



Selecting for Tolerance against Pathogens and Herbivores to Enhance Success of Reintroduction and Translocation

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Abstract: *Some species have insufficient defenses against climate change, emerging infectious diseases, and non-native species because they have not been exposed to these factors over their evolutionary history, and this can decrease their likelihood of persistence. Captive breeding programs are sometimes used to reintroduce individuals back into the wild; however, successful captive breeding and reintroduction can be difficult because species or populations often cannot coexist with non-native pathogens and herbivores without artificial selection. In captive breeding programs, breeders can select for host defenses that prevent or reduce pathogen or herbivore burden (i.e., resistance) or traits that limit the effects of parasitism or herbivory on host fitness (i.e., tolerance). We propose that selection for host tolerance may enhance the success of reintroduction or translocation because tolerant hosts generally have neutral effects on introduced pathogens and herbivores. The release of resistant hosts would have detrimental effects on their natural enemies, promoting rapid evolution to circumvent the host resistance that may reduce the long-term probability of persistence of the reintroduced or translocated species. We examined 2 case studies, one on the pathogenic amphibian chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]) and the other on the herbivorous cactus moth (*Cactoblastis cactorum*) in the United States, where it is not native. In each case study, we provide recommendations for how captive breeders and managers could go about selecting for host tolerance. Selecting for tolerance may offer a promising tool to rescue hosts species from invasive natural enemies as well as new natural enemies associated with climate change-induced range shifts.*

Keywords: artificial selection, *Batrachochytrium dendrobatidis*, *Cactoblastis cactorum*, cactus moth, captive breeding, chytridiomycosis, non-native species, tolerance

Selección de Tolerancia a Patógenos y Herbívoros para Incrementar el Éxito de la Reintroducción y la Translocación

Resumen: *Algunas especies no tienen suficientes defensas contra el cambio climático, enfermedades infecciosas emergentes y especies no nativas porque no han sido expuestas a estos factores a lo largo de su historia evolutiva, y esto puede disminuir su probabilidad de persistencia. El éxito de la reproducción en cautiverio y de la reintroducción puede ser difícil porque las especies o poblaciones a menudo no pueden coexistir con patógenos y herbívoros no nativos sin selección artificial. En programas de reproducción en cautiverio, los criadores pueden seleccionar defensas que prevengan infecciones o herbivoría o que reducen la carga de patógenos o herbívoros atacando directamente al patógeno o herbívoro (i.e., resistencia) o atributos que limitan los efectos del parasitismo o la herbivoría sobre la adaptabilidad del huésped (i.e., tolerancia).*

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Paper submitted August 16, 2011; revised manuscript accepted January 13, 2012.

Proponemos que la selección de tolerancia del huésped puede incrementar el éxito de los programas de reintroducción o translocación. Nuestra hipótesis es que los huéspedes tolerantes generalmente tienen efectos neutros sobre patógenos y herbívoros introducidos. En contraste, planteamos la hipótesis que los huéspedes resistentes tienen efectos perjudiciales sobre los patógenos y herbívoros porque promueven la evolución rápida para sortear la resistencia del huésped, lo cual puede reducir la probabilidad de persistencia a largo plazo de la especie reintroducida o translocada. Examinamos dos casos de estudio, uno en el hongo quitridio patógeno de anfibios (*Batrachochytrium dendrobatidis* [Bd]) y el otro en la polilla herbívora (*Cactoblastis cactorum*) en los Estados Unidos, donde es no nativa. Con anfibios, la tolerancia a Bd puede provenir de respuestas inmunológicas o de atributos fisiológicos que mantienen la función osmorguladora. En cactus, la tolerancia a la herbivoría puede ser mediada por incremento en la producción de brotes o por redistribución de energía de las raíces a la reproducción. El conocimiento de la importancia relativa de la tolerancia de huéspedes versus la resistencia podría tener implicaciones para taxa de plantas y animales amenazados.

Palabras Clave: *Batrachochytrium dendrobatidis*, *Cactoblastis cactorum*, especie no nativa, polilla del cacto, quitridiomycosis, reproducción en cautiverio, selección artificial, tolerancia

Introduction

Some species have insufficient defenses against introduced pathogens and predators because they have not been exposed to these organisms over evolutionary time. As species continue to shift their distributions naturally in response to climate change or as their distributions are changed artificially through translocation, they will be more likely to be exposed to non-native pathogens and predators to which they lack evolved defenses (Hoegh-Guldberg et al. 2008).

When species are unable to survive in their natural habitat because of an introduced pathogen or herbivore, conservation efforts, such as captive breeding, are often used to reduce the threat of extinction (Ebenhard 1995). The eventual introduction of captive-bred individuals to their natural habitat (Griffiths & Pavajeau 2008) or their translocation to another area thought to be sufficient habitat (Hoegh-Guldberg et al. 2008) is an inherent element of captive breeding programs. Artificial selection in captive breeding programs is often an important tool because natural selection may not occur fast enough to protect hosts from rapid exposure to novel pathogens or herbivores.

An unresolved question, however, is how to implement a successful reintroduction or translocation program for species that cannot presently coexist with introduced pathogens or herbivores. In the disease and plant biology literature, strategies for coexisting with pathogens (here, pathogens include micro- and macroparasites) and herbivores are dichotomized into resistance and tolerance (e.g., Strauss & Agrawal 1999; Nunez-Farfan et al. 2007; Raberg et al. 2007). *Resistance* refers to defenses that reduce pathogen or herbivore burden by either preventing infection or herbivory or directly attacking the pathogen or herbivore (e.g., immune responses to pathogens, herbivore-detering toxins); consequently, resistance has a direct adverse effect on the pathogen or herbivore. Resistance is typically measured as the inverse of pathogen abundance or herbivory given

a constant exposure to the pathogen or herbivore. Tolerance reduces the fitness consequences of a particular pathogen load or level of herbivory and thus, unlike resistance, it is hypothesized to have either a neutral or positive effect on the pathogen or herbivore (Miller et al. 2006). Tolerance is typically measured as the slope of the relation between host fitness (or a proxy measure of fitness) and pathogen or herbivore abundance (Fig. 1) (Raberg et al. 2007; Raberg et al. 2009). Hence, reintroduction and translocation programs can artificially select for individuals that are resistant or tolerant to a pathogen or herbivore or reintroduce or translocate the species without selection.

Given that resistance has a direct adverse effect on pathogens and herbivores, but tolerance does not, resistance and tolerance can impose different selection pressures on pathogens and herbivores (Roy & Kirchner

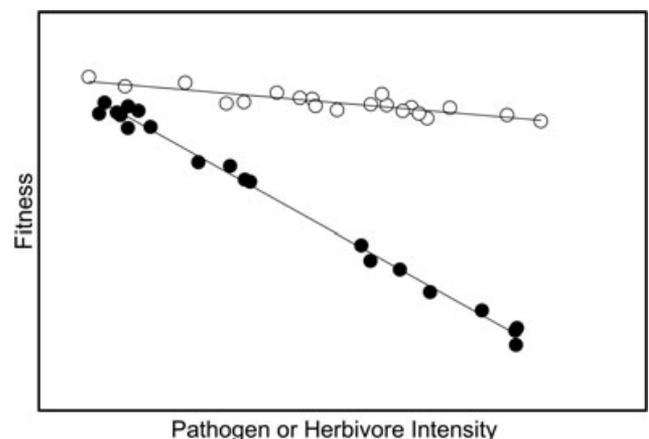


Figure 1. Comparison of the tolerance responses of individuals of 2 species or genotypes when exposed to a pathogen or herbivore (slope of lines is tolerance). Fitness and pathogen or herbivore intensity increase on each axis from the origin. Individuals of the species represented by the closed circles are less tolerant than those represented by the open circles.

2000) that may affect the success of reintroductions and translocations. For instance, to breed resistant individuals, one selects for traits that provide countermeasures (i.e., an action to oppose, neutralize, or retaliate against some other action) to pathogens or herbivores. Given that pathogens and most herbivores (e.g., insects) have shorter generation times than their hosts, host evolution would likely lag behind the countermeasures of the pathogens or herbivores. This evolutionary lag could result in more virulent pathogens or herbivores. (Here, we define *virulence* as the per capita effects of a pathogen or herbivore.)

In contrast, tolerance is expected to increase pathogen or herbivore abundance without increasing per capita virulence because high virulence may lead to rapid host mortality that would compromise transmission success (Frank 1996). Thus, tolerant hosts should have neutral effects on pathogens or herbivores (Roy & Kirchner 2000). Hence, selectively releasing hosts that are tolerant to pathogens or herbivores may maximize the potential success of reintroduction and translocation programs because there should be little selection for parasite or herbivore countermeasures and there could even be selection for decreased virulence. Hence, selection for host tolerance could improve the chances of long-term persistence of reintroduced or translocated species. Recent publications highlight potential applications of the resistance-tolerance framework to the study of animal species (Rohr et al. 2010; Johnson et al. 2011), but this framework has not been applied in a conservation context.

We considered the potential effects of selecting for resistance or tolerance to introduced pathogens and herbivores before a species is reintroduced or translocated on reintroduction success. We sought to encourage a thorough consideration of artificial selection of resistance versus tolerance in reintroduction programs, given that much of the emphasis in the literature is on selecting for resistance. We examined 2 case studies, a pathogen and a non-native insect herbivore. Our discussion is applicable to the reintroduction or translocation of many species threatened by non-native pathogens or herbivores, whether the reintroduction or translocation is driven by climate change or other mechanisms.

Amphibian Declines

Approximately 40% of amphibians are believed to be threatened with extinction (Stuart et al. 2004). Although numerous factors are driving their declines (Collins & Storfer 2003; Rohr & Raffel 2010), infectious diseases are increasingly implicated (Daszak et al. 2003). For example, the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) causes chytridiomycosis and is recognized as the proximate driver of many amphibian declines worldwide (e.g., Berger et al. 1998; Lips et al. 2006; Vredenburg et al. 2010). There is currently no vaccine for chytridiomy-

cosis, so conservation strategies focus on management of host populations. Captive breeding was explicitly recommended in the International Union for Conservation of Nature (IUCN) global amphibian action plan as a priority conservation initiative in response to the spatial and temporal spread of Bd epidemics (Gascon et al. 2007).

As a result of efforts by the IUCN Amphibian Ark network and other conservation initiatives, hundreds of threatened amphibian species are being bred in zoos, aquariums, and other locations around the world until a mechanism to successfully reintroduce the species into habitats in which Bd occurs is found (Gascon et al. 2007). These captive-breeding programs have been effective in conserving some species of amphibians (Griffiths & Pava-jeau 2008). However, the release of captive bred frogs without artificial selection is not an effective approach to reintroductions if Bd is present at the release site. For example, Stockwell et al. (2008) released 850 tadpoles of *Litoria* spp. into 3 ponds in Australia as part of a reintroduction effort. Within 13 months, all the introduced individuals died and mortality was attributed to Bd infection (Stockwell et al. 2008).

Reintroductions of amphibians that have been selectively bred are not novel (e.g., Mendelson et al. 2006; Gascon et al. 2007; Woodhams et al. 2011). However, previous work did not clearly differentiate whether resistance or tolerance to the pathogen was the ultimate goal of the selection (but see Woodhams et al. [2011]). The focus of selective-breeding research has been primarily on traits that confer Bd resistance (Voyles et al. 2011), such as antimicrobial peptides and commensal skin bacteria (Woodhams et al. 2007; Harris et al. 2009). This emphasis on resistance appears to conflict with evolutionary theory. Given that Bd potentially evolves faster than its hosts (approximate 4-day generation time), reintroducing resistant hosts could drive a rapid increase in Bd virulence. The increase in virulence, in turn, could eventually lead to a new outbreak of Bd that might increase the probability of extirpation of both the reintroduced host species and the host species that persisted after the first Bd outbreak. Selection for Bd tolerance, coupled with other reintroduction or translocation strategies, could greatly improve the probability of persistence of these threatened taxa. We assume amphibians that persist after a Bd outbreak and populations that are currently not declining due to Bd are tolerant to Bd and thus would persist if there were an increase in Bd prevalence after the release of native amphibian species that had been artificially selected for tolerance. It would be important to test this assumption before selecting for tolerance.

This recommendation poses the question – How do we select for amphibian tolerance to Bd? We suggest taking advantage of the fact that there is typically more variation among than within amphibian clutches for most traits. Hence, to test for tolerance differences among clutches, one could expose a subset of individuals per clutch to

Bd, swab their skin for Bd upon death, and test whether the slope of the relation between time of death and Bd load differs among clutches (the steeper the slope, the lower the tolerance; Fig. 1). Time to death is important because Bd infections in amphibians can be reduced or eliminated if body temperature is elevated (Chatfield & Richards-Zawacki 2011). Hence, the longer they survive with a Bd infection, the greater the probability they will encounter micro- or macrohabitats warm enough to clear the infection. Once the most tolerant clutches are identified, they could be bred. This process could then be iterated with each new generation until it is deemed that there is sufficient tolerance of Bd (i.e., traits that imply increased fitness at a given Bd load) to attempt a reintroduction. Given that many captive amphibian species have long generation times (>3 years), this process may not provide an immediate solution. Nevertheless, it may transform captive-breeding programs into more proactive endeavors.

A potential criticism of this approach is that it requires a subset of each clutch to be killed by Bd. This may not be necessary if reliable early signs of Bd-induced pathology or morbidity are identified. Once these signs are detected, the temperature could be increased immediately to allow for clearing of the infection (Chatfield & Richards-Zawacki 2011), or individuals could be treated with antifungal medications (Pessier & Mendelson 2010). Two promising early signs of Bd-induced morbidity are increases in amphibian metabolic rate and in levels of corticosterone, the primary amphibian stress hormone. Peterson et al. (2011) demonstrated that, compared with asymptomatic frogs, frogs symptomatic for chytridiomycosis exhibited significant increases in metabolic rate and corticosterone several weeks before they died. These measures could provide a warning of morbidity and steps could be taken to clear individuals of the infection. Metabolic rate can be measured easily through respiration analysis, and there are now relatively straightforward and inexpensive enzyme-linked immunosorbent assays that quantify amphibian corticosterone in plasma, urine, and feces (Narayan et al. 2010; McMahon et al. 2011). Detection of these and other early signs of chytridiomycosis could be used to prevent Bd-induced mortality.

An alternative approach is to identify traits of amphibians that could reliably predict their tolerance to Bd without pathogen exposure. Although some data are available on immune mechanisms that provide resistance to Bd (Voyles et al. 2011), there are few data on traits associated with tolerance. However, knowledge of the etiology of mortality due to Bd infections may provide information on mechanisms that promote tolerance. Typical amphibian pathology of Bd infection includes epidermal hyperplasia (Berger et al. 1998), which disrupts electrolyte transport in the amphibian epidermis and eventually leads to cardiac arrest (Voyles et al. 2009). Infected amphibians seem to have lower than expected levels of

inflammation and immunological resistance to Bd infection (Berger et al. 1998), which may reflect the lack of co-evolution. Given that some amphibian species appear to be tolerant to Bd (e.g., Parker et al. 2002; Davidson et al. 2003; Savage et al. 2011), some as-yet-undefined tolerance mechanisms must exist. Such mechanisms could be immunological, including responses involved in wound healing, such as production of type 2 T helper cells (Allen & Wynn 2011), or responses that reduce inflammation and reduce the activity of other immune cells, such as regulatory T cells (e.g., Sears et al. 2011). Furthermore, given the role of osmoregulatory dysfunction in the pathogenesis of chytridiomycosis, physiological responses that maintain these functions, despite Bd infection, may also promote tolerance. Likewise, a specific repertoire of major histocompatibility complex (MHC) receptors may increase host tolerance without the autoimmune consequences of less-specific MHC repertoires (Kurtz et al. 2006).

Cactus Moth

The cactus moth (*Cactoblastis cactorum*) is native to parts of eastern South America. It was introduced to Australia in 1925 to control non-native prickly pear (*Opuntia* spp.) that had colonized hundreds of thousands of hectares and thus made livestock grazing impossible (Dodd 1940). Within a few years after the release of the cactus moth, the abundance and distribution of prickly pear had decreased by 99.6%. Subsequently the cactus moth was introduced to South Africa, India, Hawaii, and Ascension Island to control non-native prickly pear.

Although the introduction of the cactus moth for biological control was successful in many parts of the world, it did not achieve its objective in North America. In 1957 the cactus moth was introduced to the Lesser Antilles to control native prickly pear on rangelands (Simmonds & Bennett 1966), but the cactus moth spread throughout the Greater Antilles and eventually colonized the United States. In 1989 the cactus moth was detected in the Florida Keys and since has spread northward to South Carolina and westward to Louisiana (Rose et al. 2011) and was recently discovered on cacti located off of the Yucatan peninsula of Mexico. On the basis of its rate of spread, it is projected to inhabit much of Mexico and the southwestern United States (Stiling 2002).

In Florida the cactus moth is believed to threaten persistence of 2 extremely rare cacti: the jumping cactus (*Opuntia triancanthos*) and the semaphore cactus (*Conseola corallicola*). Both species are endemic to the Florida Keys and exist in populations of between 15 and 100 adult plants on just 1 or 2 islands (Stiling 2010). In 1990 botanists collected a series of juvenile cacti around the base of all known adult semaphore cacti and propagated individuals in a greenhouse by breaking off pads and potting them. They did not artificially select for any

traits. During subsequent years, this greenhouse population was the source of 96, 180, 240, and 125 cacti planted on various islands in the Florida Keys in 1996, 1998, 2000, and 2002, respectively (Stiling 2010). The cacti planted in 2000 and 2002 were colonized by the cactus moth within 2 years and experienced substantial mortality. By 2008, 3.3% of the transplanted cacti were alive. The unsuccessful establishment of transplanted cacti is similar to the fate of many translocated endangered plant species for which no artificial selection occurred. For example, between 1910 and 1960, 13 plant species listed as endangered under the U.S. Endangered Species Act were reintroduced onto public lands in Hawaii, and none survived (Mehroff 1996). In general, few plants reintroduced into their natural habitat persist (Pavlik et al. 1993).

Selection of plant traits for tolerance of non-native herbivores (e.g., the ability of plants to regenerate and reproduce after herbivory) in captive-breeding programs may increase the success of introduction or reintroduction (Nunez-Farfan et al. 2007). For example, in the Caribbean, the Spanish lady (*Opuntia triancantha*) and the erect prickly pear (*Opuntia stricta*) have persisted for 50 years after the release of the cactus moth, despite the moths feeding on up to 44% of the cacti (Pemberton & Liu 2007). In Florida, the erect prickly pear and the eastern prickly pear (*Opuntia humifusa*) have survived over 6 years, during which time some plants have been colonized each year by the cactus moth (P.S., unpublished data). Together, these data suggest that some individuals of prickly pear cacti are tolerant of herbivory by cactus moths. Plant tolerance to herbivory can take several forms, such as increased net photosynthetic rate after herbivory, increased branching or tillering (production of shoots that grow from the initial seedling) after release from apical dominance, or energy reallocation from roots to aboveground production or reproduction (Strauss & Agrawal 1999). Reproduction of prickly pear cacti often involves the growth of new plants from fallen pads. Thus, a tendency to rapidly abscise stressed pads, such as those colonized by cactus moth larvae (as observed in other cacti [Stiling et al. 2004]), could increase tolerance for herbivory and could be selected for in captivity. In South Africa, the release of cactus moths to control non-native prickly pear cacti was not as successful as in Australia because in South Africa many plants dropped pads following herbivory by cactus moths and new plants became established (Hoffmann et al. 1998a,b). Thus, the assemblage of prickly pear cacti shifted from relatively few, large plants to more abundant, smaller plants (Hoffmann et al. 1998a,b).

Selection for tolerance may be effective not only as a reactive but as a proactive measure to reduce the probability of extirpations. For instance, given its present rate of spread (Rose et al. 2011), cactus moths will likely reach the mainland of Mexico and the southwestern United States. Artificially selecting for and releasing strains of

tolerant cacti in advance of the arrival of the cactus moth may prevent the extirpation or extinction of endemic cacti. This work may be facilitated by identifying rare endemic cacti species, testing the tolerance of different genotypes to cactus moths, and shipping tolerant plants to locations where the moth is already present.

Caveats

There are a variety of ecological risks that need to be considered before choosing to conduct selection-and-release programs, and it is important to weigh the risks against the possibility of extinction. For example, it is possible that pathogen or herbivore abundance will increase within a population following the introduction of tolerant hosts, which could result in pathogen levels exceeding host thresholds and increasing host mortality. Moreover, an overall increase in pathogen or herbivore abundance could facilitate the spread of those species beyond the area occupied by tolerant hosts. However, it is also possible that after a pathogen or herbivore outbreak, the surviving hosts may be the tolerant individuals, which would minimize the risk of reintroducing tolerant hosts into the environment. Although most theory suggests that tolerance should not select for increased pathogen virulence, alternative evolutionary scenarios exist and any change in host responses could shift the optimal virulence of a pathogen (Little et al. 2010). Thus, any trait associated with pathogen virulence could change in response to host defenses.

Another potential concern is that the translocation of species bred in captivity may introduce new pathogens and herbivores to the reintroduction or translocation site (Hoegh-Guldberg et al. 2008). Also, increased tolerance may result in trade-offs with other important traits, such as competitive abilities. For example, late flowering time and decreased leaf longevity, which are thought to increase tolerance to herbivory (reviewed in Strauss & Agrawal 1999), may decrease a plant's ability to attract pollinators and thus affect its ability to compete with other species. If selection for tolerance is genetically correlated with unfavorable traits, traits that confer tolerance could decrease long-term population viability.

Before the widespread implementation of tolerance-selection programs, we recommend first selecting for tolerance in species that are experiencing rapid population declines or are already highly endangered. We recommend using adaptive management strategies to enhance the likelihood of success. This could entail releasing both resistant and tolerant hosts at multiple sites followed by monitoring of the entire community (e.g., Rohr et al. 2007) to determine which approach is associated with long-term persistence of the reintroduced species and no adverse, unintended effects on other species. This process may increase the probability of success of artificial selection and release programs and improve the

probability of survival of host species from present and future non-native pathogens and herbivores as a result of range shifts associated with climate change.

Acknowledgments

We thank T. Raffel and L. Martin for discussions on the topic of resistance and tolerance.

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