apply this mechanism to *Dunaliella*, we first amplify the SQD2 promoter and DGTT4 cDNA using PCR. After that, we insert them into plasmids containing a drug resistance marker. We keep the *D. salina* cells, plasmids and the buffer (15M glycerol, 30mM Tris-Cl pH 7.5, 6mM CaCl2) on ice for 5 min. Then we proceed with electroporation (recommended 1000V/cm at a capacitance of 220 μF). After that, the cells are kept on ice for 5 min and are afterwards added in a liquid culture medium for 24h. Next, the cells are plated on a medium containing antibiotics for selection. After we selected the cells that took up the plasmid, we put them under the same conditions as the *Dunaliella* control group (we may have to shock the control group at 1000V/cm too, because electroporation seems to trigger some TAG formation due to stress). We keep both the control group and the treatment group under the same (P-starved) conditions and see whether the TAG concentration increased because of the inserted plasmid.

D. salina would be cultured in modified Johnson's medium as described in Lv et al., 2016 at the salinity of 6% NaCl. The phosphorus deprivation condition would be achieved by replacing KH2PO4 with KCl.

MASS CULTIVATION

For the mass cultivation and harvest of *Dunaliella salina* it is suggested to use the vertical flat plate photobioreactor (Khadim et al., 2018). The cultivation would be performed under semicontinuous mode with inoculum concentration of OD680 = 0.1, light illumination of 100μmol/m2s, and aeration of 1L/min. The composition of the culture medium would follow the one used in the experiments. The cells would be harvested by flocculation using NaOH or FeCl3 (Pirwitz et al., 2015). It is also suggested to perform extraction with SDEP (Simultaneous Distillation and Extraction Process) method (Dejoye Tanzi et al., 2013).

CONCLUSION

Metabolic engineering through genetic manipulation represents a promising strategy for the overproduction of algal oils (TAGs or other lipids). Further understanding of TAG production is essential for achieving higher oil yield and is necessary for the biofuel industry. Through this experiment, we wanted to test whether the *Dunaliella* cells accumulate more TAGs under P starved conditions when introduced to the SQD2 promoter and DGTT4 cDNA.

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3A10

Increasing Homology Directed Repair Efficiency by Fusion of Cas9 with BRCA1-Derived Domains

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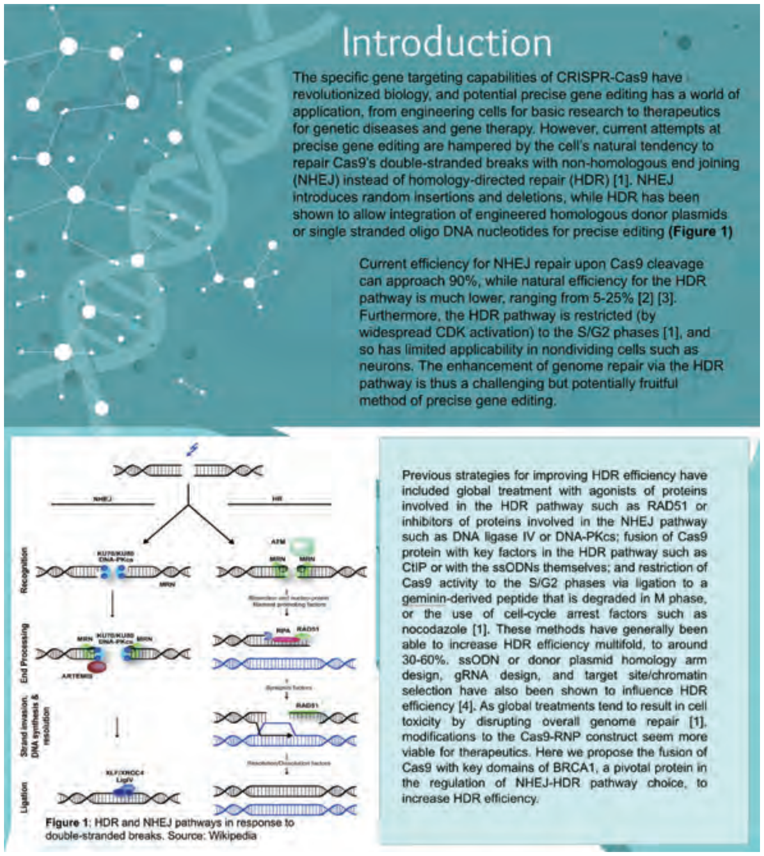


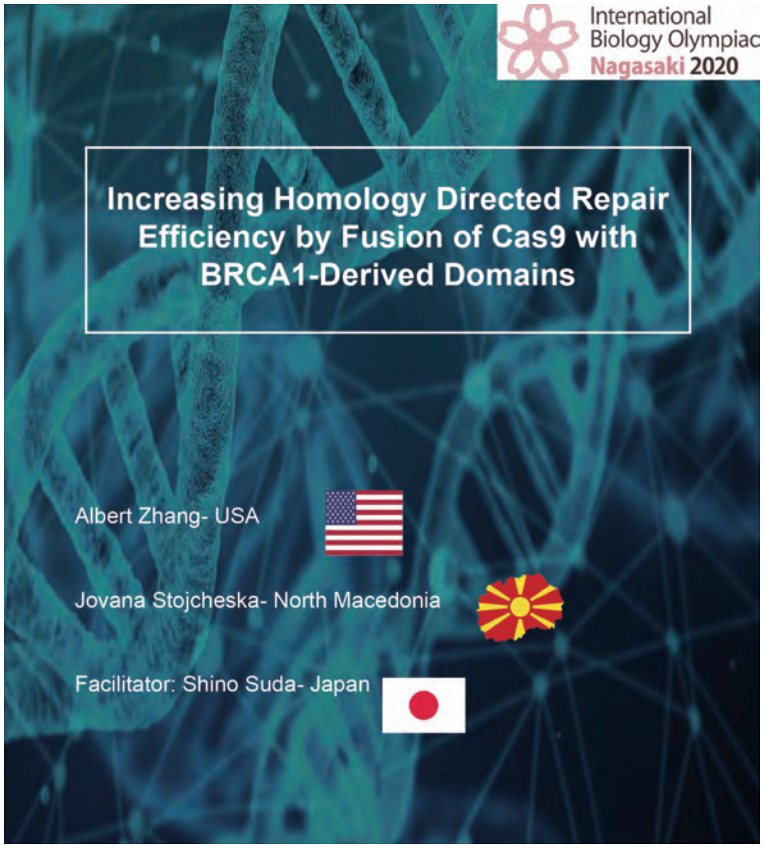
Introduction

The specific gene targeting capabilities of CRISPR-Cas9 have revolutionized biology, and potential precise gene editing has a world of application, from engineering cells for basic research to therapeutics for genetic diseases and gene therapy. However, current attempts at precise gene editing are hampered by the cell's natural tendency to repair Cas9's double-stranded breaks with non-homologous end joining (NHEJ) instead of homology-directed repair (HDR) [1]. NHEJ introduces random insertions and deletions, while HDR has been shown to allow integration of engineered homologous donor plasmids or single stranded oligo DNA nucleotides for precise editing **(Figure 1)**

Current efficiency for NHEJ repair upon Cas9 cleavage can approach 90%, while natural efficiency for the HDR pathway is much lower, ranging from 5-25% [2] [3]. Furthermore, the HDR pathway is restricted (by widespread CDK activation) to the S/G2 phases [1], and so has limited applicability in nondividing cells such as neurons. The enhancement of genome repair via the HDR pathway is thus a challenging but potentially fruitful method of precise gene editing.

Figure 1: HDR and NHEJ pathways in response to double-stranded breaks. Source: Wikipedia

Previous strategies for improving HDR efficiency have included global treatment with agonists of proteins involved in the HDR pathway such as RAD51 or inhibitors of proteins involved in the NHEJ pathway such as DNA ligase IV or DNA-PKcs; fusion of Cas9 protein with key factors in the HDR pathway such as CtIP or with the ssODNs themselves; and restriction of Cas9 activity to the S/G2 phases via ligation to a geminin-derived peptide that is degraded in M phase, or the use of cell-cycle arrest factors such as nocodazole [1]. These methods have generally been able to increase HDR efficiency multifold, to around 30-60%. ssODN or donor plasmid homology arm design, gRNA design, and target site/chromatin selection have also been shown to influence HDR efficiency [4]. As global treatments tend to result in cell toxicity by disrupting overall genome repair [1], modifications to the Cas9-RNP construct seem more viable for therapeutics. Here we propose the fusion of Cas9 with key domains of BRCA1, a pivotal protein in the regulation of NHEJ-HDR pathway choice, to increase HDR efficiency.



International Biology Olympiad Nagasaki 2020

Increasing Homology Directed Repair Efficiency by Fusion of Cas9 with BRCA1-Derived Domains

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BRCA1 & HDR/NHEJ

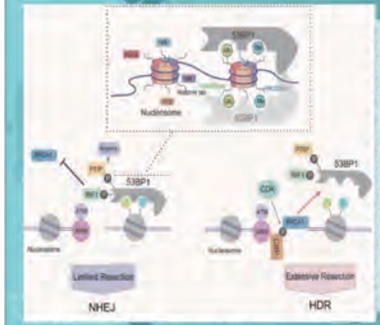

Figure 2: Proposed 53BP1/BRCA1 regulation of NHEJ/HDR pathway choice [1]

Although not fully understood, the central regulatory point between the NHEJ or HDR pathways is the competition between 53BP1 and BRCA1 [5] **(Figure 2).** Binding to chromatin markers of DNA damage (H4K20Me2 and H2AK15Ub), phosphorylated 53BP1 recruits artemis (an end-processing nuclease that is key to NHEJ initiation) via interaction with PTIP, and complexes with RIF to both prevent the HDR-initiating DNA end-resection and antagonize BRCA1. On the other hand, beginning in S-phase, BRCA1 can (i) complex with activated CtIP to block 53BP1-RIF1 interaction, (ii) recruit UHRF1, an E3 ligase that ubiquitinylates RIF1 for degradation, and (iii) complex with BARD1 to become an E3 ligase that ubiquitinylates H2A, attracting SMARCAD1, a protein that removes 53BP1 from the damage site [1]. 53BP1 and BRCA1 thus compete for binding to the DNA-damage site to initiate NHEJ or HDR, respectively. Fusing BRCA1 with Cas9 to enhance its activity is thus attractive towards increasing HDR efficiency. Indeed, mutant cells with hyperexpressed or hyperactive BRCA1 have been shown to have increased HDR efficiency [6].

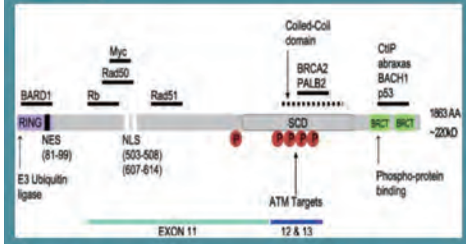

Figure 3: Structure and function of exon regions on the BRCA1 gene [7].

As BRCA1 is a comparatively large protein of size 220kDa, it is much more practical to fuse certain fragments or domains of BRCA1 to Cas9. Of particular interest are (i) the N-terminal RING domain, a 109-amino acid domain that binds to BARD1 and has the E3 ligase activity, and (ii) the C-terminal BRCT domain, a 214-amino acid domain that binds to both CtIP and UHRF1 [7] **(Figure 3).** It will be important to study how some combination of these domains, or modifications of them, can retain effectiveness in activating the HDR pathway when fused to Cas9.

Experimental methods

1

Cas9-BRCA1 fusion proteins will be produced with standard cloning procedures (construction on plasmids followed by protein expression/purification), with focus on the RING and BRCT domains as previously mentioned.

2

Cas9-RNPs will then be constructed with standard procedures, and introduced into host cells via electroporation. For primary experiments to determine if the fusion protein can increase HDR efficiency, the gRNAs will be designed with common software and target the AAVS1 locus, a common site for CRISPR-Cas9 studies [8].

3

The host cells will be a model human fibroblast cultures. If these experiments show significant effectiveness, gRNAs will be expanded to target different loci.

4

Host cells will also be expanded to other commonly studied cell types such as hIPSCs, rat zygotes, HeLa cells, and Jurkat cells, as both these factors generally impact HDR efficiency as well [1].

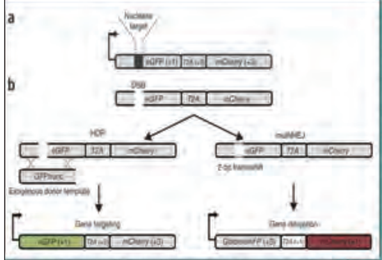

Figure 4: (a) Structure of TLR locus (b) Result of repair under either HDR or NHEJ pathways to produce eGFP or mCherry-expressing cells [9]

The control group for assays of NHEJ/HDR efficiency will be cells with introduction of unmodified Cas9-RNP. The experimental groups will have introduction of Cas9-BRCA1 fusion protein. For one set of experiments, the donor DNA for HDR will be transfected plasmids with GFP-expressing regions. HDR assays will be performed by FACS to determine GFP positivity/plasmid integration rate, and NHEJ assays will be performed by DNA extraction, PCR, and sequencing around the AAVS1 locus to determine presence of imprecise mutations. In the other set of experiments, ssODNs according to the traffic light reporter (TLR) system [9] will serve as donors. The commonly-used TLR system **(Figure 4)** enables FACS analysis to determine both NHEJ and HDR rates: integration of donor ssODN (indicative of HDR) results in eGFP expression, while disruption of the AAVS1 locus (indicative of NHEJ) results in mCherry expression.

After data collection, important statistics for analysis will include absolute HDR efficiency (calculated as percentage of cells that undergo HDR), and the HDR/NHEJ efficiency ratio (calculated as HDR efficiency/NHEJ efficiency).

CONCLUSION

Expanding on previous studies regarding Cas9 fusion proteins, here we propose the fusion of Cas9 with BRCA1-derived domains to increase HDR efficiency for Cas9-cleaved DNA. BRCA1 is a pivotal regulator in the HDR-NHEJ control pathway, and the relevant interactions for this function are restricted to two relatively small domains, thus making it attractive for fusion with Cas9.

Looking forward, there still exists significant challenges for achieving precise gene editing with CRISPR-Cas9. While each of previously described methods have been moderately successful, their effects are not necessarily additive when combined (for example, CtIP-Cas9-geminin fusion proteins did not show significant improvement over CtIP-Cas9) [7]. The increases in HDR efficiency also tend to be quite variable over different cell types and targeted loci.

Furthermore, there has not been much progress on overcoming the widespread (and perhaps insurmountable) inhibition of HDR in the M and G1 phases. Increased understanding of the NHEJ and HDR pathways may prove critical to better manipulation of their relative probabilities. Other researchers have bypassed HDR entirely and studied the linking of transposases to Cas9, another possible source of homologous recombination [10].

Whatever the method, precise gene editing technology has vast applications, at the least including targeted transformation of cell culture genomes, precise insertion of gene therapeutics into human tissues, and direct correction of human genetic diseases or manipulation of the human genome. If precise gene editing can be applied practically, efficiently, and safely, it could truly realize the new era of biology that the CRISPR-Cas9 system implicates.

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3B01 Treating melanoma with the liposomal CRISPR/Cas9 ointment

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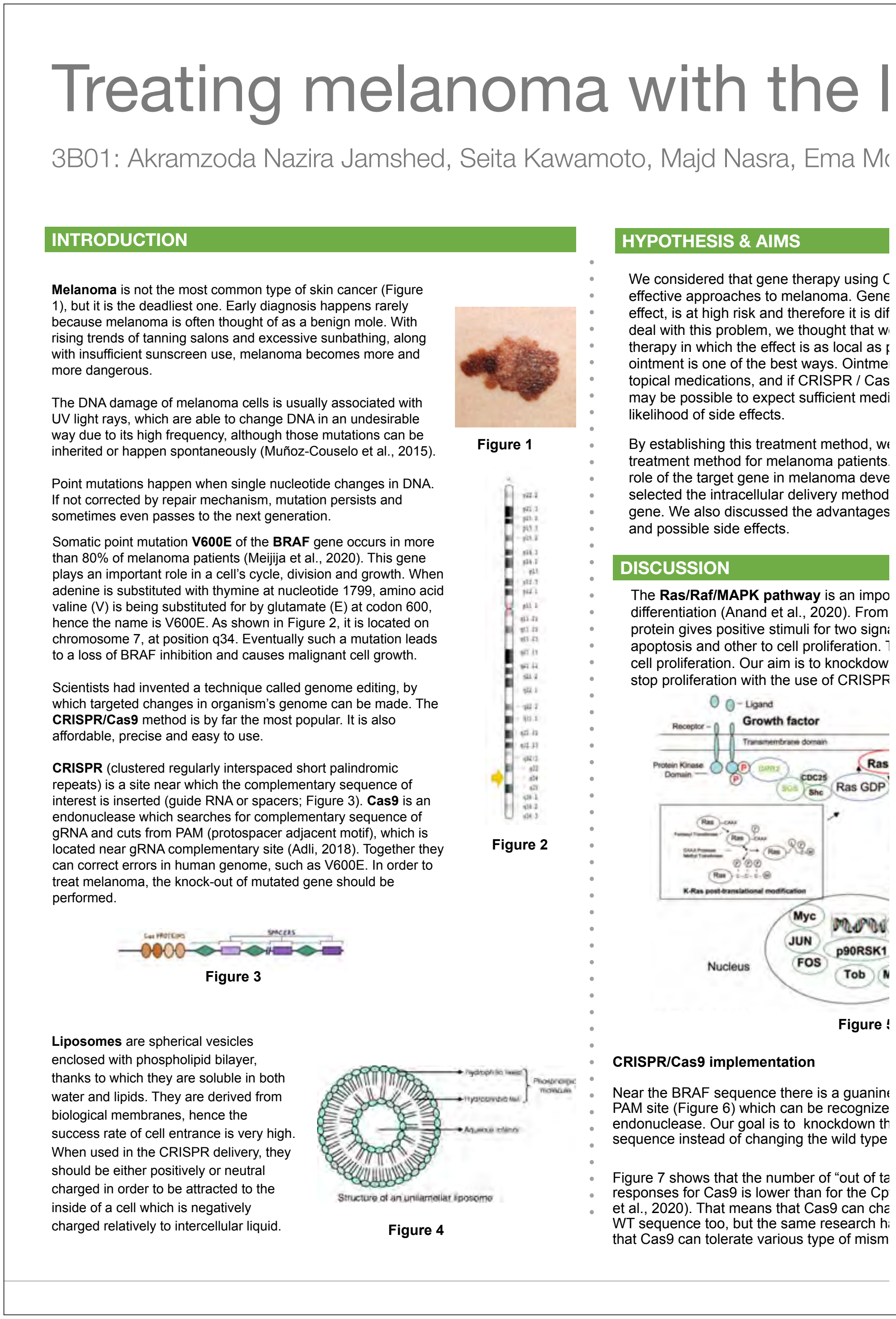
The IBO2020 President Award



Treating melanoma with the l

3B01: Akramzoda Nazira Jamshed, Seita Kawamoto, Majd Nasra, Ema Mo

INTRODUCTION

Melanoma is not the most common type of skin cancer (Figure 1), but it is the deadliest one. Early diagnosis happens rarely because melanoma is often thought of as a benign mole. With rising trends of tanning salons and excessive sunbathing, along with insufficient sunscreen use, melanoma becomes more and more dangerous.

Figure 1

The DNA damage of melanoma cells is usually associated with UV light rays, which are able to change DNA in an undesirable way due to its high frequency, although those mutations can be inherited or happen spontaneously (Muñoz-Couselo et al., 2015).

Point mutations happen when single nucleotide changes in DNA. If not corrected by repair mechanism, mutation persists and sometimes even passes to the next generation.

Somatic point mutation **V600E** of the **BRAF** gene occurs in more than 80% of melanoma patients (Meijija et al., 2020). This gene plays an important role in a cell's cycle, division and growth. When adenine is substituted with thymine at nucleotide 1799, amino acid valine (V) is being substituted for by glutamate (E) at codon 600, hence the name is V600E. As shown in Figure 2, it is located on chromosome 7, at position q34. Eventually such a mutation leads to a loss of BRAF inhibition and causes malignant cell growth.

Scientists had invented a technique called genome editing, by which targeted changes in organism's genome can be made. The **CRISPR/Cas9** method is by far the most popular. It is also affordable, precise and easy to use.

CRISPR (clustered regularly interspaced short palindromic repeats) is a site near which the complementary sequence of interest is inserted (guide RNA or spacers; Figure 3). **Cas9** is an endonuclease which searches for complementary sequence of gRNA and cuts from PAM (protospacer adjacent motif), which is located near gRNA complementary site (Adli, 2018). Together they can correct errors in human genome, such as V600E. In order to treat melanoma, the knock-out of mutated gene should be performed.

Figure 2

Figure 3

Liposomes are spherical vesicles enclosed with phospholipid bilayer, thanks to which they are soluble in both water and lipids. They are derived from biological membranes, hence the success rate of cell entrance is very high. When used in the CRISPR delivery, they should be either positively or neutral charged in order to be attracted to the inside of a cell which is negatively charged relatively to intercellular liquid.

Figure 4

HYPOTHESIS & AIMS

We considered that gene therapy using C
effective approaches to melanoma. Gene
effect, is at high risk and therefore it is dif
deal with this problem, we thought that w
therapy in which the effect is as local as p
ointment is one of the best ways. Ointme
topical medications, and if CRISPR / Cas
may be possible to expect sufficient medi
likelihood of side effects.

By establishing this treatment method, we
treatment method for melanoma patients.
role of the target gene in melanoma deve
selected the intracellular delivery method
gene. We also discussed the advantages
and possible side effects.

DISCUSSION

The **Ras/Raf/MAPK pathway** is an impo
differentiation (Anand et al., 2020). From
protein gives positive stimuli for two signa
apoptosis and other to cell proliferation. T
cell proliferation. Our aim is to knockdow
stop proliferation with the use of CRISPR

Figure 5

CRISPR/Cas9 implementation

Near the BRAF sequence there is a guanine
PAM site (Figure 6) which can be recognize
endonuclease. Our goal is to knockdown th
sequence instead of changing the wild type

Figure 7 shows that the number of "out of ta
responses for Cas9 is lower than for the Cp
et al., 2020). That means that Cas9 can cha
WT sequence too, but the same research h
that Cas9 can tolerate various type of mism

e liposomal CRISPR/Cas9 ointment

a Moskatelo

International
Biology Olympiad
Nagasaki 2020

y using CRISPR/Cas9 is one of the most
a. Gene therapy, which has the systemic
e it is difficult to apply to humans. In order to
ht that we should design a form of gene
local as possible. We propose that using an
. Ointments are already widely used as
PR / Cas9 can be mixed with ointments, it
ent medical effects while reducing the

thod, we aim to provide a safe and effective
patients. It could also be used to analyze the
ma development. Therefore, in this study, we
method of CRISPR / Cas9 and the target
antages and disadvantages of this treatment

an important pathway in cell growth, division and
0). From the Figure 5, we can see that the Raf
two signal transduction pathways: one that leads to
eration. The V600E mutation specifically amplifies
ockdown the Raf sequence in cancer cells and
CRISPR/Cas9.

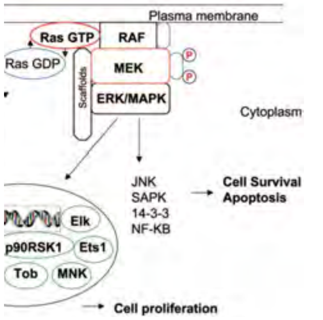


Figure 5

a guanine-rich NGG
ecognized by Cas9
kdown the mutated
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“out of target”
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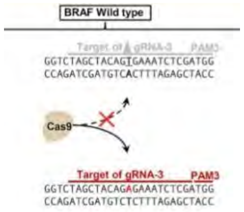

Figure 6

DISCUSSION

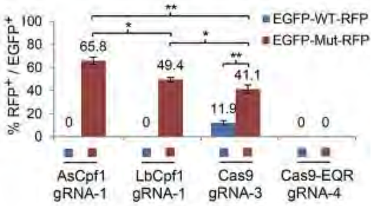

Figure 7

Why liposomes?
Liposomes (Figure 8) make great transporters because they can transport both hydrophilic and hydrophobic substances. Also, they are very unlikely to cause immune reaction.

Which way should liposomes be designed?
CRISPR/Cas9 is hydrophilic and can easily be transported by a liposome. Our idea is to put a modified liposome with CRISPR/Cas9 into an ointment. Also, glycoproteins on the surface should be used as receptors. Hence, liposomes should have specific topical ligands which would bind to cancer cell receptors. An example of overexpressed receptor on melanoma cells is melanocortin receptor-1 (Rosenkranz, 2020).

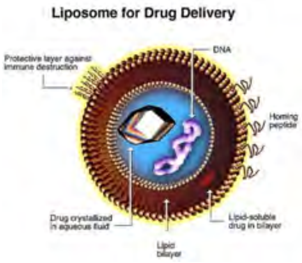

Figure 8

What type of ointment would deliver most efficiently?
An ointment should have consistency that would make application easy. Also, it must be made of non-toxic materials. Due to amphiphilic properties of liposomes, it does not matter whether material is hydrophilic or not.

We could use a hectorite gel which has already been used to deliver CRISPR/Cas9 (Niu et al., 2020). Hectorite is soft, greasy, alergene-free white clay mineral occurring in volcanic ash and tuff. If needed, hectorite would be mixed with oils to achieve ideal viscosity.

What are advantages and disadvantages of liposome CRISPR/Cas9 ointment?
In comparison to other delivery methods, the ointment seems to be the most convenient solution. It provides local delivery of gene modifying tools into the cancer cells which minimizes off-target effects. Furthermore, a patient can apply it at home, which comes in handy during pandemic times.

Properties of liposomes make them look like a promising CRISPR delivery agent. The ointment will not harm the healthy skin, unless patients are allergic to specific ingredients, hence they should be tested before. The synthesis of liposomes is low cost compared to the price of alternative delivery methods such as gold nanoparticles.

On the other hand, liposomes can be absorbed through the skin and can pass through blood vessels. Also, there are very few studies about this way of CRISPR delivery, hence its safety and success rate is questionable. Additionally, many studies on both animals and humans should be done, which might arise ethical dilemmas.

Multiple studies have confirmed the relationship between melanoma development and the V600E mutation. However, it is not present in 100% of melanoma cases, hence a patient should be tested for the mutation prior to the therapy. Furthermore, cancer is generally caused by many genes. Therefore, even if this mutation is corrected, there are a lot of other genes which may cause melanoma growth.

CONCLUSIONS

With a described method, it could be possible to stop melanoma from spreading which is of great because skin cancer is notorious for its metastatic potential. However, it has both good and bad sides. We agreed that advantages of our idea outweigh disadvantages. Finally, we came to a conclusion that this method should be tested on animals first since there are no records of the CRISPR/Cas9 melanoma ointment study found in scientific papers.

Pros

- Application is simple and can be done at home
- There should not be any immunological reactions which would inactivate the drug or cause any harm to the patient’s body
- Liposomes should lead to a high delivery rate and minimize off-target effects
- Liposomes are cheap compared to other delivery options

Cons

- Patients should be tested for the BRAF mutation first (cost approx. 400 USD) before the therapy (Dalal et al., 2018)
- Unexpected off-target responses can still happen
- Application of the ointment might be messy
- A lack of medical supervision may lead to dangerous consequences.

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3B03 Combating disease and human enhancement

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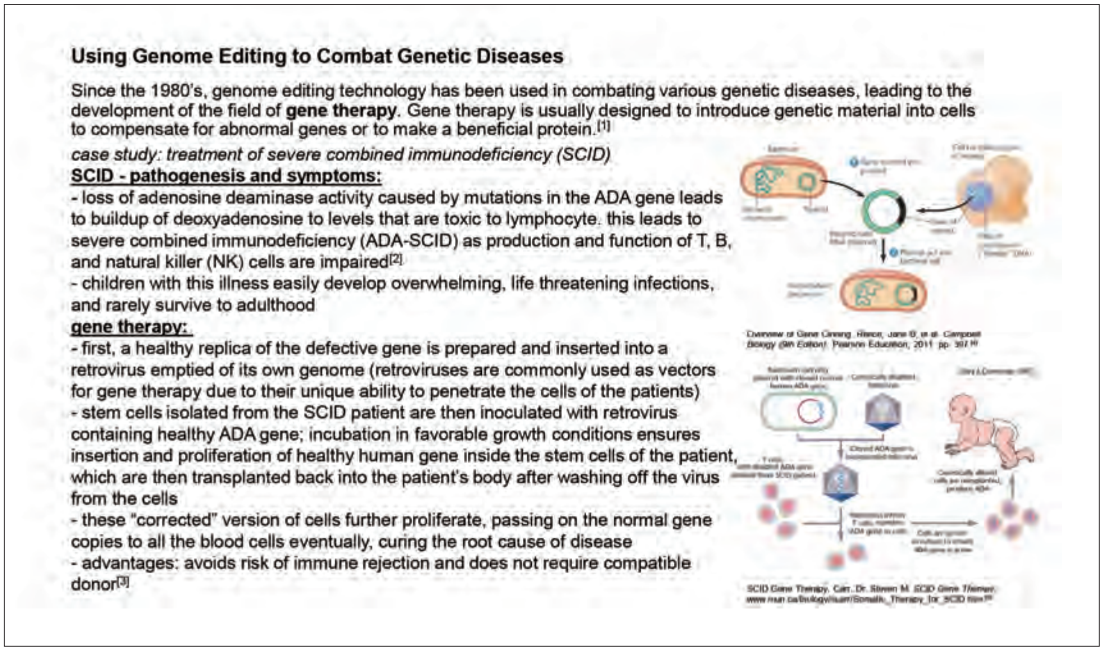
Combating disease and human enhancement

A scientific and socio-ethical approach

IBO Group Project 2020, Group 3B03
Ren Kanehisa
Kate Lu
Martin Rahe
Group Facilitator: Danai Theou



Using Genome Editing to Combat Genetic Diseases

Since the 1980's, genome editing technology has been used in combating various genetic diseases, leading to the development of the field of **gene therapy**. Gene therapy is usually designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein.[1]

case study: treatment of severe combined immunodeficiency (SCID)

SCID - pathogenesis and symptoms:

- loss of adenosine deaminase activity caused by mutations in the ADA gene leads to buildup of deoxyadenosine to levels that are toxic to lymphocyte. this leads to severe combined immunodeficiency (ADA-SCID) as production and function of T, B, and natural killer (NK) cells are impaired[2]
- children with this illness easily develop overwhelming, life threatening infections, and rarely survive to adulthood

gene therapy:

- first, a healthy replica of the defective gene is prepared and inserted into a retrovirus emptied of its own genome (retroviruses are commonly used as vectors for gene therapy due to their unique ability to penetrate the cells of the patients)
- stem cells isolated from the SCID patient are then inoculated with retrovirus containing healthy ADA gene; incubation in favorable growth conditions ensures insertion and proliferation of healthy human gene inside the stem cells of the patient, which are then transplanted back into the patient's body after washing off the virus from the cells
- these "corrected" version of cells further proliferate, passing on the normal gene copies to all the blood cells eventually, curing the root cause of disease
- advantages: avoids risk of immune rejection and does not require compatible donor[3]

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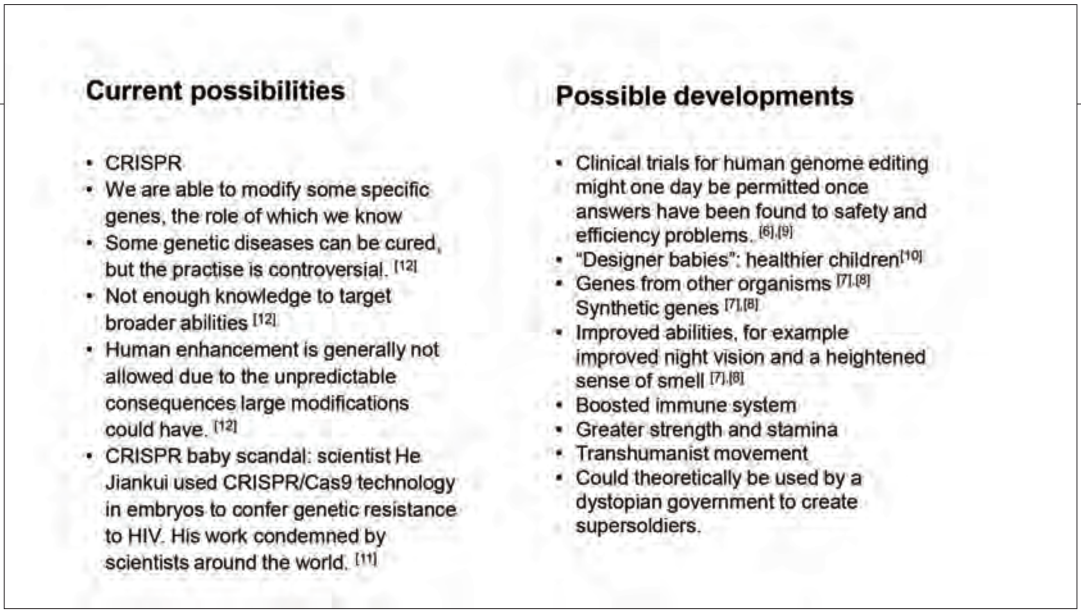
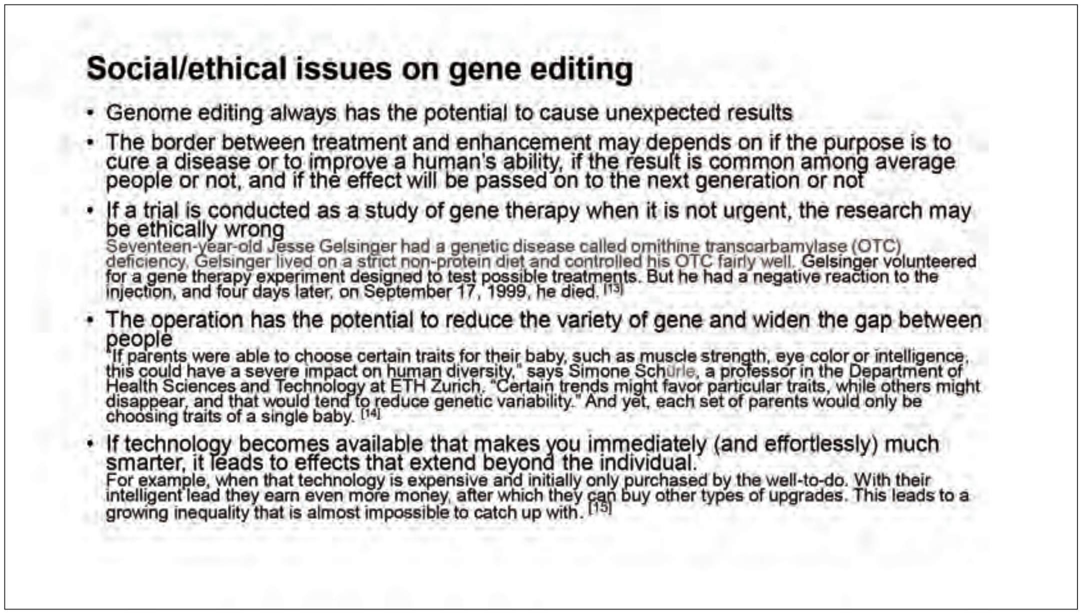


Current possibilities

- CRISPR
- We are able to modify some specific genes, the role of which we know
- Some genetic diseases can be cured, but the practise is controversial. [12]
- Not enough knowledge to target broader abilities [12]
- Human enhancement is generally not allowed due to the unpredictable consequences large modifications could have. [12]
- CRISPR baby scandal: scientist He Jiankui used CRISPR/Cas9 technology in embryos to confer genetic resistance to HIV. His work condemned by scientists around the world. [11]

Possible developments

- Clinical trials for human genome editing might one day be permitted once answers have been found to safety and efficiency problems. [6],[9]
- "Designer babies": healthier children[10]
- Genes from other organisms [7],[8] Synthetic genes [7],[8]
- Improved abilities, for example improved night vision and a heightened sense of smell [7],[8]
- Boosted immune system
- Greater strength and stamina
- Transhumanist movement
- Could theoretically be used by a dystopian government to create supersoldiers.



Social/ethical issues on gene editing

- Genome editing always has the potential to cause unexpected results
- The border between treatment and enhancement may depends on if the purpose is to cure a disease or to improve a human's ability, if the result is common among average people or not, and if the effect will be passed on to the next generation or not
- If a trial is conducted as a study of gene therapy when it is not urgent, the research may be ethically wrong
  Seventeen-year-old Jesse Gelsinger had a genetic disease called ornithine transcarbamylase (OTC) deficiency. Gelsinger lived on a strict non-protein diet and controlled his OTC fairly well. Gelsinger volunteered for a gene therapy experiment designed to test possible treatments. But he had a negative reaction to the injection, and four days later, on September 17, 1999, he died. [13]
- The operation has the potential to reduce the variety of gene and widen the gap between people
  "If parents were able to choose certain traits for their baby, such as muscle strength, eye color or intelligence, this could have a severe impact on human diversity," says Simone Schürle, a professor in the Department of Health Sciences and Technology at ETH Zurich. "Certain trends might favor particular traits, while others might disappear, and that would tend to reduce genetic variability." And yet, each set of parents would only be choosing traits of a single baby. [14]
- If technology becomes available that makes you immediately (and effortlessly) much smarter, it leads to effects that extend beyond the individual.
  For example, when that technology is expensive and initially only purchased by the well-to-do. With their intelligent lead they earn even more money, after which they can buy other types of upgrades. This leads to a growing inequality that is almost impossible to catch up with. [15]

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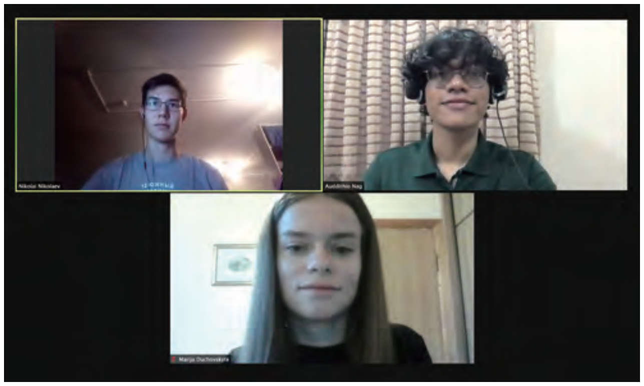
3B04 Proposing a XIST-based in vivo treatment for trisomy-related disorders



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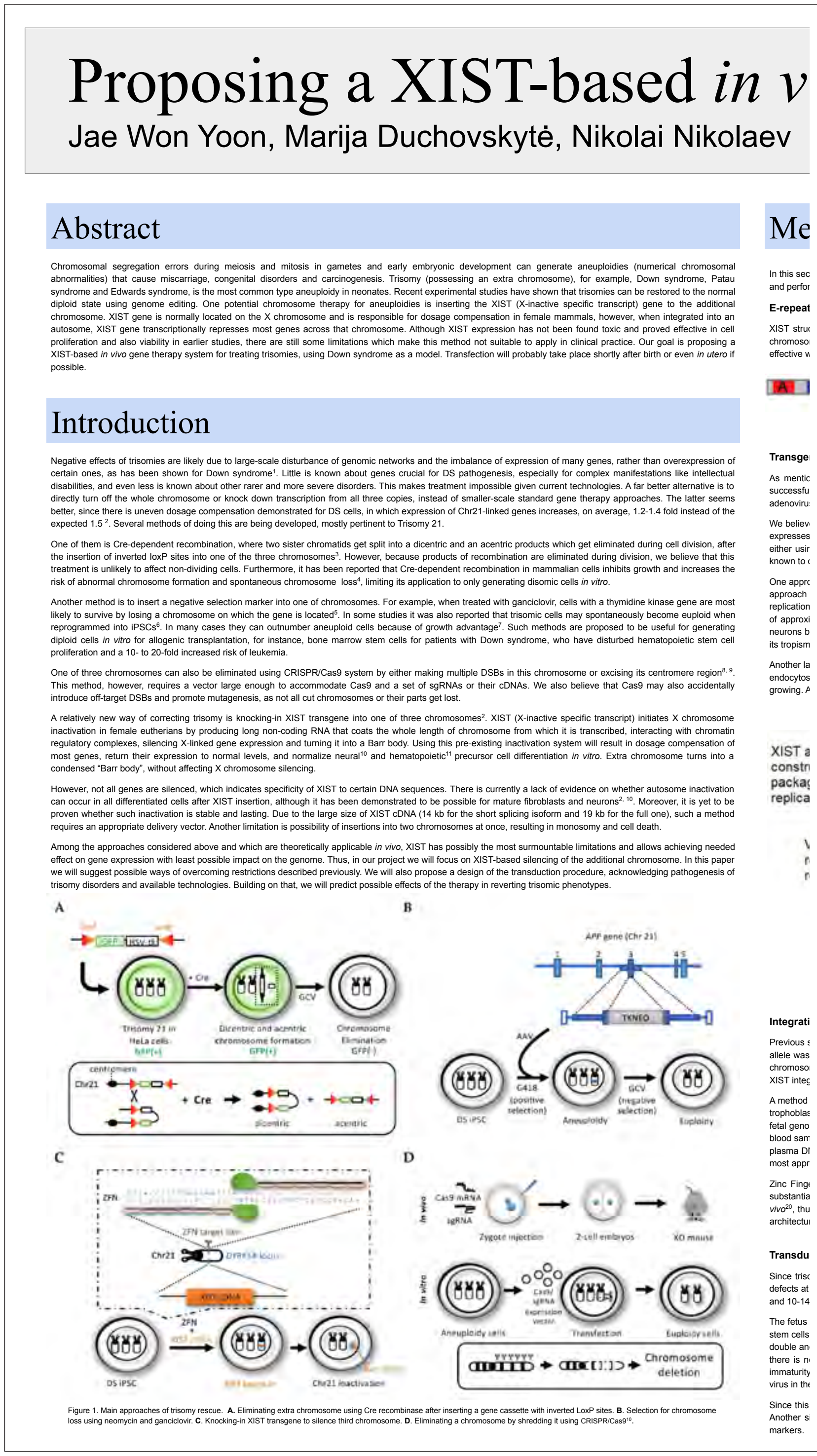

Proposing a XIST-based *in v*

Jae Won Yoon, Marija Duchovskytė, Nikolai Nikolaev

Abstract

Chromosomal segregation errors during meiosis and mitosis in gametes and early embryonic development can generate aneuploidies (numerical chromosomal abnormalities) that cause miscarriage, congenital disorders and carcinogenesis. Trisomy (possessing an extra chromosome), for example, Down syndrome, Patau syndrome and Edwards syndrome, is the most common type aneuploidy in neonates. Recent experimental studies have shown that trisomies can be restored to the normal diploid state using genome editing. One potential chromosome therapy for aneuploidies is inserting the XIST (X-inactive specific transcript) gene to the additional chromosome. XIST gene is normally located on the X chromosome and is responsible for dosage compensation in female mammals, however, when integrated into an autosome, XIST gene transcriptionally represses most genes across that chromosome. Although XIST expression has not been found toxic and proved effective in cell proliferation and also viability in earlier studies, there are still some limitations which make this method not suitable to apply in clinical practice. Our goal is proposing a XIST-based *in vivo* gene therapy system for treating trisomies, using Down syndrome as a model. Transfection will probably take place shortly after birth or even *in utero* if possible.

Introduction

Negative effects of trisomies are likely due to large-scale disturbance of genomic networks and the imbalance of expression of many genes, rather than overexpression of certain ones, as has been shown for Down syndrome[1]. Little is known about genes crucial for DS pathogenesis, especially for complex manifestations like intellectual disabilities, and even less is known about other rarer and more severe disorders. This makes treatment impossible given current technologies. A far better alternative is to directly turn off the whole chromosome or knock down transcription from all three copies, instead of smaller-scale standard gene therapy approaches. The latter seems better, since there is uneven dosage compensation demonstrated for DS cells, in which expression of Chr21-linked genes increases, on average, 1.2-1.4 fold instead of the expected 1.5 [2]. Several methods of doing this are being developed, mostly pertinent to Trisomy 21.

One of them is Cre-dependent recombination, where two sister chromatids get split into a dicentric and an acentric products which get eliminated during cell division, after the insertion of inverted loxP sites into one of the three chromosomes[3]. However, because products of recombination are eliminated during division, we believe that this treatment is unlikely to affect non-dividing cells. Furthermore, it has been reported that Cre-dependent recombination in mammalian cells inhibits growth and increases the risk of abnormal chromosome formation and spontaneous chromosome loss[4], limiting its application to only generating disomic cells *in vitro*.

Another method is to insert a negative selection marker into one of chromosomes. For example, when treated with ganciclovir, cells with a thymidine kinase gene are most likely to survive by losing a chromosome on which the gene is located[5]. In some studies it was also reported that trisomic cells may spontaneously become euploid when reprogrammed into iPSCs[6]. In many cases they can outnumber aneuploid cells because of growth advantage[7]. Such methods are proposed to be useful for generating diploid cells *in vitro* for allogenic transplantation, for instance, bone marrow stem cells for patients with Down syndrome, who have disturbed hematopoietic stem cell proliferation and a 10- to 20-fold increased risk of leukemia.

One of three chromosomes can also be eliminated using CRISPR/Cas9 system by either making multiple DSBs in this chromosome or excising its centromere region[8, 9]. This method, however, requires a vector large enough to accommodate Cas9 and a set of sgRNAs or their cDNAs. We also believe that Cas9 may also accidentally introduce off-target DSBs and promote mutagenesis, as not all cut chromosomes or their parts get lost.

A relatively new way of correcting trisomy is knocking-in XIST transgene into one of three chromosomes[2]. XIST (X-inactive specific transcript) initiates X chromosome inactivation in female eutherians by producing long non-coding RNA that coats the whole length of chromosome from which it is transcribed, interacting with chromatin regulatory complexes, silencing X-linked gene expression and turning it into a Barr body. Using this pre-existing inactivation system will result in dosage compensation of most genes, return their expression to normal levels, and normalize neural[10] and hematopoietic[11] precursor cell differentiation *in vitro*. Extra chromosome turns into a condensed "Barr body", without affecting X chromosome silencing.

However, not all genes are silenced, which indicates specificity of XIST to certain DNA sequences. There is currently a lack of evidence on whether autosome inactivation can occur in all differentiated cells after XIST insertion, although it has been demonstrated to be possible for mature fibroblasts and neurons[2, 10]. Moreover, it is yet to be proven whether such inactivation is stable and lasting. Due to the large size of XIST cDNA (14 kb for the short splicing isoform and 19 kb for the full one), such a method requires an appropriate delivery vector. Another limitation is possibility of insertions into two chromosomes at once, resulting in monosomy and cell death.

Among the approaches considered above and which are theoretically applicable *in vivo*, XIST has possibly the most surmountable limitations and allows achieving needed effect on gene expression with least possible impact on the genome. Thus, in our project we will focus on XIST-based silencing of the additional chromosome. In this paper we will suggest possible ways of overcoming restrictions described previously. We will also propose a design of the transduction procedure, acknowledging pathogenesis of trisomy disorders and available technologies. Building on that, we will predict possible effects of the therapy in reverting trisomic phenotypes.

Figure 1. Main approaches of trisomy rescue. **A.** Eliminating extra chromosome using Cre recombinase after inserting a gene cassette with inverted LoxP sites. **B**. Selection for chromosome loss using neomycin and ganciclovir. **C**. Knocking-in XIST transgene to silence third chromosome. **D**. Eliminating a chromosome by shredding it using CRISPR/Cas9[10].

n vivo treatment for trisomy-related disorders

laev

Methods

In this section we will discuss possible ways to preprocess XIST, deliver it to cells *in vivo*, insert it into a chromosome effectively, make sure there are no multiple insertions and perform gene therapy.

E-repeat and escaping silencing

XIST structure and interactions have been extensively studied during last years. It was recently discovered that the E-repeat in its last exon is insufficient for overall chromosome silencing but is necessary for maintaining proper level of expression of genes that escape repression[7]. It is possible that removing it will make silencing more effective without negative side effects.

Figure 2. Human mature 19-kb XIST RNA consisting of 6 exons; conserved repeats A-F involved in protein recruitment and chromosome covering.

Transgene delivery

As mentioned earlier, XIST has several splicing isoforms including 19 kb-long and 14 kb - short lncRNA. The latter is proved to be capable of silencing, as it was successfully used for the inactivation of Chr21[2]. However, its size with homology shoulders and nuclease still makes delivery with traditional gene therapy vectors such as adenovirus, AAV or lentivirus impossible.

We believe Herpes simplex virus (HSV) can be used for this purpose. HSV allows delivery of 30 to 100 kb of dsDNA into the nucleus. However, during its lytic cycle, HSV expresses 3 classes of genes: immediate early (IE), early and late. Producing any of IE genes is enough for a vector to be cytotoxic. This obstacle can be overcome by either using the latent state of the virus to express transgenes or depriving the vector backbone of all IE genes. Since the latent state of HSV is not well studied and is known to occur only in neurons, we believe the latter is more feasible.

One approach to cancel viral gene transcription would be by introducing conditional mutations, thus making viral transcription impossible in a transfected organism. Another approach is to transfer those genes into a complementing cell line. The end point of such transfer is an amplicon vector—a plasmid containing transgenes, viral origin of replication and packaging signals, amplified in a cell line containing viral genome that is made unable to be packaged in a capsid. This allows an exceedingly large capacity of approximately 100 kb[13]. Such an approach is also convenient for engineering surface glycoproteins and proteins of a vector: WT HSV infects epithelium cells and neurons by first binding to heparin sulfate on the cell surface and then to HVEM (a member of TNF receptor family), nectin 1, nectin 2 or 3-O-sulfated heparin sulphate, so its tropism has to be broadened.

Another large dsDNA virus used in gene therapy is Baculovirus. It infects Hymenoptera and is initially unable to replicate in mammalian cells. Baculovirus enters the cell via endocytosis, phagocytosis or macropinocytosis, binding with lipid rafts[14]. Though not as well documented, the list of cell types known to be susceptible to infection is growing. Although transduction of cells of hematopoietic origin is inefficient, it can be boosted by pseudotyping and integrating cell-penetrating peptides into its surface.

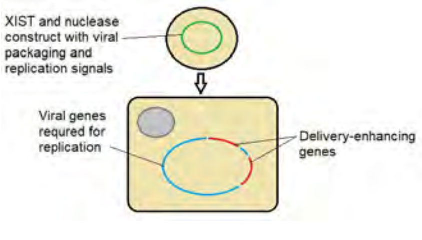

Figure 3. Approximate scheme of vector generation system

The main limitation of using such vector is cellular antiviral response caused by lots of unmethylated CpGs in a large viral DNA molecule, which also often appears in cytosol (unlike HSV genome which is protected by a capsid). Moreover, there were reports that the virus may alter transcription in the host cell. Those problems can be solved in a fashion similar to one described earlier: by creating a complementing cell line with viral genes that cannot be encapsulated. For example, infecting a cell with baculovirus and then excising its replication and packaging signals—this will make recombination with a transgene-carrying plasmid impossible as well. A big insertion may also make it unable to fit in the capsid. After that, transfecting the cell line with a desired construct will provide us with a safe transfection vector.

For these reasons, we believe amplicon-like technology is the most flexible, universal and safe approach for getting a vector for delivery of XIST construct. The downside, however, is low viral particle yield. Because baculovirus doesn't infect humans, it is more preferable (see *Transduction procedure*).

Integration into the genome

Previous studies have shown that XIST transgene insertion into the DYRK1A locus in two and three alleles occurred effectively, while the transgene insertion into only one allele was rare[1]. Furthermore, silencing one of three chromosomes can potentially reveal recessive or imprinting disorders, particularly when both remaining homologous chromosomes are inherited from the same parent (in case of uniparental disomy)[15]. These problems could be solved by chromosome sequencing, finding unique sites for XIST integration and designing target specific Zinc Finger Nucleases (ZFNs).

A method we deem suitable for choosing proper site for XIST insertion could be targeted haplotyping. It relies on cell-free fetal DNA (cffDNA) that originates from placental trophoblasts and can be detected in maternal blood. Although the size of cfDNA is only 150-300 bp, the entire fetal genome is still represented and cffDNA can be used for fetal genome sequencing, reducing the risk of fetal loss and maternal morbidity associated with invasive prenatal testing[16]. This non-invasive diagnosis technique requires blood samples from both parents in order to targeted locus amplifying of selected sequence. Targeted sequencing of cffDNA from maternal plasma, comparing the maternal plasma DNA sequencing data with the parental genomic DNA data and using a series of bioinformatics filters enable predicting fetal gene inheritance[17, 18]. In this way, the most appropriate chromosome for XIST knock-in could be picked and multiple insertion or uniparental disomy could be avoided.

Zinc Finger Nucleases (ZFNs) can be designed to target desired sequences up 36 bp and it is highly specific because a sequence of this size will usually display substantial divergence from even the most alike genomic site[19]. Moreover, ZFNs have the ability to carry large genes such as XIST[2] and can be used for gene insertion *in vivo*[20], thus, it is suitable for gene therapy. Although off-target cleavages of ZFNs sometimes occur, precision of these nucleases can be improved by alterations of ZFNs architecture[19].

Transduction procedure

Since trisomy harms the individual most during prenatal development, the biggest effect would be achieved by in utero transduction. Not only will it limit development of defects at an early stage, but it will also greatly increase efficacy of transfection[21]. Trisomies usually can be detected during 9-10 weeks of gestation using cffDNA analysis and 10-14 weeks using chorionic villus sampling, so injection of vector in the umbilical vein can take place in the beginning of the second trimester.

The fetus has a significantly lower cell count, so effective transduction will be able to be achieved with low concentrations of viral particles. Moreover, many of its cells are stem cells, which are highly susceptible to transfection and integration of transgenes into their genome due to high division rate (unfortunately, this also increases the risk of double and triple insertions, so careful choice of integration site is required). These cells are also proved to be able to silence chromosomes with newly added XIST genes; there is no lack of such data unlike for differentiated cells (see *Discussion*). The immaturity of the blood-brain barrier will make delivery to brain more effective, while immaturity of the immune system will lower the chances of inflammation and strong immune response against the virus. However, the fetus may acquire tolerance for this virus in the future, so we believe it is better to use a virus that never infects humans, i.e. Baculovirus.

Since this procedure is invasive, there is a risk of pregnancy loss or infection[22], but it is quite low as injection in umbilical vein are ultrasound-guided, routine procedures.. Another significant risk is possibly transfecting mother's cells. We think this may probably be excluded in case of targeting the vector to stem cells or cellular trisomic markers.

Discussion

Possible applications and benefits

While new methods and technologies are constantly being proposed, developed and used for curing genetic disorders caused by single-gene mutations or defects, a class of trisomy-related diseases still does not respond to any treatment, despite affecting about 0.3% of live births and accounting for 35% of spontaneous abortions[23]. A logical step of solving this problem is bringing gene therapy to higher, chromosomal scale, which we believe will be done in the recent future.

In this project we have proposed a system of Baculovirus-based delivery and ZFN-based integration of XIST into the genome of the fetus in the beginning of the second trimester of gestation via injection into the umbilical vein. We believe it can be capable of reducing or even eliminating most disorders caused by non-mosaic presence of a full third autosome. More specifically, in the case of Down Syndrome, based on existing observations we can predict that normalizing expression of (a) CAF1A, CBS and GART may improve DNA synthesis and repair and thus general growth; (b) DYRK1A will improve neural cells differentiation and mental development; (c) ETS2 will improve hematopoiesis (remove increased risk of leukemia); (d) CRYA1 will reduce risk of cataracts; and (e) IFNAR and SOD1 will improve immune system functioning[24]. However, preventing heart defects and thymus underdevelopment is unlikely as they mostly develop during the first trimester.

Other human full trisomies are highly lethal during gestation and first years of life and thus much less studied. Proposed therapy may increase viability in this case, but it is hard to evaluate the effect.

What also limits possibility of such evaluations is small amount of data describing development of pathologic phenotypes, especially during intrauterine development. From this perspective, a system of delivery and integration of XIST into cells *in vivo* is a highly valuable tool. Inducibly silencing the third chromosome during different stages of development, for example, in primate models, can provide researchers with a lot of information and shed a new light on pathogenesis of aneuploidies.

Remaining restrictions and new challenges

Suggested XIST-based gene therapy for treating trisomies *in vivo* overcomes several current limitations, however, this method still requires some refinements.

First, XIST integration is only suitable for correcting full trisomies and it is unable to cure partial trisomies. Partial trisomy occurs when an additional chromosome fragment is inserted into the other chromosome[24], therefore, knocking-in XIST would result in monosomy which is fatal. This problem could be solved after more closely studying XIST interactions and sequences specific for genes that do not need to be silenced, thus, further research is required.

Moreover, because the amount of cffDNA depends on the gestation period, the progression of the pregnancy, presence of maternal diseases, body weight and other factors, extracted amount of cffDNA can be not sufficient for sequencing and invasive methods like chorionic villus sampling or amniocentesis may still be needed[17].

In addition, if *in utero* transduction is applied, there is a slight chance that adverse effects on both mother and fetus may occur. It may possibly result in a miscarriage or an infection, transfection of mother's cells and higher frequency of off-target and double insertions because of rapid fetus cell division[22].

According to previous research[10], it was thought that XIST-based gene editing or chromosome silencing was impossible in somatic cells, with the exception of some limited effects in certain cancer cells or a subset of mouse hematopoietic cells[11], until it was found that human neural cells retain “chromosome plasticity” to induce formation of heterochromatin. No direct results about using XIST in other cell types exist, hence, tests of XIST-induced chromosome silencing in various cell types should be performed in the future.

Recent studies show that few genes remain active even after XIST inactivation of additional chromosomes. Moreover, there is a chance that the extra chromosome in the nucleus may disrupt nuclear organization and specific chromatin contact points and impair gene expression regulation[15]. More analysis needs to be conducted in order to determine which genes escape silencing and what are phenotypic effects of nuclear disorganization.

Much additional research is needed in order to further understand and improve XIST knocking-in for correcting trisomy and overcoming above mentioned limitations.

Conclusion

Here we have suggested what can be done to use X-inactivation system for trisomy therapy. Our proposed system is based on sequencing chromosomes of the fetus, choosing an insertion site, injecting baculoviral vector containing XIST, homology shoulders and ZFN gene into the umbilical vein for directed insertion into one of three chromosomes. Although *in utero* transduction comes with some dangers, when developed in the future it will provide valuable benefits and new possibilities, which are very much needed for trisomy therapy. We believe that it can significantly reduce the effect of trisomy on individual's health, weakening manifestations of Down Syndrome and possibly even making other trisomies viable. More research is needed, however, to increase its safety and better understand XIST functioning for further modifications.

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3B07

Nutrient Biofortification of Crops through Genetic Engineering : A Comprehensive Case Study

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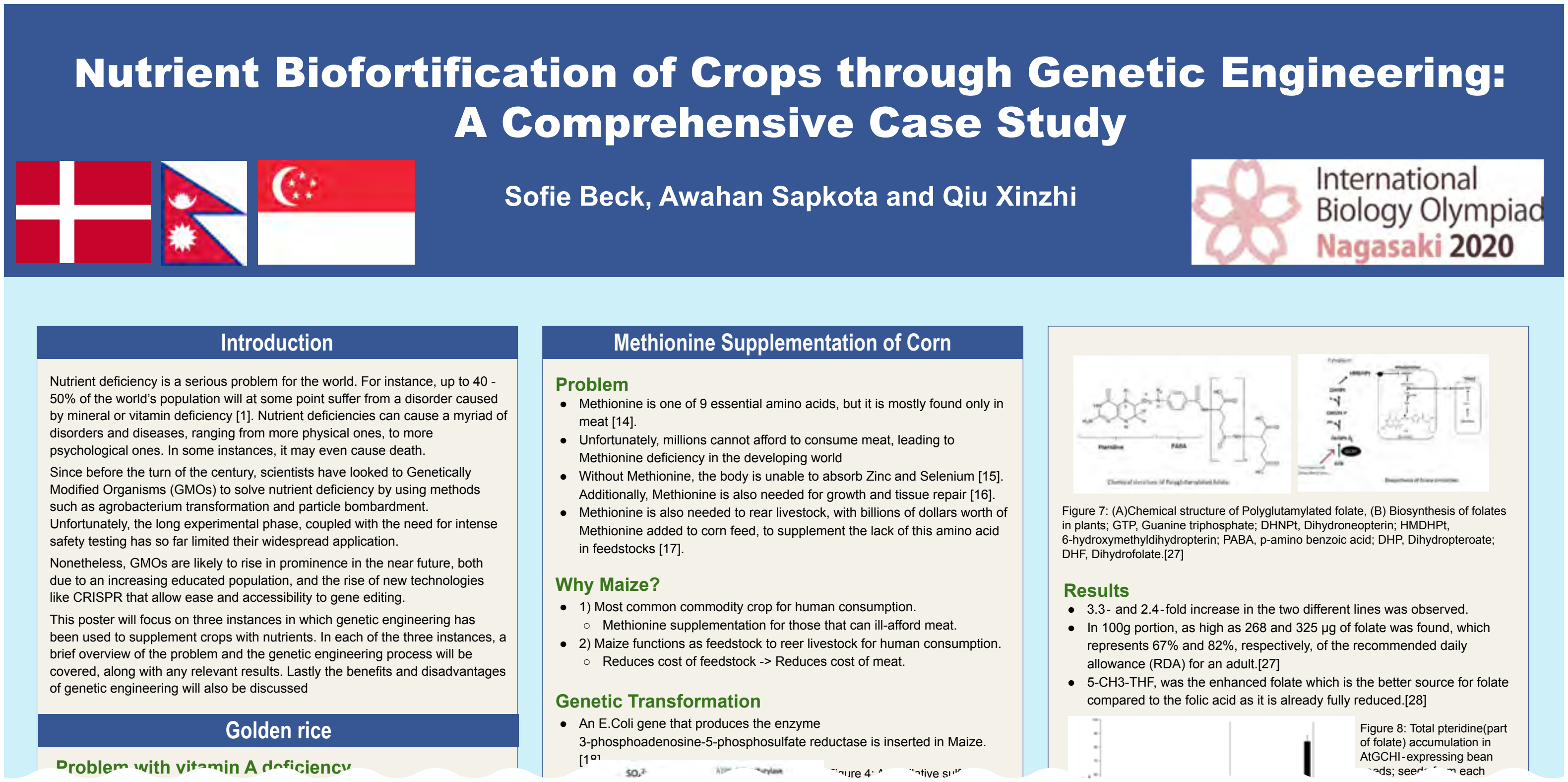

Nutrient Biofortification of Crops through Genetic Engineering: A Comprehensive Case Study

Sofie Beck, Awahan Sapkota and Qiu Xinzhi

Introduction

Nutrient deficiency is a serious problem for the world. For instance, up to 40 - 50% of the world's population will at some point suffer from a disorder caused by mineral or vitamin deficiency [1]. Nutrient deficiencies can cause a myriad of disorders and diseases, ranging from more physical ones, to more psychological ones. In some instances, it may even cause death.

Since before the turn of the century, scientists have looked to Genetically Modified Organisms (GMOs) to solve nutrient deficiency by using methods such as agrobacterium transformation and particle bombardment. Unfortunately, the long experimental phase, coupled with the need for intense safety testing has so far limited their widespread application.

Nonetheless, GMOs are likely to rise in prominence in the near future, both due to an increasing educated population, and the rise of new technologies like CRISPR that allow ease and accessibility to gene editing.

This poster will focus on three instances in which genetic engineering has been used to supplement crops with nutrients. In each of the three instances, a brief overview of the problem and the genetic engineering process will be covered, along with any relevant results. Lastly the benefits and disadvantages of genetic engineering will also be discussed

Golden rice

Problem with vitamin A deficiency

Methionine Supplementation of Corn

Problem

- Methionine is one of 9 essential amino acids, but it is mostly found only in meat [14].
- Unfortunately, millions cannot afford to consume meat, leading to Methionine deficiency in the developing world
- Without Methionine, the body is unable to absorb Zinc and Selenium [15]. Additionally, Methionine is also needed for growth and tissue repair [16].
- Methionine is also needed to rear livestock, with billions of dollars worth of Methionine added to corn feed, to supplement the lack of this amino acid in feedstocks [17].

Why Maize?

- 1) Most common commodity crop for human consumption.
  - Methionine supplementation for those that can ill-afford meat.
- 2) Maize functions as feedstock to reer livestock for human consumption.
  - Reduces cost of feedstock -> Reduces cost of meat.

Genetic Transformation

- An E.Coli gene that produces the enzyme 3-phosphoadenosine-5-phosphosulfate reductase is inserted in Maize. [18]

Figure 7: (A)Chemical structure of Polyglutamylated folate, (B) Biosynthesis of folates in plants; GTP, Guanine triphosphate; DHNPt, Dihydroneopterin; HMDHPt, 6-hydroxymethyldihydropterin; PABA, p-amino benzoic acid; DHP, Dihydropteroate; DHF, Dihydrofolate.[27]

Results

- 3.3- and 2.4-fold increase in the two different lines was observed.
- In 100g portion, as high as 268 and 325 μg of folate was found, which represents 67% and 82%, respectively, of the recommended daily allowance (RDA) for an adult.[27]
- 5-CH3-THF, was the enhanced folate which is the better source for folate compared to the folic acid as it is already fully reduced.[28]

Figure 8: Total pteridine(part of folate) accumulation in AtGCHI-expressing bean

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This poster will focus on three instances in which genetic engineering has been used to supplement crops with nutrients. In each of the three instances, a brief overview of the problem and the genetic engineering process will be covered, along with any relevant results. Lastly the benefits and disadvantages of genetic engineering will also be discussed

Golden rice

Problem with vitamin A deficiency

- Vitamin A is found in animal products and fish. It's also derived from Carotenoids found in plants.[5,6]
- Vitamin A is needed for a functioning Immune system, normal vision and reproduction.[2] Vitamin A deficiency (VAD) increases the risk of childhood infections, is the main cause of preventable blindness in children, and leads to night blindness and a higher risk of maternal mortality among pregnant women.[3]
- VAD is highest in Africa and southeast asia.[3] As many as 230 million children are at risk of clinical or subclinical VAD worldwide.[4]

Why Rice?

- Vitamin A deficiency is highest in countries where rice is the major food source[4]. A high rice consumption is found amongst poor people in south east asia. Some of their rural populations have a diet that contains up to 80% rice.[7]

Genetic transformation

- The golden rice project started more than 20 years ago. One of the first steps was to prove that the plant endosperm had the multistep carotenoid pathway needed (figure 1). [4]
- It turned out that the production of carotenoids in the endosperm is halted by the absence of certain enzymes along the pathway (see figure 2). Their job was then to fill a biosynthetic gap. [7]
- They found that the two transgenes required to make Golden rice are 1: A plant gene for phytoene synthase (PSY) which uses the plants GGPP to form phytoene, and 2: A bacterial gene that codes for carotene desaturase (CRTI) negating the need for multiple steps in the pathway. together PSY and CRTI make lycopene from the plants own GGPP. (figure 2) [7]
- The pathway beyond lycopene was found active in the wild type rice endosperm, based on the fact that, after insertion of only those two genes, α- & β-carotenes and xanthophylls where found in the GM plants. [7]

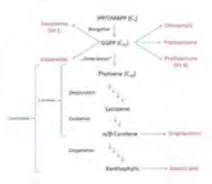


Figure 1: The carotenoid pathway. IPP: Isopentenyl-diphosphate, DMAPP: dimethyl-diphosphate, GGPP: Geranylgeranyl-diphosphate [7].

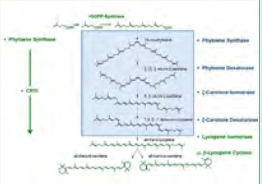


Figure 2: The inserted gene (left) and the pathway In WT rice grain (right) the enzymes in green are functional, and products and enzymes in blue, are effectively absent [7].

- In GR2, the second and final version, a PSY gene from maize is used. It wields the highest outcome of previtamin A: 37 µg/g carotenoids (where 31 µg/g was β-carotenes) In GR1 the outcome was only 1,6 µg/g. The CRTL gene is from the bacterium Pantoea ananatis.[7]
- The genes were inserted with agrobacterium, which were added to a petri dish with rice embryos to infect, successfully transferring the genes. they where then crossbred with locally used rice sorts.[8]

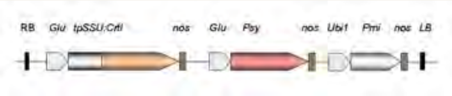


Figure 3: The gene sequence that was inserted to make GR2 [7].

Results

- 100 - 150 g of GR2 (60g dry weight) can provide 60% of the recommended intake of vitamin A.[9]
- GR2 plants give as much food as a WT plant.[8] The only meaningful biological difference is the level of beta-carotenes and other provitamin A carotenoids in the grain.[9]
- Golden rice is waiting to be approved by more countries before it's globally available. The first Asian country to approve was the Philippines.[10] The US FDA approved it in may, 2018, Canada in March 2018 and Australia and new Zealand in February 2018.[11]
- Golden rice is in the process of being released for use in Bangladesh, as the first country. but there is a hold up because of opposition to GM food. [12,13] A study was done in 2019 to further test the safety of golden rice, in an effort to appease them.[13]

- 1) Most common commodity crop for human consumption.
    - Methionine supplementation for those that can ill-afford meat.
- 2) Maize functions as feedstock to reer livestock for human consumption.
    - Reduces cost of feedstock -> Reduces cost of meat.

Genetic Transformation

- An E.Coli gene that produces the enzyme 3-phosphoadenosine-5-phosphosulfate reductase is inserted in Maize. [1

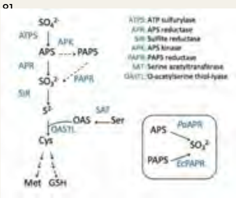


Figure 4: Assimilative sulfate reduction in plants. Sulfate is reduced to sulfite either by APS reductase (APR) via the primary (solid arrows) or an alternative secondary sulfate assimilation pathway (dashed arrows) with PAPS reductase (PAPR) [18].

- Done using agrobacterium to infect immature maize embryos.
- A few transgenic lines were produced and inbred to produce lines with the highest 10-kDa δ-zein levels (indicative of Met).

Results

- Key Result 1: 57% Increase in Met in Corn Kernels.
- Key Result 2: Feeding trial with the transgenic high-Met PE5 maize shows that the transgenic feed promotes significant weight gain compared with nontransgenic kernels.

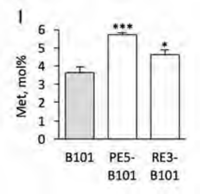
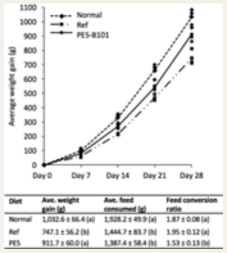


Figure 5: Met contents of transgenic plants PE5-B101 and RE3-B101 and non-transgenic plant B101 in mol% determined after protein hydrolysis and separation in a UPLC column.[18]



Diet	Ave. weight gain (g)	Ave. feed consumed (g)	Feed conversion ratio
Normal	1,032.6 ± 66.4 (a)	1,928.2 ± 49.9 (a)	1.87 ± 0.08 (a)
Ref	747.1 ± 56.2 (b)	1,444.7 ± 83.7 (b)	1.95 ± 0.12 (a)
PE5	911.7 ± 60.0 (a)	1,387.4 ± 58.4 (b)	1.53 ± 0.13 (b)

Figure 6: Feeding trial on chicks carried out with three diets consisting of corn supplemented with methionine (normal), of transgenic PE5-B101, and of the null transgenic segregant from PE5-B101. [18]

Folate Supplementation of Bean

Problem

- Folate plays an important role in the replication of DNA and normal cell formation and growth, but is not synthesized in human body and found only in plants and bacteria.[19]
- Folate malnutrition is a worldwide problem associated with the onset of megaloblastic anaemia[20], neural tube defects[21],an increased risk of cardiovascular disease, certain cancers[22] and neuropsychiatric disorders.[23]
- Annually 260,100 NTD-affected pregnancies occurring worldwide resulting in early death and lifelong disability.[24]
- NTD affected pregnancies can be prevented if women consumes enough folic acid prior to or during early pregnancy.[19]

Why Bean?

- The common bean is the most consumed legume in the world.
- It is cultivated as a subsistence crop by rural populations in which folate fortification efforts are difficult to implement.
- It already contains significant amounts of folate which makes the modifications more easier.
- It is already the subject of biofortification efforts with iron and zinc.[25]

Genetic Transformation

- Common bean is transformed by the particle bombardment, only method that has produced stable transgenic lines in this species.[26]
- The embryonic axes were bombarded with the pAHAS-AtGCHI vector.
- The vector contains the genes *Arabidopsis thaliana* GTP cyclohydrolase I (AtGCHI) and Arabidopsis thaliana acetohydroxy acid synthase(AHAS).
- The AtGCHI gene increases the folate synthesis by overexpressing one of the biosynthetic routes of folate.
- The AHAS gene was kept to confer resistance to imidazoline herbicides.
- Tungsten particles coated with 8 µg of pAHAS-AtGCHI linearized with KpnI was bombarded utilizing a high-pressure Helium microparticle acceleration system.
- The transformed embryo was placed in elongation medium 1 and then in elongation medium 2 and later it was acclimated [27]

- 3.3- and 2.4-fold increase in the two different lines was observed.
- In 100g portion, as high as 268 and 325 µg of folate was found, which represents 67% and 82%, respectively, of the recommended daily allowance (RDA) for an adult.[27]
- 5-CH3-THF, was the enhanced folate which is the better source for folate compared to the folic acid as it is already fully reduced.[28]

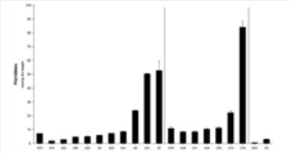


Figure 8: Total pteridine(part of folate) accumulation in AtGCHI-expressing bean seeds; seeds from each primary transgenic line are compared to Wild type(WT) seeds. 3 different lines-S(Saltillo), C(Cafe) and D(Duranga) were tested; error bars indicate standard error (SE).[27]

Discussion on Nutrient Supplementation via Genetic Engineering

Benefits

- Alleviates nutrient deficiency, especially for those in the developing world.
- Food produced with higher nutritional content.
- Lower cost incurred from health issues resulting from nutrient-deficiency
- Higher manpower productivity.

Disadvantages & opposition

- The publics fear of GMO, especially in the developing world
- Unknown long-term impacts to health.
- The ultimate cost of GM product may be more expensive than the alternate nutrient supplements.
- Potential for economic monopoly by a small number of GM companies.
- The dosage for a healthy amount, need to be figured out or problems associated with overdosage of nutrients may arise.
- The problems with transgene escaping and Hybridization with wild weeds.

Our opinion

- We agree with the notion of implementing GM foods that are deemed fit for consumption, as it will not negate or affect the choice of eating non-GM varieties by those that are more skeptical of GM.
- We believe the knowledge of GM will need to be more widespread among the public, for more to realise the benefits of safe and approved GMO food.

Conclusion

In this poster, we investigated how genetic engineering can be used for nutrient supplementation of crops. In particular, we looked at how the genetic modification of three different types of crops: Rice, Maize and the Common Bean, can tackle the deficiency of Vitamin A, Methionine and Folate, respectively. Here, the three crops were specifically chosen due to the prevalence of their consumption around the world.

In this work, besides studying the motivation behind the genetic engineering of these crops, we also looked at how the different GMOs were modified and the results behind such modification.

Our studies on Golden Rice, on Methionine supplementation in Maize and on the Folate Biofortification of Common Bean are reflections of current research, serving as glimpses into a future where GMOs can be successfully incorporated to solve nutrient deficiency, prevent medical complications, and save lives.

Although we hold much promise for such a future, there are some potential bioethical, and educational drawbacks that needs to be considered in kind. Whether it's the potential of transgenic escape, or possible long term effects on health, it is important for us to truly understand the risks behind each geneticaly engineered plant. Only then can we appropriately weight their individual benefits and disadvantages. And hopefully then can we implement the safest technologies, as literally millions of lifes, both human and wild, will depend on our decisions, whatever they may be.

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3B08

CRISPR-CAS9 IN CROP IMPROVEMENT

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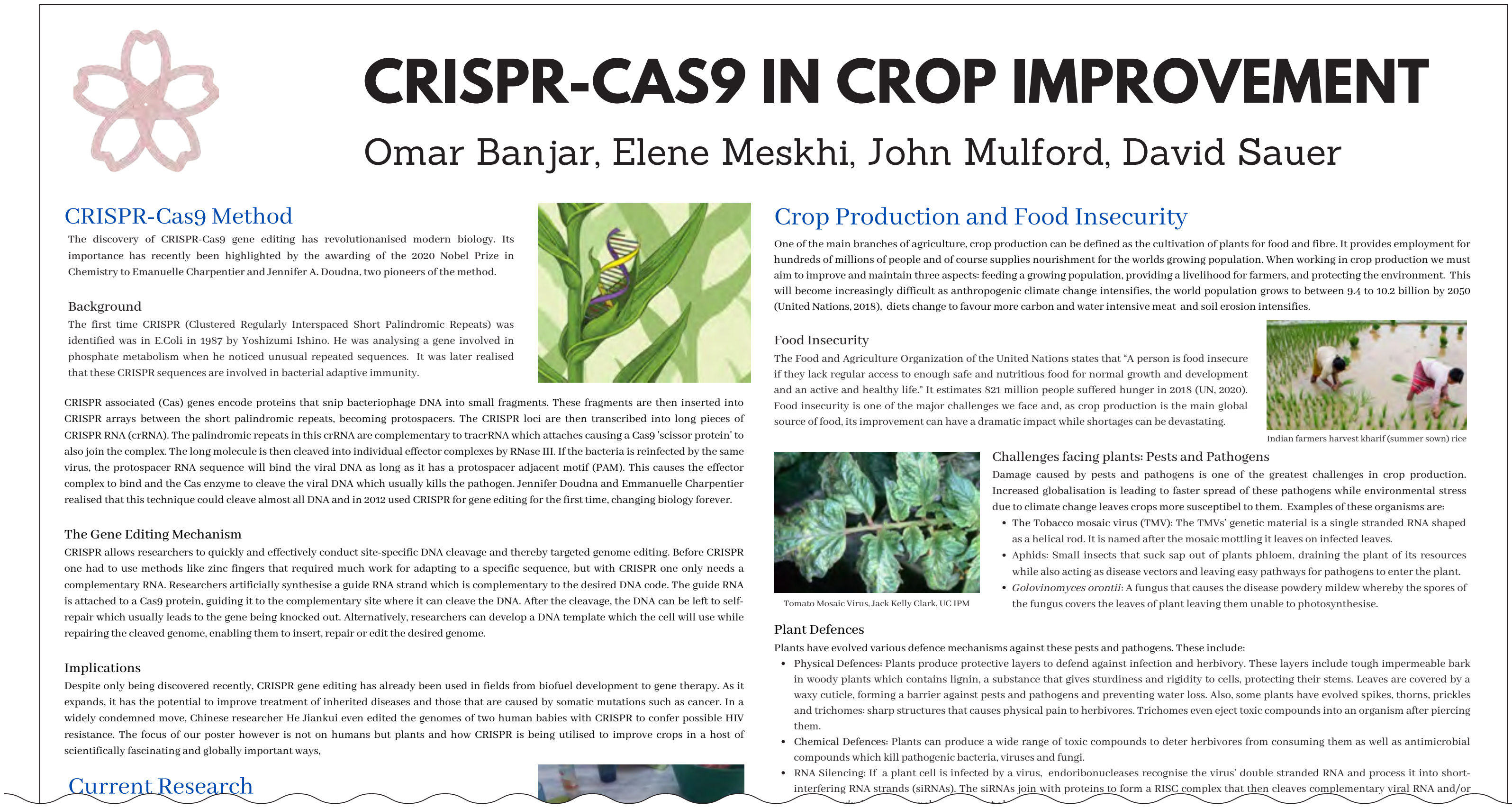

CRISPR-CAS9 IN CROP IMPROVEMENT

Omar Banjar, Elene Meskhi, John Mulford, David Sauer

CRISPR-Cas9 Method

The discovery of CRISPR-Cas9 gene editing has revolutionanised modern biology. Its importance has recently been highlighted by the awarding of the 2020 Nobel Prize in Chemistry to Emanuelle Charpentier and Jennifer A. Doudna, two pioneers of the method.

Background

The first time CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) was identified was in E.Coli in 1987 by Yoshizumi Ishino. He was analysing a gene involved in phosphate metabolism when he noticed unusual repeated sequences. It was later realised that these CRISPR sequences are involved in bacterial adaptive immunity.

CRISPR associated (Cas) genes encode proteins that snip bacteriophage DNA into small fragments. These fragments are then inserted into CRISPR arrays between the short palindromic repeats, becoming protospacers. The CRISPR loci are then transcribed into long pieces of CRISPR RNA (crRNA). The palindromic repeats in this crRNA are complementary to tracrRNA which attaches causing a Cas9 'scissor protein' to also join the complex. The long molecule is then cleaved into individual effector complexes by RNase III. If the bacteria is reinfected by the same virus, the protospacer RNA sequence will bind the viral DNA as long as it has a protospacer adjacent motif (PAM). This causes the effector complex to bind and the Cas enzyme to cleave the viral DNA which usually kills the pathogen. Jennifer Doudna and Emmanuelle Charpentier realised that this technique could cleave almost all DNA and in 2012 used CRISPR for gene editing for the first time, changing biology forever.

The Gene Editing Mechanism

CRISPR allows researchers to quickly and effectively conduct site-specific DNA cleavage and thereby targeted genome editing. Before CRISPR one had to use methods like zinc fingers that required much work for adapting to a specific sequence, but with CRISPR one only needs a complementary RNA. Researchers artificially synthesise a guide RNA strand which is complementary to the desired DNA code. The guide RNA is attached to a Cas9 protein, guiding it to the complementary site where it can cleave the DNA. After the cleavage, the DNA can be left to self-repair which usually leads to the gene being knocked out. Alternatively, researchers can develop a DNA template which the cell will use while repairing the cleaved genome, enabling them to insert, repair or edit the desired genome.

Implications

Despite only being discovered recently, CRISPR gene editing has already been used in fields from biofuel development to gene therapy. As it expands, it has the potential to improve treatment of inherited diseases and those that are caused by somatic mutations such as cancer. In a widely condemned move, Chinese researcher He Jiankui even edited the genomes of two human babies with CRISPR to confer possible HIV resistance. The focus of our poster however is not on humans but plants and how CRISPR is being utilised to improve crops in a host of scientifically fascinating and globally important ways,

Current Research

Crop Production and Food Insecurity

One of the main branches of agriculture, crop production can be defined as the cultivation of plants for food and fibre. It provides employment for hundreds of millions of people and of course supplies nourishment for the worlds growing population. When working in crop production we must aim to improve and maintain three aspects: feeding a growing population, providing a livelihood for farmers, and protecting the environment. This will become increasingly difficult as anthropogenic climate change intensifies, the world population grows to between 9.4 to 10.2 billion by 2050 (United Nations, 2018), diets change to favour more carbon and water intensive meat and soil erosion intensifies.

Food Insecurity

The Food and Agriculture Organization of the United Nations states that "A person is food insecure if they lack regular access to enough safe and nutritious food for normal growth and development and an active and healthy life." It estimates 821 million people suffered hunger in 2018 (UN, 2020). Food insecurity is one of the major challenges we face and, as crop production is the main global source of food, its improvement can have a dramatic impact while shortages can be devastating.

Indian farmers harvest kharif (summer sown) rice

Tomato Mosaic Virus, Jack Kelly Clark, UC IPM

Challenges facing plants: Pests and Pathogens

Damage caused by pests and pathogens is one of the greatest challenges in crop production. Increased globalisation is leading to faster spread of these pathogens while environmental stress due to climate change leaves crops more susceptibel to them. Examples of these organisms are:

- The Tobacco mosaic virus (TMV): The TMVs' genetic material is a single stranded RNA shaped as a helical rod. It is named after the mosaic mottling it leaves on infected leaves.
- Aphids: Small insects that suck sap out of plants phloem, draining the plant of its resources while also acting as disease vectors and leaving easy pathways for pathogens to enter the plant.
- *Golovinomyces orontii*: A fungus that causes the disease powdery mildew whereby the spores of the fungus covers the leaves of plant leaving them unable to photosynthesise.

Plant Defences

Plants have evolved various defence mechanisms against these pests and pathogens. These include:

- Physical Defences: Plants produce protective layers to defend against infection and herbivory. These layers include tough impermeable bark in woody plants which contains lignin, a substance that gives sturdiness and rigidity to cells, protecting their stems. Leaves are covered by a waxy cuticle, forming a barrier against pests and pathogens and preventing water loss. Also, some plants have evolved spikes, thorns, prickles and trichomes: sharp structures that causes physical pain to herbivores. Trichomes even eject toxic compounds into an organism after piercing them.
- Chemical Defences: Plants can produce a wide range of toxic compounds to deter herbivores from consuming them as well as antimicrobial compounds which kill pathogenic bacteria, viruses and fungi.
- RNA Silencing: If a plant cell is infected by a virus, endoribonucleases recognise the virus' double stranded RNA and process it into short-interfering RNA strands (siRNAs). The siRNAs join with proteins to form a RISC complex that then cleaves complementary viral RNA and/or

widely condemned move, Chinese researcher He Jiankui even edited the genomes of two human babies with CRISPR to confer possible HIV resistance. The focus of our poster however is not on humans but plants and how CRISPR is being utilised to improve crops in a host of scientifically fascinating and globally important ways,

Current Research


The first ever CRISPR meal: Spaghetti and roast vegetables cooked with a brassica (cabbage) deletion mutant.

CRISPR-Cas9 technologies have already begun to overtake other genome editing technologies like TALENs and ZFNs as they are simpler to design and implement, have higher success rates, are more versatile and are cheaper. The CRISPR-Cas9 technique and its derivatives have been used to edit the genomes of nearly 20 different plant species with agricultural applications from *Cucumis sativus* (cucumber) to *Linum usitatissimum* (flaxseed) (Ricroch et al., 2017). The studies undertaken fall into two main camps: functional studies in model organisms and 'proof of concept' studies which describe specific applications of CRISPR-Cas9 and its derivatives to improve crop stress tolerance, yield and nutrition. The most studied organism is *Oryza sativa* (rice), the primary crop of over half of the world population and, thanks to its small genome, a model crop for monocots (Jaganathan et al., 2018).

Functional Studies

By knocking out certain genes with Cas9 proteins, researchers can observe the loss-of-function phenotypic consequences and hence work out the function of those genes. This strategy has been used in a variety of model plants and not only helps us better understand the natural world but has direct applications to crop improvement. For example, targeted deletion of *AP1*, *SVP*, and *TFL1* genes in Arabidopsis with CRISPR-Cas9 helped elucidate their role in floral development, including branching and inflorescence type (Liu et al., 2019). CRISPR-Cas9 has also been used to inactivate genes related to nitrogen fixation symbiosis in the model legume Lotus japonicus (Wang et al., 2016). This improves our understanding of the genetics behind one of agriculture's most important processes and may allow us to edit legume genomes to improve nitrogen fixation or potentially even transfer symbiotic nitrogen fixation to non-leguminous crops (Mus et al., 2016).

Disease Resistance

CRISPR-Cas9 has been used to edit crop genomes to improve resistance to viral, bacterial and fungal pathogens. The main technique is the generation of CRISPR-mediated targeted mutations in the plants' genomes. This mostly involves modifying susceptibility genes (genes which facilitate the infection process).

- Bacterial pathogens, like rice bacterial blight, caused by *Xanthomonas oryzae pv. oryzae (Xoo)*, can decimate crops. Analysis of 63 *Xoo* strains shows each strain has one or more variants of genes coding for TALE proteins. Each TALE protein induces at least one of the three host genes *SWEET11*, *SWEET13* and *SWEET14* which encode sucrose transporters. These transporters increase rice disease susceptibility by giving *Xoo* access to nutrients from the plant's leaves. By editing the sequence of *SWEET* genes with CRISPR-Cas9, researchers were able to induce resistance to at least 95 *Xoo* strains, freeing the rice from bacterial blight (Oliva et al., 2019).
- Fungal resistance has also been conferred. Researchers in Italy and South Korea have used CRISPR-Cas9 to modify susceptibility genes in grapevines and apples, increasing resistance to the destructive fungal pathogen *Golovinomyces orontii* (powdery mildew) (Malnoy et al., 2016). CRISPR-Cas9 has even been used to edit mildew susceptibility genes in hexaploid bread wheat, conferring broad-spectrum heritable resistance to *G. orontii* (Wang et al., 2014). This is particularly impressive as all 3 homoeologous genes (homologous genes resulting from allopolyploidy) had to be edited.
- Similar methods have been used for viral pathogens. For example, the susceptibility gene *eIF4E* was disrupted in *Cucumis sativus* (cucumber), conferring resistance to a host of viruses (Chandrasekaran et al., 2016). There is also another technique available for viral resistance. As discussed, CRISPR are a family of DNA sequences which allow prokaryotes to respond to viral infection by detecting and destroying DNA from bacteriophages which have previously infected them. This ancient defence mechanism can therefore be harnessed through the integration of CRISPR-encoding sequences that target and interfere with viral DNA into the plant genome. This method was successfully trialled in *Arabidopsis* and *N. benthamiana* (a tobacco-like model plant), conferring resistance against Beet Severe Curly Top Virus (Ji et al., 2015).

Herbicide Resistance

Herbicide resistant crops allow farmers more flexibility in spraying herbicides, allowing them to apply during the growing season. This also enables the adoption of conservation tillage (leaving last year's stubble before and after planting) to reduce soil erosion. Traditionally herbicide resistance has been achieved by transformation with genes from microorganisms encoding herbicide-degrading enzymes or transformation with mutant versions of enzymes in essential biosynthetic pathways that are insensitive to the herbicide. (Han & Kim, 2019) These mutant biosynthetic genes can also be generated more quickly and precisely with CRISPR-Cas9. The watermelon acetolactate synthase *(ALS)* gene has been base-edited for example, conferring resistance against the herbicide tribenuron and likely all all sulfonylurea herbicides (which inhibit *ALS*). (Tian et al., 2018)


Six-week-old tomato plants of slmapk3 mutants (middle and right) and WT (left) exposed to heat stress

Climatic Stress Tolerance

As the effects of climate change become increasingly severe, it is more important than ever to produce crops that can survive harsh environmental conditions like drought and heat stress. CRISPR-Cas9 can help by generating improved variants of genes which assist and knocking out genes which inhibit environmental response. Researchers knocked out the negative thermoregulatory *SlMAPK3* gene in tomatoes for instance leading to mutants who, under heat stress, exhibit less severe wilting membrane damage and elevated transcription of heat stress transcription factors and heat shock proteins (Yu et al., 2019). Developments like this can help prevent deforestation and wetland draining by increasing productivity from existing agricultural land.

Improving Yield and Nutrition

Increasing crop yield will lead to greater productivity from existing land and hence reduced wetland draining and deforestation as well as higher profits for farmers. For example, researchers used CRISPR to disrupt the *SlIAA9* gene which inhibits parthenocarpy (production of seedless fruit without fertilization). This led to the rapid breeding of parthenocarpic tomatoes which respond better to fluctuating environments (as they don't require pollination) and have much greater industrial value (eg. in sauce production). (Ueta et al., 2017).

There is also scope for biofortification, the increase of crops' nutritional value. Many people globally suffer from nutrient deficiencies with a host of negative side effects. Many of these can be addressed with improved crops. For example, resistant starch (RS) is a type of indigestible starch which is thought to lead to a smaller rise in blood sugar following carbohydrate consumption and produces short-chain fatty acids which act as a prebiotic for healthy bacteria in the colon. By disrupting the function of the rice *SBEIIb* gene (involved in the branching of amylopectin) with CRISPR-Cas9 editing, scientists have produced a high-amylose and hence high-RS rice variety. If commercialised, this has the potential to reduce risk of many non-infectious diseases, such as diabetes. (Sun et al., 2017)

Group 3B08, supervised by Victor Ribeiro, October 2020

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- Chemical Defences: Plants can produce a wide range of toxic compounds to deter herbivores from consuming them as well as antimicrobial compounds which kill pathogenic bacteria, viruses and fungi.
- RNA Silencing: If a plant cell is infected by a virus, endoribonucleases recognise the virus' double stranded RNA and process it into short-interfering RNA strands (siRNAs). The siRNAs join with proteins to form a RISC complex that then cleaves complementary viral RNA and/or supresses viral protein translation. (Wu et al., 2019)
- Signalling ally species: Plants have developed a signal method to call for the help of other species in times of need, these signals can be chemical or electrical or due to movement by changing the plants inner water pressure. For example, when a plant is being infested by caterpillars it can send signals to a parasitic wasp which will come and lay its eggs in the caterpillars.

The Arms Race and Other Challenges

Plants and their pests and pathogens are constantly coevolving defences and anti-defences in an arms race with huge implications for food insecurity. Millenia of plant domestication have actually 'disarmed' our crops. Bitter-tasting chemical defences and harmful physical defences have been directly selected against while selection for large organs and higher productivity has diluted chemical defences or reduced their production where growth and defence trade off metabolically (Moreira et al., 2018). For crop production to the meet its future demands while minimising environmental damage it is vital that we produce new disease resistant crop varieties. Disease is not the only threat to crop production, however; water and phosphorous scarcity, extreme weather and soil erosion will put further stress on crop production. CRISPR gene-editing has a huge potential to address these challenges by producing crop varieties with better yield, nutrition, disease resistnace and abiotic stress tolerance.

Future Developments and Ethical Concerns

Future

Before the CRISPR revolution, scientists had to use non-targeted classical breeding approaches that took much longer and were often ineffective. As described in more detail in the Current Research section of the poster, CRISPR has already been shown to effectively improve a variety of crops in different ways from developing geminivirus resistance in tobacco relative plants to increasing amylose content in rice. It is likely that gene editing and plant breeding techniques will continue to improve as research into CRISPR edited crops grows into the next decade. With the growing world population and rapidly changing climate, the development of these crop improving technologies will probably prove to be one of the main scientific endeavours of the 21st Century.

The CRISPR editing techniques themselves continue to be improved to reduce the risk of off target DNA cleavage, improve efficiency (increasing the proportion of organisms whose genes are cleaved) and target cleavage at multiple sites. In the future we will almost certainly also see new approaches to breeding that combine the knowledge of centuries we have in classical breeding with the speed and preciseness provided by CRISPR-based technologies. Examples thereof on which research has already been conducted include (Zhou et al. 2020):

- reducing the number of „selfing steps" needed for haploidization
- allowing easy fixation of hybrid vigour by allowing plants to skip the second meiotic division
- removing self-incompatibility from certain species, which allows for inbred lines.

These breeding technologies will provide the ability to produce new lines within short periods of time, far below the usual decades required by classical breeding technologies. Furthermore, CRISPR provides great flexibility in the kind of improvements that can be made, due to its versatility and near universality. Further research is necessary for many of these technologies to become commercially viable but the race is on. As research in this field further accelerates, we expect an explosion of patent requests as proof of concept studies become commercialised. It is likely that within the next few decades CRISPR edited plants will become part of many peoples' daily diet. It is crucial before this happens that the scientific community and public engage in an honest and well-informed discussion about the potential and risks of these developments.

Ethics

A protest against agribusiness giant Monsanto.

There has been great public discourse regarding the usage of genetic engineering in food, particularly surrounding transformation of plants with genes from other species. Although, from a scientific standpoint, most concerns (such as horizontal transfer of edited genes and transfer of newly inserted genes to food consumers) are backed by little evidence, campaigners have success restrictive regulations such as a de facto moratorium on GMO approvals in the EU since 2001. Fortunately, CRISPR allows scientists to develop transgene free genetically edited crops, allaying the fears of some campaigners. As these plants do not contain genes from other organisms and could have arisen in nature, they are also not considered GMOs and hence freed from many regulations. In 2016, the US Food and Drug Agency for example declared they would not regulate a CRISPR-edited Agaricus bisporus mushroom. (Kim et al. 2016).

Nonetheless there are still some ethical objections. Some religious individuals are opposed to 'interfering with God's creation' while other people see a slippery slope to human genome editing. There are also concerns that herbicide-resistant plants could increase the use of herbicides despite all their negative effects on the environment and hence also lead to faster evolution of resistance in weeds. Herbicide-resistant seeds can also lead to monopolies of big agricultural companies who then own every step of the agricultural production, namely the seeds, the land and the herbicides. Resistances against environmental conditions might also distract from the real challenge of anthropogenic climate change.

On the other hand, herbicide resistant crops allow farmers to kill perennial weeds with herbicides and hence plough less often, reducing soil erosion. Meanwhile, genetically edited disease resistant crops may allow farmers to spray significantly less environmentally damaging pesticides such as fungicides. In addition, improving crop yield and stress tolerance allows greater productivity from existing agricultural land, reducing the need for deforestation and wetland draining. Gene editing could also lead to hardier crops which keep better and hence reduce food waste. These environmental benefits, combined with the health benefits of better nutrition and economic benefits of higher productivity provide a strong case for the development of CRISPR edited crops. As the human population continues to grow, dietary habits continue to change and anthropogenic climate change becomes more severe, CRISPR crop improvement may become increasingly viewed as not only beneficial but essential.

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3B09 Pyrethrin Synthesis via Gene Insertion in Maize to Provide Resistance Against Spiroplasma kunkelii

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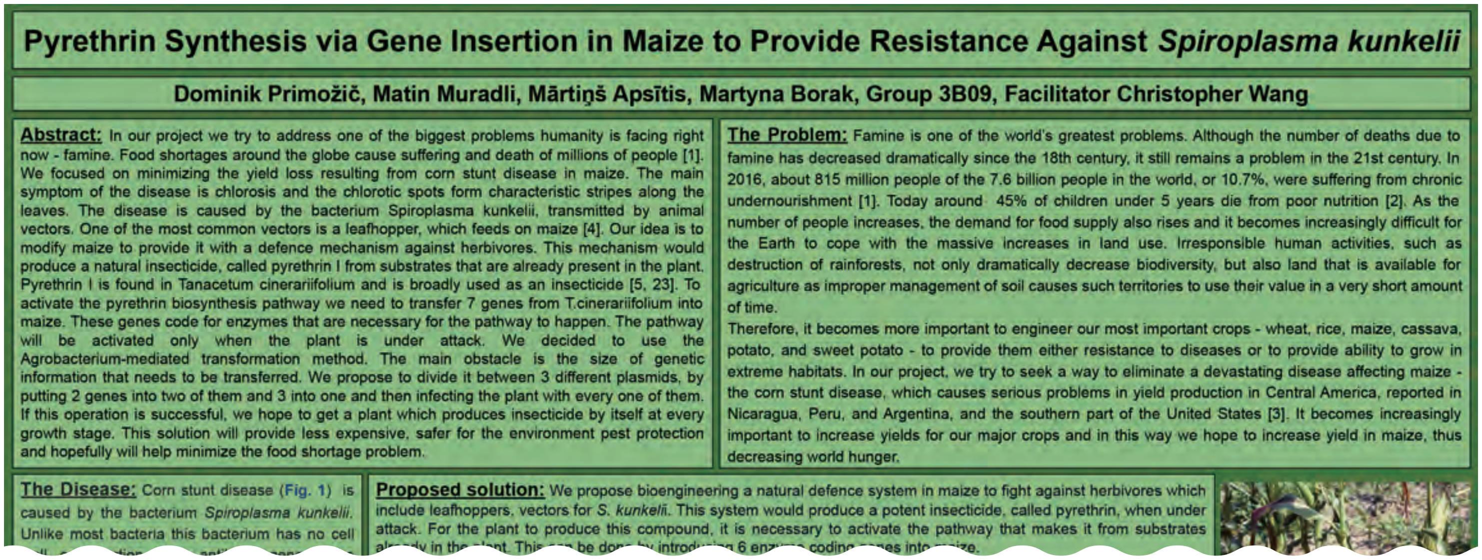

Pyrethrin Synthesis via Gene Insertion in Maize to Provide Resistance Against *Spiroplasma kunkelii*

Dominik Primožič, Matin Muradli, Mārtiņš Apsītis, Martyna Borak, Group 3B09, Facilitator Christopher Wang

Abstract: In our project we try to address one of the biggest problems humanity is facing right now - famine. Food shortages around the globe cause suffering and death of millions of people [1]. We focused on minimizing the yield loss resulting from corn stunt disease in maize. The main symptom of the disease is chlorosis and the chlorotic spots form characteristic stripes along the leaves. The disease is caused by the bacterium Spiroplasma kunkelii, transmitted by animal vectors. One of the most common vectors is a leafhopper, which feeds on maize [4]. Our idea is to modify maize to provide it with a defence mechanism against herbivores. This mechanism would produce a natural insecticide, called pyrethrin I from substrates that are already present in the plant. Pyrethrin I is found in Tanacetum cinerariifolium and is broadly used as an insecticide [5, 23]. To activate the pyrethrin biosynthesis pathway we need to transfer 7 genes from T.cinerariifolium into maize. These genes code for enzymes that are necessary for the pathway to happen. The pathway will be activated only when the plant is under attack. We decided to use the Agrobacterium-mediated transformation method. The main obstacle is the size of genetic information that needs to be transferred. We propose to divide it between 3 different plasmids, by putting 2 genes into two of them and 3 into one and then infecting the plant with every one of them. If this operation is successful, we hope to get a plant which produces insecticide by itself at every growth stage. This solution will provide less expensive, safer for the environment pest protection and hopefully will help minimize the food shortage problem.

The Problem: Famine is one of the world's greatest problems. Although the number of deaths due to famine has decreased dramatically since the 18th century, it still remains a problem in the 21st century. In 2016, about 815 million people of the 7.6 billion people in the world, or 10.7%, were suffering from chronic undernourishment [1]. Today around 45% of children under 5 years die from poor nutrition [2]. As the number of people increases, the demand for food supply also rises and it becomes increasingly difficult for the Earth to cope with the massive increases in land use. Irresponsible human activities, such as destruction of rainforests, not only dramatically decrease biodiversity, but also land that is available for agriculture as improper management of soil causes such territories to use their value in a very short amount of time.

Therefore, it becomes more important to engineer our most important crops - wheat, rice, maize, cassava, potato, and sweet potato - to provide them either resistance to diseases or to provide ability to grow in extreme habitats. In our project, we try to seek a way to eliminate a devastating disease affecting maize - the corn stunt disease, which causes serious problems in yield production in Central America, reported in Nicaragua, Peru, and Argentina, and the southern part of the United States [3]. It becomes increasingly important to increase yields for our major crops and in this way we hope to increase yield in maize, thus decreasing world hunger.

The Disease: Corn stunt disease (Fig. 1) is caused by the bacterium *Spiroplasma kunkelii*. Unlike most bacteria this bacterium has no cell

Proposed solution: We propose bioengineering a natural defence system in maize to fight against herbivores which include leafhoppers, vectors for *S. kunkelii*. This system would produce a potent insecticide, called pyrethrin, when under attack. For the plant to produce this compound, it is necessary to activate the pathway that makes it from substrates already in the plant. This can be done by introducing 6 enzyme coding genes into maize.

The Disease: Corn stunt disease (Fig. 1) is caused by the bacterium *Spiroplasma kunkelii*. Unlike most bacteria this bacterium has no cell wall, so insertion of an antibiotic gene in the plant would not help to overcome the disease (antibiotics target cell walls). The bacterium is usually transmitted from one plant to another by some kind of animal vector. One of such vectors is an insect called leafhopper (Fig. 2) that transmits *S. kunkelii* from one plant to another by feeding on the sap of phloem [4]. In our project, we focus on how to prevent leafhoppers from feeding on maize.

Figure 2: Leafhopper [21]

The Symptoms: The disease includes several symptoms and chlorosis of leaf margins is usually the first symptom of *S. kunkelii* infection, followed by reddening of tips of older leaves (some maize varieties do not redden). Small chlorotic spots appear 2-4 days later at the bases of newly developing leaves. In successive leaves above those bearing first symptoms, the chlorotic spots coalesce to form stripes that extend towards the leaf tips until entire leaves are affected. Later-emerging leaves may also develop chlorosis of the margins, yellowing or reddening, tearing, twisting, and are shortened. Plants are stunted and numerous ear shoots develop [4].

Proposed solution: We propose bioengineering a natural defence system in maize to fight against herbivores which include leafhoppers, vectors for *S. kunkelii*. This system would produce a potent insecticide, called pyrethrin, when under attack. For the plant to produce this compound, it is necessary to activate the pathway that makes it from substrates already in the plant. This can be done by introducing 6 enzyme coding genes into maize.

Pyrethrin is a natural insecticide produced by *Tanacetum cinerariifolium*. It is widely considered a human and environmentally safe yet strong compound [5]. There are 6 main types of natural pyrethrins – jasmolin I, jasmolin II, cinerin I, cinerin II, pyrethrin II, pyrethrin I, the latter is of particular interest to this project as it has been reported deadliest to insects [6]. Like all other pyrethrins, it is an ester, composed of an acid moiety, chrysanthemoyl CoA, and alcohol moiety, pyrethrolone [6]. Synthesis of acid part starts with 2-dimethylallyl diphosphate (Fig. 3), short DMADP, which is a precursor, made in the mevalonate pathway, to many compounds in many plants, including maize [7]. DMADP can then be converted into (R,R)-chrysanthemyl diphosphate with the help of bifunctional chrysanthemol synthase, short CDS [8]. The same enzyme then further catalyzes the conversion of the previously stated compound into (R,R)-chrysanthemol [8]. Chrysanthemol can then be oxidized into (R,R)-chrysanthemal by enzyme alcohol dehydrogenase 2, short ADH2, and oxidizing agent NAD+ [9]. The product can then be oxidized once more by aldehyde dehydrogenase 1 with the help of NAD+ or NADP+ and water, which acts as a base in this reaction, meaning it takes a proton from the carboxylic group, making (R,R)-chrysanthemate [8]. In the next step previous major product is activated into (R,R)-chrysanthemoyl CoA by an enzyme from the family of CoA ligases that bind CoA to negatively charged oxygen in ester group with the help of energy in ATP [8]. With these reactions the acid part is synthesized, but also a problem arises- it is not possible to naturally control when this pathway is being executed. Luckily the production of the first reactant, cis-Jasmone, for alcohol moiety is controlled by plant's defence system against herbivores [10], preventing pyrethrin to accumulate in maize when not needed or forcing maize to waste resources. Pyrethrin is photo unstable, meaning it would quickly degrade after being synthesized during a defensive response [11]. Alcohol moiety, as mentioned, starts with cis-Jasmone which is converted into Jasmolone through hydroxylation with Jasmone hydroxylase, usually CYP71AT148, short JMH [12]. Jasmolone then goes through elimination on its acyclic part to form Pyrethrolone, enzyme Cytochrome P450 Oxidoreductase CYP82Q3, short PYS, has been identified to catalyze this reaction [13]. Chrysanthemoyl group from (R,R)-chrysanthemoyl CoA, acid moiety, is then transferred from CoA to Pyrethrolone, alcohol moiety, by GDSL lipase, forming the ester known as pyrethrin I [14].

For this pathway (Fig. 3) to happen 7 enzymes are needed- CDS, ADH2, ALDH1, CoA ligase, JMH, PYS and GDSL. Genes coding for these proteins have been identified in *Tanacetum cinerariifolium* [5,12,13,14]. To activate this pathway in maize, these 7 genes need to be transferred onto plasmids, then maize has to be transformed using those plasmids and maize culture with potent defence against vectors of *S. kunkelii* can then be grown. Additionally, a special promoter sequence has be made for each of these genes, one that is sensitive to the same stimuli as that starting cis-jasmone synthesis. That way pyrethrin would only be synthesized when maize is under attack.

Figure 1: Corn stunt [20]

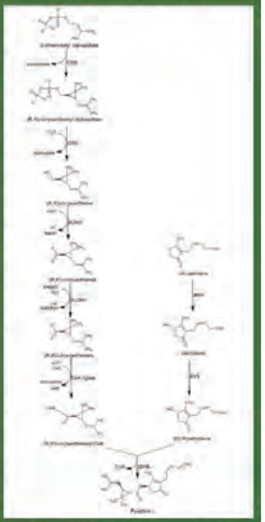

Figure 3: Pyrethrin biosynthesis pathway. Adapted from [5,8,9,12,13,14]

Method of transformation: To achieve this goal, we decided to use *Agrobacterium* - mediated plant transformation. In the past two decades the ability of *Agrobacterium* to transfer DNA to plant cells has been harnessed for the purposes of plant genetic engineering. Some of these modifications have resulted in extending the host range of the bacterium to economically important crop species. In some developed countries, a high percentage of the acreage of such economically important crops as corn, soybeans, cotton, canola, potatoes, and tomatoes is transgenic [15].

The molecular basis of genetic transformation of plant cells by *Agrobacterium* is to transfer from the bacterium into the plant nuclear genome a region of a large tumor-inducing (Ti) or rhizogenic (Ri) plasmid resident in *Agrobacterium*. Ti plasmids are on the order of 200 to 800 kbp in size [16]. The transferred DNA (T-DNA) is referred to as the T-region when located on the Ti or Ri plasmid. T-regions generally represent less than 10% of the Ti plasmid [17]. The processing of the T-DNA from the Ti plasmid and its subsequent export from the bacterium to the plant cell result in large part from the activity of virulence (vir) genes carried by the Ti plasmid [18]. T-regions are defined by T-DNA border sequences. These borders are 25 bp in length and highly homologous in sequence. They flank the T-region in a directly repeated orientation [19]. However, Ti plasmids are very large and T-DNA regions do not generally contain unique restriction endonuclease sites not found elsewhere on the Ti plasmid. Therefore, one cannot simply clone a gene of interest into the T-region. To overcome this obstacle a number of strategies to introduce foreign genes into the T-DNA have been developed. One of them being a binary-vector system (Fig. 4); the replicon harboring the T-region constituted the binary vector, whereas the replicon containing the vir genes became known as the vir helper. The vir helper plasmid generally contained a complete or partial deletion of the T-region, rendering strains containing this plasmid unable to incite tumors [15].

Thus, we think that the 7 previously mentioned genes can be transferred using this system. We propose making 3 plasmids which are similar in size - 2 containing 2 genes and one containing 3. The issue here might pose to be the size of these plasmids. Hence transformation would need to be closely monitored to see if it was successful.

Besides, a unique sequence has to be made for each of the genes which is susceptible for the same stimuli as that triggering cis-jasmone synthesis. Additionally, the genes have to be inserted into plasmids in the correct orientation in between the two borders. Then, via *Agrobacterium*- mediated transformation, these genes will be transferred into maize and should work functionally.

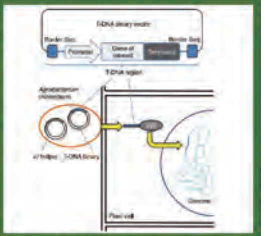

Figure 4: *Agrobacterium* - mediated plant transformation [22]

Pyrethrins' safety: Pyrethrins have been used as insecticides since the 1950s. Thus, their presence in plants would not be new for the ecosystems nor humans. Some insecticides containing pyrethrins can be used in organic agriculture [23]. Pyrethrins are also used in head lice treatment. Drugs containing pyrethrin are approved by the FDA as over-the-counter medications and can be used on children 2 years of age or older [24]. Studies on pyrethrin I have shown low mammalian toxicity. Majority of the administered dose was excreted in unmetabolized form. The bioavailable dose is easily metabolized and does not accumulate in mammalian tissues [25]. Although pyrethrins' toxicity is low, some negative effects may occur as a result of direct contact with the substance. Most injuries are associated with occupational contact. When pyrethrin has been inhaled or has had contact with skin, dermatitis or anaphylactic type of reaction might appear. Most cases resulted from allergenic properties of pyrethrin, rather than its direct toxicity. With massive doses administered orally, nervous system symptoms may occur, including excitation and convolutions leading to paralysis and even death. Immediately Dangerous to Life or Health (IDLH) dose of pyrethrin I amounts to 5,000 mg/m3 [26]. Pyrethrin I is classified as "Not Likely To Be Carcinogenic To Humans: At Doses That Do Not Cause A Mitogenic Response In The Liver" by the US Environmental Protection Agency [27]. Pyrethrins are non-toxic to other mammals and birds, but highly toxic to honey bees and aquatic fauna [23].

Pyrethrins are highly unstable in the presence of light and air. They are decomposed by light and oxidized by air, with loss of their insecticidal activity [26]. Half-lives are 11.8 hours in water and 12.9 hours on soil surfaces. On potato and tomato leaves, less than 3% remained after 5 days. In the absence of light, pyrethrin I breaks down more slowly in water. Half-lives of 14 to 17 days have been reported. When water was more acidic, pyrethrin I did not readily break down. Pyrethrins that enter the water do not dissolve well but tend to bind to sediment. Half-lives of pyrethrin I in sediment are 10.5 to 86 days [23].

Benefits of the project: Providing plants with the ability to produce insecticides by themselves will eliminate the need to spray them. Furthermore, plants' will have constant protection, as they will have their own defensive system, opposed to those which are dependent on insecticide sprayings. Considering instability of pyrethrin when exposed to daylight, thus the need of frequent sprayings, modifying maize to produce pyrethrin by itself will decrease the cost of cultivation. Modified maize will synthesize pyrethrin only when under attack, in damaged parts [10], so the concentration of pyrethrin in grains will be low. It means that no toxicity symptoms should appear after ingesting these grains. Additionally, there will be less pyrethrin in the leaves, compared to sprayed leaves, so it will be safer to feed livestock with remaining parts of the plant. Our solution is also more ecologically friendly, because little to no pyrethrin would be released to the environment and if it was, it would be quickly degraded [23]. Although pyrethrin I is highly toxic to honey bees, these insects would not be affected, because they do not feed on maize or even pollinate it.

Finally, the solution proposed by our team will protect maize from herbivores, such as leafhoppers. If the population of leafhoppers is reduced, the incidence of corn stunt disease will decrease. With the disease eliminated, there should be a significant increase in grain yield. This way the famine problem and environmental issues regarding pest control can be addressed.

References:
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4A02 Investigating the effects of species and habitat on the color response of birds

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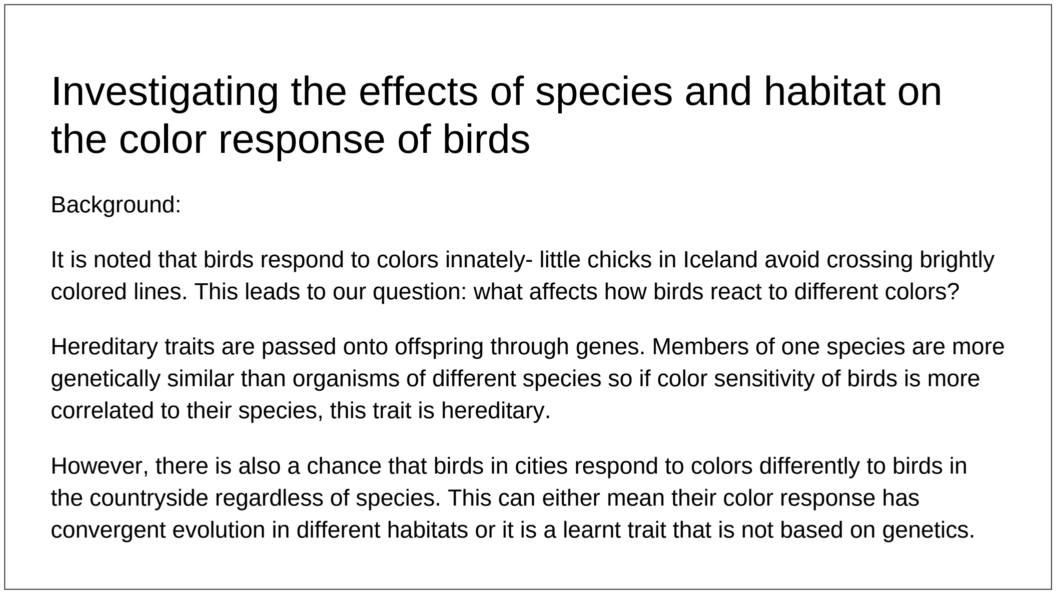
The Uniqueness Award



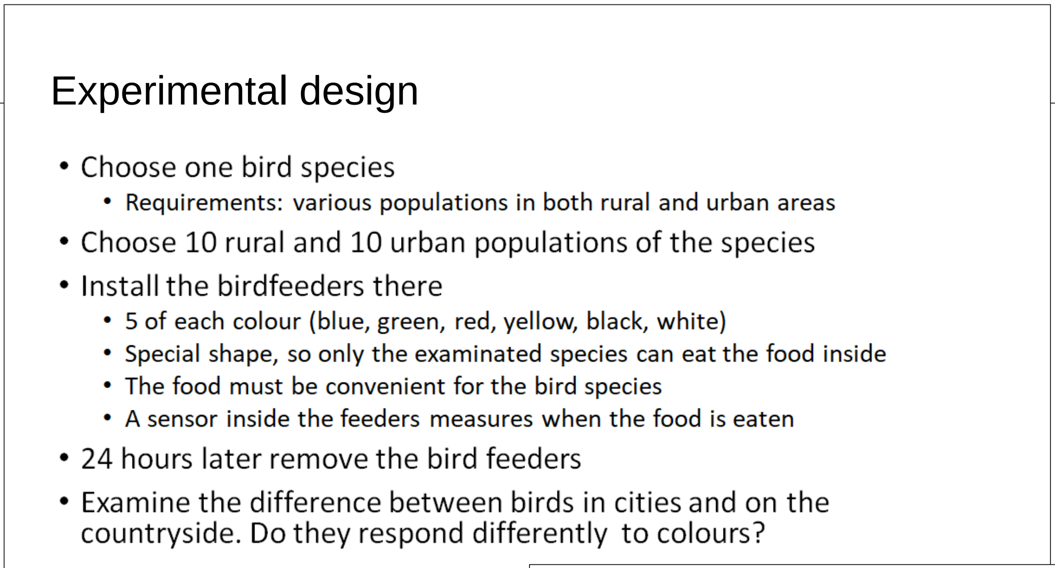
Investigating the effects of species and habitat on the color response of birds

Background:

It is noted that birds respond to colors innately- little chicks in Iceland avoid crossing brightly colored lines. This leads to our question: what affects how birds react to different colors?

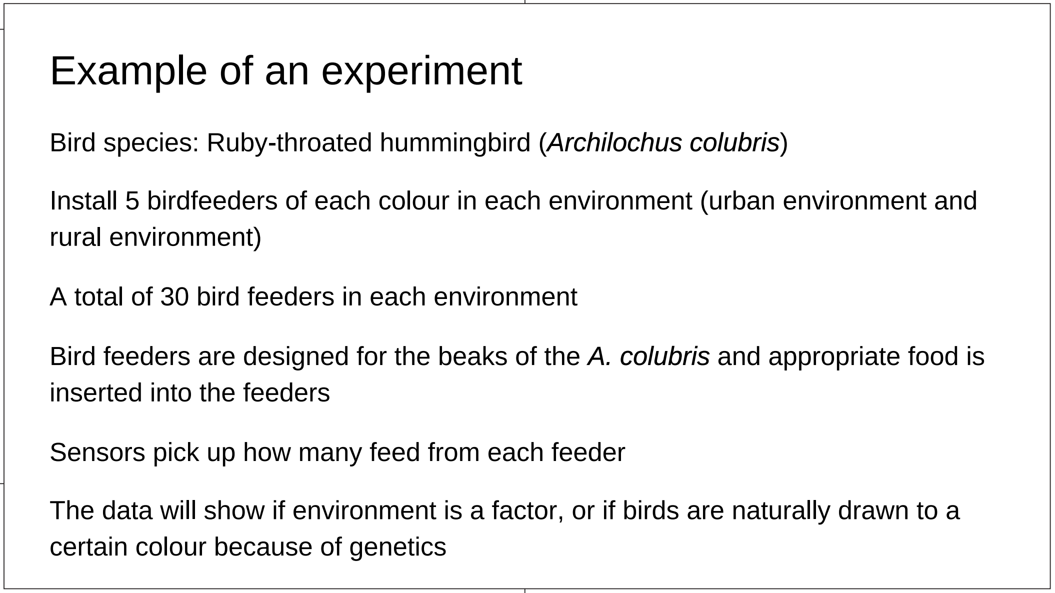
Hereditary traits are passed onto offspring through genes. Members of one species are more genetically similar than organisms of different species so if color sensitivity of birds is more correlated to their species, this trait is hereditary.

However, there is also a chance that birds in cities respond to colors differently to birds in the countryside regardless of species. This can either mean their color response has convergent evolution in different habitats or it is a learnt trait that is not based on genetics.



Experimental design

- Choose one bird species
  - Requirements: various populations in both rural and urban areas
- Choose 10 rural and 10 urban populations of the species
- Install the birdfeeders there
  - 5 of each colour (blue, green, red, yellow, black, white)
  - Special shape, so only the examinated species can eat the food inside
  - The food must be convenient for the bird species
  - A sensor inside the feeders measures when the food is eaten
- 24 hours later remove the bird feeders
- Examine the difference between birds in cities and on the countryside. Do they respond differently to colours?



Example of an experiment

Bird species: Ruby-throated hummingbird (*Archilochus colubris*)

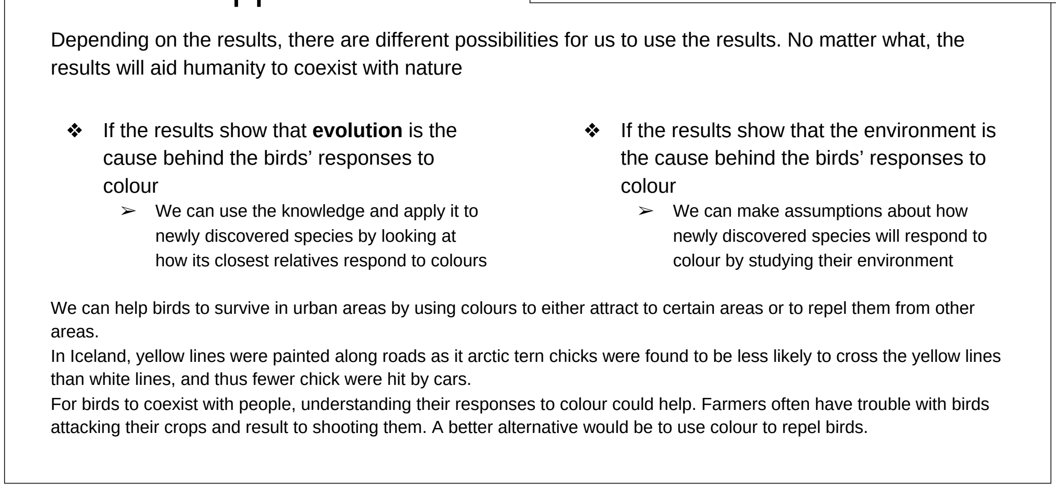
Install 5 birdfeeders of each colour in each environment (urban environment and rural environment)

A total of 30 bird feeders in each environment

Bird feeders are designed for the beaks of the *A. colubris* and appropriate food is inserted into the feeders

Sensors pick up how many feed from each feeder

The data will show if environment is a factor, or if birds are naturally drawn to a certain colour because of genetics



Real life application

Depending on the results, there are different possibilities for us to use the results. No matter what, the results will aid humanity to coexist with nature

- If the results show that **evolution** is the cause behind the birds' responses to colour
  - We can use the knowledge and apply it to newly discovered species by looking at how its closest relatives respond to colours
- If the results show that the environment is the cause behind the birds' responses to colour
  - We can make assumptions about how newly discovered species will respond to colour by studying their environment

We can help birds to survive in urban areas by using colours to either attract to certain areas or to repel them from other areas.
In Iceland, yellow lines were painted along roads as it arctic tern chicks were found to be less likely to cross the yellow lines than white lines, and thus fewer chick were hit by cars.
For birds to coexist with people, understanding their responses to colour could help. Farmers often have trouble with birds attacking their crops and result to shooting them. A better alternative would be to use colour to repel birds.

4A03 Introduction and Microevolution of New Gut Bacterial Species : An Eco-Evo-Devo Approach

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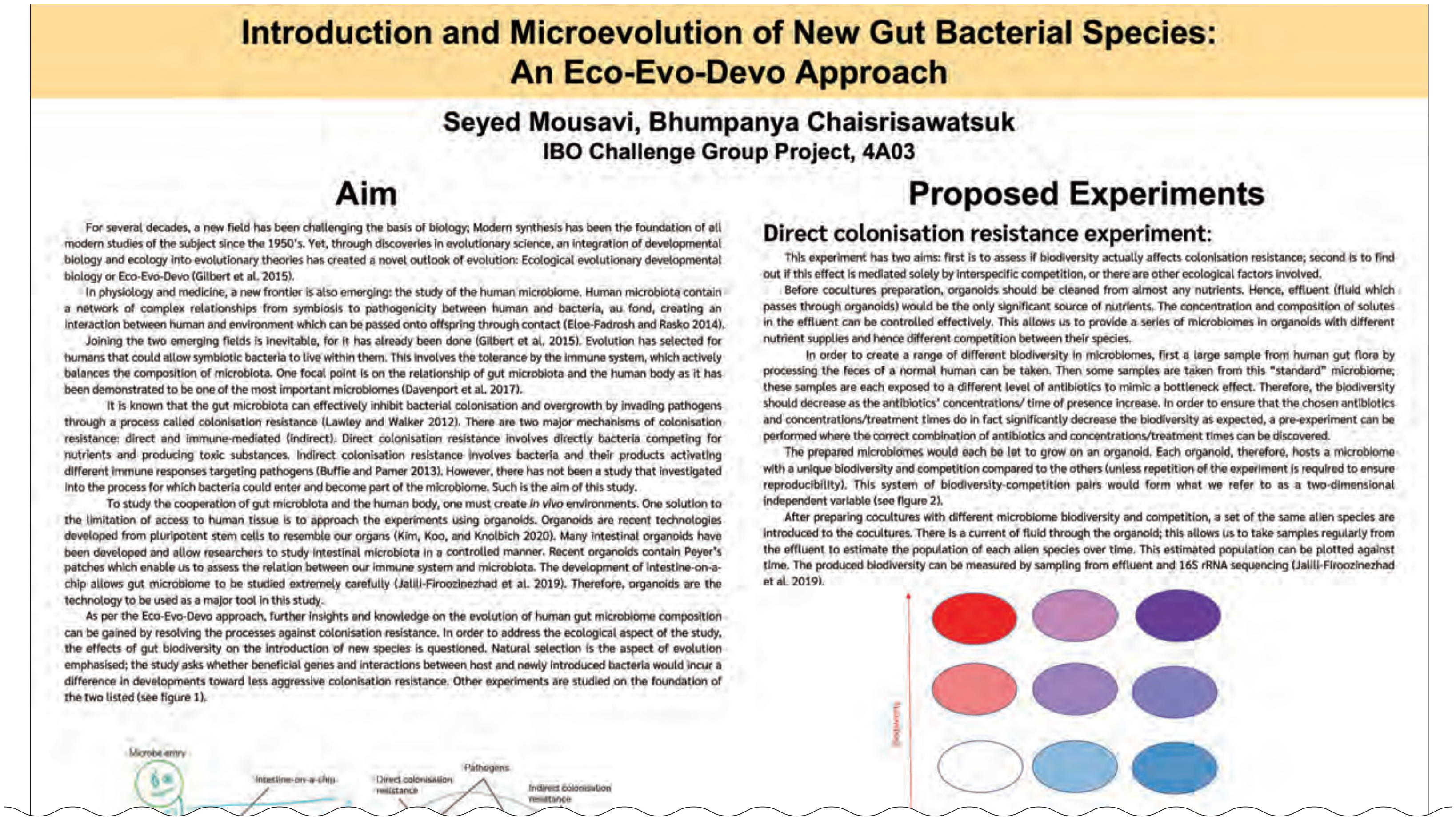

Introduction and Microevolution of New Gut Bacterial Species: An Eco-Evo-Devo Approach

Seyed Mousavi, Bhumpanya Chaisrisawatsuk

IBO Challenge Group Project, 4A03

Aim

For several decades, a new field has been challenging the basis of biology; Modern synthesis has been the foundation of all modern studies of the subject since the 1950's. Yet, through discoveries in evolutionary science, an integration of developmental biology and ecology into evolutionary theories has created a novel outlook of evolution: Ecological evolutionary developmental biology or Eco-Evo-Devo (Gilbert et al. 2015).

In physiology and medicine, a new frontier is also emerging: the study of the human microbiome. Human microbiota contain a network of complex relationships from symbiosis to pathogenicity between human and bacteria, au fond, creating an interaction between human and environment which can be passed onto offspring through contact (Eloe-Fadrosh and Rasko 2014).

Joining the two emerging fields is inevitable, for it has already been done (Gilbert et al. 2015). Evolution has selected for humans that could allow symbiotic bacteria to live within them. This involves the tolerance by the immune system, which actively balances the composition of microbiota. One focal point is on the relationship of gut microbiota and the human body as it has been demonstrated to be one of the most important microbiomes (Davenport et al. 2017).

It is known that the gut microbiota can effectively inhibit bacterial colonisation and overgrowth by invading pathogens through a process called colonisation resistance (Lawley and Walker 2012). There are two major mechanisms of colonisation resistance: direct and immune-mediated (indirect). Direct colonisation resistance involves directly bacteria competing for nutrients and producing toxic substances. Indirect colonisation resistance involves bacteria and their products activating different immune responses targeting pathogens (Buffie and Pamer 2013). However, there has not been a study that investigated into the process for which bacteria could enter and become part of the microbiome. Such is the aim of this study.

To study the cooperation of gut microbiota and the human body, one must create *in vivo* environments. One solution to the limitation of access to human tissue is to approach the experiments using organoids. Organoids are recent technologies developed from pluripotent stem cells to resemble our organs (Kim, Koo, and Knolbich 2020). Many intestinal organoids have been developed and allow researchers to study intestinal microbiota in a controlled manner. Recent organoids contain Peyer's patches which enable us to assess the relation between our immune system and microbiota. The development of intestine-on-a-chip allows gut microbiome to be studied extremely carefully (Jalili-Firoozinezhad et al. 2019). Therefore, organoids are the technology to be used as a major tool in this study.

As per the Eco-Evo-Devo approach, further insights and knowledge on the evolution of human gut microbiome composition can be gained by resolving the processes against colonisation resistance. In order to address the ecological aspect of the study, the effects of gut biodiversity on the introduction of new species is questioned. Natural selection is the aspect of evolution emphasised; the study asks whether beneficial genes and interactions between host and newly introduced bacteria would incur a difference in developments toward less aggressive colonisation resistance. Other experiments are studied on the foundation of the two listed (see figure 1).

Proposed Experiments

Direct colonisation resistance experiment:

This experiment has two aims: first is to assess if biodiversity actually affects colonisation resistance; second is to find out if this effect is mediated solely by interspecific competition, or there are other ecological factors involved.

Before cocultures preparation, organoids should be cleaned from almost any nutrients. Hence, effluent (fluid which passes through organoids) would be the only significant source of nutrients. The concentration and composition of solutes in the effluent can be controlled effectively. This allows us to provide a series of microbiomes in organoids with different nutrient supplies and hence different competition between their species.

In order to create a range of different biodiversity in microbiomes, first a large sample from human gut flora by processing the feces of a normal human can be taken. Then some samples are taken from this "standard" microbiome; these samples are each exposed to a different level of antibiotics to mimic a bottleneck effect. Therefore, the biodiversity should decrease as the antibiotics' concentrations/ time of presence increase. In order to ensure that the chosen antibiotics and concentrations/treatment times do in fact significantly decrease the biodiversity as expected, a pre-experiment can be performed where the correct combination of antibiotics and concentrations/treatment times can be discovered.

The prepared microbiomes would each be let to grow on an organoid. Each organoid, therefore, hosts a microbiome with a unique biodiversity and competition compared to the others (unless repetition of the experiment is required to ensure reproducibility). This system of biodiversity-competition pairs would form what we refer to as a two-dimensional independent variable (see figure 2).

After preparing cocultures with different microbiome biodiversity and competition, a set of the same alien species are introduced to the cocultures. There is a current of fluid through the organoid; this allows us to take samples regularly from the effluent to estimate the population of each alien species over time. This estimated population can be plotted against time. The produced biodiversity can be measured by sampling from effluent and 16S rRNA sequencing (Jalili-Firoozinezhad et al. 2019).

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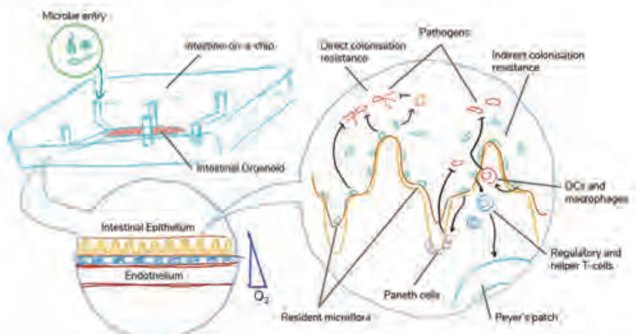

Figure 1. General picture of the experiments. Inoculation of selected microbes into the intestine-on-a-chip or other proposed organoids whereby a system in circulation with environment through fluid flow via bloodstream (endothelium) or intestinal fluid. O_2 gradient is created to replicate the anoxic condition of human intestine. In the intestinal microbiome resides human normal flora or other specific combinations, according to each experimental design. The mechanisms of direct colonisation resistance involve competing for nutrients and producing inhibitory substances, while those of indirect colonisation resistance include, for instance, activation of dendritic cells (DCs, CD103+) and macrophages (CX3-CR1+), activation of helper T-cells (T_H17), and activating Paneth cells.

Hypothesis

As mentioned above, colonisation resistance can be mediated by the immune system of host (indirect) or interspecific competition (direct). Hence, it can seem trivial that, during the coevolution of host-gut microbiota, natural selection has acted by both of these direct and indirect means to determine the composition of the flora. However, there can be some questions with non-trivial answers:

1. How long does it take for the host immune system to evolve in order to accept or reject a species from the microbiota?
2. How significant the roles of ecological factors, other than the interspecific competition, are in affecting the composition of microbiota?

Even though these two questions seem to need further investigations to be answered, we can indeed have some strong hypotheses regarding what the answers should be. For example, we can hypothesise that one generation of a human cannot provide sufficient time for evolution to select for a composition of microbiota. Event though the hypermutation and selection in germinal centres, such as those in the Peyer's patches in gut organoids, can be affected by some probable microbiota-host interactions to allow growth and fixation of a species, these information (such as the sequence of variable chain of antibodies) cannot be passed through to the next generation of humans.

It is also trivial to hypothesise many ecological factors such as biodiversity of the flora are, through affecting interspecific competition, related to direct colonisation resistance. However, one question can be if there are some ecological factors which can affect the colonisation independent to the interspecific competition.

In this poster, it has been investigated if the host immune system can evolve (of course without heritability) to select for its gut microbiome composition. It has been hypothesised that the immune system can undergo a microevolution to select for some species, depending on their phenotypes.

Another experiment devised here is trying to assess the validity of our hypothesis, that some ecological factors such as biodiversity are involved in determining the gut flora. It is hypothesised that biodiversity affects the interspecific competition, which in turn alters the direct colonisation resistance. However, can biodiversity alter the colonisation of introduced species independent to competition? We have hypothesized that all the ecological factors such as biodiversity affect the colonisation of new pieces by altering the degree of interspecific species. However, the possibility of the existence of some other ecological factors which act on the flora without altering the competition between its species is not denied.

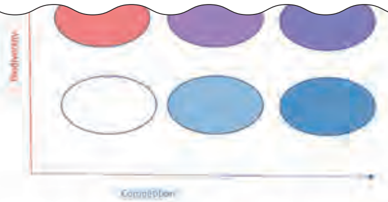

Figure 2. Two dimensional independent variable. In this experiment, competition and biodiversity are both independent variables; having said that, they can be interpreted as ordered pairs of one independent variable and span a two dimensional space. The growth curves of alien species are measured in each section in this space. Therefore, we can analyse how the effect of biodiversity is related to the level of competition. Each coloured ellipse represents an organoid with controlled level of competition and biodiversity.

Indirect colonisation resistance experiment:

The main aim of this experiment as stated, is to assess the role of beneficial genes in first colonisation of unfamiliar bacterial species. There are 1 main experiment and 1 control experiment as follow:

Main Experiment:

Selection of bacteria for mutant construction.

To create a mutant, we must first select the appropriate candidate for such gene insertions. In this study, we consider using commensal bacterial species of major gut microbial populations: Bacteroidetes, Firmicutes and Proteobacteria. All of which are selected by methods of relatedness to normal flora of human intestines and overlap of optimal conditions and organoid environment. One must be very discreet to choose bacterial species which do not usually inhabit and is not familiar to the human intestine. It is suggested to select certain species from lower vertebrate gastrointestinal tract to maintain genetic differences yet is optimal to the environment of the human gut. On the subject of pathogenicity, commensals are preferred, although pathogens are possible for study.

Construction of insertion mutants.

For the selected bacterial species, it is hoped that the scientific society has a great understanding of its genome. An insertion mutant is to be created through genetic engineering as appropriate by using DNA cloning with expression vectors relying on bacterial transformation. In this case, the study uses Bacteroides fragilis PSA (ZPS) operon as the subject of cloning. CRISPR-Cas9 insertion mutagenesis could also be performed. The mutant will then be cultured in isolation.

Bacterial inoculation in organoids.

After the preparation of intestinal organoids, whether as intestine-on-a-chip or in Matrigel according to the protocols, bacterial cocultures derived from human intestine normal flora will be introduced. They will be kept in condition for 3 days to allow settlement, then a series of single species introduction will be enacted for each set of organoids: mutated species with PSA operon, an untreated variant of species which mutants are derived, and Bacteroides fragilis with functioning PSA operon. We will measure the parameters the following 2 months and assess the evolutionary patterns. The parameters include population size, time until colonization, duration of colonisation, immune system mobilisation, and composition of microbiota.

Metagenomics.

Bacterial population size and composition will be evaluated through metagenomics by collecting samples of 16S rRNA from outflowing fluid and comparing with the prokaryotic database.

Immune response evaluation.

Products of the organoid immune system will also be evaluated by studying the outflowing fluid where the concentration of interleukins, interferons, immunoglobulins, and other immune-related secretions will be determined. Quantification of such a large repertoire of specific proteins requires Western blotting and absorbance methods, or mass spectrometry.

Control experiment:

Comparison of mutated and natural biosynthesis of PSA

Mutated species with PSA operon will be compared to Bacteroides Fragilis, in order to determine the result of mutation. Both species will be cultured in isolation separately where the concentration of PSA in culture broths will be quantified. We also require the comparison of mutant and Bacteroides fragilis in organoids with regulatory T cells (Treg FoxP3+), whereby we measure the size of T cell population and concentration of Interleukin-10 (IL-10) in following days.

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4B02 Human Evolution

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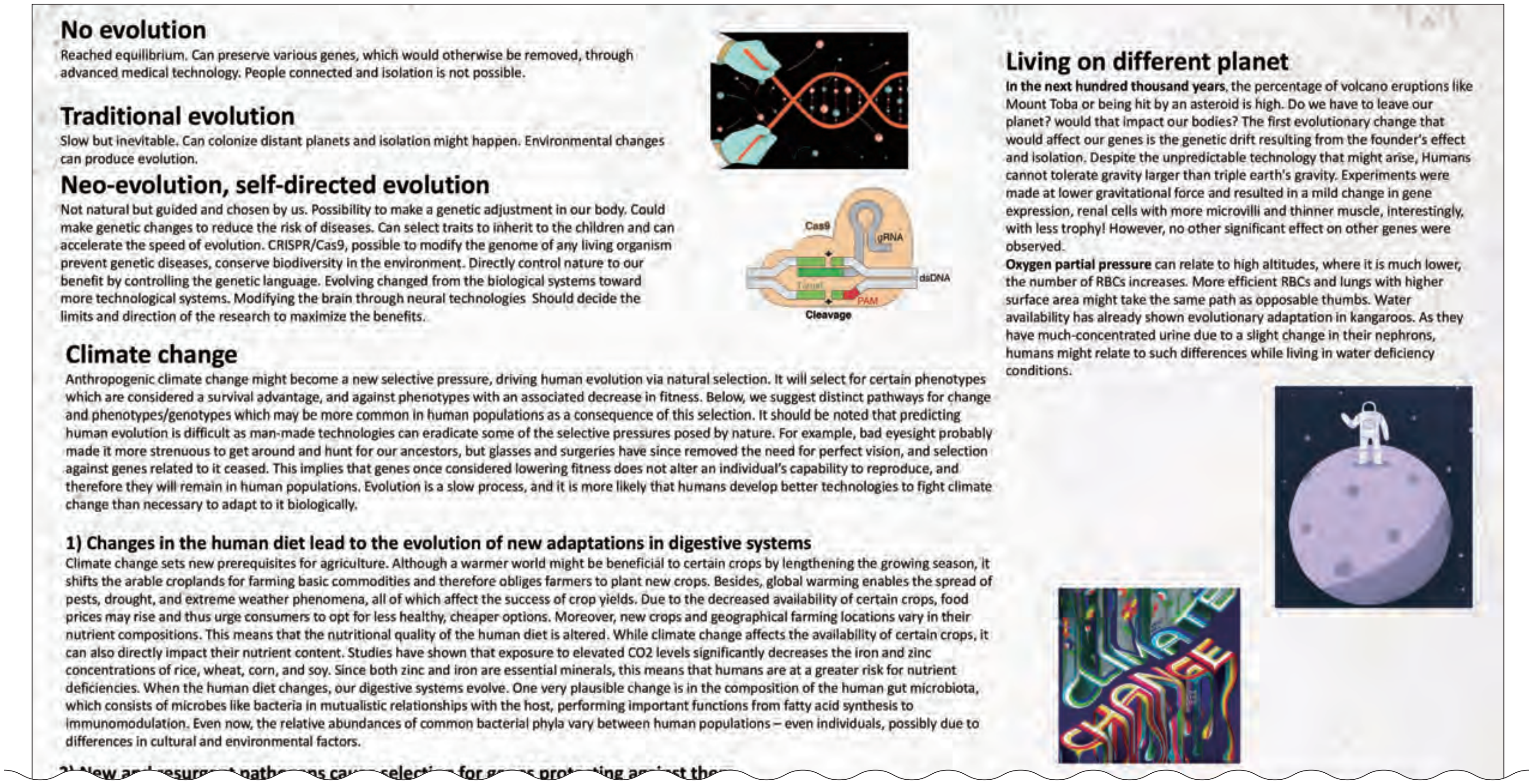


No evolution

Reached equilibrium. Can preserve various genes, which would otherwise be removed, through advanced medical technology. People connected and isolation is not possible.

Traditional evolution

Slow but inevitable. Can colonize distant planets and isolation might happen. Environmental changes can produce evolution.

Neo-evolution, self-directed evolution

Not natural but guided and chosen by us. Possibility to make a genetic adjustment in our body. Could make genetic changes to reduce the risk of diseases. Can select traits to inherit to the children and can accelerate the speed of evolution. CRISPR/Cas9, possible to modify the genome of any living organism prevent genetic diseases, conserve biodiversity in the environment. Directly control nature to our benefit by controlling the genetic language. Evolving changed from the biological systems toward more technological systems. Modifying the brain through neural technologies Should decide the limits and direction of the research to maximize the benefits.

Climate change

Anthropogenic climate change might become a new selective pressure, driving human evolution via natural selection. It will select for certain phenotypes which are considered a survival advantage, and against phenotypes with an associated decrease in fitness. Below, we suggest distinct pathways for change and phenotypes/genotypes which may be more common in human populations as a consequence of this selection. It should be noted that predicting human evolution is difficult as man-made technologies can eradicate some of the selective pressures posed by nature. For example, bad eyesight probably made it more strenuous to get around and hunt for our ancestors, but glasses and surgeries have since removed the need for perfect vision, and selection against genes related to it ceased. This implies that genes once considered lowering fitness does not alter an individual's capability to reproduce, and therefore they will remain in human populations. Evolution is a slow process, and it is more likely that humans develop better technologies to fight climate change than necessary to adapt to it biologically.

1) Changes in the human diet lead to the evolution of new adaptations in digestive systems

Climate change sets new prerequisites for agriculture. Although a warmer world might be beneficial to certain crops by lengthening the growing season, it shifts the arable croplands for farming basic commodities and therefore obliges farmers to plant new crops. Besides, global warming enables the spread of pests, drought, and extreme weather phenomena, all of which affect the success of crop yields. Due to the decreased availability of certain crops, food prices may rise and thus urge consumers to opt for less healthy, cheaper options. Moreover, new crops and geographical farming locations vary in their nutrient compositions. This means that the nutritional quality of the human diet is altered. While climate change affects the availability of certain crops, it can also directly impact their nutrient content. Studies have shown that exposure to elevated CO2 levels significantly decreases the iron and zinc concentrations of rice, wheat, corn, and soy. Since both zinc and iron are essential minerals, this means that humans are at a greater risk for nutrient deficiencies. When the human diet changes, our digestive systems evolve. One very plausible change is in the composition of the human gut microbiota, which consists of microbes like bacteria in mutualistic relationships with the host, performing important functions from fatty acid synthesis to immunomodulation. Even now, the relative abundances of common bacterial phyla vary between human populations – even individuals, possibly due to differences in cultural and environmental factors.

2) New and resurgent pathogens cause selection for genes protecting against the

Living on different planet

In the next hundred thousand years, the percentage of volcano eruptions like Mount Toba or being hit by an asteroid is high. Do we have to leave our planet? would that impact our bodies? The first evolutionary change that would affect our genes is the genetic drift resulting from the founder's effect and isolation. Despite the unpredictable technology that might arise, Humans cannot tolerate gravity larger than triple earth's gravity. Experiments were made at lower gravitational force and resulted in a mild change in gene expression, renal cells with more microvilli and thinner muscle, interestingly, with less trophy! However, no other significant effect on other genes were observed.

Oxygen partial pressure can relate to high altitudes, where it is much lower, the number of RBCs increases. More efficient RBCs and lungs with higher surface area might take the same path as opposable thumbs. Water availability has already shown evolutionary adaptation in kangaroos. As they have much-concentrated urine due to a slight change in their nephrons, humans might relate to such differences while living in water deficiency conditions.

immunomodulation. Even now, the relative abundances of common bacterial phyla vary between human populations – even individuals, possibly due to differences in cultural and environmental factors.

2) New and resurgent pathogens cause selection for genes protecting against them

Consider malaria and multiple other tropical diseases; as our planet warms, pathogens can begin to exploit new regions, becoming an issue for the developed nations. Not only can climate change affect existing pathogens, but it can also lead to the emergence of new ones. Many pathogens and their vectors benefit from a temperature rise since it speeds up their development. However, it also seems to shorten the lifespans of some pathogens and their hosts. If pathogens can adapt to higher temperatures, it could imply that climate change selects for ones expressing a higher level of heat tolerance. Because of the tight co-evolutionary relationship between certain parasites and their human hosts, there is pressure to select for traits enhancing our survival. In the case of malaria, there are already pre-existing human genotypes that can give an individual resistance to the disease. Since Plasmodium parasites infect red blood cells, alterations to structures essential for their normal function protect the cells from invasions. This explains why selection has preserved the alleles for sickle-cell disease in countries suffering from malaria, as heterozygous individuals have greater fitness than both homozygous forms (sickled red blood cells are destroyed more rapidly, preventing the parasites from replicating in them). This heterozygote advantage, a form of balancing selection, might be the reason the frequency of the sickle-cell allele increases in the future when malaria spreads. This same reasoning can be applied to genetic blood disorders like thalassemia. Natural resistance to diseases like Lyme and others with no licensed vaccines will be advantageous in the future and can be considered to increase one's fitness. Due to coevolution, humans will develop better defenses against them. If we take an example from the evolutionary past of our species, one of the defenses our ancestors evolved was inflammation in response to infection. However, some studies have linked this inflammatory response to an increased vulnerability to autoimmune and other immune-related diseases, and certain aspects of our future lifestyles may make us even more susceptible to them.

3) Mass migrations merge human populations and gene pools

Current human populations around the globe have accumulated differences in physical traits through a combination of natural selection and innovations. These populations have remained open, enabling gene flow from one pool to another. However, as we will start seeing more and more environmental refugees fleeing from areas that have become inhabitable as a result of climate change, mass migrations begin to merge the populations even quicker. One implication of this is narrower phenotypic diversity amongst humans or more specifically greater similarity in skin color. Humans have evolved a variety of complexions ranging from pale to dark, each reflecting a different level of eumelanin pigmentation which protects from mutagenic UV rays. Skin color is a polygenic trait, controlled by a multitude of different genes, and because of this, parents tend to have offspring whose skin tone is their intermediate. Consequently, there may be fewer people with either extremity (i.e. dark or light skin) due to gene flow.

4) Humans will evolve new techniques for regulating body temperature

Humans are endothermic homeotherms, meaning we are capable of generating our own heat and our core temperature stays fairly constant. Climate change disrupts our thermoregulatory mechanisms and makes it more difficult for our bodies to lose excess heat since the ambient air temperature is on the rise and high humidity prevents effective cooling via sweat evaporation. A possible adaptation to this, by Bergmann's and Allen's ecogeographical rules, is evolving taller and slimmer bodies: longer limbs have more surface area for heat dissipation, and a slimmer body produces less heat. Studies have proved that Bergmann's rule, which holds that body size is inversely proportional to temperature, is already applicable to humans living on different hemispheres.

The Future

The Past

Agriculture When agriculture arose, it did not merely affect our diet. New food sources resulted in many evolutionary changes, like lower bone density by 20% due to a less mobile lifestyle, animal domestication led to lactase persistence, and the exposer of new devastating disease made genetic resistance evolve. Now, Europeans have a genetic difference related to skin color. Light skin color was needed to compensate for vitamin D deficiency in their diet. Keeping in mind that natural mutations and random shuffling of genes have a greater impact on the variation of human genes than natural selection.

Agriculture was the first step for humans to learn how to adapt the environment to their needs. It allowed the human population to grow massively by settling down in one area. Even though large density made the spread of infectious diseases easier, it gave humans a chance to enhance collective learning, which improved human knowledge in many aspects. Thus, they had better medical care. Advanced communication and science gave better ways of harvesting energy, leading to the industrial revolution.

Evolutionary adaptation, sexual and natural selection, and genetic drift with Homo Sapiens populations. Humans' culture might have accelerated human evolution.

Archaic admixture

Homo heidelbergensis: Neanderthals, Denisovans, Homo Sapiens later met and interbred. Tibetans, Melanesians, and Australian Aboriginals 3-5% of Denisovan DNA. Indonesians, Papua New Guineans interbred with Denisovans between 15,000 to 30,000 years ago. East Asians inherited more Neanderthal DNA than Europeans. Sherpas of Nepal inherited EPAS1 from the Denisovans to breathe easily at high altitude.

Upper Paleolithic, or the Late Stone Age(50,000-12,000)

Cold climate: heavily built, flat and broad noses, straight hair, larger cranial volume. Warm climate: thicker lips, narrow and protruding noses, curly hair. East Asian variant of EDAR gene 35,000 years ago: more sweat glands, teeth, thicker hair, less breast tissue. Light skin in Europeans and East Asians is due to KITLG, ASIP. Brains seem shrinking over the last twenty thousand years: becoming less intelligent, or lower levels of aggression.

Holocene(12,000)

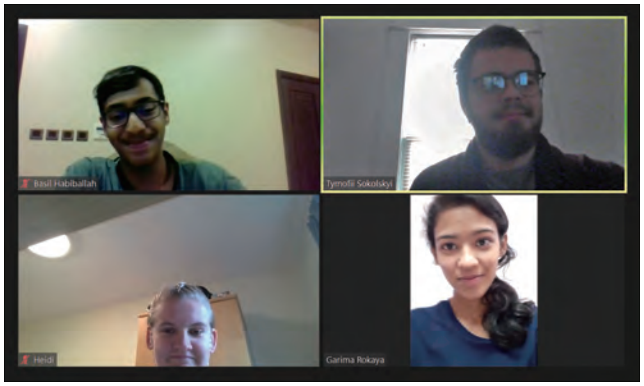
Neolithic, New Stone Age. Brown eyes change to blue eyes in regions where amounts of light are limited, OCA2 gene
Jonathan Pritchard: 700 regions of the human genome shaped by natural selection between 15,000 and 5,000 years ago. Senses of smell and taste, skin color, digestion, bone structure, and brain function. Explain why people from different parts of the globe can be so different even though most of their DNAs are identical.

Sources:

Human Evolution

The Authors: Oona Kurola Geono Kim Zainab Al-alawi

4B03 Evolution of Neurodegenerative Diseases



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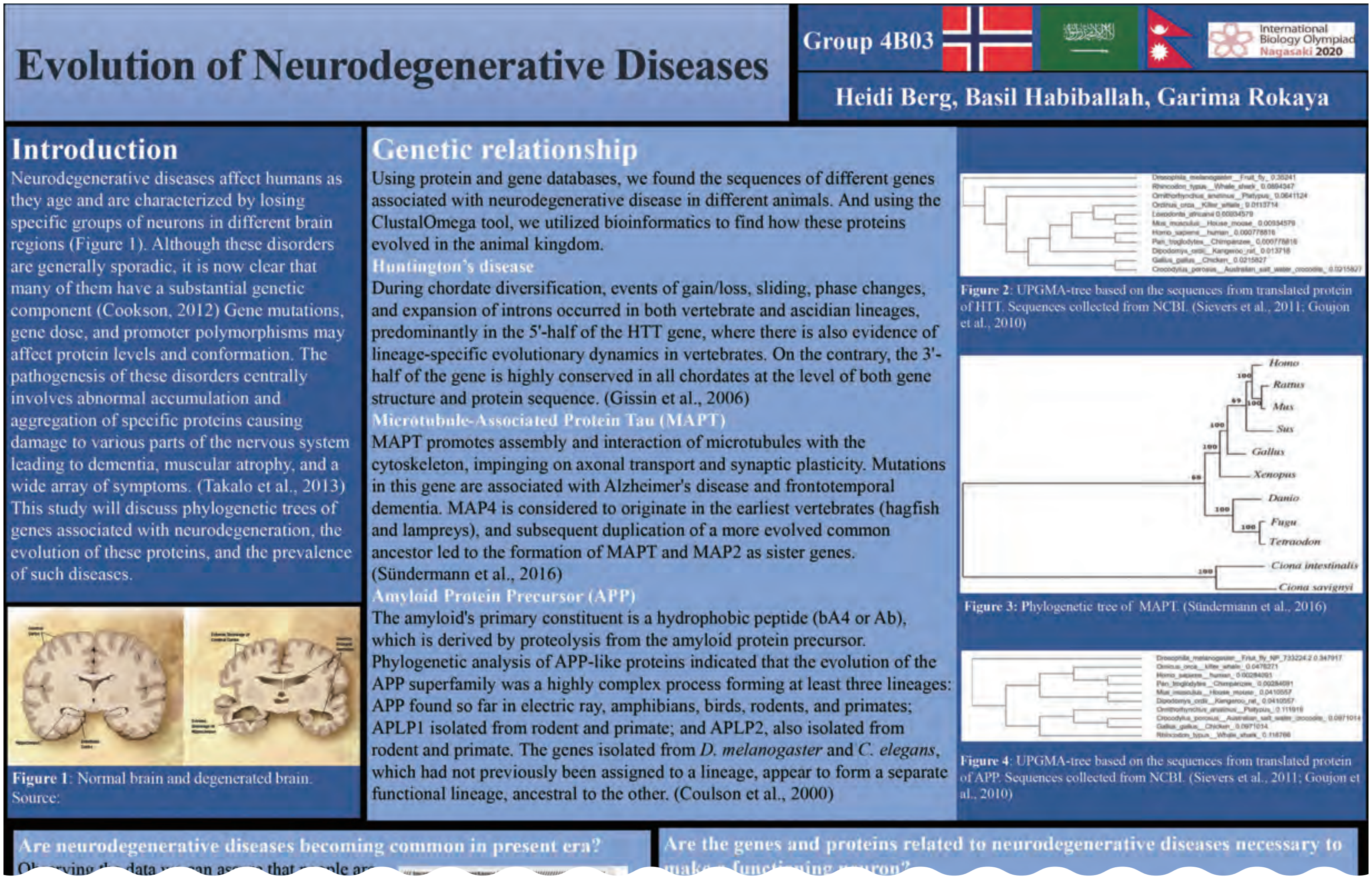
Garima Rokaya (Nepal)

Heidi Berg (Norway)

The Awards of Excellence



Evolution of Neurodegenerative Diseases

Group 4B03

Heidi Berg, Basil Habiballah, Garima Rokaya

Introduction

Neurodegenerative diseases affect humans as they age and are characterized by losing specific groups of neurons in different brain regions (Figure 1). Although these disorders are generally sporadic, it is now clear that many of them have a substantial genetic component (Cookson, 2012) Gene mutations, gene dose, and promoter polymorphisms may affect protein levels and conformation. The pathogenesis of these disorders centrally involves abnormal accumulation and aggregation of specific proteins causing damage to various parts of the nervous system leading to dementia, muscular atrophy, and a wide array of symptoms. (Takalo et al., 2013) This study will discuss phylogenetic trees of genes associated with neurodegeneration, the evolution of these proteins, and the prevalence of such diseases.

Figure 1: Normal brain and degenerated brain. Source:

Genetic relationship

Using protein and gene databases, we found the sequences of different genes associated with neurodegenerative disease in different animals. And using the ClustalOmega tool, we utilized bioinformatics to find how these proteins evolved in the animal kingdom.

Huntington's disease

During chordate diversification, events of gain/loss, sliding, phase changes, and expansion of introns occurred in both vertebrate and ascidian lineages, predominantly in the 5'-half of the HTT gene, where there is also evidence of lineage-specific evolutionary dynamics in vertebrates. On the contrary, the 3'-half of the gene is highly conserved in all chordates at the level of both gene structure and protein sequence. (Gissin et al., 2006)

Microtubule-Associated Protein Tau (MAPT)

MAPT promotes assembly and interaction of microtubules with the cytoskeleton, impinging on axonal transport and synaptic plasticity. Mutations in this gene are associated with Alzheimer's disease and frontotemporal dementia. MAP4 is considered to originate in the earliest vertebrates (hagfish and lampreys), and subsequent duplication of a more evolved common ancestor led to the formation of MAPT and MAP2 as sister genes. (Sündermann et al., 2016)

Amyloid Protein Precursor (APP)

The amyloid's primary constituent is a hydrophobic peptide (bA4 or Ab), which is derived by proteolysis from the amyloid protein precursor. Phylogenetic analysis of APP-like proteins indicated that the evolution of the APP superfamily was a highly complex process forming at least three lineages: APP found so far in electric ray, amphibians, birds, rodents, and primates; APLP1 isolated from rodent and primate; and APLP2, also isolated from rodent and primate. The genes isolated from *D. melanogaster* and *C. elegans*, which had not previously been assigned to a lineage, appear to form a separate functional lineage, ancestral to the other. (Coulson et al., 2000)

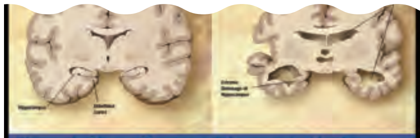
Figure 2: UPGMA-tree based on the sequences from translated protein of HTT. Sequences collected from NCBI. (Sievers et al., 2011; Goujon et al., 2010)

Figure 3: Phylogenetic tree of MAPT. (Sündermann et al., 2016)

Figure 4: UPGMA-tree based on the sequences from translated protein of APP. Sequences collected from NCBI. (Sievers et al., 2011; Goujon et al., 2010)

Are neurodegenerative diseases becoming common in present era?

Are the genes and proteins related to neurodegenerative diseases necessary to make a functioning neuron?



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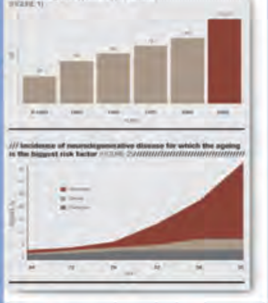

Figure 5: The image shown above gives that average life expectancy has increased over the hundreds of years which eventually loped the rate of neurodegenerative diseases in human. (Avila, 2018)

Observing the data we can assure that people are more prone to be affected by neurodegenerative diseases. However, the main reason for this is lifestyle, as that has been evolved highly compared to our ancestors in the past. People now consume high sugar diets, alcohol, high fat diets, etc.
About 25% of human body cholesterol is found in the brain. Since, cholesterol levels in brain influences the synthesis and toxicity of amyloid beta peptide which eventually accumulate in our brain and initiates neurodegeneration (Popa-Wagner et al, 2018) . However, the average life expectancy of human in present context is increasing year by year due to better medical health facilities. Humans are aging up to high average life expectancy rate as compared to past who previously used to live for just 30/40/50 years if we go half century back (Avila, 2018) as shown in figure 5. Therefore, with the increasing life expectancy and aging more people have been known to been affected by neurodegenerative disorders in present.

What is being done to find a cure to neurodegenerative diseases in future?

After the introduction of CRISPR gene editing in bioengineering medical field, we came to know that it has already been able to treat Huntington's disease up to some extent. It's effect on human has been drastically reduced by editing the genes that produce the protein responsible for neurodegeneration (Hueng Chuen Fan et al, 2013). However, we haven't found any basis for treating Alzheimer's like extreme neurodegenerative diseases.

Cannibalism which is recently been forwarded as an approach by scientists and researchers so escape from neurodegenerations has basis for believing after the tribe from Papua New Guinea who consumed their relatives brain at funeral developed resistance from neurodegenerative disorders (https://www.google.com.np/amp/s/amp.theguardian.com/science/2015/jun/10/brains-helped-papua-new-guinea-tribe, 2015). This is striking example of Darwinian evolution in humans, the epidemic of prion diseases selecting a single genetic change has found to provide complete protection against an invariantly fatal dementia. More research is yet to be done.

Are the genes and proteins related to neurodegenerative diseases necessary to make a functioning neuron?

Some of the genes and proteins associated with neurodegenerative diseases – like the SNCA gene and its protein α-synuclein – do not exist in invertebrates even though they have functional nervous system as well. This indicates that these genes are *not* required to make a functional neuron (Cookson, 2012).

What beneficial physiological function might proteins related to neurodegeneration have?

Since the proteins associated with neurodegeneration not necessarily are crucial for a functioning nervous system, it is logical to ask whether they have a beneficial physiological role in addition to the pathological one. To continue with α-synuclein, it is important in vesicle trafficking, synaptic transmission, and regulating the relationship between ER and mitochondria (Ottolini et al., 2017). α-synuclein also relates to plasticity (George et al., 1995) and neuroprotection when exposed to chronic oxidative stress (Quilty et al., 2006). Since humans are a long-lived-species, protection against such stress in combination with neuroplasticity may be important for the brain's functioning throughout the entire life. In that case, α-synuclein might actually have been selected for. This has led to a fine balance between expressing enough α-synuclein so it is beneficial for the brain, but not too much so that it cause neurodegeneration and is harmful itself (Cookson, 2012).

A common argument is that genes related to neurodegeneration are often said to be residues, but is that always the case?

To answer this, using a specific point mutation in the SNCA gene is a good example. Changing the amino acid in position 53 in the translated protein from alanine to threonine, is known to cause Parkinson's disease in humans (Polymeropoulos et al., 1997). However, threonine is the common variant in other vertebrates like mouse, cow and chicken, and also in New World primates. Old World monkeys and Great apes have on the other hand, alanine as their common variant (Hamilton 2004). This divergence between the primates is estimated to have happen about 35 million years ago. In evolutionary terms, 35 million years is not long. This indicates that Ala53 is not a residue (Cookson, 2012). Figure 6 shows the relationship between some species according to α-synuclein. We can clearly see that the protein is more similar between Great apes and Old World monkeys, compared to New World primates, even though this is just the unweighted one, so the amino acid in position 53 count just as much as the others.

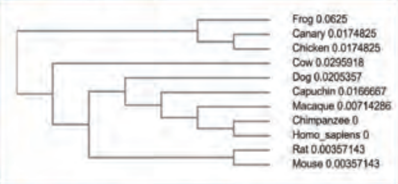

Figure 6: UPGMA-tree of α-synuclein. Sequences collected from NCBI. (Sievers et al., 2011; Goujon et al., 2010)

Neurodegeneration primarily refers to the loss of function of neurons due to over accumulation of specific proteins. Neurodegenerative diseases are mostly observed in humans along with other animals with aging. However, some of the proteins related to neurodegeneration, clearly have an important beneficial physiological role and are not just residues. Further defining how various genes and gene variants cause changes in aging brains that then lead to neurodegenerative diseases will enable doctors to diagnose the disease earlier and make new treatments.

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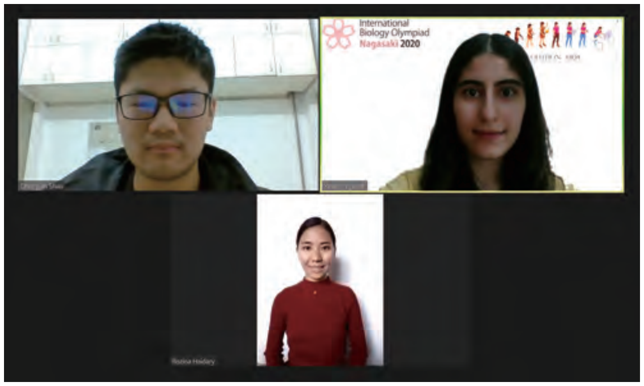
4B04 EVOLUTION



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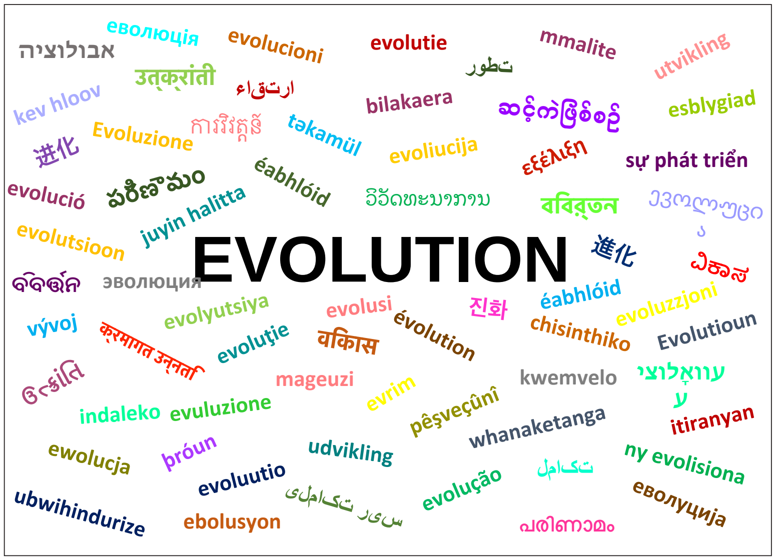

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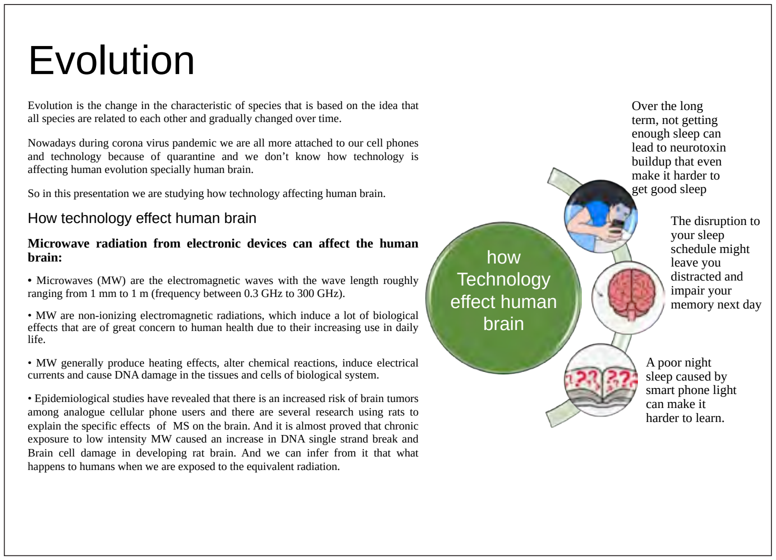


Evolution

Evolution is the change in the characteristic of species that is based on the idea that all species are related to each other and gradually changed over time.

Nowadays during corona virus pandemic we are all more attached to our cell phones and technology because of quarantine and we don't know how technology is affecting human evolution specially human brain.

So in this presentation we are studying how technology affecting human brain.

How technology effect human brain

Microwave radiation from electronic devices can affect the human brain:

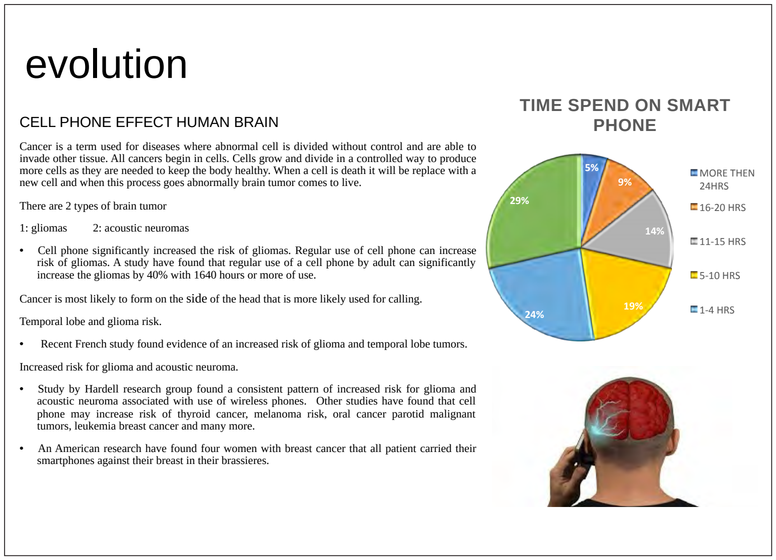
- Microwaves (MW) are the electromagnetic waves with the wave length roughly ranging from 1 mm to 1 m (frequency between 0.3 GHz to 300 GHz).
- MW are non-ionizing electromagnetic radiations, which induce a lot of biological effects that are of great concern to human health due to their increasing use in daily life.
- MW generally produce heating effects, alter chemical reactions, induce electrical currents and cause DNA damage in the tissues and cells of biological system.
- Epidemiological studies have revealed that there is an increased risk of brain tumors among analogue cellular phone users and there are several research using rats to explain the specific effects of MS on the brain. And it is almost proved that chronic exposure to low intensity MW caused an increase in DNA single strand break and Brain cell damage in developing rat brain. And we can infer from it that what happens to humans when we are exposed to the equivalent radiation.

how Technology effect human brain

Over the long term, not getting enough sleep can lead to neurotoxin buildup that even make it harder to get good sleep

The disruption to your sleep schedule might leave you distracted and impair your memory next day

A poor night sleep caused by smart phone light can make it harder to learn.



evolution

CELL PHONE EFFECT HUMAN BRAIN

Cancer is a term used for diseases where abnormal cell is divided without control and are able to invade other tissue. All cancers begin in cells. Cells grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When a cell is death it will be replace with a new cell and when this process goes abnormally brain tumor comes to live.

There are 2 types of brain tumor

1: gliomas 2: acoustic neuromas

- Cell phone significantly increased the risk of gliomas. Regular use of cell phone can increase risk of gliomas. A study have found that regular use of a cell phone by adult can significantly increase the gliomas by 40% with 1640 hours or more of use.

Cancer is most likely to form on the side of the head that is more likely used for calling.

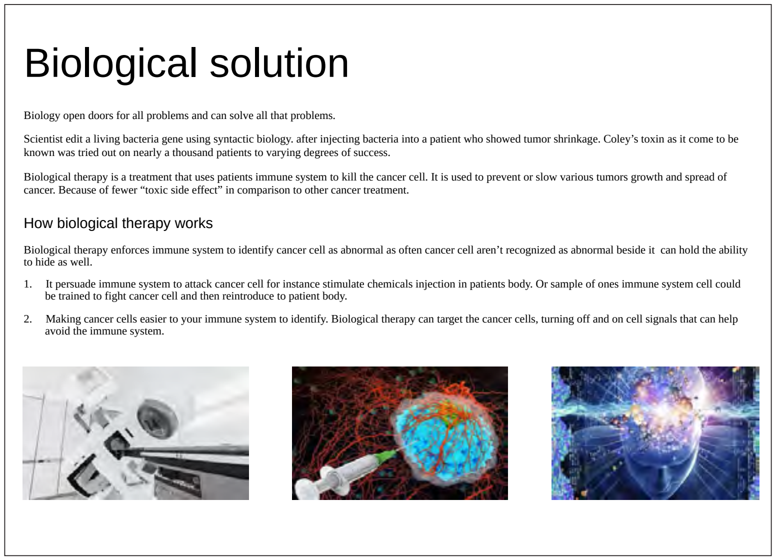
Temporal lobe and glioma risk.

- Recent French study found evidence of an increased risk of glioma and temporal lobe tumors.

Increased risk for glioma and acoustic neuroma.

- Study by Hardell research group found a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones. Other studies have found that cell phone may increase risk of thyroid cancer, melanoma risk, oral cancer parotid malignant tumors, leukemia breast cancer and many more.
- An American research have found four women with breast cancer that all patient carried their smartphones against their breast in their brassieres.

TIME SPEND ON SMART PHONE



Biological solution

Biology open doors for all problems and can solve all that problems.

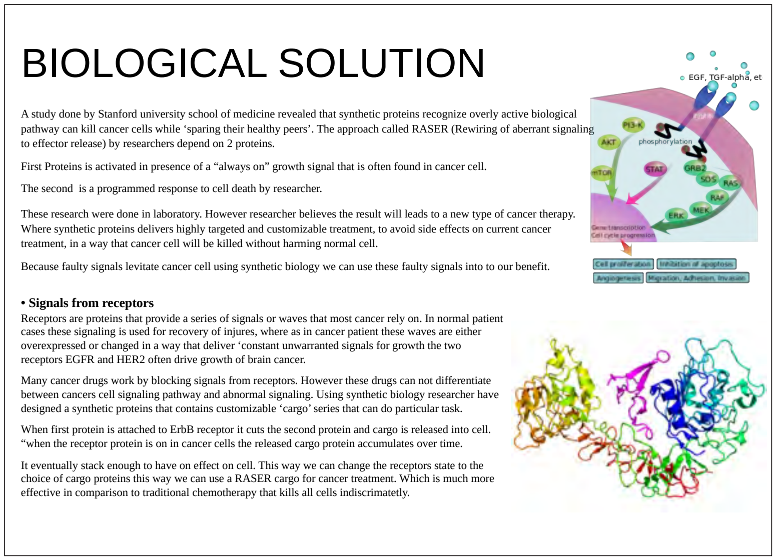
Scientist edit a living bacteria gene using syntactic biology. after injecting bacteria into a patient who showed tumor shrinkage. Coley’s toxin as it come to be known was tried out on nearly a thousand patients to varying degrees of success.

Biological therapy is a treatment that uses patients immune system to kill the cancer cell. It is used to prevent or slow various tumors growth and spread of cancer. Because of fewer “toxic side effect” in comparison to other cancer treatment.

How biological therapy works

Biological therapy enforces immune system to identify cancer cell as abnormal as often cancer cell aren’t recognized as abnormal beside it can hold the ability to hide as well.

1. It persuade immune system to attack cancer cell for instance stimulate chemicals injection in patients body. Or sample of ones immune system cell could be trained to fight cancer cell and then reintroduce to patient body.
2. Making cancer cells easier to your immune system to identify. Biological therapy can target the cancer cells, turning off and on cell signals that can help avoid the immune system.



BIOLOGICAL SOLUTION

A study done by Stanford university school of medicine revealed that synthetic proteins recognize overly active biological pathway can kill cancer cells while ‘sparing their healthy peers’. The approach called RASER (Rewiring of aberrant signaling to effector release) by researchers depend on 2 proteins.

First Proteins is activated in presence of a “always on” growth signal that is often found in cancer cell.

The second is a programmed response to cell death by researcher.

These research were done in laboratory. However researcher believes the result will leads to a new type of cancer therapy. Where synthetic proteins delivers highly targeted and customizable treatment, to avoid side effects on current cancer treatment, in a way that cancer cell will be killed without harming normal cell.

Because faulty signals levitate cancer cell using synthetic biology we can use these faulty signals into to our benefit.

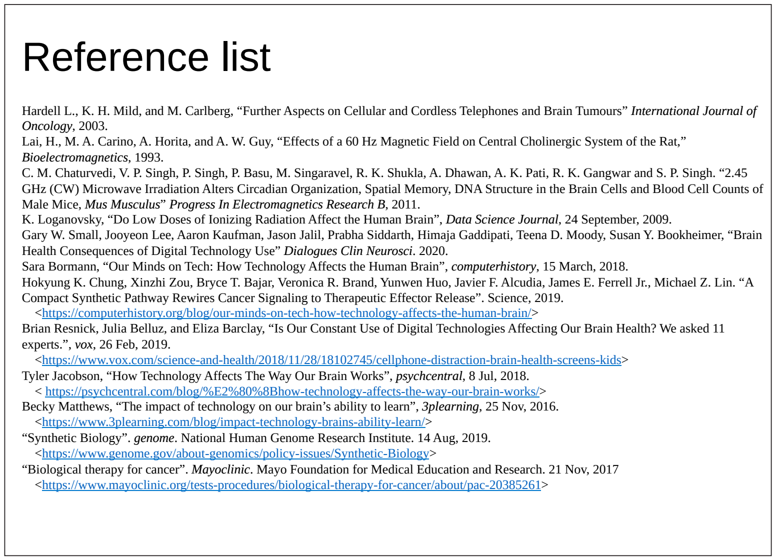
• Signals from receptors

Receptors are proteins that provide a series of signals or waves that most cancer rely on. In normal patient cases these signaling is used for recovery of injures, where as in cancer patient these waves are either overexpressed or changed in a way that deliver ‘constant unwarranted signals for growth the two receptors EGFR and HER2 often drive growth of brain cancer.

Many cancer drugs work by blocking signals from receptors. However these drugs can not differentiate between cancers cell signaling pathway and abnormal signaling. Using synthetic biology researcher have designed a synthetic proteins that contains customizable ‘cargo’ series that can do particular task.

When first protein is attached to ErbB receptor it cuts the second protein and cargo is released into cell. “when the receptor protein is on in cancer cells the released cargo protein accumulates over time.

It eventually stack enough to have on effect on cell. This way we can change the receptors state to the choice of cargo proteins this way we can use a RASER cargo for cancer treatment. Which is much more effective in comparison to traditional chemotherapy that kills all cells indiscrimatetly.



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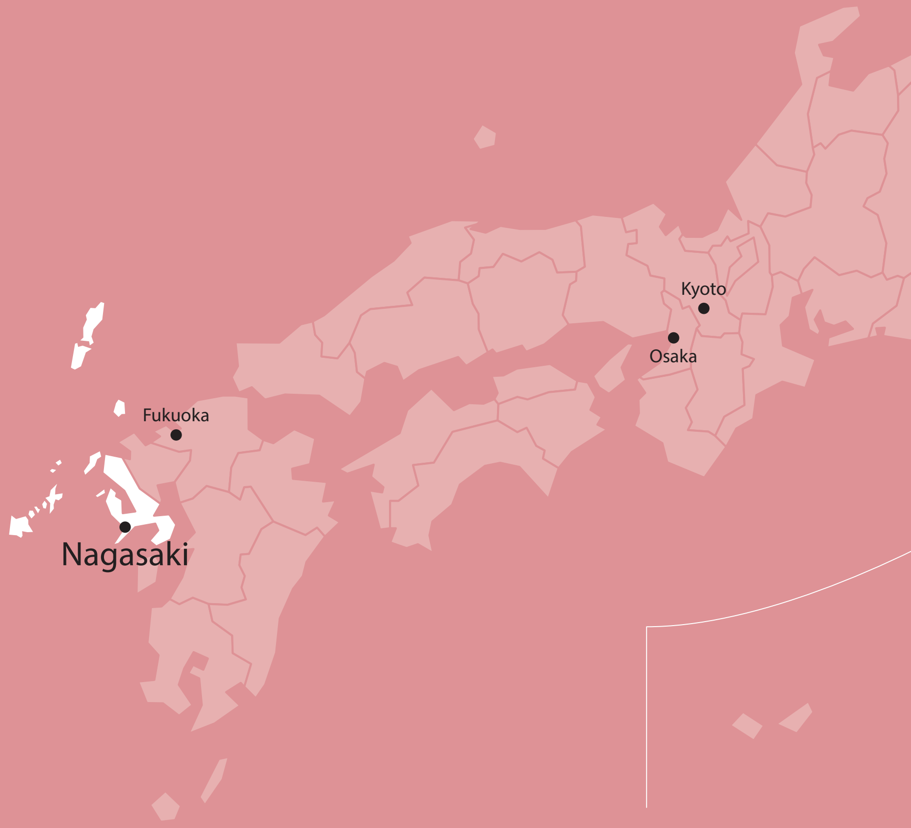
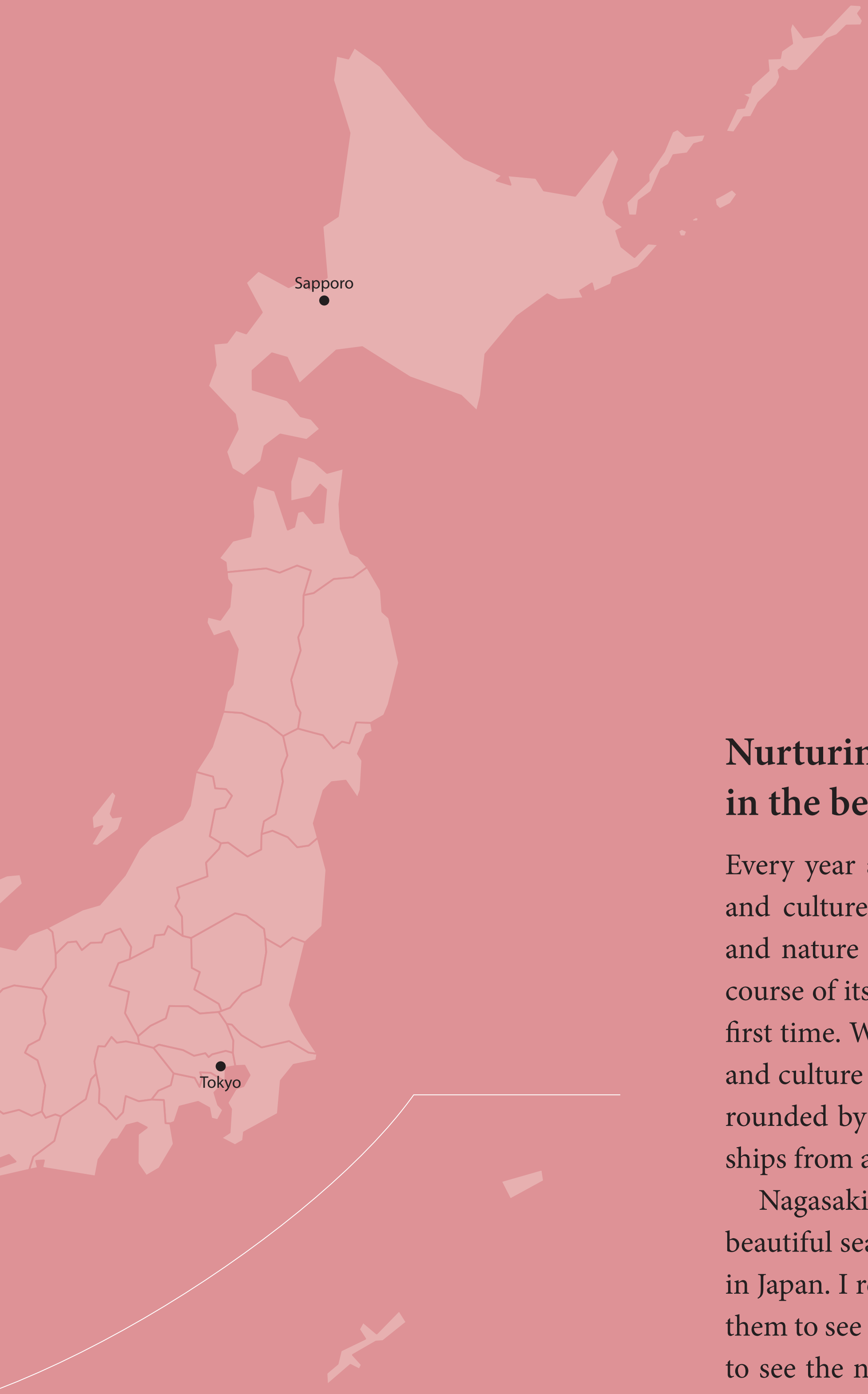
The 31st

IBO 2020 Nagasaki, Japan (Cancelled)

Thoughts Behind IBO2020 Nagasaki





Nurturing biology lovers and their friendships in the beautiful nature of Nagasaki

Every year at IBO, I become acquainted with the people, nature, and culture of the host country. I learn the traditions, wisdom, and nature of each country that have accumulated over the long course of its history. Most students would've come to Japan for the first time. We wanted all students to fully experience the traditions and culture of Japan, which were nurtured in a humid climate surrounded by the sea, and to foster biology lovers and their friendships from all over the world.

Nagasaki, the planned venue for this year, is surrounded by a beautiful sea and is a perfect environment for one's first experience in Japan. I really wanted students to see this beautiful sea. I wanted them to see the sunset over the sea. I thought it would be wonderful to see the nature of Nagasaki in the background of the memories that fostered friendships with friends from all over the world. But then, the coronavirus came to us.

Hiroshi Wada

About the Logo

International
Biology Olympiad
Nagasaki 2020

Our logo is inspired by *mizuhiki*, a traditional Japanese craft where colored rice paper cords are woven, knotted, and braided into intricate patterns and designs. The cords are conventionally made from washi, a type of unique, durable, and versatile paper made in Japan. Often used to commemorate special events or life milestones, you can find *mizuhiki* at traditional weddings (where it can be seen decorating a woman's hair), at holidays and festivals (where it often adorns New Year's decorations), and on washi envelopes that contain money or cards to mark a time of celebration or grief. It can also be used to help tie up the hair of sumo wrestlers or decorate the wigs of kabuki actors.

The type of knot featured on *mizuhiki* varies depending on the message one wishes to convey. For our *mizuhiki*-inspired logo, we chose to feature five strings, which represent the five rings of the Olympics. The cords are woven together to form a cherry blossom, which, aside from being an important national cultural symbol, also represents the concept of encounters, farewells, and strong, warm bonds. At IBO 2020, we strive to create an event where future world-leading biologists can gather, nurture deep friendships, and inspire each other to better the world.

(Kentaroh Honda, IBO2007 Former Competitor)

Schedule

Date	Competitors	Jury
3 July (Fri)	**Reception** Venue: Hotel Nikko Huis Ten Bosch **Opening Ceremony and Welcome Party** Venue: Arkas Sasebo, Sasebo City, Nagasaki	
4 July (Sat)	**Lab Instructions** **Exam Rehearsal** **Cultural Workshop** Nagasaki International University (NIU)	**Practical Exam Translation** Arkas Sasebo
5 July (Sun)	**Practical Exams** NIU	**Theoretical Exam Translation** Arkas Sasebo
6 July (Mon)	**Cultural Workshop (cont'd)** NIU	**Theoretical Exam Translation** Arkas Sasebo
7 July (Tue)	**Theoretical Exam** NIU	**IBO Educational Conference:** On the relevant use of new technologies in Life Sciences Education **Cultural Workshop** Arkas Sasebo
	Cultural Night (SASEBO Night)	
8 July (Wed)	**Excursion #1** **Atomic Bomb Hypocenter Park** **Unzen Volcano, etc.** Nagasaki City & Unzen City	**Excursion** **Choose one from "Nagasaki City," "Volcano," and "Ocean"** Nagasaki Prefecture
9 July (Thu)	**Excursion #2** **White Beach SASEBO** – Scientific Activity of Shore Exploration in the Biology Olympiad Shirahama Beach, Sasebo	**Results Review, Ranking** **General Assembly Meeting** Arkas Sasebo
10 July (Fri)	**White Beach SASEBO** **Poster Session** Arkas Sasebo	**Free**
	Closing Ceremony and Farewell Party Arkas Sasebo and Hotel Flags Kujukushima Nagasaki	
11 July (Sat)	**Departure Day**	

White Beach SASEBO

Seaside **A**ctivity and **S**hore **E**xploration in the **B**iology **O**lympiad

Opportunity to collaborate internationally over nature exploration

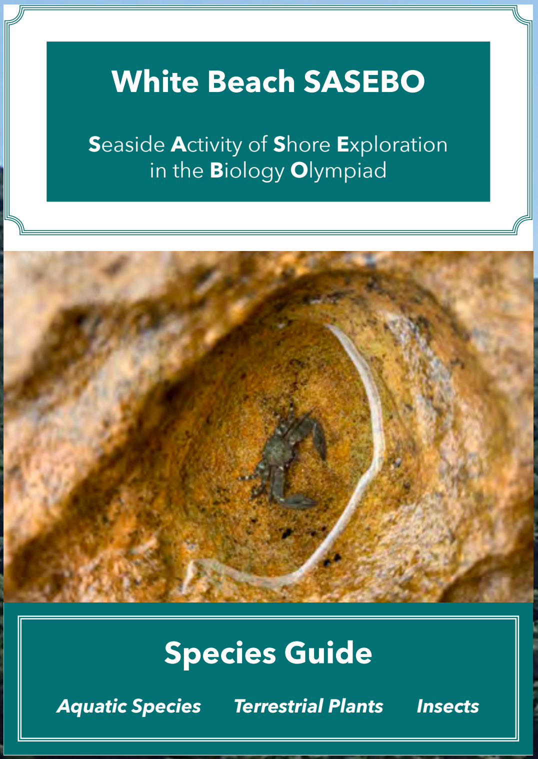
Our planet is suffering. We must do something now, but nobody is a superhero; our problems are beyond the ability of any single person. It is time to collaborate with each other, and we thought it is not a bad thing if this IBO could provide our smart students one of their first opportunities for international collaboration. This idea excited us a lot.

We also believed that by sharing a common goal, the students might be able to cultivate deeper relationships. Students who are too shy to play card games with other competitors may be much more excited in the field. Considering those goals, we planned our project, White Beach SASEBO, where groups of students would make presentations stemming from their experiences in the field. The venue, Nagasaki, was an ideal place for that.

In the field, I wanted students to experience that real nature is far beyond the textbook. We wanted them to feel that they knew very little about biology in comparison to the many questions we still need to answer. We decided that the task of the students would be to find research questions. In science, finding a question is more important than finding an answer. In fact, in many cases, when you find a question, you already know its answer. Changing unknown unknowns to known unknowns is more important than changing known unknowns to known knowns!

Although we couldn't do this in Nagasaki this year, let's explore nature more! (Hiroshi Wada)

White Beach SASEBO—Species Guide



White Beach SASEBO

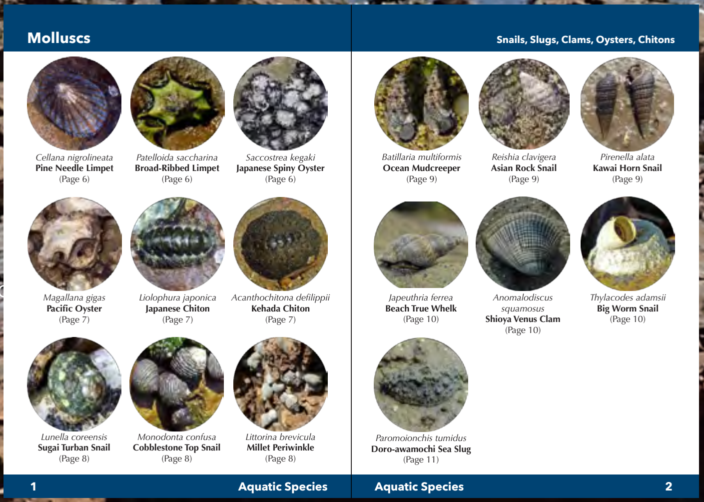
Seaside **A**ctivity of **S**hore **E**xploration
in the **B**iology **O**lympiad

Species Guide

Aquatic Species *Terrestrial Plants* *Insects*

In preparation for the international fieldwork activity at Shirahama Beach in Sasebo City, Nagasaki, IBO2020 created a species guide that introduced aquatic and terrestrial species found locally in the area.

Creator: Siri McGuire, IBO2020 Secretariat Office
In collaboration with: Yoshiharu Kawachino



Molluscs

Cellana nigrolineata
Pine Needle Limpet
(Page 6)

Patelloida saccharina
Broad-Ribbed Limpet
(Page 6)

Saccostrea kegaki
Japanese Spiny Oyster
(Page 6)

Magallana gigas
Pacific Oyster
(Page 7)

Liolophura japonica
Japanese Chiton
(Page 7)

Acanthochitona defilippii
Kehada Chiton
(Page 7)

Lunella coreensis
Sugai Turban Snail
(Page 8)

Monodonta confusa
Cobblestone Top Snail
(Page 8)

Littorina brevicula
Millet Periwinkle
(Page 8)

1 Aquatic Species

Snails, Slugs, Clams, Oysters, Chitons

Batillaria multiformis
Ocean Mudcreeper
(Page 9)

Reishia clavigera
Asian Rock Snail
(Page 9)

Pirenella alata
Kawai Horn Snail
(Page 9)

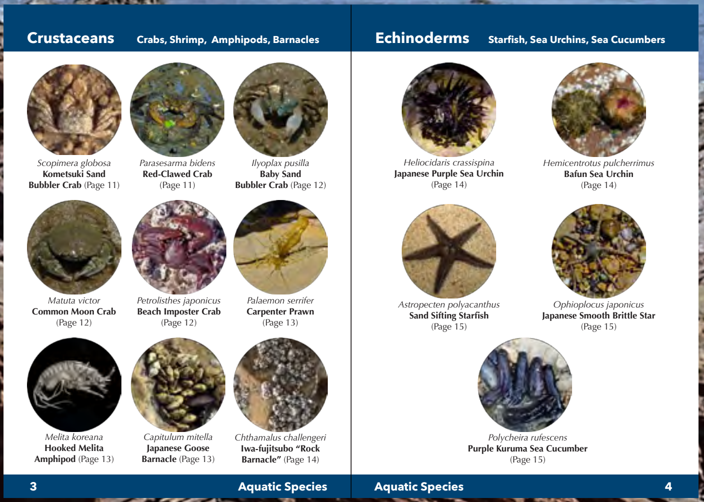
Japeuthria ferrea
Beach True Whelk
(Page 10)

Anomalodiscus squamosus
Shioya Venus Clam
(Page 10)

Thylacodes adamsii
Big Worm Snail
(Page 10)

Paromoionchis tumidus
Doro-awamochi Sea Slug
(Page 11)

Aquatic Species 2



Crustaceans Crabs, Shrimp, Amphipods, Barnacles

Scopimera globosa
Kometsuki Sand Bubbler Crab (Page 11)

Parasesarma bidens
Red-Clawed Crab
(Page 11)

Ilyoplax pusilla
Baby Sand Bubbler Crab (Page 12)

Matuta victor
Common Moon Crab
(Page 12)

Petrolisthes japonicus
Beach Imposter Crab
(Page 12)

Palaemon serrifer
Carpenter Prawn
(Page 13)

Melita koreana
Hooked Melita Amphipod (Page 13)

Capitulum mitella
Japanese Goose Barnacle (Page 13)

Chthamalus challengeri
Iwa-fujitsubo "Rock Barnacle" (Page 14)

3 Aquatic Species

Echinoderms Starfish, Sea Urchins, Sea Cucumbers

Heliocidaris crassispina
Japanese Purple Sea Urchin
(Page 14)

Hemicentrotus pulcherrimus
Bafun Sea Urchin
(Page 14)

Astropecten polyacanthus
Sand Sifting Starfish
(Page 15)

Ophioplocus japonicus
Japanese Smooth Brittle Star
(Page 15)

Polycheira rufescens
Purple Kuruma Sea Cucumber
(Page 15)

Aquatic Species 4

Cnidarians Jellyfish, Sea Anemones

Aurelia aurita
Moon Jellyfish (Page 16)

Actinia equina
Beadlet Anemone (Page 16)

Bristleworms

Spirobranchus kraussii
Blue Coral-Worm (Page 16)

Sabellidae
Feather Duster Worm (Page 17)

Tunicates

Aplidium pliciferum
Manju Sea Squirt (Page 17)

Algae

Padina arborescens
Ocean Fan Algae (Page 17)

5 Aquatic Species

Cellana nigrolineata
Pine Needle Limpet
マツバガイ (Matsubagai)

Where to find it: On rocky shores of the upper intertidal zone. Usually attached to rock surfaces and the cracks in rocks.

What it looks like: It has a flat or slightly conical shell. The shells are 5 cm long with radial black lines on the surface that look like pine tree needles.

More about this species: It is one of the largest and most common species of limpets found in Japan. They feed by grazing or scraping the algae off of rocks using a tongue-like structure called a radula.

Patelloida saccharina
Broad-Ribbed Limpet
ウノアシガイ (Unoashigai)

Where to find it: Attached to rock surfaces near the high tide line along the coast.

What it looks like: The shell is typically 3-4 cm long and is flat to slightly conical in shape. The shell has a distinct pattern that resembles the foot of a cormorant.

More about this species: When the tide is low, they do not move, and when the tide is high, they go around and search for food. Like other limpets, they possess a particularly strong radula.

Saccostrea kegaki
Japanese Spiny Oyster
ケガキ (Ke-gaki)

Where to find it: Attached to rocks in the intertidal zone along the coast or near a river mouth.

What it looks like: The shell is usually about 10 cm long. It is characterized by the many spine-like projections it has on its shell.

More about this species: The spiny projections on the oyster shells are quite sharp. Please watch your step to avoid injury.

Aquatic Species 6

Magallana gigas
Pacific Oyster
マガキ (Ma-gaki)

Where to find it: On top of a rock or stone in the intertidal zone along the coast of an ocean inlet or an estuary.

What it looks like: The shell length is usually about 10 cm. It can be distinguished from *Saccostrea kegaki* by the lack of spiny projections on its shell.
Its shell edges are very sharp.

More about this species: Though native to the Pacific coast of Asia, this species has been introduced in North and South America, Australia, and Africa. In some places it is known as an invasive species.

Liolophura japonica
Japanese Chiton
ヒザラガイ (Hizara-gai)

Where to find it: Clinging to rock surfaces along the ocean coast.

What it looks like: It is about 6 cm long, with a shell divided into eight pieces called "valves" that are arranged in a row and surrounded by a skirt called a girdle.

More about this species: Chitons are a primitive type of mollusk and often resemble living fossils. In Japanese, the name means "kneecap shellfish" because if you remove it from the rock, it will curl like a knee.

Acanthochitona defilippii
Kehada Chiton
ケハダヒザラガイ (Kehada-hizara-gai)

Where to find it: Attached to rocks along rocky ocean coasts in the Western Pacific.

What it looks like: This species is 4-5 cm long with rows of spines on its surface. Its 8 plates or "valves" appear narrower than the Japanese Chiton (see above) and are surrounded by a thick skirt or "girdle."

More about this species: This species grazes on algae attached to rocks using a tongue-like structure called a radula.

7 Aquatic Species

Lunella coreensis
Sugai Turban Snail
スガイ (Su-gai)

Where to find it: On top of a stone or in the gaps between stones on the beach.

What it looks like: Its shell is about 2.5 cm in diameter and is usually covered with green algae (*Pseudocladophora conchopheria*).

More about this species: Snails of this family (Turbinidae) are called "turban snails" and have thick shell lids called operculums that are made mostly of calcium carbonate (compare with "top snails" like *Mondonta confusa* below).

Monodonta confusa
Cobblestone Top Snail
イシダタミガイ (Ishidatami-gai)

Where to find it: On top of a stone or in the gaps between stones on the beach.

What it looks like: Adult shells are about 2.5 cm in both length and diameter. It has a characteristic cobblestone pattern on its shell which gives it its name.

More about this species: When the tide rises, they become active and feed on algae. Unlike the the calcium-carbonate shell lids of turban snails, top snails (Trochidae) have a corneous shell lid made from a protein called conchiolin.

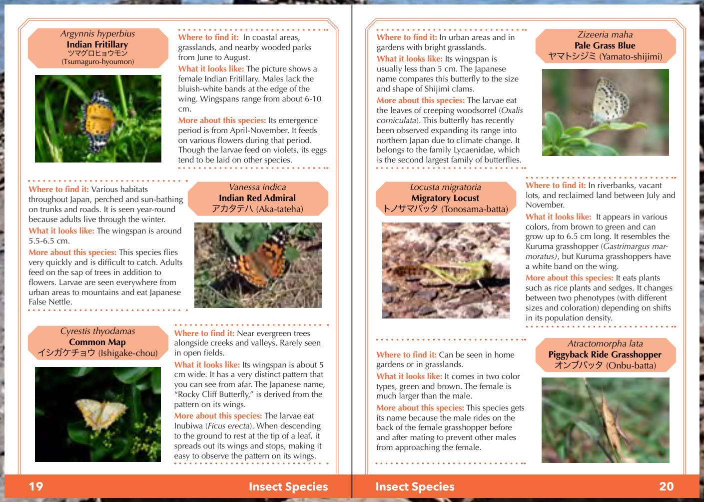
Littorina brevicula
Millet Periwinkle
タマキビガイ (Tamakibi-gai)

Where to find it: Living in groups on top of rocks or in the concave areas of rock in the intertidal zone.

What it looks like: Its shell is about 1.7 cm long, 1.4 cm in diameter, and has small, millet-like bumps on its surface.

More about this species: They usually live on land, are resistant to drying, and dislike water. At high tide, they go along the wet rocky surface eating seaweed.

Aquatic Species 8



Argynnis hyperbius
Indian Fritillary
ツマグロヒョウモン
(Tsumaguro-hyoumon)

Where to find it: In coastal areas, grasslands, and nearby wooded parks from June to August.

What it looks like: The picture shows a female Indian Fritillary. Males lack the bluish-white bands at the edge of the wing. Wingspans range from about 6-10 cm.

More about this species: Its emergence period is from April-November. It feeds on various flowers during that period. Though the larvae feed on violets, its eggs tend to be laid on other species.

Vanessa indica
Indian Red Admiral
アカタテハ (Aka-tateha)

Where to find it: Various habitats throughout Japan, perched and sun-bathing on trunks and roads. It is seen year-round because adults live through the winter.

What it looks like: The wingspan is around 5.5-6.5 cm.

More about this species: This species flies very quickly and is difficult to catch. Adults feed on the sap of trees in addition to flowers. Larvae are seen everywhere from urban areas to mountains and eat Japanese False Nettle.

Cyrestis thyodamas
Common Map
イシガケチョウ (Ishigake-chou)

Where to find it: Near evergreen trees alongside creeks and valleys. Rarely seen in open fields.

What it looks like: Its wingspan is about 5 cm wide. It has a very distinct pattern that you can see from afar. The Japanese name, "Rocky Cliff Butterfly," is derived from the pattern on its wings.

More about this species: The larvae eat Inubiwa (*Ficus erecta*). When descending to the ground to rest at the tip of a leaf, it spreads out its wings and stops, making it easy to observe the pattern on its wings.

19 Insect Species

Zizeeria maha
Pale Grass Blue
ヤマトシジミ (Yamato-shijimi)

Where to find it: In urban areas and in gardens with bright grasslands.

What it looks like: Its wingspan is usually less than 5 cm. The Japanese name compares this butterfly to the size and shape of Shijimi clams.

More about this species: The larvae eat the leaves of creeping woodsorrel (*Oxalis corniculata*). This butterfly has recently been observed expanding its range into northern Japan due to climate change. It belongs to the family Lycaenidae, which is the second largest family of butterflies.

Locusta migratoria
Migratory Locust
トノサマバッタ (Tonosama-batta)

Where to find it: In riverbanks, vacant lots, and reclaimed land between July and November.

What it looks like: It appears in various colors, from brown to green and can grow up to 6.5 cm long. It resembles the Kuruma grasshopper (*Gastrimargus marmoratus)*, but Kuruma grasshoppers have a white band on the wing.

More about this species: It eats plants such as rice plants and sedges. It changes between two phenotypes (with different sizes and coloration) depending on shifts in its population density.

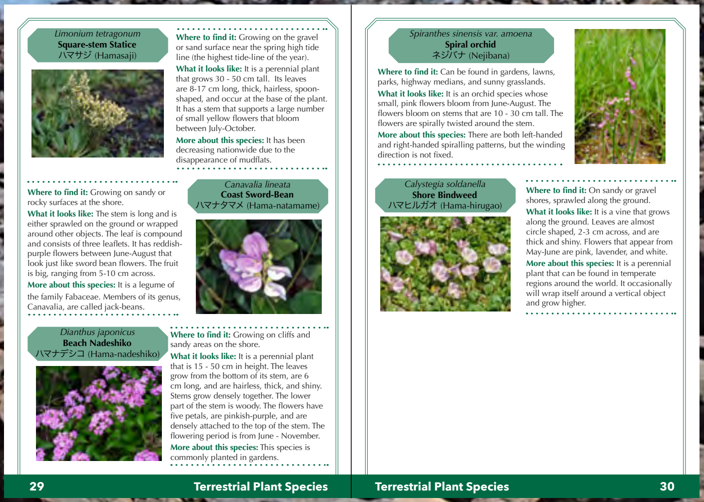
Atractomorpha lata
Piggyback Ride Grasshopper
オンブバッタ (Onbu-batta)

Where to find it: Can be seen in home gardens or in grasslands.

What it looks like: It comes in two color types, green and brown. The female is much larger than the male.

More about this species: This species gets its name because the male rides on the back of the female grasshopper before and after mating to prevent other males from approaching the female.

Insect Species 20



Limonium tetragonum
Square-stem Statice
ハマサジ (Hamasaji)

Where to find it: Growing on the gravel or sand surface near the spring high tide line (the highest tide-line of the year).

What it looks like: It is a perennial plant that grows 30 - 50 cm tall. Its leaves are 8-17 cm long, thick, hairless, spoon-shaped, and occur at the base of the plant. It has a stem that supports a large number of small yellow flowers that bloom between July-October.

More about this species: It has been decreasing nationwide due to the disappearance of mudflats.

Canavalia lineata
Coast Sword-Bean
ハマナタマメ (Hama-natamame)

Where to find it: Growing on sandy or rocky surfaces at the shore.

What it looks like: The stem is long and is either sprawled on the ground or wrapped around other objects. The leaf is compound and consists of three leaflets. It has reddish-purple flowers between June-August that look just like sword bean flowers. The fruit is big, ranging from 5-10 cm across.

More about this species: It is a legume of the family Fabaceae. Members of its genus, Canavalia, are called jack-beans.

Dianthus japonicus
Beach Nadeshiko
ハマナデシコ (Hama-nadeshiko)

Where to find it: Growing on cliffs and sandy areas on the shore.

What it looks like: It is a perennial plant that is 15 - 50 cm in height. The leaves grow from the bottom of its stem, are 6 cm long, and are hairless, thick, and shiny. Stems grow densely together. The lower part of the stem is woody. The flowers have five petals, are pinkish-purple, and are densely attached to the top of the stem. The flowering period is from June - November.

More about this species: This species is commonly planted in gardens.

29 Terrestrial Plant Species

Spiranthes sinensis var. amoena
Spiral orchid
ネジバナ (Nejibana)

Where to find it: Can be found in gardens, lawns, parks, highway medians, and sunny grasslands.

What it looks like: It is an orchid species whose small, pink flowers bloom from June-August. The flowers bloom on stems that are 10 - 30 cm tall. The flowers are spirally twisted around the stem.

More about this species: There are both left-handed and right-handed spiralling patterns, but the winding direction is not fixed.

Calystegia soldanella
Shore Bindweed
ハマヒルガオ (Hama-hirugao)

Where to find it: On sandy or gravel shores, sprawled along the ground.

What it looks like: It is a vine that grows along the ground. Leaves are almost circle shaped, 2-3 cm across, and are thick and shiny. Flowers that appear from May-June are pink, lavender, and white.

More about this species: It is a perennial plant that can be found in temperate regions around the world. It occasionally will wrap itself around a vertical object and grow higher.

Terrestrial Plant Species 30

Japan Cultural Workshop

We planned to welcome you with a variety of cultural workshops and activities that were carefully designed to showcase many aspects unique to Japanese culture. Each workshop booth was going to be staffed by Nagasaki International University volunteers and former Japan Biology Olympiad competitors (SCIBO ➔ page 202).

Cultural Workshop Special Activity

Tea Ceremony

Overview

In this workshop, you would have participated in and learned about sado, which is a traditional Japanese tea ceremony. However, sado is about much more than tea: according to Nagasaki International University, the host of this workshop, sado contains many aspects of Japanese culture, including calligraphy, flower arrangement, incense, and pottery. For the tea ceremony experience, you also would have learned how to sit in the traditional seiza style on a tatami floor, both of which are unique to Japanese culture.

Sado and Nagasaki International University (NIU)

The hospitality, grace, and dignity one can learn from the tea ceremony is the "spirit of NIU," and is an embodiment of the university's founding principles. Because of this, NIU requires students to take a course about the history, meaning, and importance of the tea ceremony, so that students can take that "spirit" into whatever fields or professions they choose.

Cultural Workshop Special Activity

Zazen Meditation

Overview

In this special activity, we planned for you to first experience zazen (which means "seated meditation") at a zazen hall in Saihoji Temple, located within Sasebo City. According to the vice priest of the temple, this is an activity that people of all religious beliefs can enjoy. After the zazen experience, we planned to show you some facilities of the temple. The temple is also in possession of some samurai armor, which some people would have had the opportunity to try on and take photos with. Afterwards, we planned to treat you to the temple's special Japanese premium soft cream with some Japanese fruit juice.

Buddhism and Zen in Japan

Buddhism has had a large influence on Japanese history and culture ever since it was introduced to Japan in the 6th century. Participation in its traditions is common among the vast majority of people in Japan. Weddings, funerals, memorial services, celebrations, and zazen sessions are some of the many services that Buddhist temples provide for people in their communities.

Zen Buddhism is one of the many schools of Buddhism found in Japan. Zen emphasizes the importance of meditative practice to gain insight into the nature of existence. Around the 13th century, Master Dogen, a Japanese Buddhist monk, established the Soto branch of Zen Buddhism, which is the largest of the three major schools of Zen found in Japan. Among many examples of Soto Zen temples in Japan is Saihoji Temple, where one of IBO2020's Cultural Workshop Special Activities would have taken place.

Japan Cultural Workshop

Wearing Traditional Clothes

Competitors would have been able to choose and wear various traditional clothes of Japan, such as kimono, yukata, Judo wear, and Kendo (traditional sword-fighting) gear. In addition, they would have been able to take some photos at a special photo booth and bring them home as souvenirs.

Experiencing Japanese Calligraphy

Competitors would have been shown how to write their own names in Kanji (Chinese characters) based on a sample written and provided by a master calligrapher.

Nagasaki in Kanji

Playing Traditional Games in Japan

Competitors would have played traditional Japanese games, such as Shogi, Hanafuda, Hyakunin-isshu, Kendama, Daruma-otoshi, Hagoita, and Koma with the help of Japanese university volunteers.

Making Traditional Crafts in Japan

Competitors would have made small traditional crafts from Japan, such as Kumihimo, Mizuhiki, Origami, Tsumami-zaiku, and brought them home as souvenirs.

Learning Japanese Dance

Originating in the Okinawa and Amami regions of Japan, Eisa is one of the most well-known and popular traditional dances in Japan. With the instructions of local dancers at NIU, competitors would have learned how to dance Eisa, with a possibility to perform on stage during the cultural night.

Excursions

Competitor Excursion

Along with Hiroshima, Nagasaki is one of two cities in the world that experienced the atomic bombing. To remember this, we planned the competitor's excursion to first visit the Hypocenter Park (the exact place where the bomb was dropped) and the Nagasaki Atomic Bomb Museum.

After that, competitors were going to visit Mt. Unzen, an active volcano in southern Nagasaki, and the Shimabara castle at the foot of the volcano.

Jury Excursion

To accommodate the various interests of our worldwide jury members, we prepared three different excursion packages to enjoy the beautiful nature and culture of Nagasaki.

After visiting the Nagasaki Atomic Bomb Museum, jury members could have chosen to visit:

1. The Kujukushima Archipelago and Shirahama Beach, where competitors would have conducted their fieldwork activity,
2. Mt. Unzen, an active volcano, and Shimabara Castle, or
3. Tanada (step-like rice fields) and a hidden, historic pottery town in the mountains.

Hypocenter Park (the exact place where the bomb was dropped)

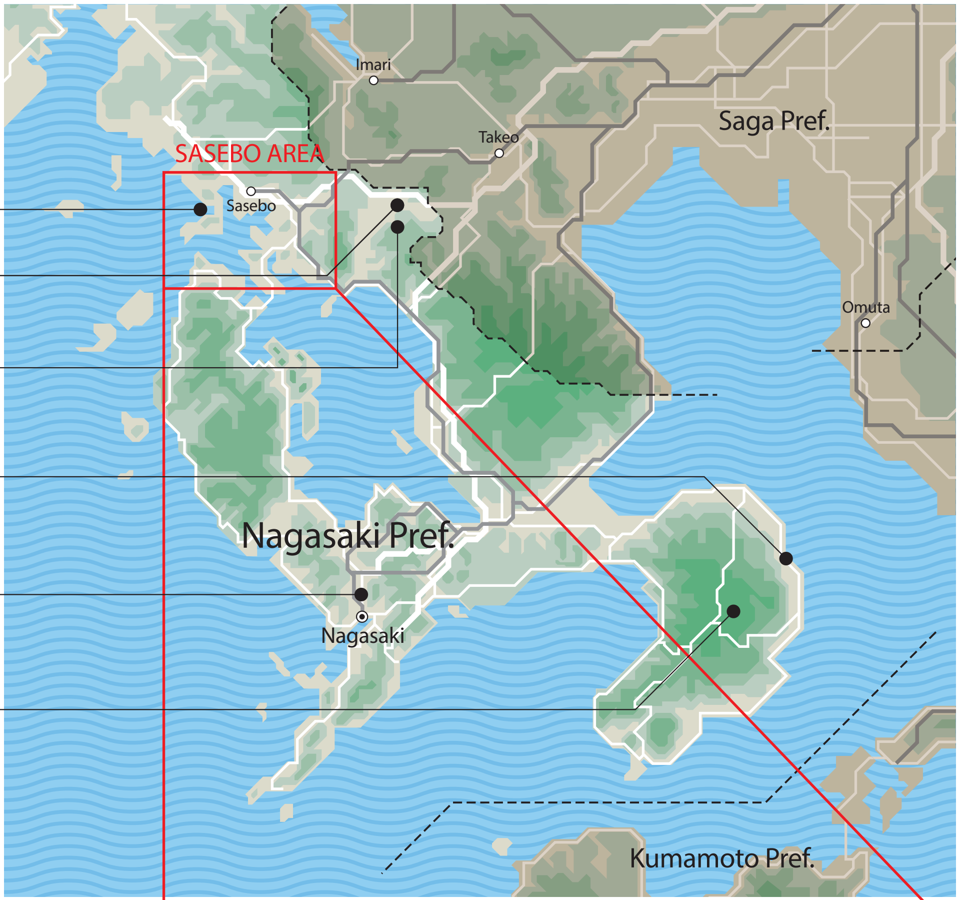
Mt. Unzen, an active volcano

The Kujukushima Archipelago

Shimabara Castle

Tanada (Step-like Rice Fields)

NAGASAKI MAP



The Kujukushima Archipelago

Historic Pottery Town

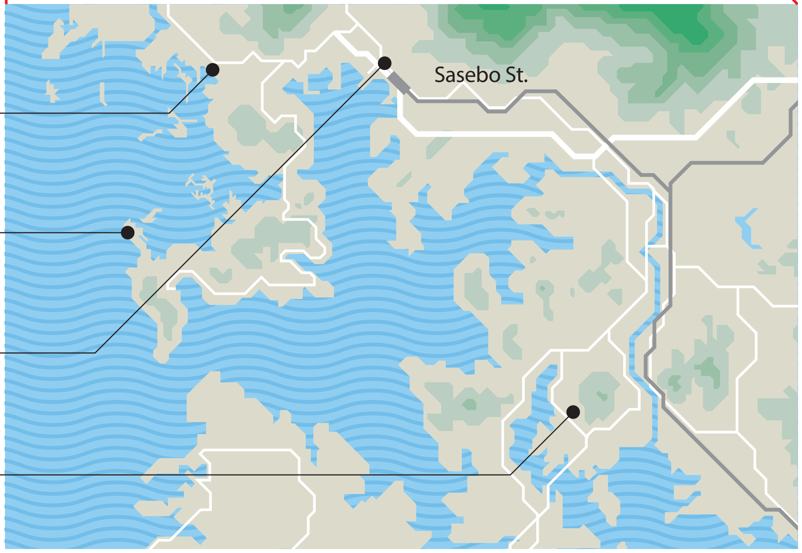
Tanada (Step-like Rice Fields)

Shimabara Castle

Hypocenter Park
Nagasaki Atomic Bomb Museum

Mt. Unzen

SASEBO AREA MAP



Farewell party venue

Hotel Flags Kujukushima Nagasaki

White Beach SASEBO venue

The Saikai National Park

Jury meeting and opening ceremony venue

Arkas Sasebo

Exam venue

Nagasaki International University

About International Volunteers

After years of participation in the IBO, one thing became clear to us: the positive impact of IBO alumni on the IBO community and how important it is for the future of this event. However, the opportunities for IBO alumni to "come back" to the event were limited; some became jury members after a while, but only a certain amount could do that every year.

When we were thinking about a good way to involve more IBO alumni in the event, we learned that the IBO2019 in Hungary recruited some of their team guides internationally, mostly from IBO alumni. We were delighted to hear the news and thought that we could continue and expand this trend during our event. This idea became more realistic when we realized that it was impossible to find enough Japanese volunteers due to their conflicting academic responsibilities; the IBO2020 period was not going to overlap with the summer vacation of Japanese university students.

There were some obstacles that initially concerned us. First, Japan was located far away from a lot of participating countries, which made their travel expenses extremely high, especially during summer. Second, some organizers showed concern that internationally recruited volunteers would not be able to properly introduce Japanese culture, which was traditionally one of the main duties of team guides in the IBO.

In late July of 2019, more than 11 months prior to the IBO2020, we announced the opportunity to the alumni community. Despite our concerns, this quickly received a lot of interests, not only from the alumni, but also from some former volunteers in the previous IBOs. In the end, we received 133 applications from more than 35 countries and regions across the world. By February 2020, our volunteer coordinators accepted 69 volunteers by reviewing all of their applications and interviewing more than 100 of them via Skype.

It is worth noting that every single applicant was full of passion (and even love, for some) towards IBO. Many of them, if not all, wrote on their applications that the IBO was "the best week of their life." As the host country, we would like to once again show our utmost appreciation to such enthusiasm and strongly believe that the IBO community must cherish it as our wonderful asset.

After reading the applications, nobody in our organization was concerned about their lack of knowledge in Japanese culture. Rather, we were extremely excited that our international team guides would be able to act as a role model for competitors, not only by sharing their advanced biological knowledge, but also by offering some emotional support as someone who had been through the same nerve-wrecking IBO process.

By the time we accepted all the volunteers, however, the COVID-19 pandemic was starting to take over the entire world. Due to a series of sudden changes in our plan caused by the rapidly changing situation, we had to admit that we burdened the international volunteers greatly at multiple occasions. At every step of this painful process up until the cancellation of the IBO2020 Nagasaki, we appreciated their patience and understanding towards our operations.

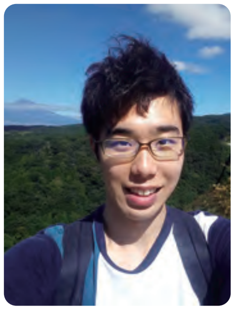
Because of all the pleasant interactions with the volunteers, we kept seeking ways to still involve them in our new event, the remotely held IBO Challenge 2020. When we decided to organize the International Group Project following the exam, we didn't think twice to create a supporting position and offer it to the accepted IBO2020 volunteers. Even though their responsibilities were vastly different from the team guide position, more than half of them registered to become facilitators for the group project.

We cannot stress enough that the IBO alumni are the future of this wonderful IBO community. Although we could not host a physical event this year, we truly hope that we can keep offering them active roles in our community.

(Taiga Araki, IBO2012 Former Competitor)

Team Guide Leaders


Ayaka Eguchi


Eichiro Kanatsu


Fumika Hemmi

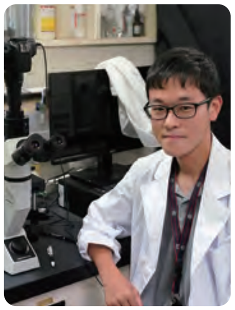

Midori Kajitani


Riho Horie

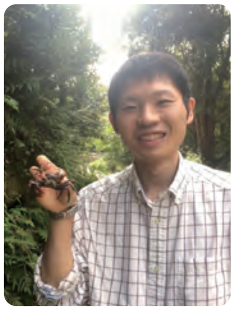
Ryota Takemoto


Shino Suda


Shusuke Atoji


Tomoyuki Wakashima


Uzuki Horo


Yuiki Kondo


Yuki Koshida
IESO2017 Former Competitor

About International Volunteers

Team Guides


Alexandru Golic
(Sweden)


Alfred Petersson
(Sweden)


Alisia Zink
(Germany)


Chun-Wei Liu
(Chinese Taipei)


David Barnabas Balogh
(Hungary)


Dumitrita Ungureanu
(Moldova)


Elena Lacroix
(Belgium)


Hsiang Ting Wu
(Chinese Taipei)


Ioannis Stouras
(Greece)


Iskra Jovanovska
(North Macedonia)


Jessica Law
(Australia)

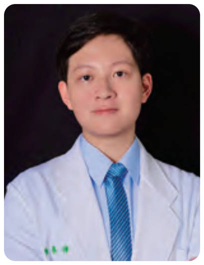

Kai-Na Chiu
(Chinese Taipei)

Katherine Lister
(UK)

Magdy Mekdad
(Romania)

Otso Lauri Tapio Nieminen
(Finland)

Shermane Yun Wei Lim
(Singapore)



Tai-Yi Chen
(Chinese Taipei)

Zack Dominic Orlina
(Philippines)

Zi Lin Wang
(New Zealand)

Aino Kilpeläinen (Finland)
Alkmini Zania (Greece)
Anna Li (Hungary)
Chen-Yu Lu (Chinese Taipei)
Daniel Istvan Papvari (Hungary)
Dilshan Weerasinghe (Sri Lanka)
Dóra Katalin Juhász (Poland)
Harper Kirschner-Sroka (Bulgaria)
Ivan Georgiev Georgiev (Poland)
Jaromir Hunia (Poland)
Joseph Aguilar (USA)
Ma Luisa Aurora Saenz Pascual (Philippines)
Muhammad Salman (Pakistan)
Saad Khan (Pakistan)
Sai Campbell (Australia)
Shristi Kunwar (Nepal)
Sin-Yuan Chien (Chinese Taipei)
Vahini Jessica Moodley (South Africa)
Zipei Tan (China)

About International Volunteers

Jury Guides


Yotaro Sueoka


Ren Ishida

Workshop Guides (SCIBO Volunteers)

SCIBO: Student committee of International Biology Olympiad in JAPAN


Ayami Yamanaka

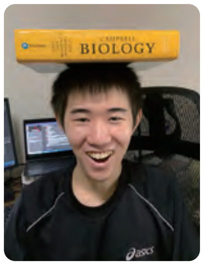

Ayana Tanino


Haruki Ishida


Kazuyuki Sanada

Kohei Oshima

Appreciate the moment.



Kou Takahashi


Masahiro Sakono


Mito Hotta


Ryo Suda

Find wonder in every day!

Executive Committee Members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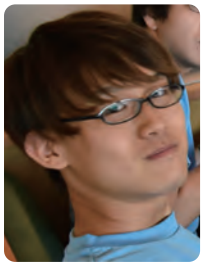
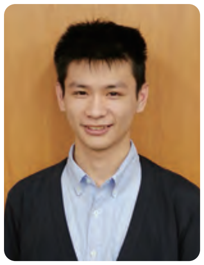
Ryota Akino

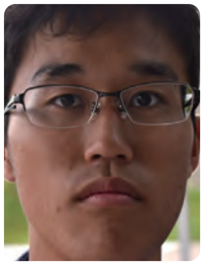

Ryotaro Fukue


Masato Shibuya M. D.


Jun Yatsu


Shigetaka Toba

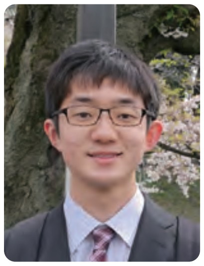

Shunsuke Ono


Takaya Koga

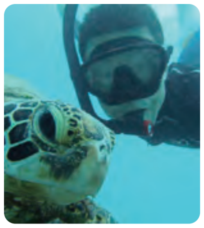
Catch the dream.


Kyoko Matsuzaki


Toshitaka Imaki


Yu Suzuki


Yuki Tukakoshi


Yuya Takeshita

Overcoming Obstacles for the Success of the IBO Challenge 2020

This year's IBO, the 31st International Biology Olympiad 2020 Nagasaki, Japan, was held as a remote event called the IBO Challenge 2020. We sincerely congratulate all the students who participated in this event after many preliminary rounds.

Originally, the event was scheduled for July 3-7, 2020, with Nagasaki International University (Sasebo City, Nagasaki Prefecture) as the main venue. However, since February, the COVID-19 pandemic spread rapidly across the globe. Under such circumstances, the IBO Organizing Committee thought about how to hold this year's International Biology Olympiad. By the end of March, discussions with the IBO2020 Organizing Committee confirmed that the event would be held as a remote contest, not in Nagasaki.

Although the type of event changed drastically, we were still committed to fulfilling the four objectives we set ourselves as the host country: (1) provide an opportunity for the young generation of the world to test their abilities, (2) provide a place for the continuity of IBO activities and student-oriented challenges, (3) provide a place to encounter new aspects of biology, and (4) develop next-generation human resources and revitalization through international exchange. With these objectives in mind, we finalized the content of the remote event in close cooperation with the IBO Steering Committee.

However, this was the first attempt by a host country to conduct an IBO international contest remotely. Because of this, we faced many issues, such as fraud prevention and fairness when testing, differing communication conditions and environments, operational management challenges due to time differences, and remotely held practical exams without any lab equipment and materials.

We were able to overcome all of them with the cooperation of all the staff members of the Secretariat Office and faculty members nationwide. The contest was held with the participation of 53 countries and regions, which far exceeded the initial estimations. We would like to express our sincere gratitude to the students, jury members, and all the people concerned from each country for their participation during the extremely difficult situation caused by the spread of COVID-19.

Students, you are the hope of our society. Please keep doing your best. Please take on a big challenge. I look forward to seeing the success of all the students.

Dr. Makoto Asashima
President, the IBO2020 Organizing Committee
浅島 誠

IBO Trophy
The IBO Trophy, a complimentary gift from Her Royal Highness, Krom Luang Naradhiwas Rajanagarindra, to circulate from one IBO host country to another annually.

From the Secretariat Office

The Right People in the Right Place

The IBO2020 Secretariat Office was established in April of 2018 and will soon end its short, three-year duty in March 2021. Thanks to our beloved Ryoichi, we enjoyed the privilege of using an office in the Tokyo University of Science, located in the heart of Tokyo. On the fifth floor of the same building, Ryoichi was working relentlessly on the duties of being the IBO Chairman as well as his own experiments. Under the director, Mitsuko Kudo, four staff members of the Secretariat Office played a supportive role, each utilizing their own expertise: Taiga Araki and Siri McGuire for international outreach and coordination, Ryoko Utsumi for logistical operations, and Kimiko Takeuchi for accounting.

Most of you may recognize the name "Taiga" at this point. He has been a part of the Japan Biology Olympiad community since participating as a contestant in IBO2012 in Singapore. I officially recruited him in the summer of 2019 when he was working at a tech company in Tokyo after graduating from university in the USA. Since he was already looking for another opportunity in his life, he gladly joined the office - fate really does exist! Utilizing his English ability, he took care of a wide variety of tasks, such as coordinating the registration process, publishing exam guidelines and timetables, and most importantly, communicating with country coordinators and facilitators. His experience as a former IBO contestant and his attention to detail were a great help in the organization of this event.

There was another IBO alumnus who greatly contributed to the event: Kentaroh Honda, a former contestant of IBO2007 in Canada. While working full-time at a different company, he generously devoted his entire summer vacation to building and coordinating all exam-related websites and platforms. To minimize the learning curve of participants, he built the whole system by combining existing services. During the exam period, he managed the system both calmly and accurately, which led to the success of the event. In fact, he was the very person who proposed the idea of operating the event based on each participating country's local time zone. Even though that meant the organizers had to stay up several nights in a row, he prioritized the convenience of the participants. When the jury members of some countries made logistical mistakes prior to the exam, he worked particularly hard so that their competitors could still take the exam. His passion and love toward the event as an IBO alumnus deserves special recognition.

Ryoko, who supported our logistical operations, gave birth to her daughter while preparing for the event! Even while being pregnant, she found time to support the Secretariat Office in various ways. Originally, she was in charge of the cultural workshop during IBO2020 in Nagasaki. Once we shifted to the remote event, she handled the logistical operations of the International Group Project while raising her daughter. Every night after the baby fell asleep, she sacrificed her precious sleeping time to work on tasks like summarizing the progress of all the groups and creating a post-event report.

Siri, our only staff member with native English, enjoyed working on many English-related tasks, from researching and writing the 'Japonica' species guide to coordinating all international volunteers. Although it was unfortunately not used, a species guide for a beautiful beach in Sasebo, where the group fieldwork activity was going to take place, was her wonderful work as well. She was in charge of proofreading (and sometimes creating) our English documents for both on-site and remote events. Thanks to Siri and Taiga, the official language of IBO2020 became native

English. As a director, I was extremely satisfied with this achievement.

Our support staff member for accounting, Kimiko, fully utilized her expertise to efficiently and accurately handle the financial aspects of this event, which other staff members, including myself, had little knowledge about. Although an event like this tends to experience some financial issues, it seems like this wasn't the case for IBO2020 because of her.

I, the director of the office, specialize in communicating life science research to the public. My life goal is to entertain as many people as possible through the proper communication of scientific papers. To achieve this goal, I have previously used books, websites, movies, exhibitions, and many other mediums. However, organizing an event this big was relatively new for me.

The part I cared about the most as the director was delivering the organizers' great passion for the event to the participants in a visible way. I hope I succeeded in that task. As the office nears its end, my stress level is finally starting to decrease. While organizing the event was rewarding, I may have to admit that sometimes I needed time to forget about all the stress and enjoy working on things like designing event gifts or editing this yearbook.

At the beginning of the preparation for IBO2020, I defined the goals of the International Biology Olympiad as 1) high-quality biology examination, 2) the international exchange of like-minded youth, and 3) immersive cultural experiences in the host country. We are confident that we achieved the first goal, and hope that we fulfilled the second one through the online International Group Project. As for the third goal, we struggled to achieve this because of the nature of a remotely held event.

We truly wished that we could welcome you all in Nagasaki. Our exam venue, Nagasaki International University, was extremely generous and cooperative, and was looking forward to hosting students from many countries from across the world. Moreover, we believed that Nagasaki was a wonderful city in which to host IBO because of its rich and diverse natural features, from the ocean (Kujukushima) to the mountains (Mr. Fugen). This


Mitsuko Kudo


Taiga Araki


Ryoko Utsumi

is in addition to its unique culture and history, such as the tea ceremony (Chinshin-Ryu ceremony), pottery (Hasami-yaki), and its well-known history of the atomic bomb. I would like to show our deep and sincere appreciation once again to all of the stakeholders in Nagasaki, especially to Nagasaki Prefecture, Sasebo City, and Nagasaki International University.

That being said, I believe you still had some other opportunities to experience Japan during the event. The accurate and detail-oriented operation of the event with only five staff members (all sleep-deprived, even!) is very much like Japan, just like our dead-accurate train timetables. This could be the biggest "Japanese cultural experience" we could provide through this event.

Last, but not least, I would like to thank our president, Dr. Makoto Asashima. Getting enough funding for this kind of huge international event is always tricky and can be full of people acting for their own personal financial and/or political gains. However, he never cut corners and raised enough donations solely through his pure passion and enthusiasm for the event. Even when we experienced financial problems due to the cancellation of the on-site competition, he never gave up, just because he didn't want to disappoint our competitors who were looking forward to their turn to participate in IBO. On top of this, he was such a pleasant person to work with. Once again, I would like to express my utmost appreciation and respect to him!

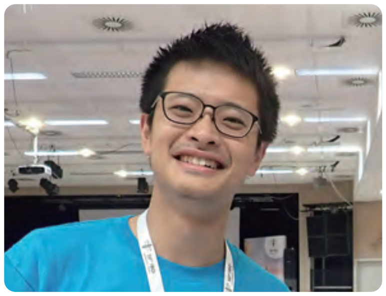
Even though our IBO Challenge 2020 was held remotely, a lot of countries and regions participated in the event. We would like to truly thank the IBO Steering Committee, the IBO Office, and all the NBO organizations around the world.

IBO spirit never fades!

Mitsuko Kudo
Director, the IBO2020 Secretariat Office

Kimiko Takeuchi

Siri McGuire



Kentaroh Honda

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The 31st International Biology Olympiad 2020 Nagasaki Organizing Committee

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Japan Science Foundation

■ 共催団体 Co-host

国立研究開発法人 科学技術振興機構
Japan Science and Technology Agency

長崎国際大学
Nagasaki International University

生物科学学会連合
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Co-host (Only IBO Challenge 2020 International Group Project)

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Vice-Presidents	Dr. Kenichiro Nakashima	President, Nagasaki International University
	Dr. Hiroshi Wada	Professor at Tsukuba University
Advisors	Dr. Akito Arima*	President of Science Olympiads Promotion Council, Former Minister of Education, Dean of Musashi Academy of Nezu Foundation *Dr. Arima passed away on December 6th, 2020. He was a long-time leader of the entire Japanese scientific community, and his contribution to the scientific Olympiads, regardless of the subject, was tremendous as well. We would like to express our deepest condolences to his family.
	Mr. Sadayuki Sakakibara	Chairman of Japan Science Foundation
	Dr. Hideo Mori	Professor Emeritus, the University of Tokyo
Members	Mr. Naoki Abe	Administrative Director of Kyushu Bunka Gakuen and Nagasaki International University
	Dr. Yuichiro Anzai	Advisor of Japan Society for the Promotion of Science, Former Dean of Keio University
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	Mr. Mitsuru Imoto	CEO, Nippon Wealth Life Insurance Company Limited
	Mr. Tsutomu Ueki	Standing Director, Japan Science Foundation
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	Dr. Mitsuo Ochi	President, Hiroshima University
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	Dr. Ryuzo Sakakibara	Head of Nagasaki International University Faculty of Pharmaceutical Sciences
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	Dr. Seizo Miyata	Chairman of The Frontier Salon Foundation, Professor Emeritus at Tokyo University of Agriculture and Technology

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Members	**Dr. Chiaki Mukai**	Specially-appointed Vice-Dean at Tokyo University of Science, Former Astronaut
	Mr. Masao Murakoshi	CEO, Murakoshi Holdings, Inc.
	Dr. Kimiko Murofushi	President, Ochanomizu University
	Dr. Mamoru Mori	Director at National Museum of Emerging Science and Innovation, Former Astronaut
	Dr. Akiyoshi Wada	Advisor at IBO Japan Committee

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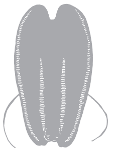
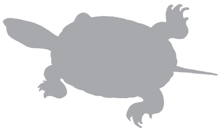


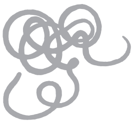
Leucothea japonica

Lotus japonicus

Luehdorfia japonica

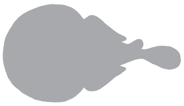


Mauremys japonica

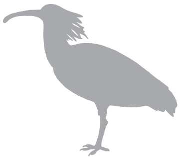


Meyersia japonica

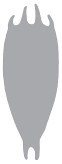
Mimobdella japonica



Narke Japonica



Nipponia nippon

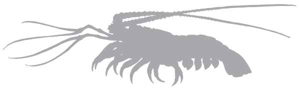


Notholca japonica

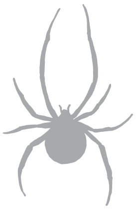
Omphalotus japonicus

Osmunda japonica

Oxycomanthus japonicus



Panulirus japonicus

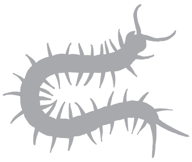


Parasteatoda japonica

Perophora japonica

Prasiola japonica

Saccharina japonica



Scolopendra subspinipes japonica

Ulnaria japonica

For IBO2020, we selected some species that contained “japonica” in their scientific name and used them as design motifs. We attempted our best to include species from as many phylogenic groups as possible while keeping the selection interesting. We hope you will enjoy learning about them.

Just like this, it would be interesting to find species that contain your country or region’s name in it.

International
Biology Olympiad 2020
Nagasaki, Japan


Lotus japonicus

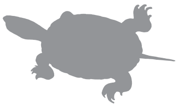

Osmunda japonica


Conocephalum japonicum

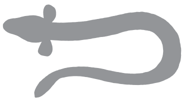

Branchiostoma japonicum

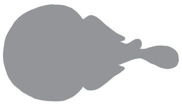

Mimobdella japonica

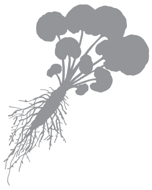

Dugesia japonica

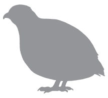

Mauremys japonica


Hyla japonica

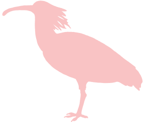

Anguilla japonica


Narke Japonica


Eutrema japonicum


Coturnix japonica

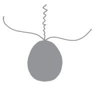
IBO 2020
Supplementary Materials

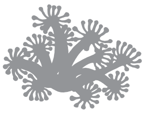

Nipponia nippon


Omphalotus japonicus


Ephebe japonica

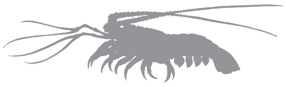

Aspergillus japonicus


Fibrocapsa japonica

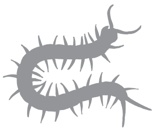

Alveopora japonica


Meyersia japonica

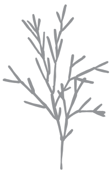

Oxycomanthus japonicus

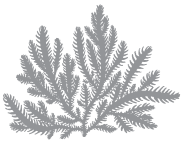

Panulirus japonicus


Notholca japonica


Scolopendra subspinipes japonica


Perophora japonica


Cladophora japonica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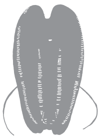
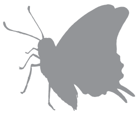
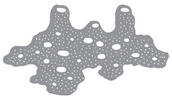
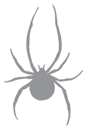

Leucothea japonica

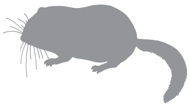

Hydroglyphus japonicus


Luehdorfia japonica

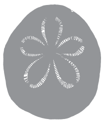

Halichondria japonica


Parasteatoda japonica


Hyleoglomeris japonica


Glirulus japonicus

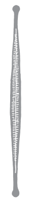

Saccharina japonica

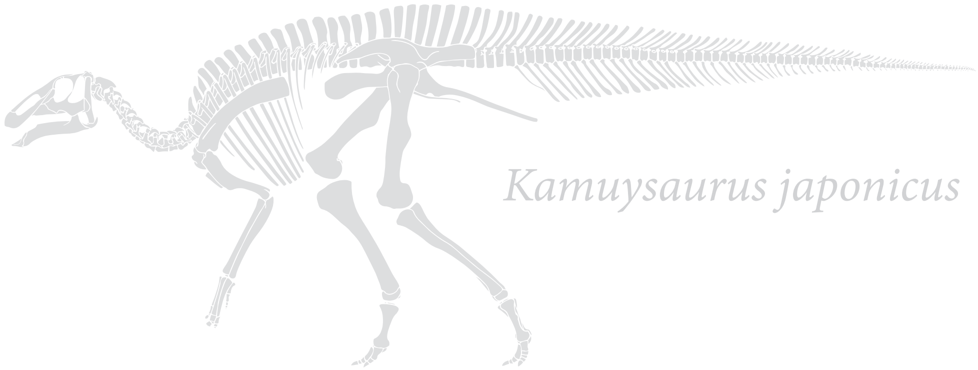

Clypeaster japonicus


Prasiola japonica


Cryptomeria japonica


Corbicula japonica


Ulnaria japonica



Kamuysaurus japonicus

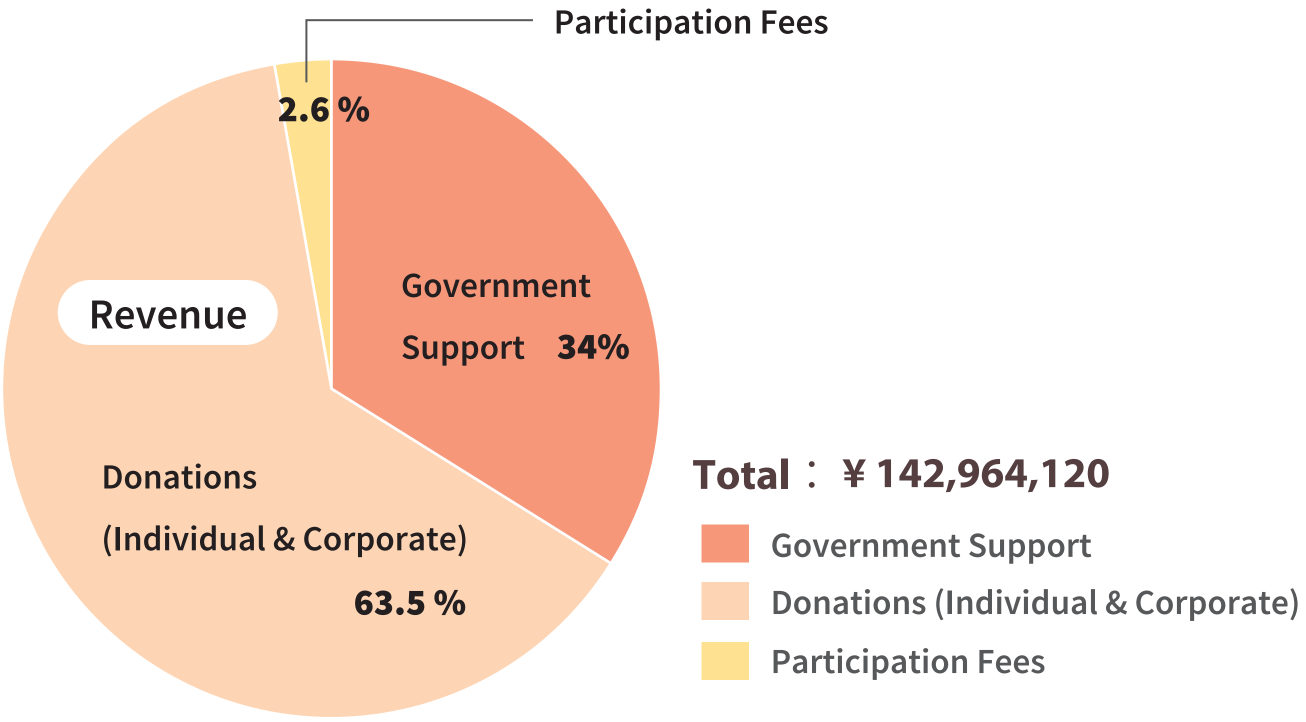
1. IBO 2020 Budget

Revenue

Government Support:

Over the course of three years (2018 to 2020), the Japanese government funded the IBO2020/IBO Challenge 2020 with a 1:1 match-funding.

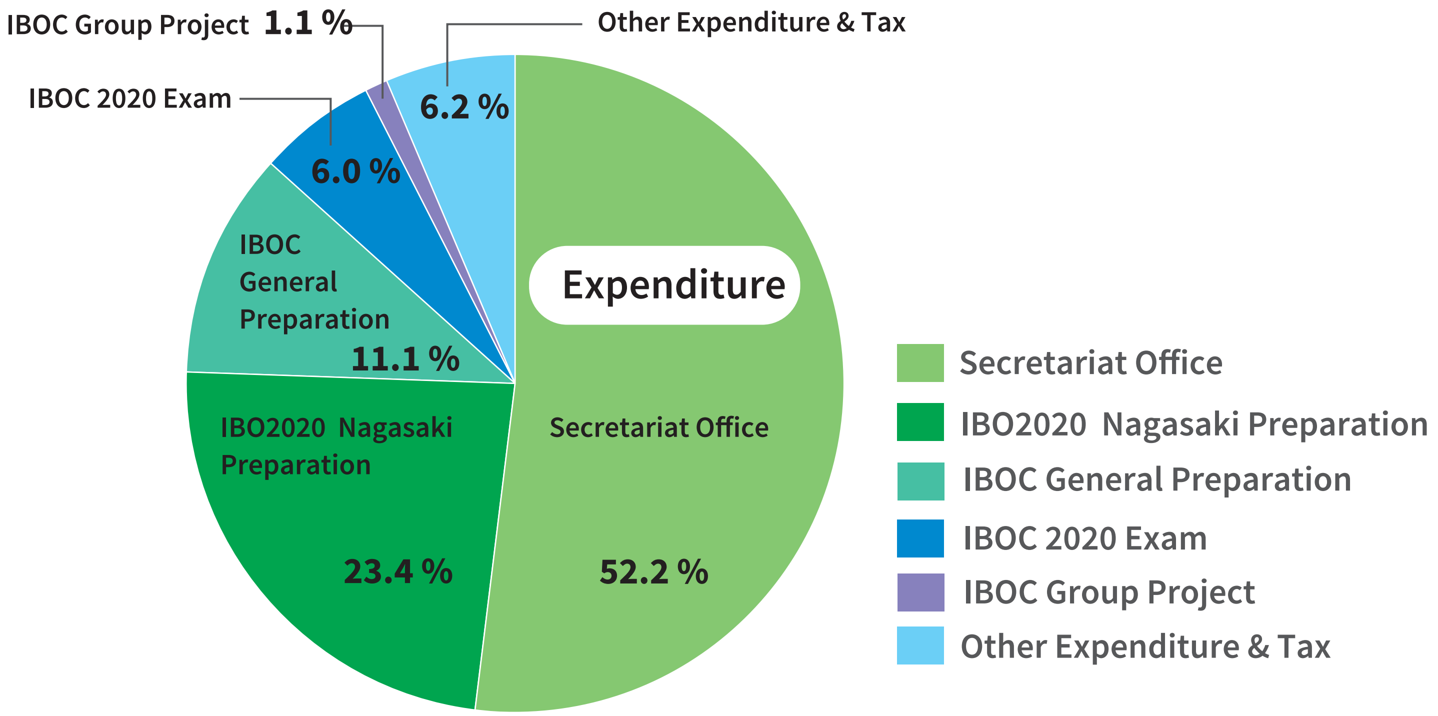
Donations: We received donations from 80 organizations, including local governments of our event venue, as well as 23 individuals.



Expenditure

20% of our total expenditure was spent on the remotely-hosted IBO Challenge 2020.

The operational cost of the secretariat office accounted for more than half of the expenditure.

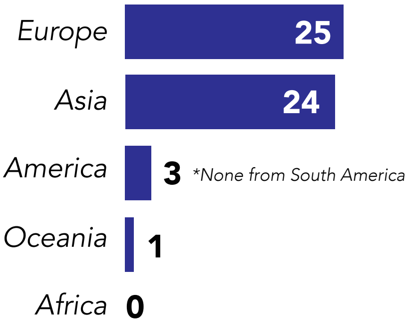


2. General Data

Participating Countries/Regions

Category	Count
Both Exam and Group Project	47
Only Group Project	3
Exam Observer + Group Project	2
Exam Observer	1
Total	53

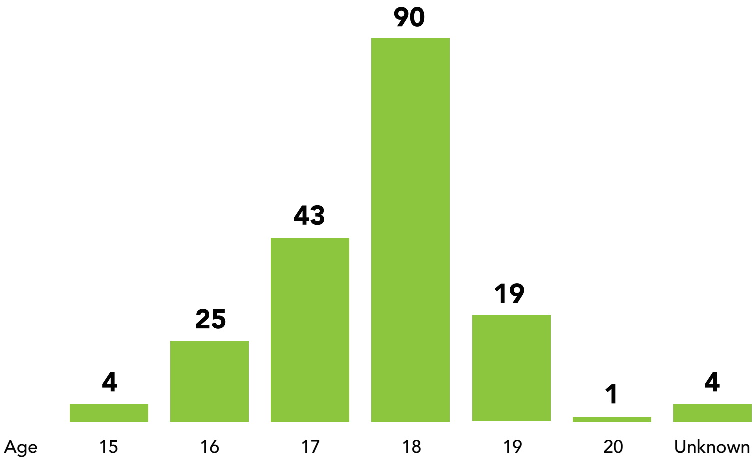
Regional Distributions



Participants by Categories

Category	Part 1 Exam	Part 2 Int'l Group Project	Total
Competitors	186	16	202
Jury Members	202	N/A	202
Exam Supervisors	45	N/A	45
Project Facilitators	N/A	37	37
Total	433	53	486

Age Distributions of Competitors



3. Exam Venues & Supervisions

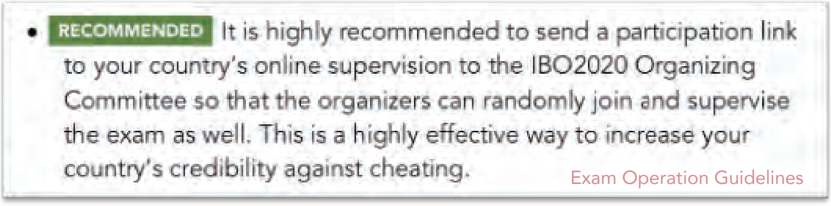
Venues by Supervision Styles

	Supervision Style			Total
	On-site	Both	Online	
# of Countries and Regions	**31**	**9**	**7**	**47**
Breakdowns	**22**: Schools **9**: Others	Various combinations	**3**: Schools **24**: Home (unit: person)	-
How Competitors Read the Questions	**2**: Computer Screen **14**: Print **15**: Both	Various combinations	Only Computer Screen Allowed	-

Venues within Each Country

		Number of Venue(s) within One Country/Region					**Total** Competitors
		1 Venue	2 Venues (3 + 1)	2 Venues (2 + 2)	3 Venues	4 Venues	
Supervision Styles of Countries/Regions	On-site	123 (31)	15 (5)		2 (1)		140
	Both		2 (2)	4 (1)	2 (1)	6 (2)	14
	Online		3 (3)			29 (8)	32
Total (Counties/Regions)		31	5	1	1	9*	186 (47)

Online Supervision by the Organizers (Optional)



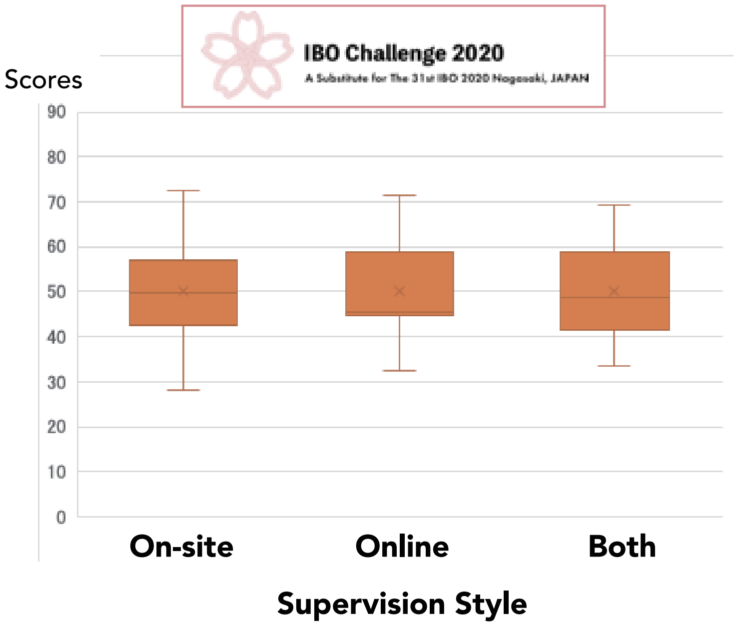
- RECOMMENDED It is highly recommended to send a participation link to your country's online supervision to the IBO2020 Organizing Committee so that the organizers can randomly join and supervise the exam as well. This is a highly effective way to increase your country's credibility against cheating.

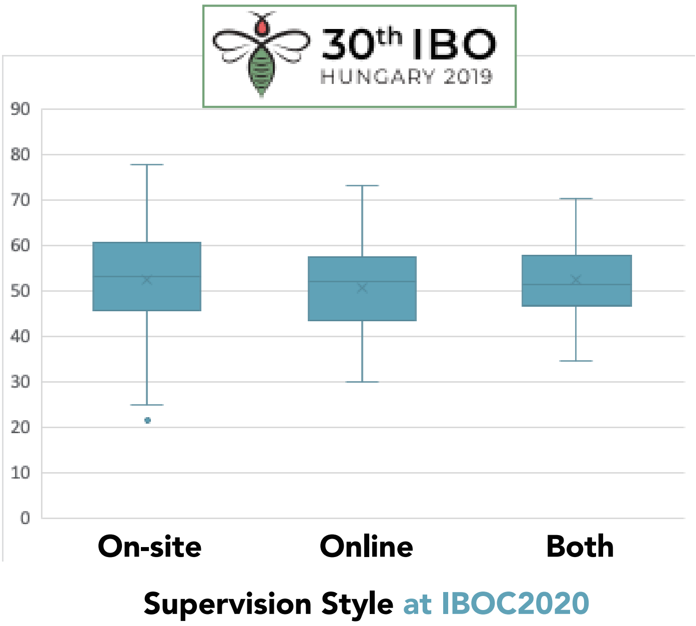
Exam Operation Guidelines

	Supervision Style			Total
	On-site	Both	Online	
Link Provided	**19**	**7**	**5**	**31**
Link NOT Provided	12	2	2	16
Total	31	9	7	47

IBO2020 supervised all 31 countries/regions

4. Effect of Remote Examination

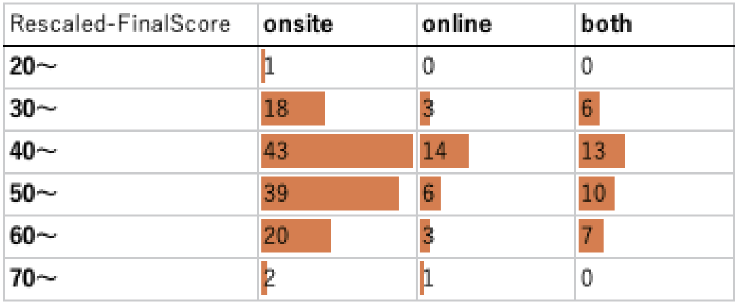




No Statistical Effects of Remote Examination
on the Exam Scores

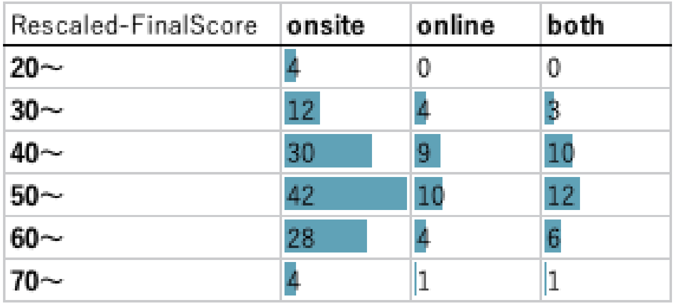
5. Supervision Style and Scores

IBO Challenge 2020
A Substitute for The 31st IBO 2020 Nagasaki, JAPAN



Rescaled-FinalScore	onsite	online	both
20~	1	0	0
30~	18	3	6
40~	43	14	13
50~	39	6	10
60~	20	3	7
70~	2	1	0

30th IBO
HUNGARY 2019



Rescaled-FinalScore	onsite	online	both
20~	4	0	0
30~	12	4	3
40~	30	9	10
50~	42	10	12
60~	28	4	6
70~	4	1	1

6. IBOC2020 Medal Counts

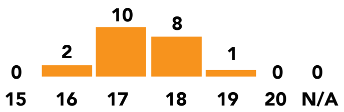
Gold 21

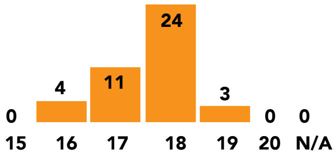
Silver 42

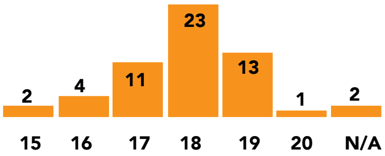
Bronze 56

Merit
11

Age Distribution







7. More Feedback from Organizers

Ideal Timing/Duration of the Event

Exam: Same timing as a regular IBO (everybody is available)
Group Project: Short and intensive style (1-2 weeks) is better
    Fair amount of competitors dropped out due to their conflicting responsibilities.

More Supervision is Possible

More countries were comfortable with the online supervision protocols than we expected.
It may be possible to have more strict rules about supervision.
    Example: Two cameras (overall and desk) for each competitor

Technical Operations of the Exam

Strategic combination of existing services was enough to organize this remote IBO event.
    No need to build a special application or system.
A host country needs at least several staff members who are comfortable with current technologies.
    Google services, basic web/server management, AWS, etc.
Backups are always important!
    Backup staff members
    Backup servers, storages, submission tools…
    Emergency tech support

Exam Quality is Important (even more so)

Good quality exams require less communications and discussions during the Online Jury Meeting.
Good quality exams enable competitors to stay focused.
    Less questions asked during the exam; makes supervision easier, even online.
In a remote event, quality of exams directly influences the competitor experiences.
    Exams need to help competitors encounter the new aspects of biology.

Special Thanks to

IBO Challenge 2020 Jury Members, IBO Challenge 2020 Exam Supervisors,
IBO Steering Committee, International Subgroup Members, and International Volunteers