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Confronting inconsistencies in the amphibian-chytridiomycosis system: implications for disease management

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ABSTRACT

Chytridiomycosis, caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*), is one of the largest threats to wildlife and is putatively linked to the extirpation of numerous amphibians. Despite over a decade of research on *Bd*, conflicting results from a number of studies make it difficult to forecast where future epizootics will occur and how to manage this pathogen effectively. Here, we emphasize how resolving these conflicts will advance *Bd* management and amphibian conservation efforts. We synthesize current knowledge on whether *Bd* is novel or endemic, whether amphibians exhibit acquired resistance to *Bd*, the importance of host resistance *versus* tolerance to *Bd*, and how biotic (e.g. species richness) and abiotic factors (e.g. climate change) affect *Bd* abundance. Advances in our knowledge of amphibian–chytrid interactions might inform the management of fungal pathogens in general, which are becoming more common and problematic globally.

Key words: alternative hosts, *Batrachochytrium dendrobatidis*, climate change, conservation, dilution effect, emerging infectious disease, fungal pathogen.

CONTENTS

I. INTRODUCTION

Infectious diseases of humans and wildlife are increasing at an unprecedented rate and place a significant burden on global economies, ecosystem function, and human health (Jones *et al.*, 2008). Outbreaks of pathogenic fungi are of particular concern because they have caused some of the largest mortality events in modern times and pose a disproportionately greater threat to plant and animal biodiversity than any other group of pathogens (Fisher *et al.*, 2012). For example, fungal infections are putatively responsible for 'white nose syndrome' in North American bat species, 'colony collapse disorder' in bees, and chytridiomycosis in amphibians –all of which have caused

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Fig. 1. A dead field-collected adult Southern leopard frog (*Rana sphenocephala*) heavily infected with the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; seen in the insert at 1000× magnification). *Bd* infections are associated with the epidermis of metamorphic amphibians and cause mortality from cardiac arrest.

substantial host population declines (reviewed in Fisher *et al.*, 2012). Amphibians, in particular, have experienced an unrivaled loss of biodiversity with approximately one-third of species threatened with extinction (Stuart *et al.*, 2004). Although several factors contribute to amphibian declines, some declines and extinctions are linked to the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*; Fig. 1) (Stuart *et al.*, 2004). *Bd* is found on six of the seven continents (Fig. 2) and is associated with widespread amphibian declines on each continent on which it occurs (Fisher, Garner & Walker, 2009).

Since the description of *Bd* nearly 15 years ago (Longcore, Pessier & Nichols, 1999), substantial efforts have been made to gain a basic understanding of the host–pathogen ecology of this system, as emphasized in recent reviews on *Bd* transmission (Kilpatrick, Briggs & Daszak, 2010) and emergence (Fisher *et al.*, 2009). Nevertheless, a substantial portion of amphibian–*Bd* ecology remains controversial because of inconsistencies and gaps in the literature. Conflicting results make it difficult to identify general patterns of chytridiomycosis outbreaks and implement successful disease management.

Highlighting and scrutinizing inconsistencies across studies can be the source of new ideas that can enhance our understanding of ecological systems and could lead to novel management approaches. As an example, the strong association between *Bd* and the keratinized tissues of amphibians (Kilpatrick *et al.*, 2010) has been assumed by many researchers to indicate that *Bd* specializes on amphibians. By contrast, several researchers have hypothesized that alternative hosts or vectors of *Bd* might exist (Laurance, McDonald & Speare, 1996; Rachowicz *et al.*, 2005), but identifying non-amphibian hosts has been elusive. Recent work, however, demonstrated that *Bd* can infect living crayfish and that *Bd*-infected crayfish could transmit *Bd* to non-infected tadpoles (McMahon *et al.*, 2013). Moreover, in the field, crayfish presence was the best predictor of whether a wetland supported *Bd*-infected amphibians, accounting for more variation than tadpole densities, amphibian richness, wetland area, and bullfrog presence (a known reservoir host for *Bd*) (McMahon *et al.*, 2013). These findings might offer insights into the spatial distribution of *Bd*, the persistence of *Bd* in the absence of amphibians, and discrepancies in the rates of *Bd* spread among geographic localities (e.g. Lips *et al.*, 2006, 2008). Furthermore, they suggest that controlling the abundance of alternative hosts may be an important aspect of managing chytridiomycosis. As with this example, resolving other conflicting findings or concepts within the amphibian–*Bd* literature might offer new hope for imperiled amphibians.

We highlight some frontiers in amphibian–*Bd* research by reviewing the conflicts surrounding the following topics: (*i*) *Bd* as a novel or endemic pathogen; (*ii*) how species richness affects *Bd* abundance; (*iii*) whether *Bd* abundance is increased or decreased by human impacts on the landscape; (*iv*) how climate change impacts *Bd* outbreaks; (*v*) the role of host resistance *versus* tolerance to *Bd*; and (*vi*) whether there is an acquired immune response to *Bd*. For each topic, we describe how resolving the conflict could enhance chytridiomycosis management and amphibian conservation.

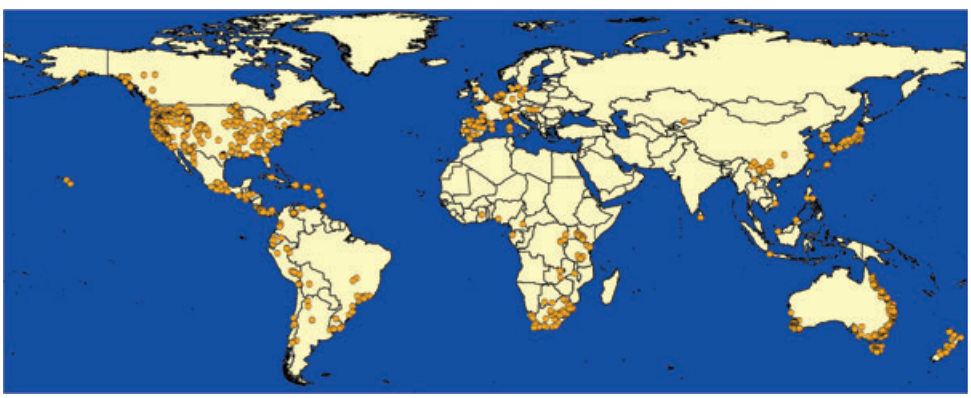

Fig. 2. The known global distribution of *Batrachochytrium dendrobatidis* (*Bd*). Each dot ($N = 1829$) represents a positive sample from precise geographic coordinates obtained from the Global *Bd*-Mapping Project (http://www.bd-maps.net/, accessed 23 October 2011) and supplemented with publsihed and unpublished literature. Image courtesy of Xuan Liu.

II. NOVEL *VERSUS* ENDEMIC PATHOGEN HYPOTHESES

The debate on whether *Bd* is (*i*) a novel pathogen expanding its geographic range and encountering hosts that lack evolved defences, or (*ii*) a widespread pathogen whose emergence was triggered by a change in the host, pathogen, or environment, is a key conflict in the chytridiomycosis literature. While each hypothesis has garnered some support (reviewed in Skerratt *et al.*, 2007; Fisher *et al.*, 2009), some of the strongest evidence for either hypothesis comes from studies documenting the spread of *Bd* across different spatial scales to areas where it did not exist previously (Lips *et al.*, 2006; Vredenburg *et al.*, 2010). Analyses of population genetics on smaller scales (i.e. within continent) show reduced heterozygosity in locations where *Bd* is hypothesized to have invaded recently (Velo-Anton *et al.*, 2012) and the recent whole-genomic analyses of 20 globally distributed *Bd* isolates showed a phylogenetic structure parsimonious with multiple global introductions of *Bd* (Farrer *et al.*, 2011), each supporting the novel pathogen hypothesis. However, the proposed *Bd* phylogeny from Rosenblum *et al.* (2013) suggests that the evolutionary history of *Bd* is more ancient than would be expected if it was a novel pathogen but lacks the within-clade signature that would be expected if *Bd* was an endemic pathogen. Resolving this conflict will require large-scale studies that utilize whole-genomic analyses of geographically diverse *Bd* isolates, specifically *Bd* isolates from under-sampled geographic localities, to identify patterns of global genome diversity.

Determining whether *Bd* is a novel or endemic pathogen has important implications for amphibian conservation, because disease management strategies depend on this distinction. If *Bd* is a novel pathogen, then the prevailing conservation strategy is to minimize its spread to unexposed populations (e.g. Skerratt *et al.*, 2007). By contrast, conservation approaches for an endemic pathogen usually involve managing biotic and abiotic cofactors that might favour chytridiomycosis outbreaks (e.g. Rachowicz *et al.*, 2005). Even if *Bd* is a novel pathogen, there are important cofactors that can affect its reproductive rate (R_0), transmission, outbreaks, and severity (many of which are discussed in this review). Hence, these two hypotheses are not mutually exclusive, as suggested by a recent study documenting that both human-assisted dispersal (i.e. propagule pressure) and environmental cofactors (realized niche) account for unique variation in the global distribution of *Bd* (Liu, Rohr & Li, 2013). Nevertheless, knowing the relative importance of each would help to target limited conservation resources.

III. BIODIVERSITY

One hypothesis regarding the relationship between biodiversity and *Bd* abundance is that increased amphibian species richness will reduce the risk of chytridiomycosis, because the relative abundance of competent host species (i.e. highly susceptible species in which the pathogen can effectively proliferate) will decrease as a function of increased biodiversity (the 'dilution effect'; Keesing *et al.*, 2010). Although recent experimental evidence confirms that the dilution effect can occur in the amphibian–*Bd* system (Searle *et al.*, 2011), evidence from sampling in natural populations offers conflicting results. Liu *et al.* (2013) found that amphibian species richness was not a significant predictor of *Bd* prevalence at the global scale. By contrast, Venesky *et al.* (accepted) provide support for the dilution effect hypothesis within the USA. Gaining a better understanding of how frequently the dilution effect occurs in nature, the scales at which biodiversity decreases chytridiomycosis risk, and the importance of species richness relative to other factors that influence *Bd* prevalence (e.g. climate; Rohr & Raffel, 2010) would help inform decisions regarding amphibian conservation.

Knowing when and where the dilution effect occurs in the amphibian–chytridiomycosis system and the specific traits of disease-amplifying or -diluting species (species that increase or decrease the abundance of a pathogen, respectively) would help to identify systems at risk from pathogen exposure and help to target management efforts. For instance, if certain amphibian and non-amphibian species are known *Bd* diluters or amplifiers (Venesky *et al.*, accepted), then populations of these species could be augmented or culled, respectively, as a tool to manage chytridiomycosis. Further, if host diversity reduces the risk posed by *Bd*, then surveys of amphibian diversity could be used to identify habitats and communities at the greatest risk from *Bd* exposure. Lastly, if host biodiversity is important for reducing *Bd* transmission, chytridiomycosis outbreaks might cause a positive feedback loop whereby the outbreak reduces amphibian species richness further increasing the severity of the epizootic. If so, early intervention for a *Bd* outbreak would be necessary to prevent any positive feedback from gaining steam.

IV. HUMAN IMPACTS

It is likely that humans have assisted in the global spread of *Bd*, which appears to have been recently introduced to at least two continents (Lips *et al.*, 2006; Fisher *et al.*, 2009), possibly from intentional or unintentional translocations of hosts (Farrer *et al.*, 2011). Although humans are likely moving *Bd*, the effects of human-induced landscape change on *Bd* remains controversial. Three studies found a positive relationship between human population density and *Bd* presence (Adams *et al.*, 2010; Murray *et al.*, 2011; Rohr, Halstead & Raffel, 2011). By contrast, *Bd* prevalence and infection intensity were found to be lower at deforested sites relative to sites with minimal anthropogenic habitat change (Van Sluys & Hero, 2009; Becker & Zamudio, 2011). Similarly, Raffel *et al.* (2010) found lower levels of *Bd* in red-spotted newts (*Notophthalamus viridescens*) in ponds whose margins had fewer overhanging trees, consistent with the relationship between

deforestation and *Bd* prevalence in Becker & Zamudio (2011) and Van Sluys & Hero (2009). Paradoxically, habitat loss, such as tree and shrub removal, has directly contributed to amphibian declines (Becker *et al.*, 2007), but might also reduce *Bd* outbreaks. Resolving whether this conflict is simply one of spatial scale or whether human-altered landscapes actually decrease *Bd* prevalence has important consequences for amphibian conservation. For instance, there might be a trade-off between restoring a habitat and increasing the prevalence of *Bd*; understanding the interactions between habitat loss and chytridiomycosis would allow practitioners better to predict and adaptively manage geographic areas at greatest risk for this disease.

V. CLIMATE CHANGE

The notion that global climate change will affect chytridiomycosis has garnered considerable attention in the literature (e.g. Pounds *et al.*, 2006). The primary support for this hypothesis (Pounds *et al.*, 2006) came under scrutiny because the correlation between increasing air temperature and amphibian extinctions was temporally confounded (Rohr *et al.*, 2008). Recent studies provide support for less confounded associations among El Niño intensity, temperature variability, *Bd* outbreaks, and amphibian extinctions (Rohr & Raffel, 2010), and for a causal link between temperature variability and increased chytridiomycosis (Raffel *et al.*, 2013). Nevertheless, skepticism remains about the relationship between climate change, temperature variability and chytridiomycosis (http://dotearth.blogs.nytimes.com/2012/08/15/on-frogs-fungi-climate-and-headlines/).

Temperature is considered one of the most important environmental factors driving *Bd* epizootics (Kilpatrick *et al.*, 2010; Rohr & Raffel, 2010). Since global climate change is projected to influence temperature variability at monthly and diurnal time scales (Easterling *et al.*, 2000), it is important to test how changing global temperatures will influence the spread of *Bd*. Increasing temperatures might be expected to decrease *Bd* prevalence because *Bd* is sensitive to high temperatures (Piotrowski, Annis & Longcore, 2004) and amphibians are generally more resistant to *Bd* at higher temperatures (Woodhams *et al.*, 2008; Raffel *et al.*, 2013). However, *Bd*-associated declines in the Neotropics were more common following high-temperature years (Rohr & Raffel, 2010), suggesting that increased temperatures associated with climate change might actually increase *Bd* outbreaks. Recent findings regarding the effects of temperature variability on *Bd*-related amphibian declines showed that increased maximum temperatures were positively correlated with the magnitude of temperature drops from 1 month to the next. These temperature drops were, in turn, more predictive of *Atelopus* declines than temperature increases (Raffel *et al.*, 2013). This result is consistent with work demonstrating that drops in temperature trigger the release of *Bd* zoospores (Woodhams *et al.*, 2008) and reduce the ability of amphibians to mount an antimicrobial skin peptide-based immune response and induce a more pronounced inflammatory reaction associated with higher *Bd* burdens (Ribas *et al.*, 2009).

Apparently contradictory results for the temperature-dependence of *Bd* infection might also result from a failure to account for temperature-dependent amphibian immune responses. Temperature-dependent growth of *Bd* on frogs is often assumed to reflect *Bd* growth rates in culture (e.g. Pounds *et al.*, 2006; Woodhams *et al.*, 2008), but recent evidence suggests that temperature-dependent growth of *Bd* on frogs (assessed by measuring *Bd* loads on amphibian skin using quantitative polymerase chain reaction) can be very different than in culture (Raffel *et al.*, 2013). Understanding the relationship between global climate change and *Bd* outbreaks will require manipulative experiments that test how factors associated with climate change affect *Bd* growth and transmission. Future studies that address these topics, and others, will improve our ability to forecast if and how climate change will influence patterns of chytridiomycosis.

VI. HOST RESISTANCE AND TOLERANCE

One important general question in disease biology is whether hosts respond to parasites *via* resistance mechanisms, which directly reduce parasite colonization and/or burden, or tolerance mechanisms, which reduce the negative fitness consequences of infections without reducing parasite burdens. In the amphibian–*Bd* literature, emphasis has been on mechanisms of amphibian resistance to *Bd*, such as antimicrobial peptides, commensal skin bacteria, and heat clearance (reviewed in Woodhams *et al.*, 2011). By contrast, theoretical work suggests that tolerance mechanisms against parasites might be more common than resistance mechanisms, because they do not have a detrimental effect on parasites (for additional details, see Roy & Kirchner, 2000; Venesky *et al.*, 2012). Hence, while much of the empirical research on *Bd* emphasizes host resistance, theory suggests that tolerance might be as or more important for the survival of infected amphibians.

Knowing the relative importance of host resistance *versus* tolerance to *Bd* could have important conservation implications for amphibians. A perplexing aspect of the amphibian–*Bd* system has been why some populations of amphibians persist with *Bd* infections while others are extirpated (Woodhams *et al.*, 2011). Some of this variation might be driven by disparities in the virulence of different *Bd* strains (Farrer *et al.*, 2011) or environmental cofactors, such as temperature and precipitation (Rohr *et al.*, 2008; Rohr & Raffel, 2010), but much of it might also be caused by variation in host resistance or tolerance (Savage, Sredl & Zamudio, 2011). Indeed, field data demonstrate that some amphibians remain healthy and exhibit little mortality while carrying detectable *Bd* loads whereas other amphibians die with lower *Bd* loads (Savage *et al.*, 2011), suggesting tolerance to *Bd*. Controlled laboratory experiments that decouple

resistance from tolerance [especially those controlling for differences in internal transcribed spacer (ITS1) copy number; Longo *et al.*, 2013] are needed in this host–pathogen system.

If tolerance to *Bd* is common, it could have important effects on amphibian community dynamics and the persistence of non-tolerant hosts. *Bd* infections in highly resistant amphibians should produce few zoospores when infected with *Bd* and thus should have low transmission potential. By contrast, highly tolerant amphibians might be 'super-shedders', which produce many zoospores, increase *Bd* transmission, and put non-tolerant hosts at risk (Venesky *et al.*, 2012). As such, the relative abundance of resistant and tolerant hosts within amphibian communities could either increase or decrease *Bd* transmission, with potential ramifications for the evenness and richness of amphibian communities.

A more thorough understanding of resistance and tolerance to *Bd* could also facilitate successful reintroduction of amphibian species to the wild. The IUCN global amphibian action plan recommends rescuing amphibians that face immediate threats of extinction from *Bd* and rearing those individuals in captive breeding colonies (Gascon *et al.*, 2007). In principle, artificially selecting these individuals for tolerance or resistance to *Bd* (depending on the pros and cons of each) might facilitate successful reintroduction of these species into the wild where *Bd* persists (Venesky *et al.*, 2012).

VII. ACQUIRED IMMUNITY

There is compelling evidence that the innate component of the amphibian immune system, namely antimicrobial peptides, can effectively inhibit *Bd* infections (reviewed in Rollins-Smith, 2009). However, evidence for acquired immunity to *Bd* is mixed. Two recent studies used a whole-genome approach to characterize the gene expression of *Xenopus tropicalis* after exposure to *Bd* and found that most genes associated with an adaptive immune response exhibited decreased expression compared to non-infected frogs (Ribas *et al.*, 2009; Rosenblum *et al.*, 2009). By contrast, Savage & Zamudio (2011) found that specific major histocompatibility complex II (MHC II) genes were associated with survivorship of *Lithobates yavapaiensis* after *Bd* infection. Given the role of MHC II in immune memory (e.g. expression on memory B cells; Janeway, 2008), this suggests that the presence of certain MHC alleles could enhance acquired immunity to *Bd*. There is also conflicting information regarding how amphibians respond to immunizations against *Bd*. Ramsey *et al.* (2010) immunized *X. laevis* with heat-killed *Bd* and found that immunized frogs had elevated *Bd*-specific antibodies compared to non-immunized frogs, suggesting that acquired immune defences are involved in resistance to *Bd*. Conversely, Stice & Briggs (2010) immunized *Rana muscosa* with formalin-killed *Bd* injections and did not find any evidence that immunization promoted survival or *Bd* resistance relative to non-immunized frogs.

The definitive study to test for acquired immunity, where individuals are infected and cleared several times to test whether *Bd* load and/or survival changes with the number of previous infections, has yet to be properly conducted. Recently, Cashins *et al.* (2013) used itraconazole (an antifungal) to clear frogs of their *Bd* infection and found that frogs infected with *Bd*, treated with itraconazole, and then re-exposed to *Bd* had a higher prevalence of infection compared to frogs only exposed to *Bd* once. Based on their findings, the authors suggested that previous exposure to *Bd* did not enhance *Bd* resistance and that vaccination will not be an effective tool for mitigating chytridiomycosis (Cashins *et al.*, 2013). However, frogs that were treated with itraconazole prior to their first *Bd* exposure were 41% more likely to get infected with *Bd* compared to frogs that were never exposed to itraconazole (see Table 1 in Cashins *et al.*, 2013). This indicates that itraconazole was likely immunosuppressive and that exposure to this antifungal had the opposite effect on disease resistance than what the researchers had hypothesized. Thus, the itraconazole treatment could have prevented any acquired resistance response.

If acquired immunity to *Bd* is common among amphibians, it could fundamentally change proactive and reactive management plans for chytridiomycosis. For instance, acquired immunity to *Bd* could make it possible to create a vaccine for chytridiomycosis or to use herd immunity to protect threatened populations. For example, if there is acquired immunity, managers could take a subset of a population, induce acquired immunity in the laboratory, and release them back to the wild to drive the basic reproductive rate of the parasite (R_0) below one to extirpate *Bd*. Acquired immunity might also be used as a proactive rather than reactive tool. Immunizing a proportion of amphibians ahead of a spreading *Bd* epizootic might help prevent disease outbreaks in the immunized population and stop the spread, protecting populations that were not immunized. While few models have incorporated *Bd* immunity into host population dynamics, a recent model found that successful immunization of amphibians might have the potential to save amphibian populations from *Bd*-related extinctions (Woodhams *et al.*, 2011), representing a potentially novel and effective approach to managing chytridiomycosis. However, the only published study that monitored survival after attempting to vaccinate amphibians did not suggest a benefit of vaccination (Stice & Briggs, 2010) and vaccinations against *Bd* might not be possible. Future research that tests whether differences in host species and/or *Bd* isolates influence the success of vaccination attempts is needed to understand whether vaccines against *Bd* will be successful. Research on immunizing frogs could serve as a model system for designing antifungal vaccines, which are notoriously difficult to develop (Iannitti, Carvalho & Romani, 2012).

VIII. CONCLUSIONS

(1) *Bd* is in particular need of management because its emergence as a global pandemic has had considerable effects on entire communities (Lips *et al.*, 2006). Amphibian conservation strategies often take the form of rescue missions with the goal of either translocating individuals or maintaining them in captive breeding colonies. *Ex situ* conservation programs, such as Amphibian ARK, have undoubtedly saved amphibian species from *Bd*-related extinctions; however, many of these amphibian rescue missions are only equipped to solve the immediate threat of extinction. Simply returning these rescued amphibians from captive breeding colonies to their native habitats has not been considered an effective reintroduction strategy because *Bd* remains in the environment after amphibian extinctions (Lubick, 2010) and continues to spread (Fisher *et al.*, 2012).

(2) Resolving the critical research questions discussed herein will help improve our understanding of emerging infectious diseases and could lead to novel approaches to help curb *Bd*-related amphibian declines (e.g. managing non-amphibian hosts to minimize chytridiomycosis outbreaks) and potentially transform existing conservation programs from maintaining captive assurance colonies of amphibians to successful reintroduction programmes (e.g. by selecting for tolerance instead of resistance in Amphibian ARK).

(3) Many emerging fungal pathogens share biological features, such as an ability to persist outside of their hosts, broad geographic ranges, and high virulence (Fisher *et al.*, 2012). As such, addressing gaps in the literature, such as how climate change impacts *Bd* outbreaks or whether amphibians can use acquired immune responses against *Bd*, could be useful for managing other pathogenic fungi that have only recently been discovered, such as the ascomycete fungus (*Geomyces destructans*) that causes white-nose syndrome in bats. Addressing the topics discussed herein about the factors driving fungal infections will likely be necessary for effective management of these and other emerging fungal diseases.

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