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Playing with Nature: Synthetic Biology and the Amphibian Extinction Crisis

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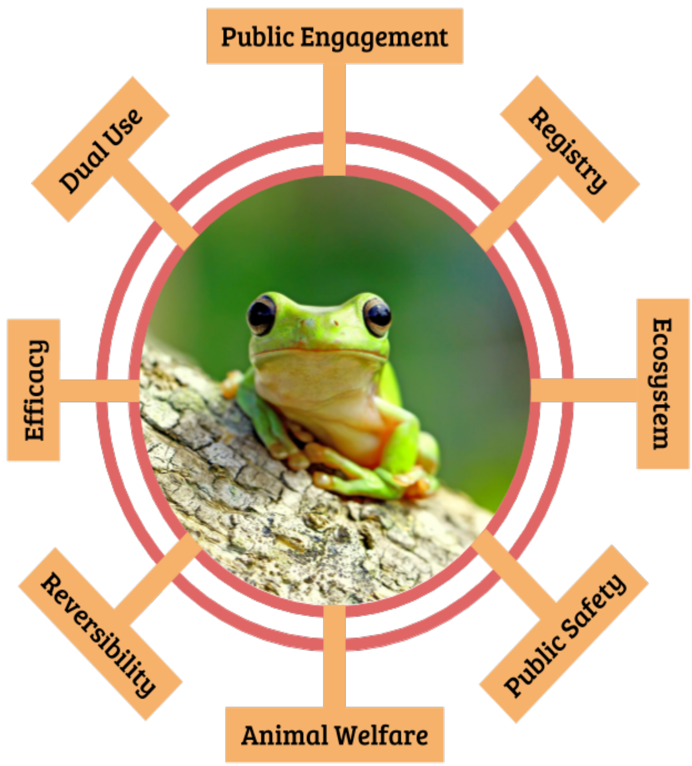
The uncontrolled spread of infectious diseases may have catastrophic worldwide consequences. Amphibian populations, including frogs, face unprecedented biodiversity loss and potential extinction due to the chytrid fungus. First detected in amphibians in the 1990s, chytridiomycosis is caused by two fungal species, *Batrachochytrium dendrobatidis* and *B. salamandrivorans,* which originated in Asia and spread by global trade and the marketing of exotic pets. Over 501 amphibian species have declined in number, 90 species are presumed extinct, and additional losses are predicted as anthropogenic trade eliminates geographic barriers and threatens biodiversity (Scheele et al., 2019). While we now understand much about the epidemiology and natural course of this disease, a treatment remains elusive. Infection control guidelines and a reduction in wildlife trade are urgently needed; in addition, emerging biosynthetic engineering tools may help mitigate the decline in frog and amphibian populations.

Although modification of species in the wild is controversial, genetic engineering techniques are becoming increasingly available and affordable. Many organizations, including *Revive and Restore* (2020), address conservation challenges through reproductive technologies and gene editing by applying them to other endangered species, including the black footed ferret, the passenger pigeon, and the heath hen. The College of Environmental Study and Forestry American Chestnut Project also conducts genetic engineering research intended to develop a blight resistant chestnut tree that may be restored to its native range (SUNY College of Environmental Science and Forestry [ESF], 2020). Similarly, amphibian research is elucidating the genes and major histocompatibility complexes that mediate disease susceptibility to chytridiomycosis. In a recent study on lowland leopard frogs infected with *B. dendrobatidis,* researchers discovered that the frogs that succumbed to the disease had a higher expression of MHC class IIβ than frogs who survived. While they predicted that increased immune gene expression would correlate with a positive disease outcome, they conversely found that surviving frogs had reduced immune gene expression compared to susceptible frogs. In addition, susceptible frogs had a larger number of IIβ expression, revealing a negative correlation between class IIβ alleles and survival. The authors infer that frogs with a stronger and sustained immune response with certain MHC variants may be less likely to survive chytridiomycosis (Savage et al., 2020). This and other functional genomics studies suggest potential genetic targets that could be utilized to confer immunity to amphibians. In addition, bioengineering and genetic engineering techniques, including CRISPR/Cas9, a gene editing tool that allows for the deletion, addition, or altering of a DNA sequence, have been proposed to develop a treatment for amphibians affected by chytridiomycosis (Grogan et al., 2018). However, there are potential risks, including, ethical concerns as humans breach natural boundaries, public disapproval, the potential to alter ecosystems in irreversible ways, and dual use concerns in which genetic engineering can be misused at an ecological or human level. It is therefore important that regulatory and policy guidelines keep pace



Applying synthetic biology to the amphibian crisis will require consideration of several biosecurity guidelines as proposed in the figure above.

PREPARED: Public Engagement, Registry, Ecosystem, Public Safety, Animal Welfare, Reversibility, Efficacy, and Dual Use

with advances in genetic engineering techniques related to preserving biodiversity (Palmer, 2020).

FIGURE 1. Guidelines for preventing harm in genetic engineering animals.

As scientists utilize emerging technologies to promote amphibian recovery, we propose that the following guidelines under the acronym PREPARED (Public Engagement, Registry, Ecosystem, Public Safety, Animal Welfare, Reversibility, Efficacy, Dual Use) be considered before performing genetic modifications to amphibians (Figure 1).

Public Engagement

Involving communities in the decision-making process that affects local ecosystems can increase public trust and support. A project that exemplifies the public openness needed in research is *Mice Against Ticks*, a community-guided synthetic biology project intended to prevent tick-borne disease by engineering a heritable immunity into the mouse that serves as a tick reservoir (Buchthal et al., 2019). By introducing antibody-encoded resistance alleles into the mouse genome, the disease transmission cycle can be broken. This project is shaped by community members on Massachusetts's islands of Nantucket and Martha's Vineyard, where community members meet and collaborate regularly with scientists starting from the inception of the project. In addition, they are continually asked to share their suggestions and concerns throughout the study development. The community collaboration not only provided development and support to the project, but also identified potential adverse ecosystem consequences that were not anticipated by the researchers (Buchthal et al., 2019).

Cultural, ethical, and historical considerations of local people potentially affected by an ecosystem change introduced by genetic engineering should also be evaluated. For example, the restoration project of the chestnut tree includes outreach meetings with elders of the Haudenosaunee Confederacy of Central and Upstate New York to discuss how to communicate about a genetically engineered chestnut. The chestnut tree contributes to many traditional family experiences, including gathering chestnuts, roasting them, and using the trunks to make cradles and coffins. The restoration of the chestnut tree reestablishes these family traditions, while employing novel interventions. The acceptance of a genetically modified tree by local residents is beneficial for its reintroduction and survival (Delborne et al., 2018).

Involving community members from local zoos, museums, institutions, and the government to address protecting amphibians from chytridiomycosis through synthetic biology will be helpful at the inception of the project. Assessing public acceptance of genetic manipulation in the local environment may facilitate identification of adverse ecological consequences and gain support from local stakeholders.

Registry

We propose the formation of an international registry system for research protocols and methods designed to apply genetic engineering to conservation efforts at an ecosystem level. An international registry was previously proposed in order to ensure that human germ-editing studies are safe (Chaib, 2019). This topic received considerable attention after the birth of genetically modified CRISPR babies in China (Nature, 2019). In addition, medical research patient registries have “facilitated reporting, retrospective and prospective research, professional development and service improvement.” (Nelson et al., 2019). FDA regulations include an Institutional Review Board (IRB) designed to review research involving human subjects, and the Institutional Animal Care and Use Committee (IACUC) designed to protect animals, but an international registry to protect ecosystems does not exist.

As proposed by the *Johns Hopkins Center for Health Security* (Nelson et al., 2019) for gene drives, the registry could encompass a four-tiered level:

- Tier 0: No registration requirement for research consisting of computer simulation
- Tier 1: Academic, Industry or Organization Research stage for genetic manipulation of animal genomes ex-vivo
- Tier 2: Field Trial stage for projects designed for field release
- Tier 3: Imminent Release stage for research ready to be released in the wild

Not only will a registry provide multiple safeguards against the potential release of dangerous genetic modifications into the wild, but it will also promote “open and responsive science, [that] flies in the face of current incentives” (Esvelt, 2016). This approach was also implemented by the *International Genetically Engineered Machine Foundation* (iGEM), which maintains a registry described as an “open community that runs and grows on the ‘Get, Give and Share’ philosophy.” The iGEM data is collected from teams and includes their research findings, which may be utilized in future synthetic biology projects and to promote academic research and education (iGEM, 2020). While risk assessments often occur at the institutional level, the potential for genetically modified organisms to spread in the environment is significant, and a national review should be performed prior to deployment. In addition, with disease as widespread as chytridiomycosis, it is possible that different interventions may spread and interact to the detriment of an individual ecosystem.

Ecosystem

Genetic engineering has the potential to rapidly and irreversibly alter ecosystems, and this risk must be evaluated when applying synthetic biology to nature. For example, self-propagating modified organisms may emigrate to other populations and alter other individuals. The environment has no boundaries, and local genetic modifications have a near endless opportunity to travel and spread through trade or migration. Potential dangers exist regarding the effects of the genetically modified species on the ecosystem and native populations, including the effect on biodiversity, keystone species, and the predator-prey imbalance. For example, if the Black Footed Ferret is engineered to become resistant to Sylvatic Plague, its usual prey, the prairie dog, may suffer a rapid population decline and disrupt the predator-prey imbalance. Furthermore, genetically modified species may become invasive, significantly altering existing communities and related species. For instance, when the cane toad was introduced to Australia, the native snakes displayed morphological adaptations which reduced their vulnerability to predation by toads. (Phillips & Shine, 2004). Before any research project is approved, the effects on the environment and surrounding population should be evaluated, realizing that not all ecosystem effects can be predicted. If frogs are genetically engineered for resistance to chytridiomycosis and are then released into the native ecosystem, the predator-prey ratios may become skewed, genes may migrate among species, and the ecosystem balance may change irreversibly.

Public Safety

Projects using synthetic biology to create disease resistance in endangered species should be evaluated for potential threats to human safety. The risk of creating animal hosts for pathogens is of concern when manipulating genetic immunity. Important considerations include determining if the intervention causes disease resistance (the ability of an animal to suppress the infection), in contrast to disease resilience (the case in which an infected host manages to live with the disease). If an intervention causes increased resilience in a target animal, the resulting population may act as a reservoir of infection for humans (Proudfoot et al., 2019). The Centers for Disease Control has identified high risk areas in which zoonotic diseases are most likely to cross over from animals to humans, and it is important to note that the creation of disease resistant animals could prevent a future pandemic (Centers for Disease Control and Prevention [CDC], 2020). As seen with the COVID-19 pandemic, zoonotic diseases are a major threat to humans that may result from human encroachment into the wild habitat, increased animal trade, and the presence of live animal markets. In addition, food insecurity is causing an increased reliance on bushmeat, with a resultant increase in zoonotic disease transmission, as was recently seen in Mongolia, where several died from plague after eating raw marmot meat (Kehrmann et al., 2020). Finally, genetic modification of animals or plants which enter the food chain need to be evaluated for consumption safety. In the case of modifying amphibians for resistance to chytridiomycosis, they may become a reservoir for disease, affecting humans and other animals if they become resilient to the disease, instead of developing resistance.

Animal Welfare

When a synthetic biology project involves changing animal DNA, steps must be taken to ensure that the intervention maintains the animal's integrity and dignity. As Colorado State University philosopher Bernard Rollin asserts, all projects should "accommodate the animal's interest when we alter an animal's telos..." (Ormandy et al., 2011). Additionally, the risk of unintended mutations, secondary diseases, and population control should be assessed and balanced with the goal of saving a species from extinction and illness burden (Ormandy et al., 2011). There is a significant risk of potential abnormalities associated with genetic changes, including adverse histological effects on organs and tissues. Although some abnormalities may not have a profound effect on animal welfare, they may significantly affect the animal's integrity, dignity, and success. It is therefore necessary for regulators to robustly study and monitor the genetically modified organisms in controlled research settings before widespread introductions, in order to mitigate risk to animal welfare. When using synthetic biology to engineer chytridiomycosis resistance in amphibians, careful measures need to be taken to avoid other genetic expressions or the creation of unintended mutations.

Reversibility

When altering an organism's genome, the DNA is permanently changed and may be inherited by future generations. With the use of a gene drive, the modified trait is inherited by all future generations, raising concern for adverse risks associated with genetic modification and highlighting the necessity for reversibility that would undo harmful changes. According to Massachusetts Institute of Technology researcher Dr. Kevin Esvelt, reversal gene drives have the capability to override the previous change and reintroduce the wild-type phenotype. The three main strategies for gene drive reversal are (1) immunization against the spread of gene drives, (2) precisely targeting subpopulations, and (3) limiting population suppression. However, if gene drives are not utilized, a reversal plan is still necessary. Finally, it is imperative to have regular monitoring of the genetically modified organism and its surrounding environment in order to determine if the reversibility strategies should be implemented to restore natural biodiversity. If genetically engineered amphibians or frogs are released into the environment, detailed surveillance should be performed and reversibility measures planned prior to release in the wild.

Efficacy

We propose a careful evaluation of the efficacy of genetic modification in comparison with less controversial techniques and to assess the viability of the strategy before significant investment. The *Chestnut Restoration Program* has deemed genetic modification to promote blight resistance to be far more effective than cross breeding with the Oriental Chestnut, a naturally fungal resistant species (SUNY College of Environmental

Science and Forestry, 2021). *Revive and Restore* focuses on enhancing biodiversity through genetic rescue and is evaluating the efficacy of genetic engineering the Black Footed Ferret to become resistant to *Yersinia pestis*, in contrast to developing an oral vaccine against the bacteria (Revive and Restore, 2020). In the amphibian, the efficacy of genetic expression to confer resistance, the ability to confer resistance to current and future generations, and the efficacy of disease containment should be described and compared with antifungal treatment, translocation methods, and selective breeding.

Dual Use

When genetic engineering is employed in nature, the potential for genetically enhancing disease resistance to animals may also create reservoirs of disease that spread through zoonotic transmission. Dr. Megan Palmer of Stanford University (2020) has asked if we can “manage the risk as quickly as we learn to manipulate life?” With the rapid advancement of technology, genetic engineering techniques are readily available and accessible to all, allowing for potential misuse. For example, it is possible to engineer an animal “superspecies” that is larger, resistant to disease, and has other favorable traits. In addition, concern has been expressed about the genetic modification of animals for their own well being or for the benefit of humans (Evans & Palmer, 2017). In order to mitigate dual use risk, we must predict and discuss adverse outcomes and have multiple layers of checks and balances.

Conclusion

Globalization and the resulting spread of fungal species has caused an unprecedented loss of amphibian life due to chytridiomycosis. A prompt reduction in wildlife trade is needed to save the biodiversity of our planet, along with the development of parallel regulation of emerging technology. As synthetic biology techniques are applied to conservation biology, we propose a set of considerations to be considered before genetic engineering is applied to conservation efforts. These considerations include engaging public input, creating a registry, evaluating the effect on the local ecosystem, assessing public safety, ensuring animal welfare, considering reversibility techniques, studying efficacy and addressing dual use concerns (PREPARED). The collaboration of molecular biologists, conservation biologists, policymakers and the general public will help us determine not only what techniques are available to save biodiversity, but also if and how these techniques should be applied in nature. By applying genetic engineering techniques in parallel with public policy, we may be able to stop the amphibian pandemic and prevent further species extinction due to chytridiomycosis.

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