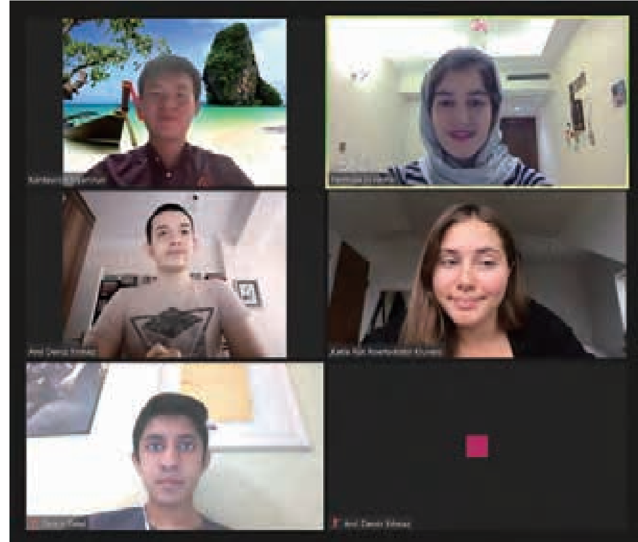
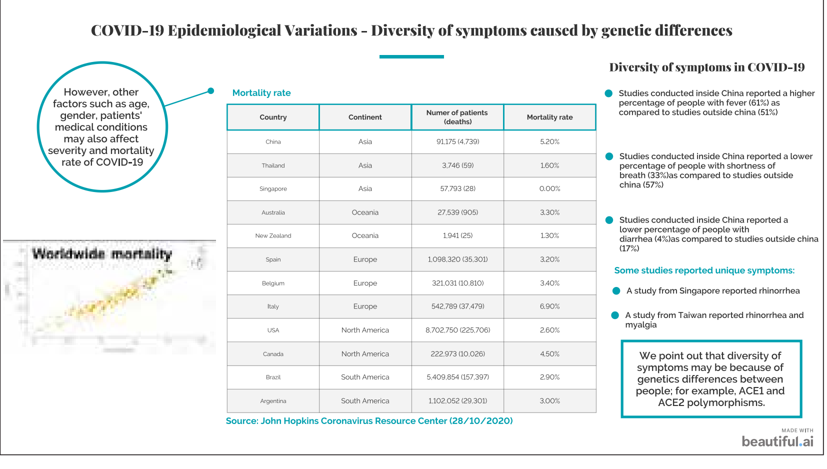
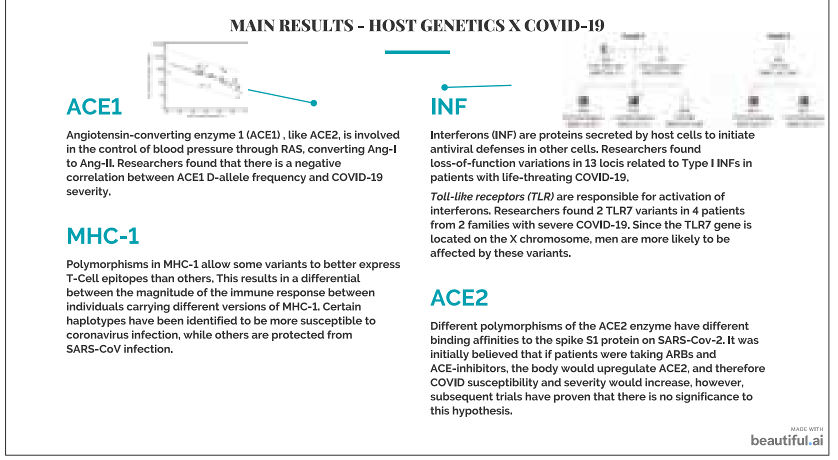
1B01 Host genetics of COVID-19





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)



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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.

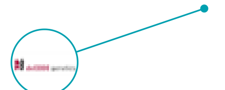
23andMe

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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References

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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

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NEW PE

Retroviruses a
for 690,000 de
human effort t
These days, a c
being frequent
patients' life ex
reservoirs in b
the entirety of
and the onset

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 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, surro 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, releas ready to infect new host cells.

Untreated HIV infection is a life-threatening health problem and many different therapies for HIV/AIDS focusing main 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.

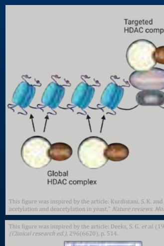
Another strategy for the permanent silencing of retroviral proviruses could be DNA methylation. There are known instruments, such as Zinc finger nucleases, TALENs or CRISPR-Cas9 platforms that can direct specific cytosine methylations[22]. However, their lack of specificity can result in off-site effects, 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.

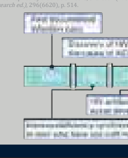
The Block-and-Lock strategy is a promising area of research, though there is much work to be done before it can be implemented as a treatment option. Currently, the greatest hurdle we have yet to overcome is the lack of specificity and risk of off-site effects, which can have unpredictable and potentially 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 inhibitor (HMT)[19]. These enzymes usu increase the expression of that 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 lor of latent provirus. Activation o of NFκB which causes increase Promising PKC agonists are bo occurring[28] and synthetic ing prostratin and phorbol-13-ste NF-κB by IKK-dependent phos degradation of IκBα[30]. In ano myristate 13-acetate (PMA) o in NF-κB activation[31].





Special thanks to:

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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.

N PERSPECTIVES IN THE TREATMENT OF HUMAN RETROVIRUSES

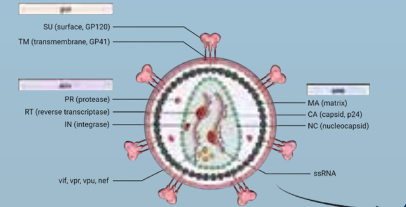
Dong Ngoc Ha, Dewey Lin, Gega Karanadze,
Tereza Maxerová, Simonas Melaika and Yerassyl Muratov

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 s 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

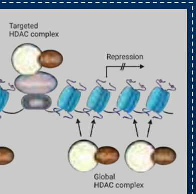
KILL

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 *vivo*[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].

Latently infected memory CD4+ T cell — 'Shock' — Active CD4+ T cell — 'Kill' — Dying infected cell — Uninfected cell
HIV protein
HIV particle



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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.

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TIMELINE OF HIV TREATMENT RESEARCH

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, limiting 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.

1 Binding
2 Fusion
Viral RNA
3 Reverse transcription
Viral DNA
4 Integration
5 Transcription
6 Translation
7 Assembly
8 Budding
9 Release & maturation

FRET
Antibody
UV exposure
"Donor"
Viral antigen
LJ001

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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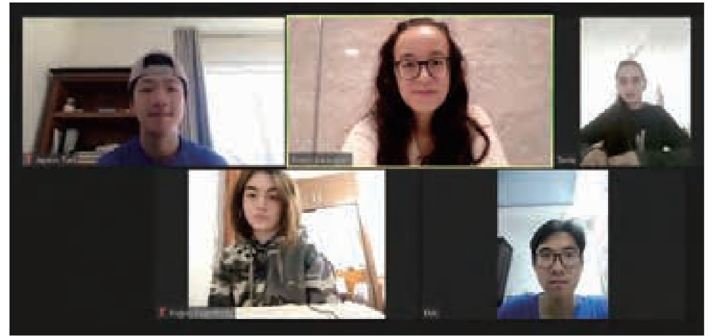
Group project
h a wonderful r their works.

References

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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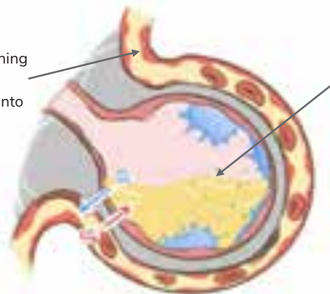
Immuno-Senescence

- 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.

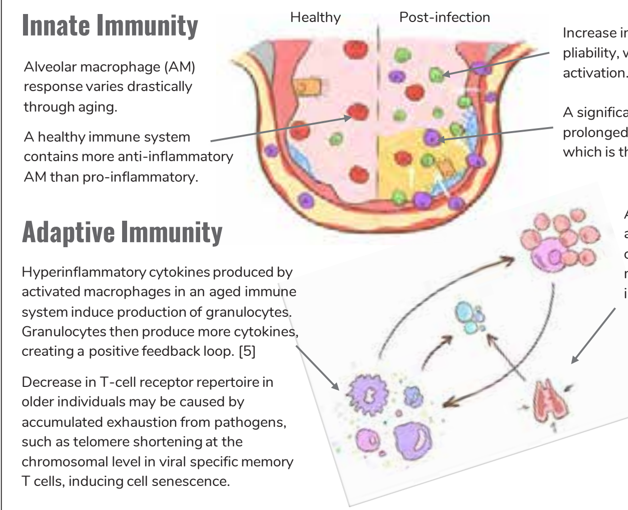
Cytokine storm then initiates microvascular clotting, causing a range of lethal symptoms like hypoxia and organ failure.

J.T.

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.

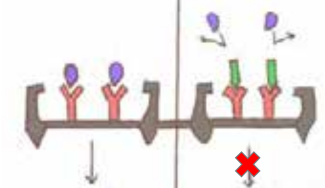
Epigenetic Factors

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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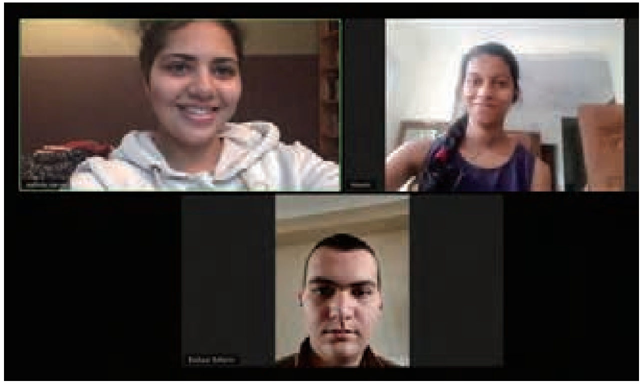
1B04 Using Biology to propose methods to prevent spread of Dengue



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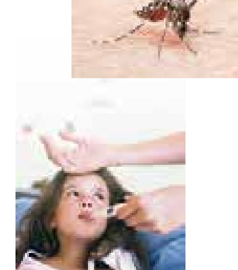
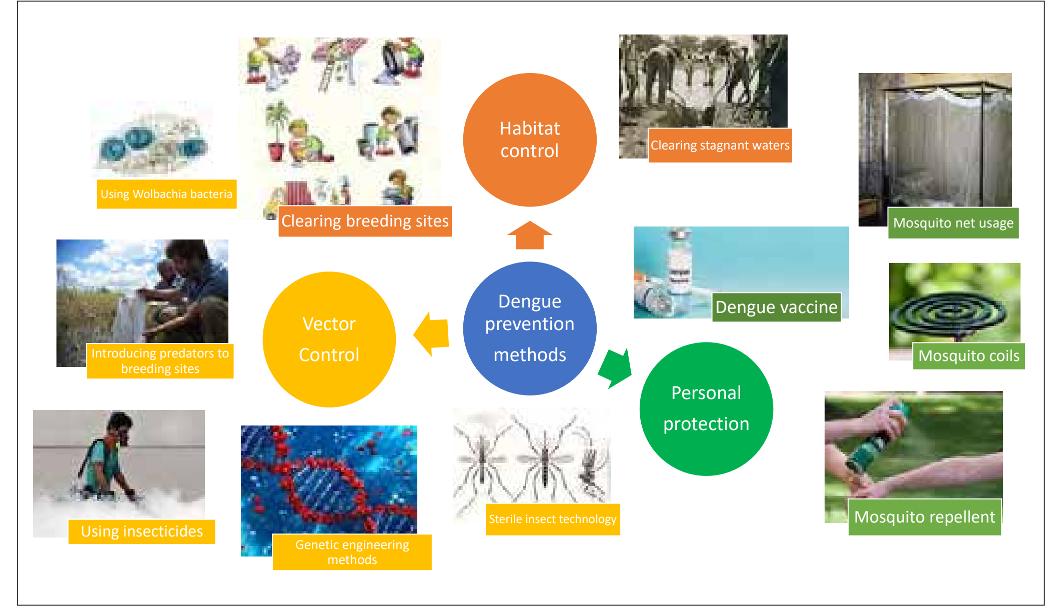
-What is dengue? A brief description-

- 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.



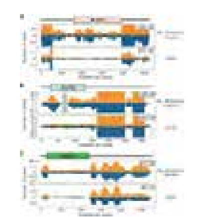
Why is prevention of dengue important?

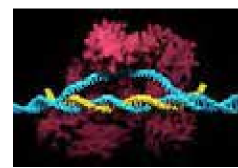
- Dengue is common in more than 120 countries, mainly in Southeast Asia, South Asia and South America. About 390 million people are infected annually and approximately 40,000 die each year.
- An efficient vaccination has not yet been introduced to prevent spread of the disease.



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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- **World Mosquito program** https://www.worldmosquitoprogram.org
- **National dengue control unit, Sri Lanka** www.dengue.health.gov.lk
- ***In silico* models for predicting vector control chemicals targeting *Aedes aegypti***
  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.

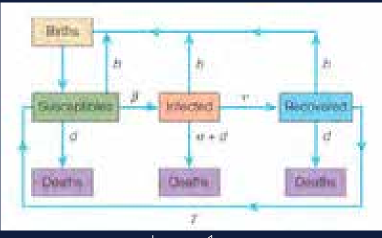

Image 1

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 + α).

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.

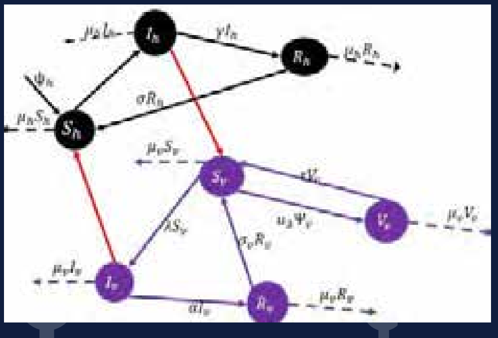
Image 2

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1B06 The Roles of Nanobiotechnology in Combatting Infectious Diseases

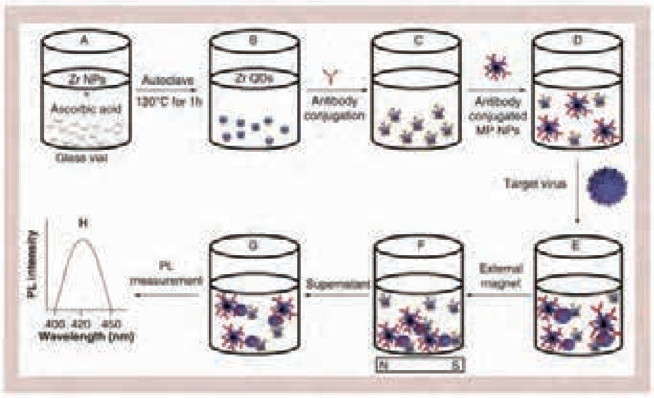

Figure 2 : One Type of Detection Procedures (1)

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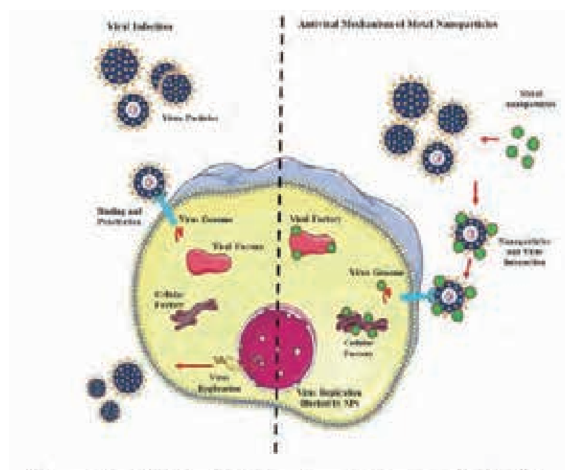

Figure 3 : Antiviral Mechanism of Nanoparticles (2)

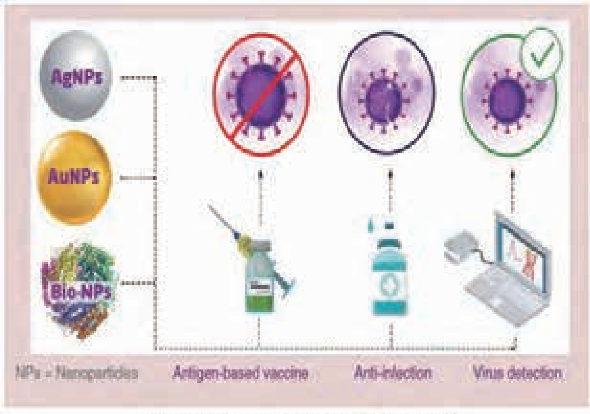

Figure 1 : Graphical Abstract (1)

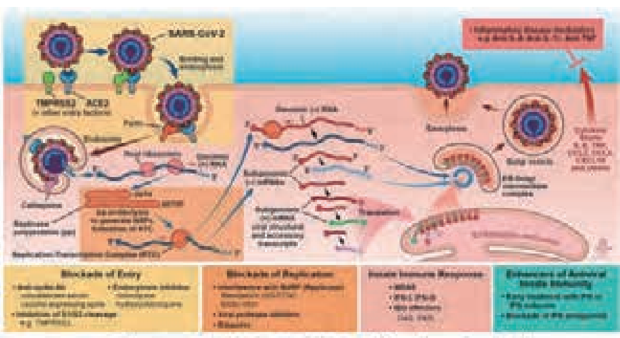

Figure 4 : SARS-COV-2 Replication Cycle (3)

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, $MnFe_2O_4$ 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 .

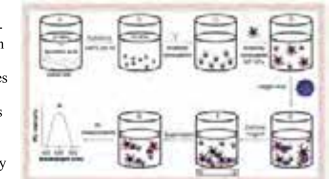

Figure 2 : One Type of Detection Procedures (1)

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

Figure 3 : Antiviral Mechanism of Nanoparticles (2)

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.

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.

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

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.

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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1B07 Infectious Diseases Finding the permanent cure for HIV?

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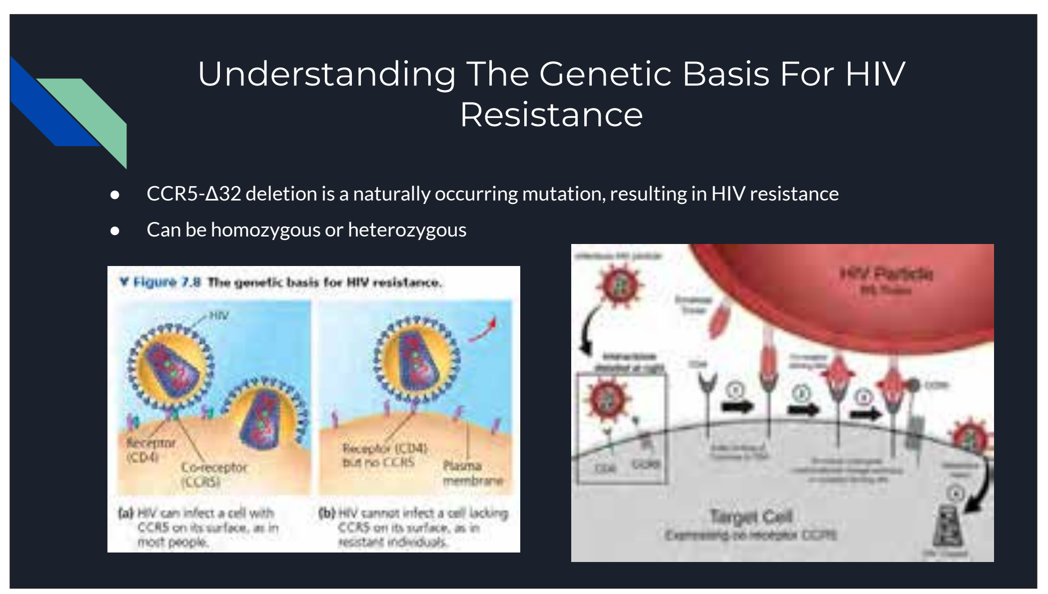
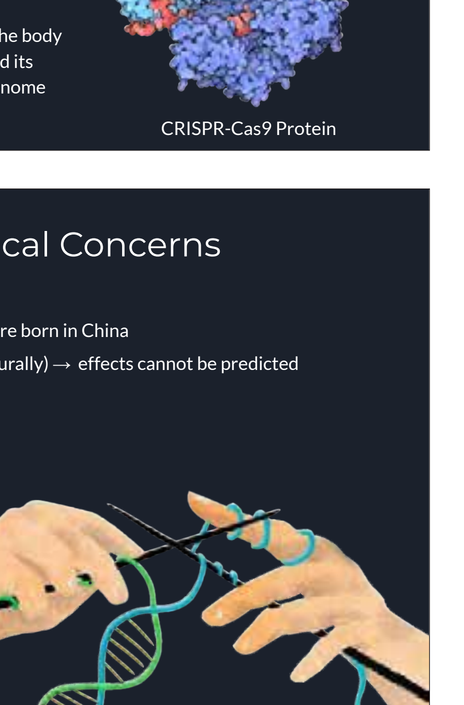


A Cure for HIV? CRISPR may be the Answer!

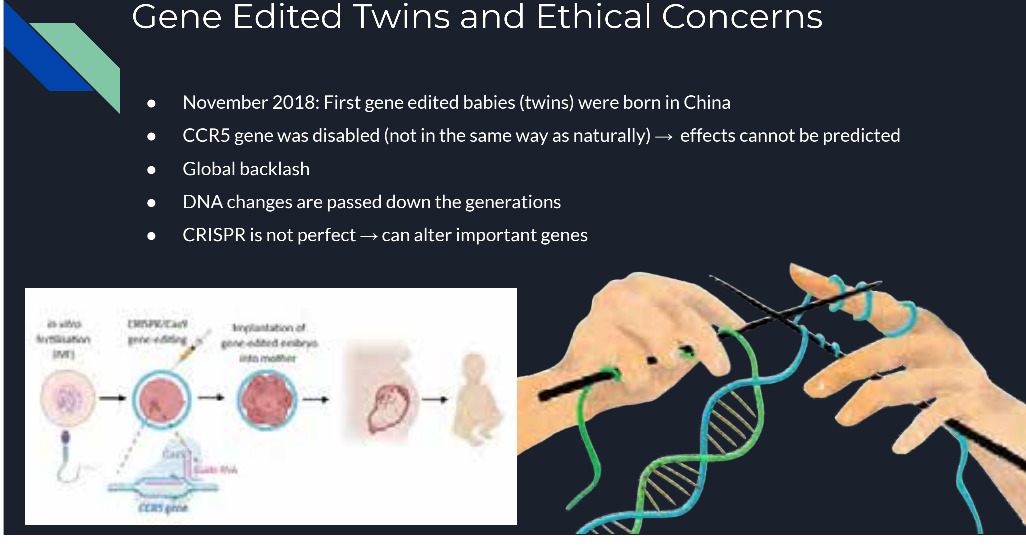
- We're thinking of creating a virus that can enter ONLY the cells which express the CCR5 receptor; the virus will act as a vector for the actual cure.
- We consider doing that by implanting the CRISPR-Cas9 and its guide RNA genes into that virus.
- The virus can't be copied in the cell so that it doesn't 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



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1B08 CAN PHAGE THERAPY SOLVE ANTIBIOTIC RESISTANCE?

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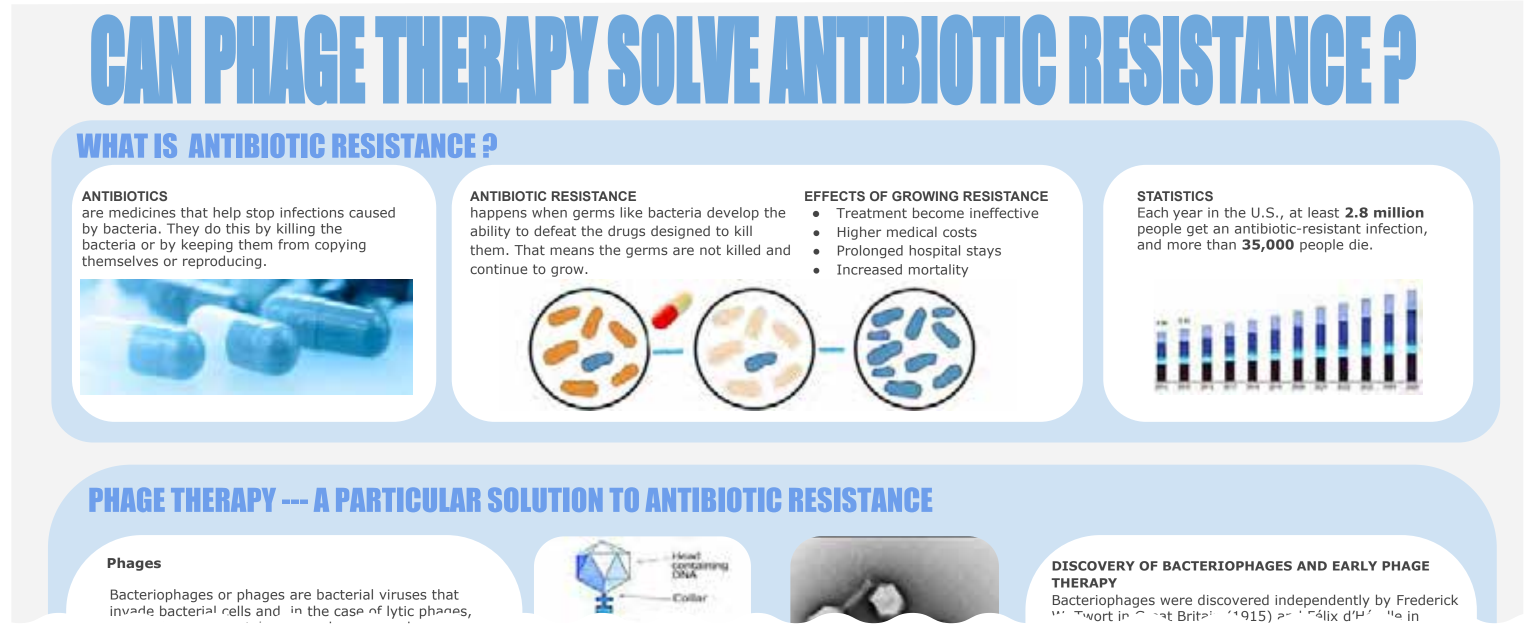


Phages

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

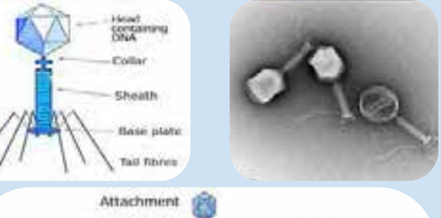
- NOT cells
- Have no cell structure
- Can't grow, move and feed outside of host cell
- Reproduce inside living cell
- Made up of a core of DNA or RNA surrounded by a protein coat (capsid).

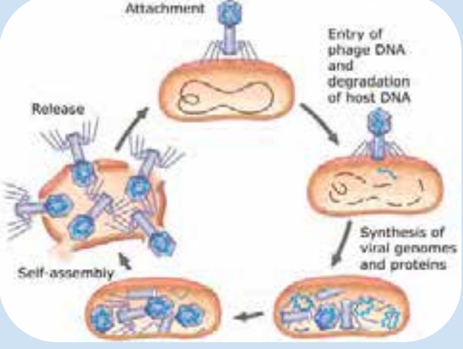
Virulent bacteriophages – Lytic cycle

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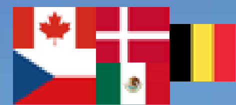
Can the physiological response to microplastic exposure be used to determine their impact on an ecosystem?



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).

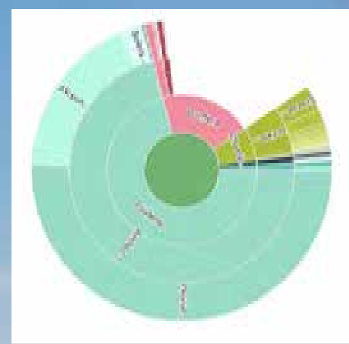


Fig. 1 : An indication of the biodiversity of Monterey Bay. GBIF.org (04 November 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.nb6jbu

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

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 linear 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.
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.
References

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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.

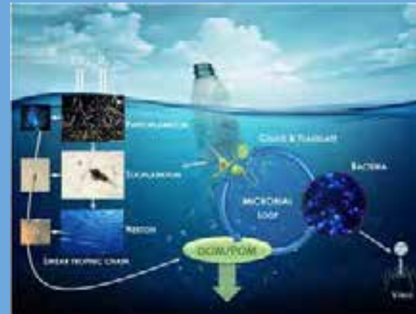

Fig. 2: picture of a potential influence microorganisms could have. [3]

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]

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].

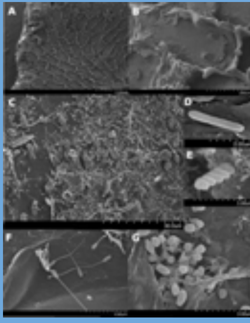

Fig. 3: SEM images of microorganisms living on pieces of microplastic. [4]

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 km²[5]. Therefore we will divide it in areas of 4 km². 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 km² areas that will divided into 100 m² 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.

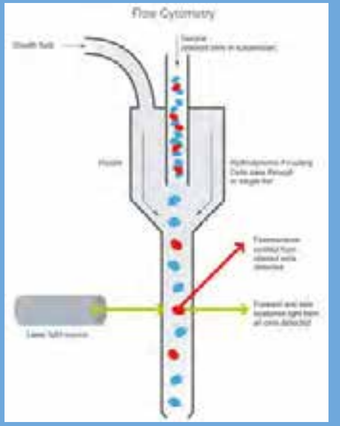

Fig. 5: A sample of stained cells will flow to a tube. Where the laser beam goes through the tube it is width enough for 1 cell at the time. A sensor will detect the differences in incoming signal per cell. [6]

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.

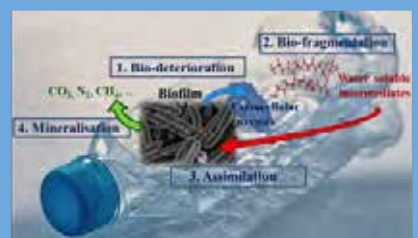
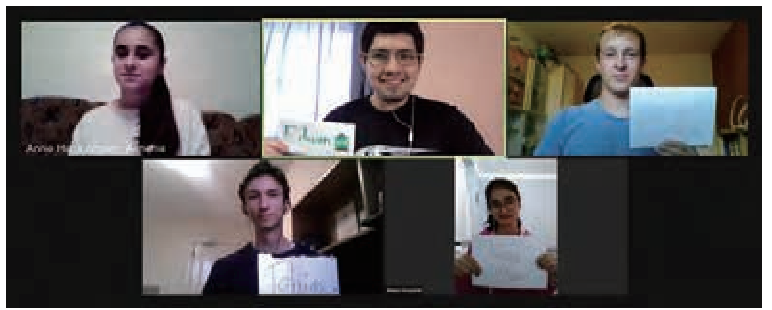


Fig. 5: Biodegradation of plastic by microorganisms [8]

2A04 Zooxanthellae Translocation



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Zooxanthellae Translocation

Promoting Coral Development of Heat Tolerance to Limit Future Bleaching Events

Anara Hussaini, Ani Harutyunyan,
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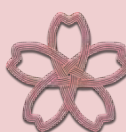

International Biology Olympiad Nagasaki 2020

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 34-36°C. 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 30°C 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 5°C 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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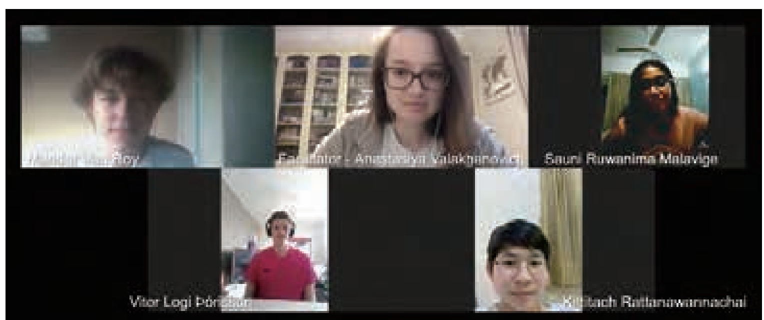
2B01 THE SMALL SAVIORS OF OUR EARTH?



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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 (CO_2) 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 CO_2 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 carbonic 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 Great Amazon Reef System

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Facilitator: Mithun Diumantha Samaranayake (Sri Lanka)

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

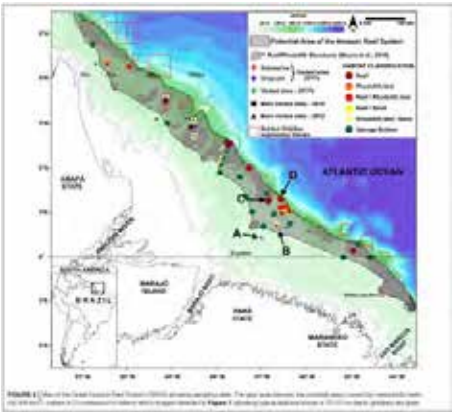
Reefs systems are typically not found at the mouth of rivers due to various factors such as high acidity, low salinity, high turbidity and turbulency. The Amazon River is *the* river, accounting for a fifth of the riverine discharge into the world's oceans. As such, it is highly unusual for GARS to be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

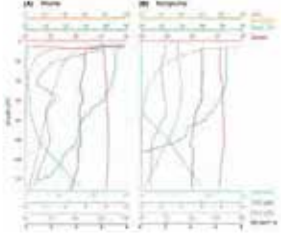
Given that this is a relatively recent discovery (first published paper in 2016), there has been

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

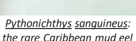
One interesting observation is that the reef is predominantly rhodoliths rather than corals. Rhodoliths are marine red algae that resemble coral. They secretes calcium carbonate structures but not attached to substrate. This is a potential explanation as to how the reef exists, due to rhodoliths being 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 *Geodia spp.* 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:



Pythonichthys sanguineus: the rare Caribbean mud eel

Lutjanus alexandrei: a newly discovered snapper species

Lepidoctopus joaquini: a newly discovered octopus species

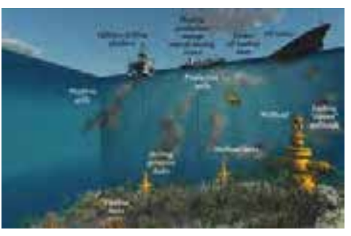
influencing the environmental conditions of the reef. Any activities upstream (e.g. logging, landslides, pollution, fertilizer runoff) may result in consequences downstream.

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:

1. Oil Drilling

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.

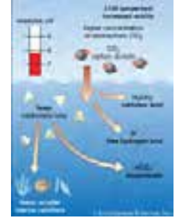
2. Overfishing

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.

3. Climate Change

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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Thinking:

ocean biodiversity

way

Problems of Ocean Biodiversity

Game structure

Introduction to the Scenarios

The Ocean Game

Created by:

Beginning Story

Overfishing

Plastics

Acidification

Ending Story

Discussion

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

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Mher Kurghinyan (Armenia)

Simon Tusnady (Hungary)

Validation of CRISPR editing

Preparing for transfusion

Discussion

Fig 2. H and h antigens

References

Fig 3. Growing and filtration of RBCs

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 synthesize 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[4].

There are already some documented engineered versions of CRISPR-Cas where they have either different PAM sequences or lower off-site activity[5,6,7]. Recently, researchers have begun to utilize rational design in protein design. Rational design, utilizing 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[8], 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[9], 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 preceding 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 – not 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 particular 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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Applications of CRISPR to mtDNA using the MITO-porter

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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

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.

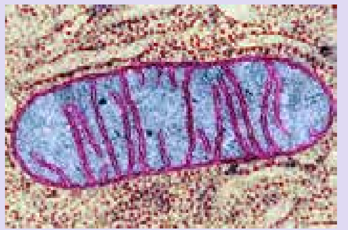

Figure 1: Normal morphology of a mitochondria observed with transmission electron microscopy [10]

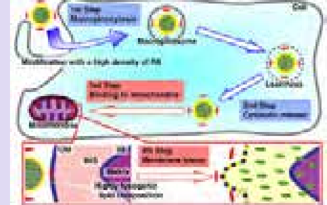

Figure 2: Brief description of how MITO porter reaches mitochondria [6]

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.

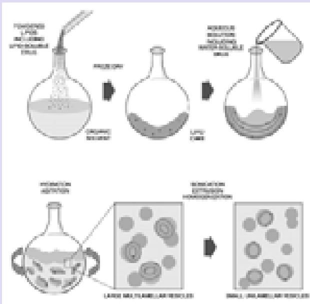

Figure 4: Liposome preparation using hydration method [11]

  - 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).

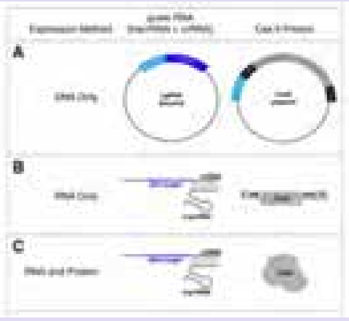

Figure 3: The three different cargo types not including the BFP DNA template [7]

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.

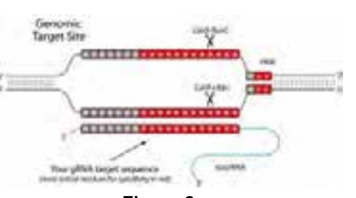

Figure 2

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**.

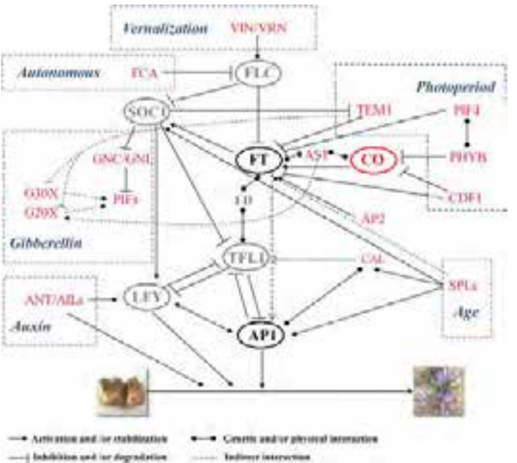

Figure 3

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.

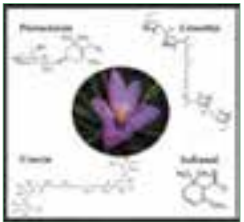

Figure 5

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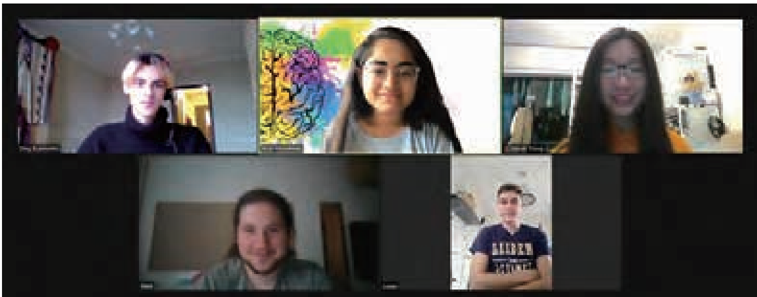
3A06 TelomExtend: A Genetic Approach to Reduce Rate of Aging by Extending Telomeres



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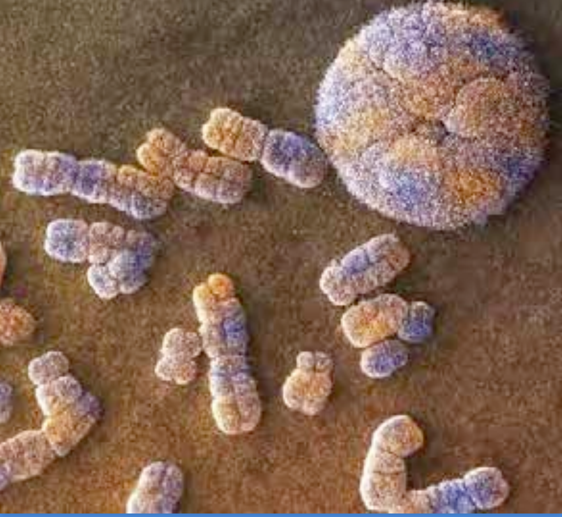

TelomExtend: A Genetic Approach to Reduce Rate of Aging by Extending Telomeres

Lucas van Osenbruggen
Máté Gulácsi
Oleg Kuzmenko
Tsang Hoi Yeung

Coordinated by *Stuti Khandwala*

2

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].

3

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]

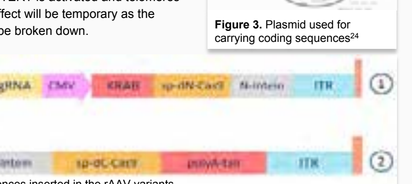

Figure 2. Crucial sequences inserted in the rAAV variants

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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.

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7

3A07+3B05 Yeast biosensor

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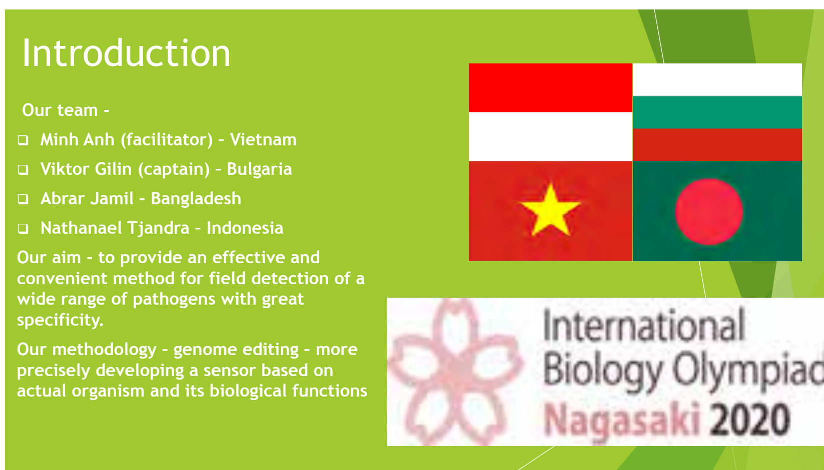
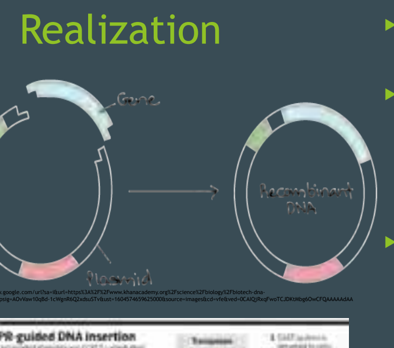


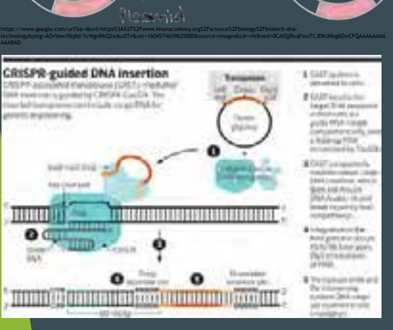
Organism biosensor

- 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



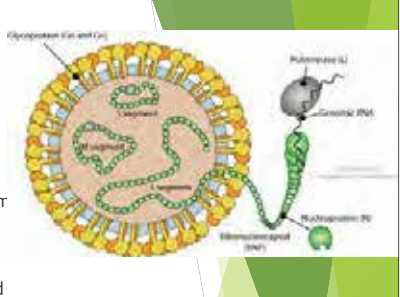


- Yeast general cloning methods can be used to transfer our STE2 homolog genes and lycopene biosynthesis genes into the yeast genome
- The insertion of functional modified versions of STE2 into the yeast genome needs to be accomplished by inserting them with TDH3 promoter (which gives the gene a high 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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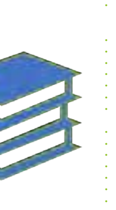

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3A09+3B02 Enhancement of Triacylglycerol Content and Mass Cultivation of Dunaliella salina

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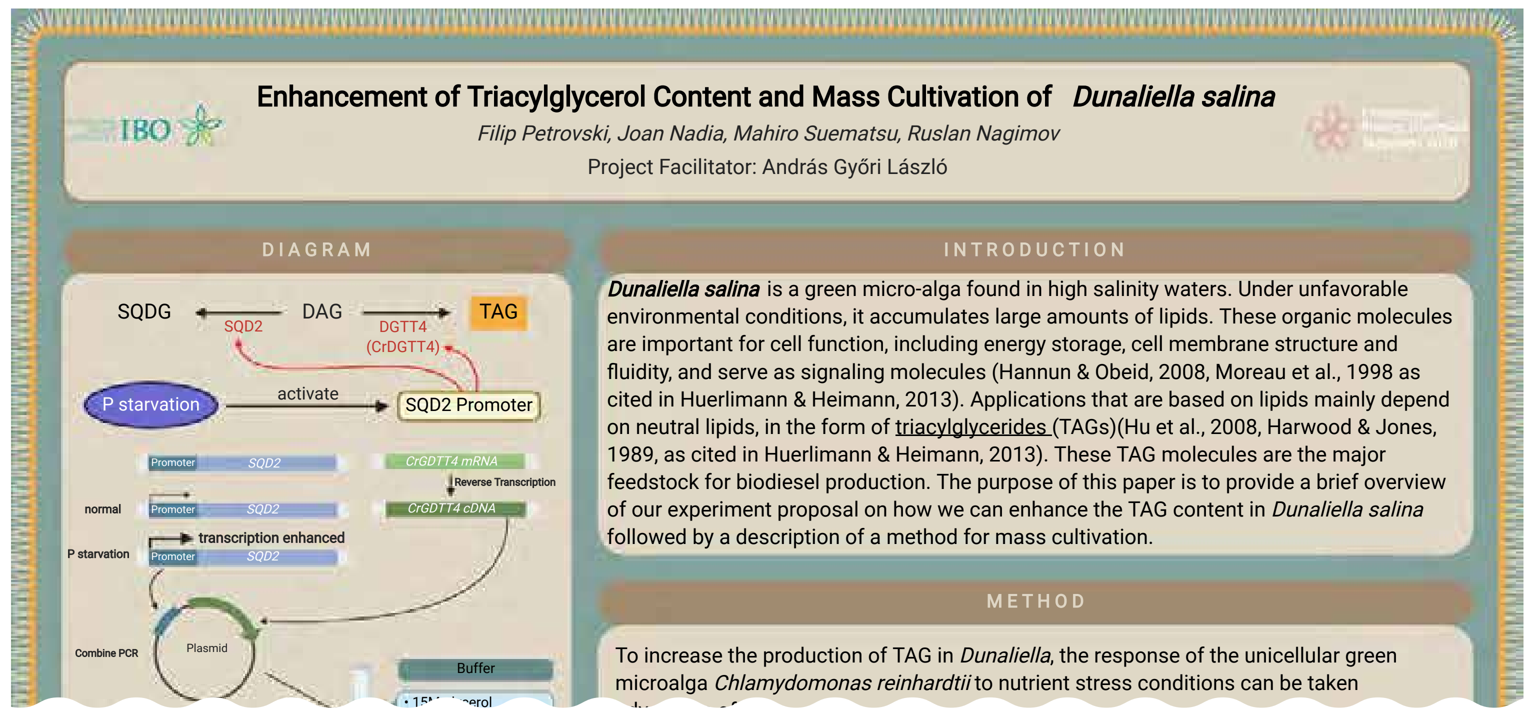

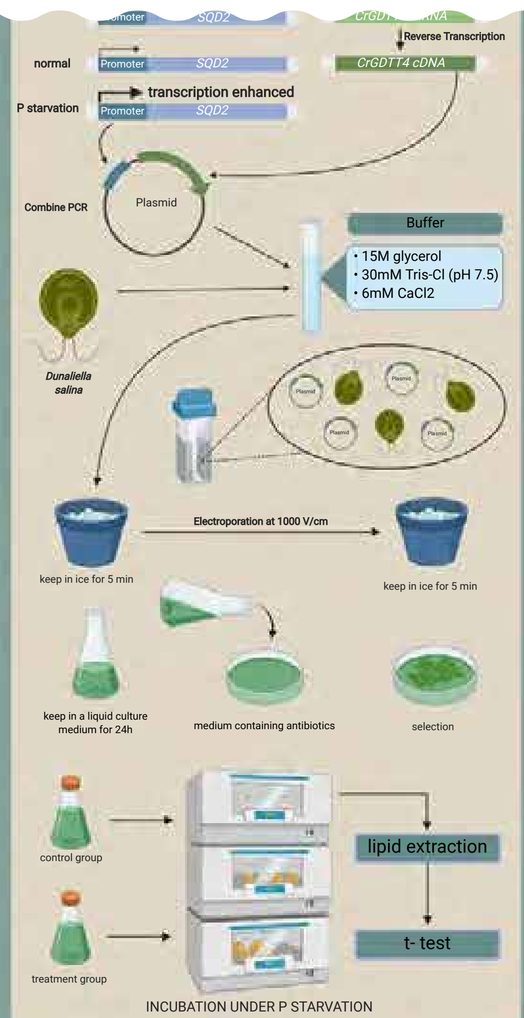

SOME FACTS

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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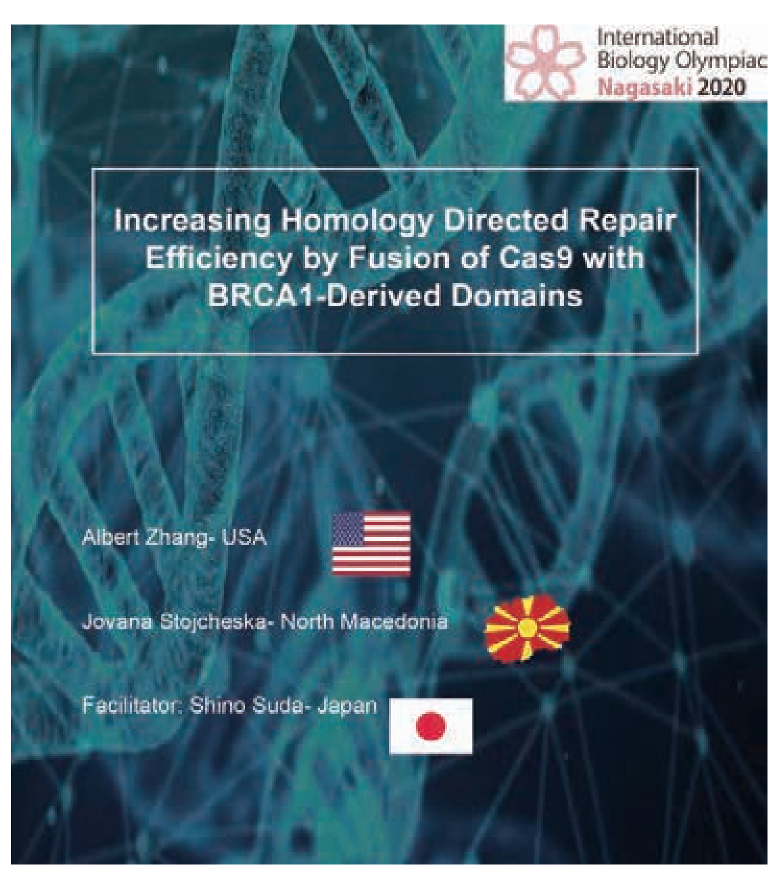
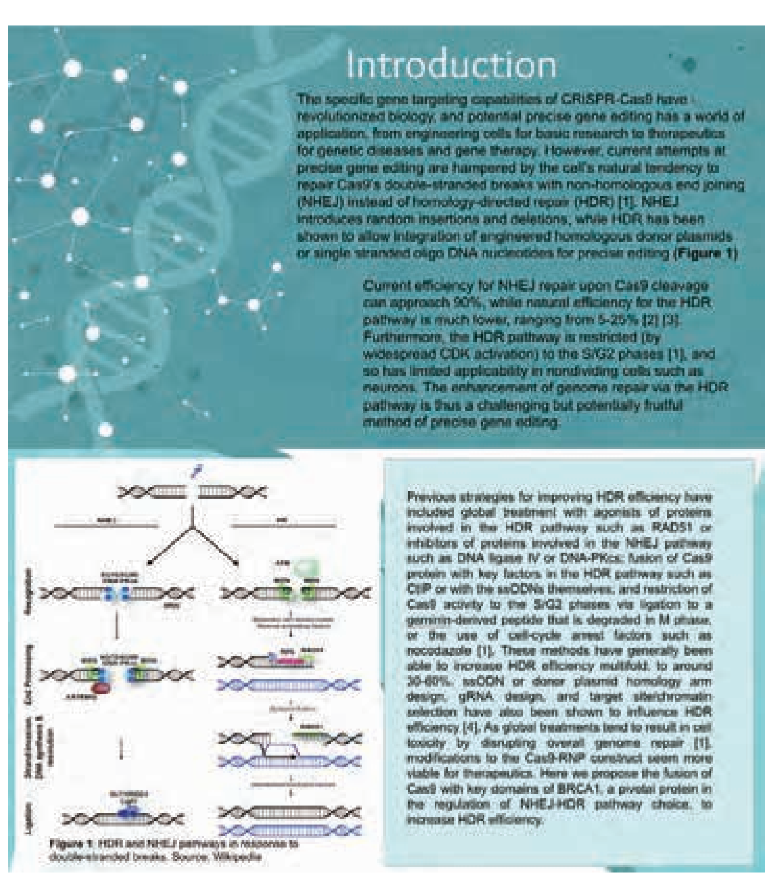
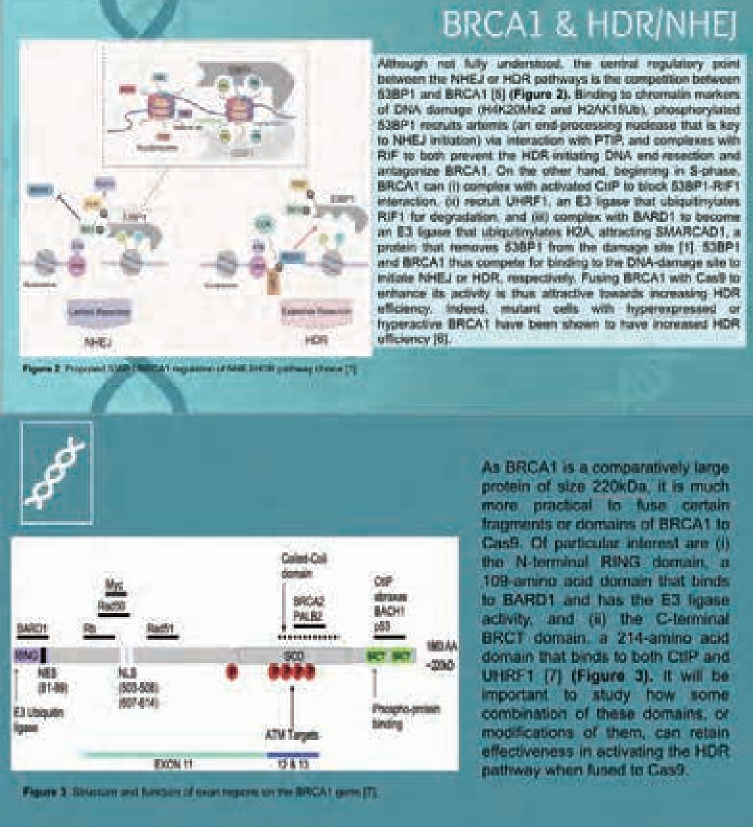
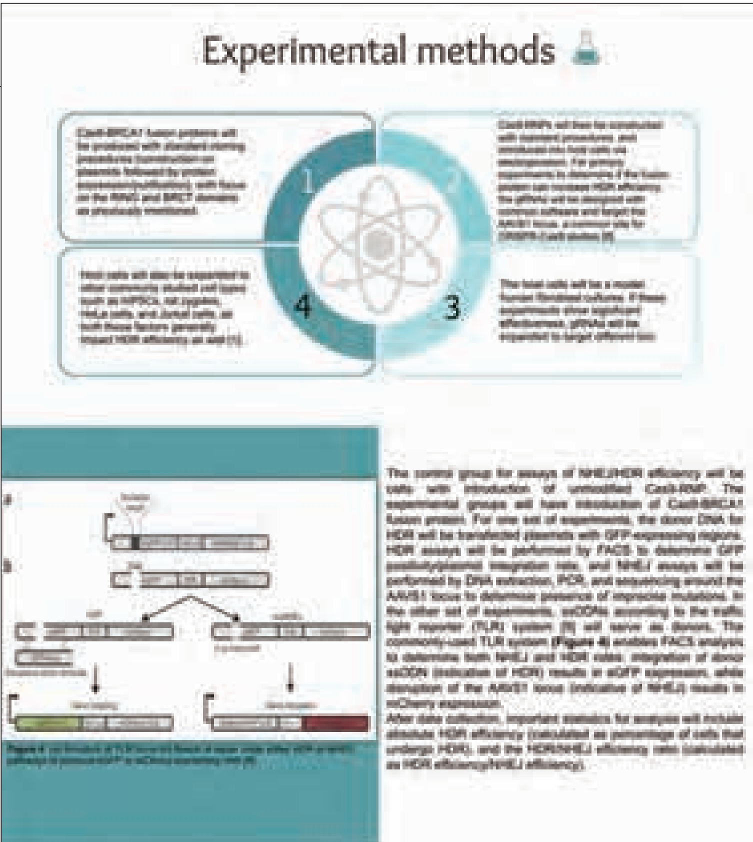






BRCA1 & HDR/NHEJ
Although not fully understood, the central regulatory point between the NHEJ or HDR pathways is the competition between 53BP1 and BRCA1 [5] (Figure 2). Binding to chromatin markers of DNA damage (H4K20Me2 and H2AK15Ub), phosphorylated 53BP1 recruits artemis (an end-processing nuclease 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].
NHEJ
HDR
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.
BARD1
Rb
Myc
Rad50
Rad51
Coiled-Coil domain
BRCA2
PALB2
CtIP
BACH1
p53
RING
SCD
BRCT
BRCT
NES
NLS
E3 Ubiquitin ligase
ATM Targets
Phospho-protein binding
EXON 11
12 & 13
Figure 3 Structure and function of exon regions on the BRCA1 gene [7].



Experimental methods
1
2
3
4
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
REFERENCES:

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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.

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).

Figure 1

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.

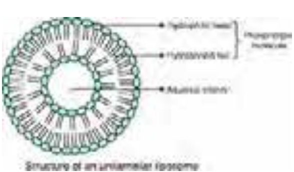

Figure 4

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

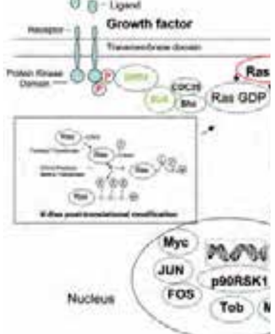

Figure 5

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 ha
that Cas9 can tolerate various type of mism

e liposomal CRISPR/Cas9 ointment

ıa Moskatelo

y using CRISPR/Cas9 is one of the most
ıa. 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

ethod, 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
vantages 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
nockdown the Raf sequence in cancer cells and
CRISPR/Cas9.

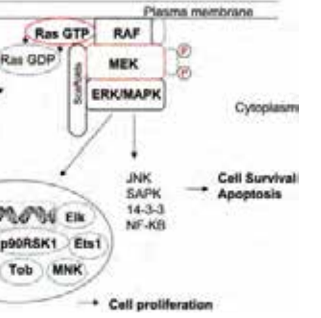

Figure 5

a guanine-rich NGG
ecognized by Cas9
kdown the mutated
wild type one.

"out of target"
r the Cpf 1 (Meijija
can change the
search has shown
of mismatches.

Figure 6

DISCUSSION

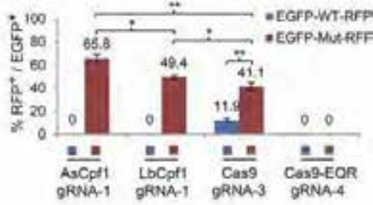

Figure 7

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).

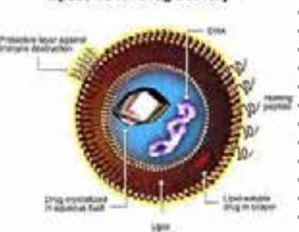

Figure 8

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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Combating disease and human enhancement

A scientific and socio-ethical approach

IBO Group Project 2020, Group 3B03
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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]

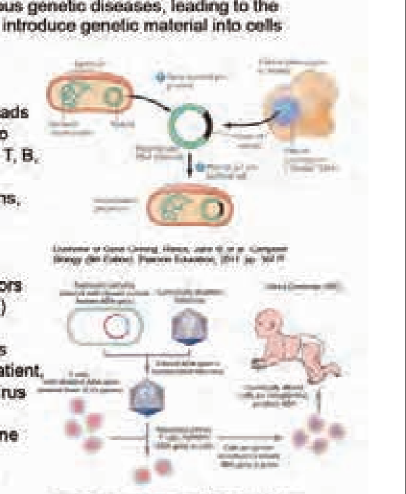
case study: treatment of severe combined immunodeficiency (SCID)

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]



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

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.

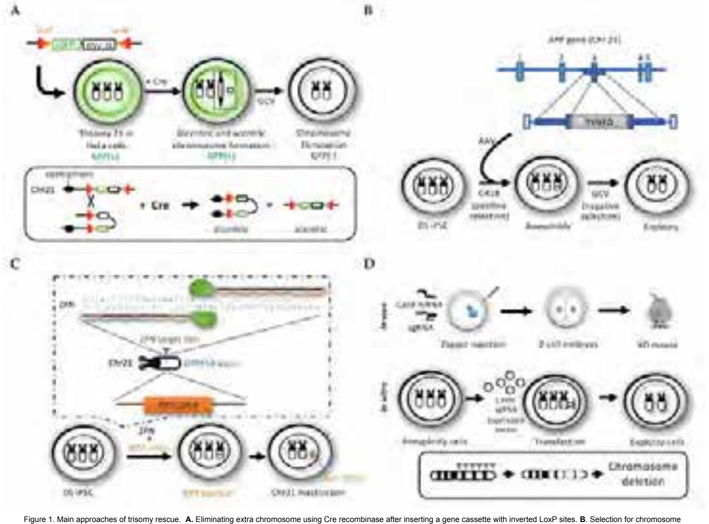

Figure 1. Main approaches of trisomy rescue. **A.** Eliminating extra chromosome using Cre recombinase after inserting a gene cassette with inverted LoxP sites. **B**. Selection for chromosome loss using neomycin and ganciclovir. **C**. Knocking-in XIST transgene to silence third chromosome. **D**. Eliminating 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.

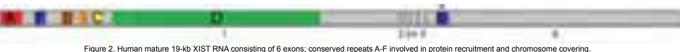

Figure 2. Human mature 19-kb XIST RNA consisting of 6 exons; conserved repeats A-F involved in protein recruitment and chromosome covering.

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.

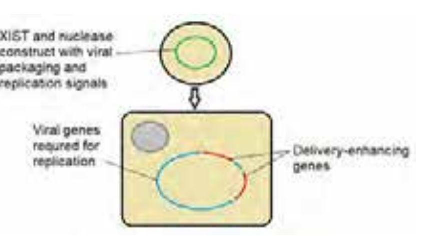

Figure 3. Approximate scheme of vector generation system

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]

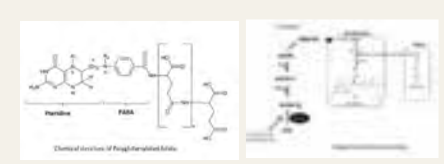

Figure 7: (A)Chemical structure of Polyglutamylated folate, (B) Biosynthesis of folates in plants; GTP, Guanine triphosphate; DHNPt, Dihydroneopterin; HMDHPt, 6-hydroxymethyldihydropterin; PABA, p-amino benzoic acid; DHP, Dihydropteroate; DHF, Dihydrofolate.[27]

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

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

- 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]

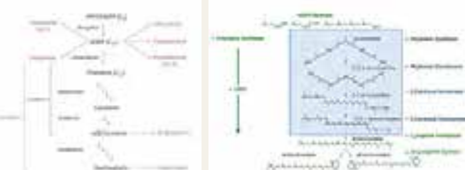

Figure 1: The carotenoid pathway. IPP: Isopentenyl-diphosphate, DMAPP: dimethyl-diphosphate, GGPP: Geranylgeranyl-diphosphate [7].

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

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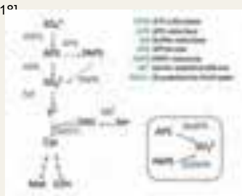

Figure 4: Assimilative sulfate reduction in plants. Sulfate is reduced to sulfite either by APS reductase (APR) via the primary (solid arrows) or an alternative secondary sulfate assimilation pathway (dashed arrows) with PAPS reductase (PAPR) [18].

- 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.

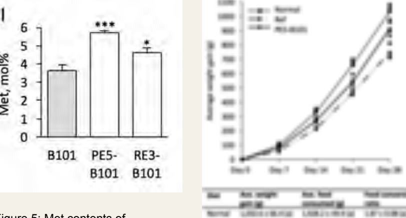

Figure 5: Met contents of transgenic plants PE5-B101 and RE3-B101 and non-transgenic plant B101 in mol% determined after protein hydrolysis and separation in a UPLC column.[18]

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]

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]

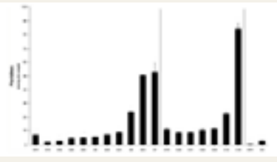

Figure 8: Total pteridine(part of folate) accumulation in AtGCHI-expressing bean seeds; seeds from each primary transgenic line are compared to Wild type(WT) seeds. 3 different lines- S(Saltillo), C(Cafe) and D(Duranga) were tested; error bars indicate standard error (SE).[27]

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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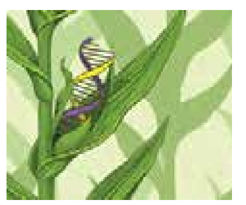
CRISPR-CAS9 IN CROP IMPROVEMENT

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,

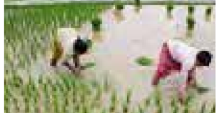
Current Research

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).

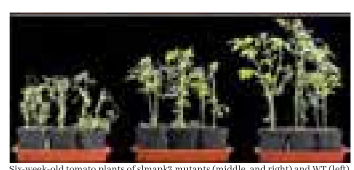
Disease Resistance

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

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 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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Dominik Primožič (Slovenia)

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

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 8 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 [9]. 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 [6]. 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 transfered 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 these 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 [5,6,8,12,13,14]

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]

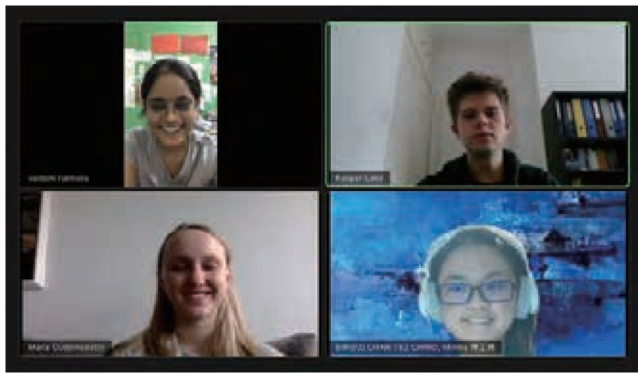
Pyrethrins' safety: Pyrethrins have been used as insecticides since the 1950s. Thus, their presence in plants would not be new for the ecosystems nor humans. Some insecticides containing pyrethrins can be used in organic agriculture [23]. Pyrethrins are also used in head lice treatment. Drugs containing 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 convulsions 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 [28]. 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.

4A02 Investigating the effects of species and habitat on the color response of birds



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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).

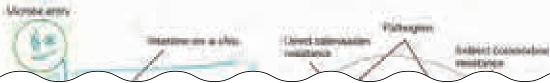
In physiology and medicine, a new frontier is also emerging: the study of the human microbiome. Human microbiota contain a network of complex relationships from symbiosis to pathogenicity between human and bacteria, au fond, creating an interaction between human and environment which can be passed onto 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 Knoblich 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:

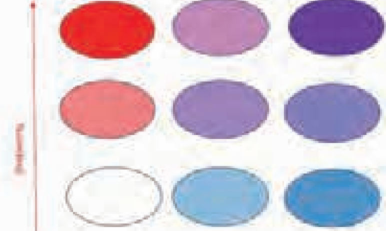
This experiment has two aims: first is to assess if biodiversity actually affects colonisation resistance; second is to find out if this effect is mediated solely by interspecific competition, or there are other ecological factors involved.

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).



the two listed (see figure 1).

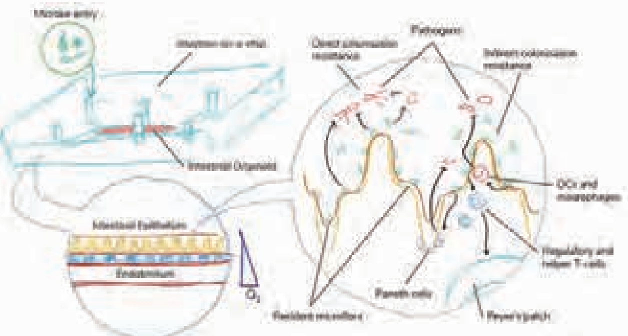


Figure 1. General picture of the experiments. Inoculation of selected microbes into the intestine-on-a-chip or other proposed organoids whereby a system in circulation with environment through fluid flow via bloodstream (endothelium) or intestinal fluid. O_2 gradient is created to replicate the anoxic 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.

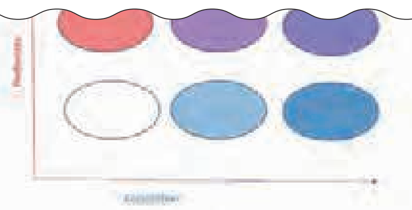


Figure 2. Two dimensional independent variable. In this experiment, competition and biodiversity are both independent variables; having said that, they can be interpreted as ordered pairs of one independent variable and span a two dimensional space. The growth curves of alien species are measured in 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

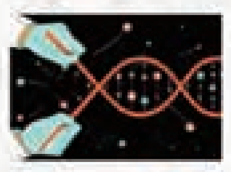
Reached equilibrium. Can preserve various genes, which would otherwise be removed, through advanced medical technology. People connected and isolation is not possible.

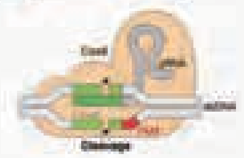
Traditional evolution

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.





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.

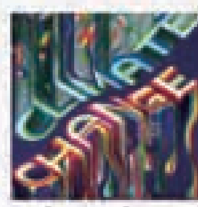
Oxygen partial pressure can relate to high altitudes, where it is much lower, the number of RBCs increases. More efficient RBCs and lungs with higher surface area might take the same path as opposable thumbs. Water availability has already shown evolutionary adaptation in kangaroos. As they have much-concentrated urine due to a slight change in their nephrons, humans might relate to such differences while living in water deficiency conditions.

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 them

[illegible]. 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

Evolutionary adaptation, sexual and natural selection, and genetic drift with Homo Sapiens populations. Humans' culture might have accelerated human evolution.

The Past

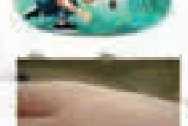
Archaic admixture

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.

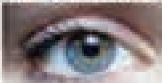
Agriculture

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 [illegible] 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.



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.

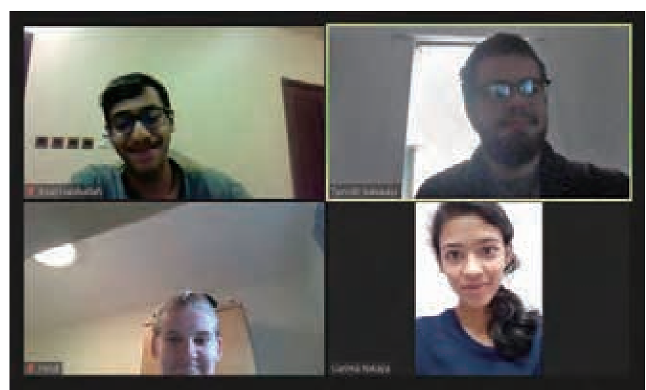
Holocene(12,000)

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.

Human Evolution

The Authors: Oona Kurola Geono Kim Zainab Al-alawi

4B03 Evolution of Neurodegenerative Diseases



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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.

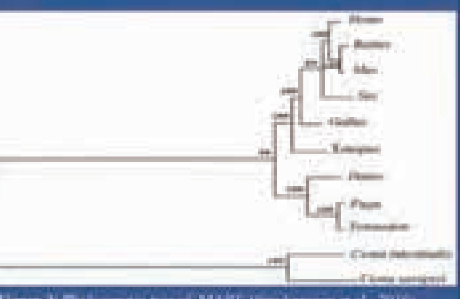

Figure 3: Phylogenetic tree of MAPT (Sündermann et al., 2016)

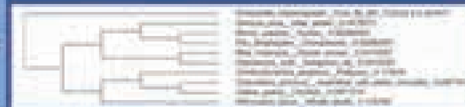

Figure 4: UPGMA-tree based on the sequences from translated protein of APP. Sequences collected from NCBI.

Are neurodegenerative diseases becoming common in present era?

Are the genes and proteins related to neurodegenerative diseases necessary to make a functioning neuron?

Figure 1: Normal brain and degenerated brain. Source:

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)

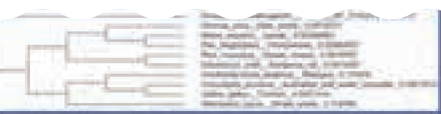

Figure 4: UPGMA-tree based on the sequences from translated protein of APP. Sequences collected from NCBI. (Sievers et al., 2011; Gouget et al., 2010)

Are neurodegenerative diseases becoming common in present era?

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.

Figure 5

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.amnh.com/...theguardian.com/science/2015/jun/10/brain-...-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; Gouget 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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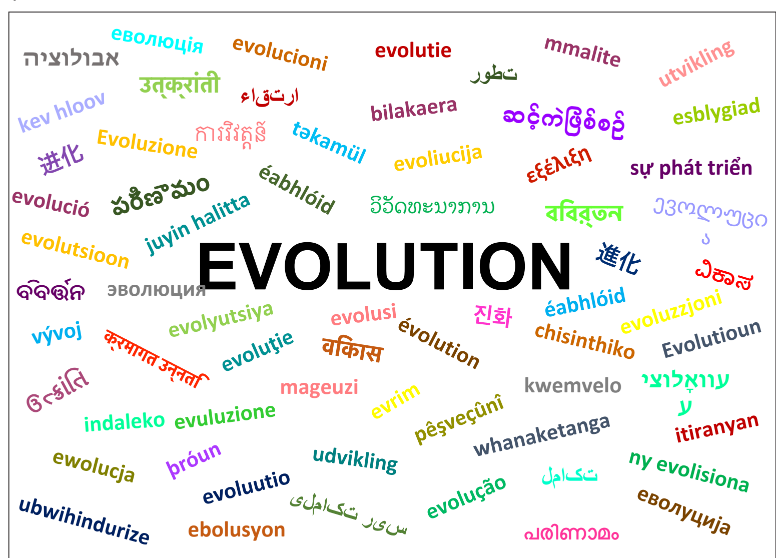


2

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.

3

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.

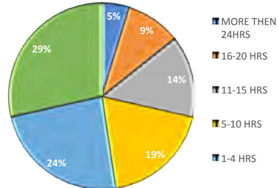
Cancer is most likely to form on the side of the head that is more likely used for calling.

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.



4

Biological solution

Biology open doors for all problems and can solve all that problems.

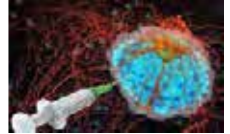
Scientist edit a living bacteria gene using syntactic biology. after injecting bacteria into a patient who showed tumor shrinkage. Coley's toxin as it come to be known was tried out on nearly a thousand patients to varying degrees of success.

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.



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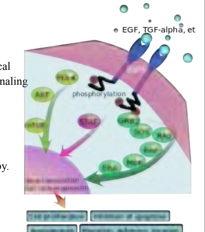
BIOLOGICAL SOLUTION



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.

6

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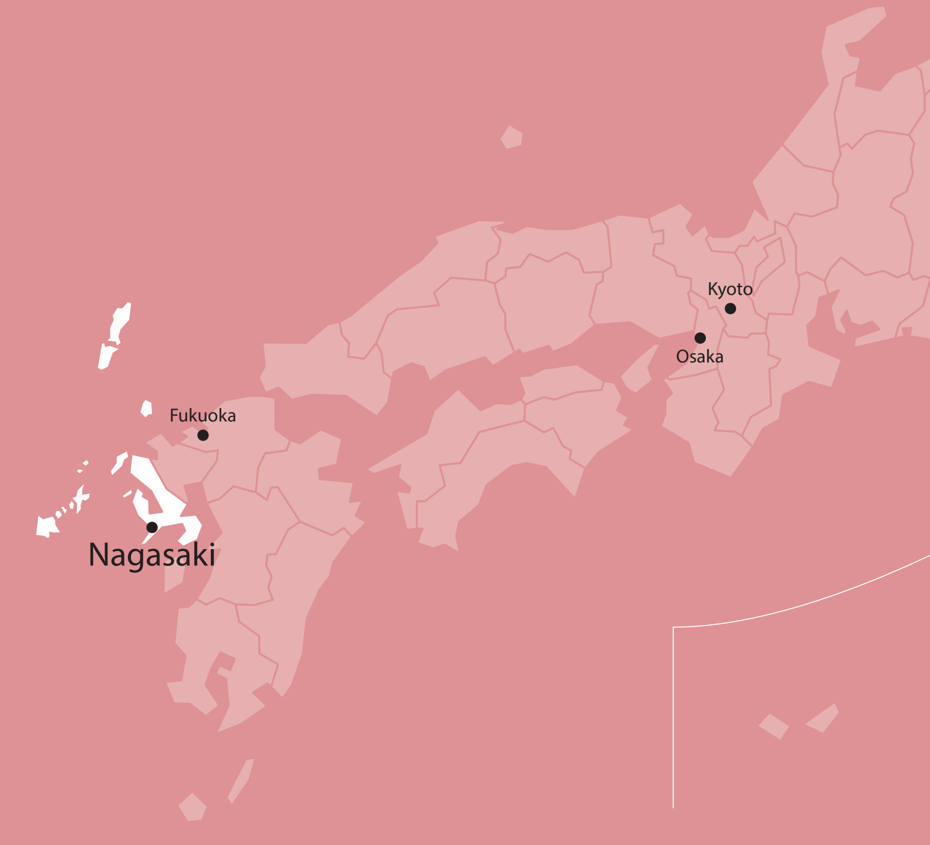
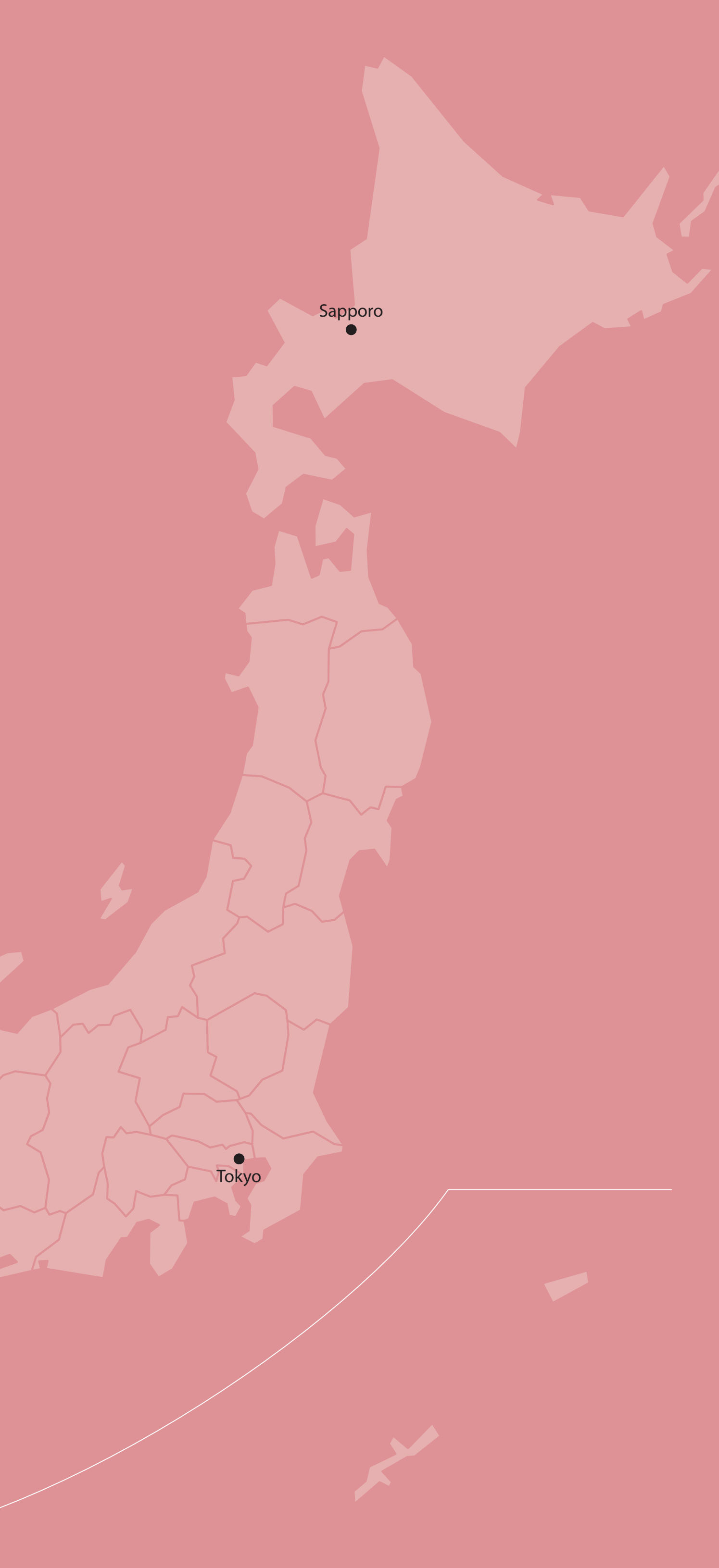
The 31st

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

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

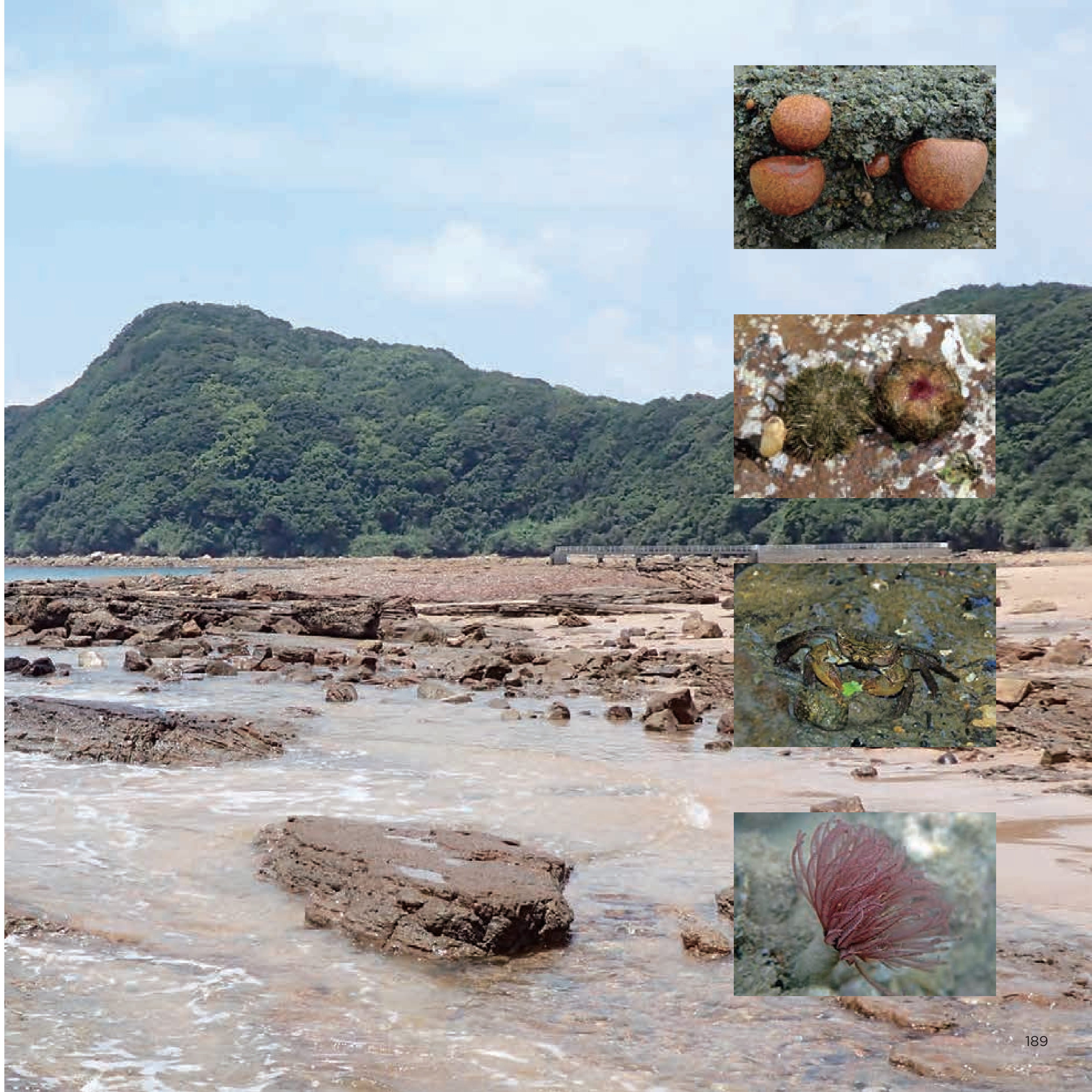
Opportunity to collaborate internationally over nature exploration

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

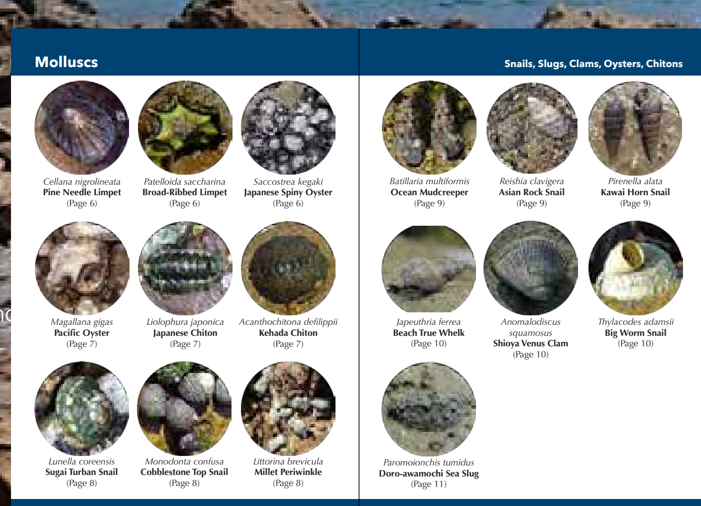
Seaside **A**ctivity of **S**hore **E**xploration in the **B**iology **O**lympiad

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



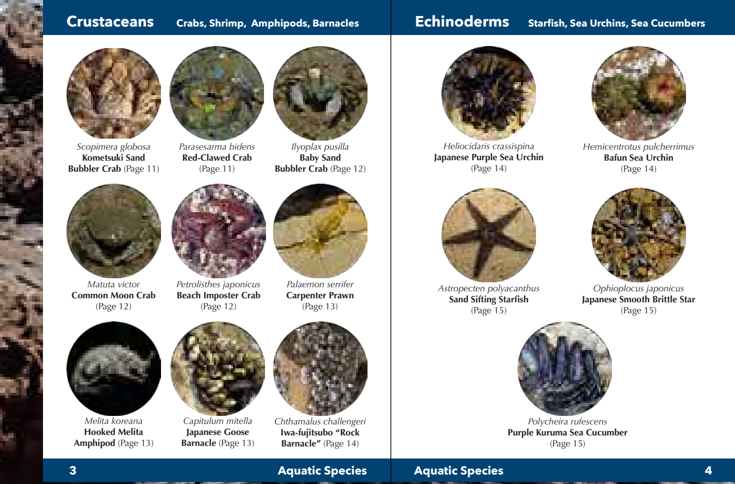
Molluscs — Snails, Slugs, Clams, Oysters, Chitons

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

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

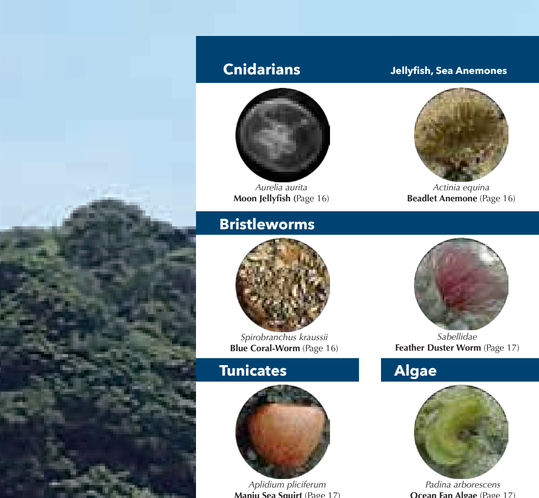
Scopimera globosa **Kometsuki Sand Bubbler Crab** (Page 11)
Parasesarma bidens **Red-Clawed Crab** (Page 11)
Ilyoplax pusilla **Baby Sand Bubbler Crab** (Page 12)
Matuta victor **Common Moon Crab** (Page 12)
Petrolisthes japonicus **Beach Imposter Crab** (Page 12)
Palaemon serrifer **Carpenter Prawn** (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)

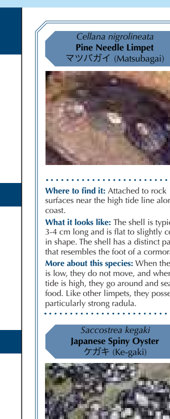
Tunicates

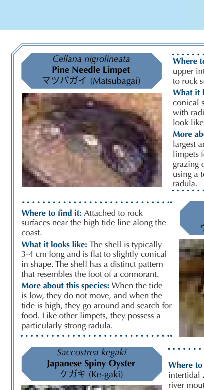
Aplidium pliciferum
Manju Sea Squirt (Page 17)

Algae

Padina arborescens
Ocean Fan Algae (Page 17)

5 Aquatic Species





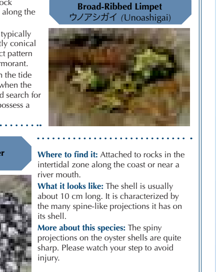
Cellana nigrolineata
Pine Needle Limpet
マツバガイ (Matsubagai)

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.

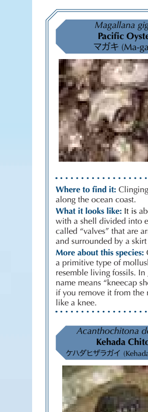
Saccostrea kegaki
Japanese Spiny Oyster
ケガキ (Ke-gaki)

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



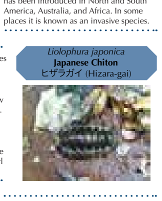
Magallana gigas
Pacific Oyster
マガキ (Ma-gaki)

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)

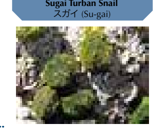
Where to find it: Attached to rocks along rocky ocean coasts in the Western Pacific.

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.

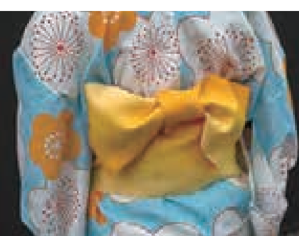
Buddhism and Zen in Japan

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.

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.

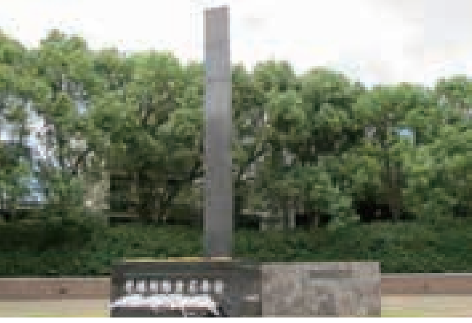
Jury Excursion

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.

Shimabara Castle



Hypocenter Park (the exact place where the bomb was dropped)

Mt. Unzen, an active volcano

The Kujukushima Archipelago

Tanada (Step-like Rice Fields)

NAGASAKI MAP

The Kujukushima Archipelago

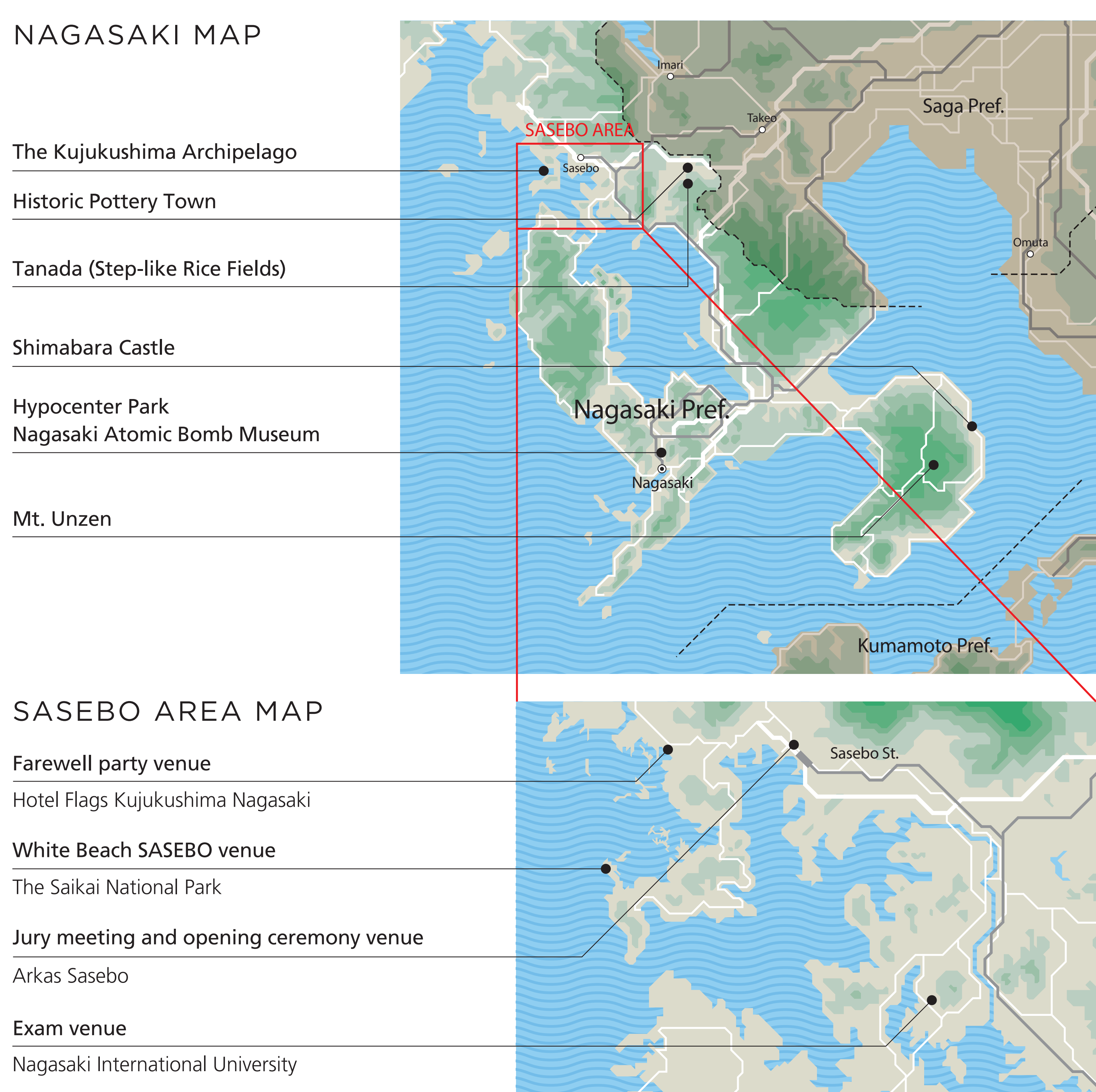
Historic Pottery Town

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.

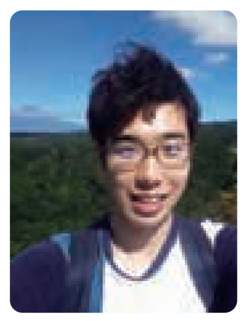
Because of all the pleasant interactions with the volunteers, we kept seeking ways to still involve them in our new event, the remotely held IBO Challenge 2020. When we decided to organize the International Group Project following the exam, we didn't think twice to create a supporting position and offer 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

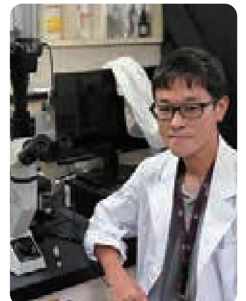
Midori Kajitani

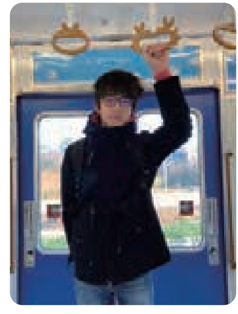
Riho Horie

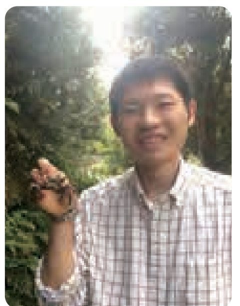
Ryota Takemoto

Shino Suda

Shusuke Atoji


Tomoyuki Wakashima


Uzuki Horo

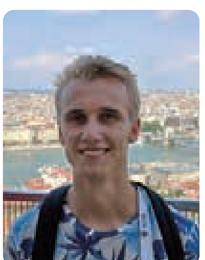

Yuiki Kondo

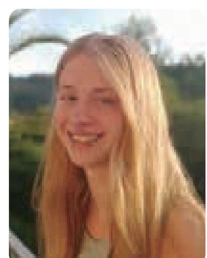
Yuki Koshida
IESO2017 Former Competitor

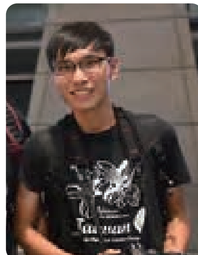
About International Volunteers

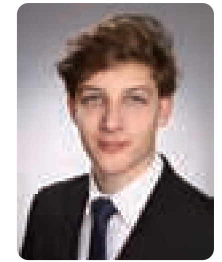
Team Guides

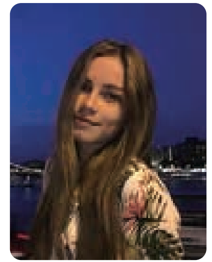
Alexandru Golic
(Sweden)

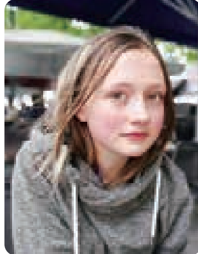

Alfred Petersson
(Sweden)

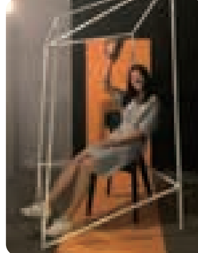

Alisia Zink
(Germany)

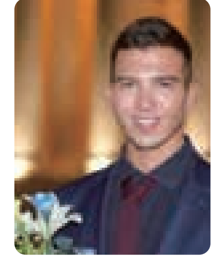

Chun-Wei Liu
(Chinese Taipei)

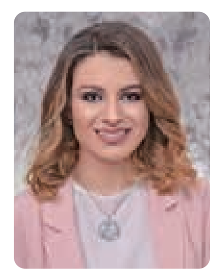

David Barnabas Balogh
(Hungary)

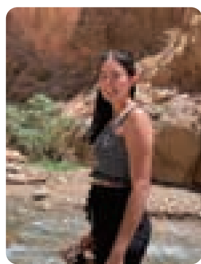

Dumitrita Ungureanu
(Moldova)


Elena Lacroix
(Belgium)

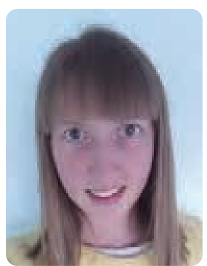

Hsiang Ting Wu
(Chinese Taipei)


Ioannis Stouras
(Greece)


Iskra Jovanovska
(North Macedonia)

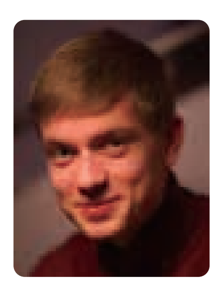

Jessica Law
(Australia)

Kai-Na Chiu
(Chinese Taipei)

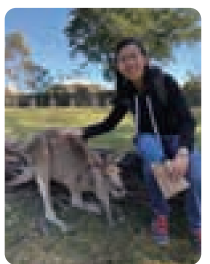


Katherine Lister
(UK)

Magdy Mekdad
(Romania)

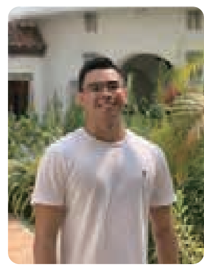


Otso Lauri Tapio Nieminen
(Finland)

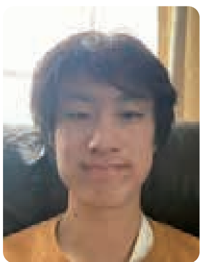


Shermane Yun Wei Lim
(Singapore)

Tai-Yi Chen
(Chinese Taipei)



Zack Dominic Orlina
(Philippines)



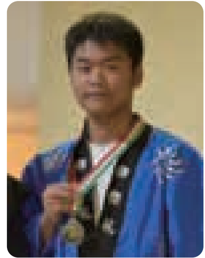
Zi Lin Wang
(New Zealand)

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

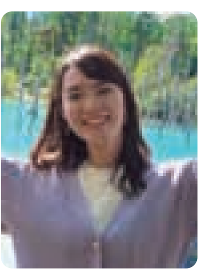
Jury Guides

Yotaro Sueoka


Ren Ishida

Workshop Guides (SCIBO Volunteers)

SCIBO: Student committee of International Biology Olympiad in JAPAN


Ayami Yamanaka

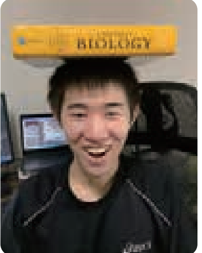
Ayana Tanino

Haruki Ishida

Kazuyuki Sanada

Kohei Oshima

Appreciate the moment.



Kou Takahashi

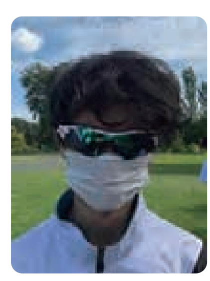
Masahiro Sakono

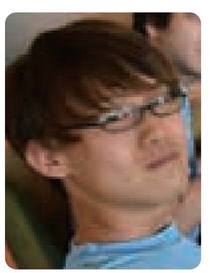
Mito Hotta

Ryo Suda

Find wonder in every day!

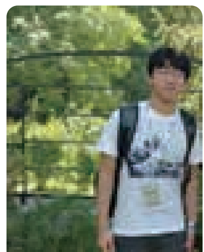
Ryohei Yufu


Ryota Akino

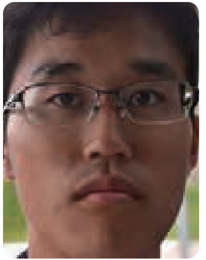

Ryotaro Fukue

Masato Shibuya M. D.

Jun Yatsu


Shigetaka Toba

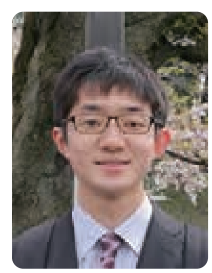
Shunsuke Ono


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

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.

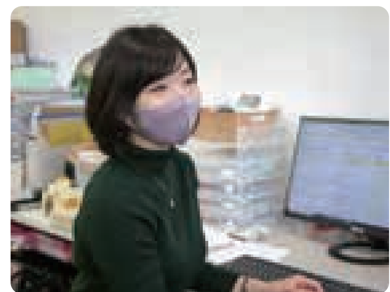
The part I cared about the most as the director was delivering the organizers' great passion for the event to the participants in a visible way. I hope I succeeded in that task. As the office nears its end, my stress level is finally starting to decrease. While organizing the event was rewarding, I may 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!

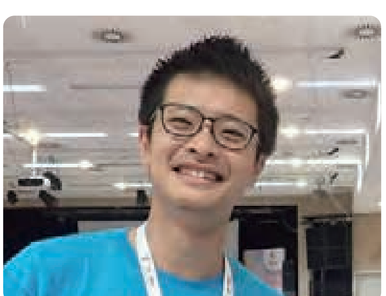
Even though our IBO Challenge 2020 was held remotely, a lot of countries and regions participated in the event. We would like to truly thank the IBO Steering Committee, the IBO Office, and all the NBO organizations around the world.

IBO spirit never fades!

Mitsuko Kudo
Director, the IBO2020 Secretariat Office

Kimiko Takeuchi

Siri McGuire


Kentaroh Honda

Organizers

■ 主催団体 Main host

第31回国際生物学オリンピック2020長崎大会 組織委員会
The 31st International Biology Olympiad 2020 Nagasaki Organizing Committee

公益財団法人 日本科学技術振興財団
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
The Union of Japanese Societies for Biological Science

■ IBO challenge 2020 International Group Project のみ共催
Co-host (Only IBO Challenge 2020 International Group Project)

公益財団法人 笹川平和財団 海洋政策研究所
The Ocean Policy Research Institute, The Sasakawa Peace Foundation

■ 後援団体 Endorsing Institutions

文部科学省
Ministry of Education, Culture, Sports, Science and Technology, Japan

厚生労働省
Ministry of Health, Labour and Welfare, Japan

長崎県
Nagasaki Prefecture, Japan

佐世保市
Sasebo City, Nagasaki

長崎県教育委員会
Nagasaki Prefecture Board of Education

東京理科大学
Tokyo University of Science

Organizing Committee

President	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
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
	Dr. Sadao Ishiwa	Deputy Director General at IBO Japan Committee
	Mr. Mitsuru Imoto	CEO, Nippon Wealth Life Insurance Company Limited
	Mr. Tsutomu Ueki	Standing Director, Japan Science Foundation
	Dr. Yoshinori Osumi	Special Emeritus Professor at Tokyo Institute of Technology, Nobel Prize winner
	Dr. Mitsuo Ochi	President, Hiroshima University
	Dr. Tadamitsu Kishimoto	Chief Director at Senri Life Science Foundation, Professor Emeritus at Osaka University
	Dr. Ryuzo Sakakibara	Head of Nagasaki International University Faculty of Pharmaceutical Sciences
	Dr. Fumimaro Takaku	President, Japan Association for Development of Community Medicine
	Dr. Tsutomu Tomioka	President of Nagasaki-Vietnam Friendship Association, Congressman
	Mr. Norio Tomonaga	Mayor of Sasebo City, Nagasaki
	Dr. Ryozo Nagai	President, Jichi Medical University
	Mr. Akiyuki Nagase	CEO, Nagase Brothers, Inc.
	Dr. Kyosuke Nagata	President, Tsukuba University
	Dr. Keiko Nakamura	Director, JT Biohistory Research Hall
	Mr. Houdou Nakamura	Governor, Nagasaki Prefecture
	Dr. Yoshihiro Hayashi	Director, National Museum of Science and Nature
	Dr. Hiroo Fukuda	Board Member and Vice-President of the University of Tokyo
	Dr. Motonori Hoshi	Professor Emeritus at Tokyo Institute of Technology
	Dr. Tasuku Honjo	Chairman of the Foundation for Biomedical Research and Innovation at Kobe, Professor Emeritus at Kyoto University, Nobel Prize winner
	Dr. Seizo Miyata	Chairman of The Frontier Salon Foundation, Professor Emeritus at Tokyo University of Agriculture and Technology

Organizing Committee

Members		
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	Mr. Masao Murakoshi	CEO, Murakoshi Holdings, Inc.
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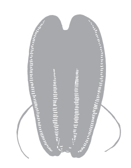

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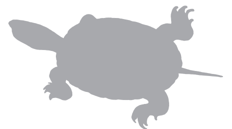
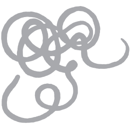
Lotus japonicus

Luehdorfia japonica

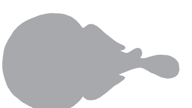


Mauremys japonica

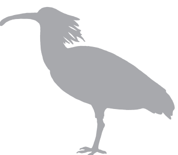


Meyersia japonica

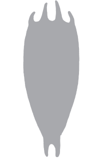
Mimobdella japonica



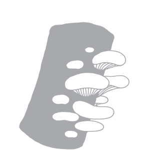
Narke Japonica



Nipponia nippon



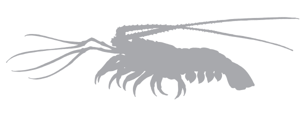
Notholca japonica



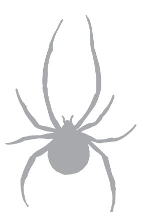
Omphalotus japonicus

Osmunda japonica

Oxycomanthus japonicus



Panulirus japonicus

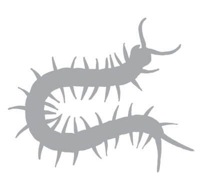


Parasteatoda japonica

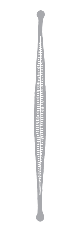
Perophora japonica

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.

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