Global Emergence of *Batrachochytrium dendrobatidis* and Amphibian Chytridiomycosis in Space, Time, and Host

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Key Words

panzootic, emerging infectious disease, chytrid

Abstract

Batrachochytrium dendrobatidis (*Bd*) is a chytrid fungus that causes chytridiomycosis in amphibians. Only named in 1999, *Bd* is a proximate driver of declines in global amphibian biodiversity. The pathogen infects over 350 species of amphibians and is found on all continents except Antarctica. However, the processes that have led to the global distribution of *Bd* and the occurrence of chytridiomycosis remain unclear. This review explores the molecular, epidemiological, and ecological evidence that *Bd* evolved from an endemic ancestral lineage to achieve global prominence via anthropogenically mediated spread. We then consider the major host and pathogen factors that have led to the occurrence of chytridiomycosis in amphibian species, populations, and communities.

Contents

Bd: *Batrachochytrium dendrobatidis*

Driver: an external, often environmental, factor that modulates the dynamics of the host/pathogen relationship

Enigmatic declines: amphibians have been long undergoing declines that are not attributable to any obvious cause: many steep population declines have been in pristine tropical or montane environments

Chytridiomycosis: the disease state caused as a result of infection by the chytrid *Bd*

INTRODUCTION

Amphibians are facing an extinction crisis that threatens up to 50% of all species (44, 115); they are the most threatened vertebrate class on the planet (1). Uniquely, the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) is now recognized as a proximate driver of many of these enigmatic declines (9, 28). First identified in 1997 and named in 1999 (67), *Bd* is widespread on all continents except Antarctica, where amphibian hosts do not occur. In many species, infection with *Bd* causes a rapidly progressing and fatal disease, chytridiomycosis, that has caused multiple-species declines in communities of amphibians (**Figure 1**). *Bd* infects over 350 amphibian species and has been implicated in driving the decline of over 200 of these species (113); in some cases the extinction of entire species in the wild has occurred as a result of disease emergence. Confirmed examples are the Australian gastric brooding frogs *Rheobatrachus* sp. (97), the Panamanian golden frog *Atelopus zeteki* (44), and the sharp-snouted day frog *Taudactylus acutirostris* (110). *Bd* therefore belongs to a select, historically important group of virulent multihost pathogens that have had profound effects on entire communities and ecosystems; other examples include West Nile virus and panzootic influenza in birds and rinderpest in African ungulates.

Bd is a basal fungal lineage in the Chytridiomycota. These fungi are characteristically aquatic and unique from other fungi in that they have a motile, flagellate zoospore (52). Many species of chytrid have been described in aquatic environments and soils as free-living or commensal organisms and as parasites of algae, invertebrates, fungi, and plants (46). Of these, *Bd* is unique in that it is one of only two Chytridiomycota that parasitizes vertebrates, and the only one to infect and develop within the keratinized epidermal cells of living amphibian skin (87–89).

Because of its unique structural and genomic traits, *Bd* has been placed as the lone member of its genus within the order *Rhizophydiales*, family *incertae sedis*. The closest relatives to *Bd* are three unnamed *Rhizophydiales* isolates (JEL122, JEL326, and JEL142), the first two of which develop inside algal cells (known as exogenous development), a feature that is likely necessary for *Bd* survival and proliferation within amphibian skin cells. Despite almost a decade of study, many facets underlying the emergence of *Bd* remain shrouded in mystery. Is *Bd* a spreading pathogen, or is the emergence of chytridiomycosis a result of changing dynamics between previously stable endemic host/pathogen associations? When and where did *Bd* arise? What factors govern the onset of chytridiomycosis? What is the genetic basis underlying the virulence of *Bd*? Why are some species of amphibian resistant to infection, some tolerant of infection, while others succumb to lethal chytridiomycosis? What is the basis of the host response to infection by *Bd*? Here we review our current understanding of the factors that

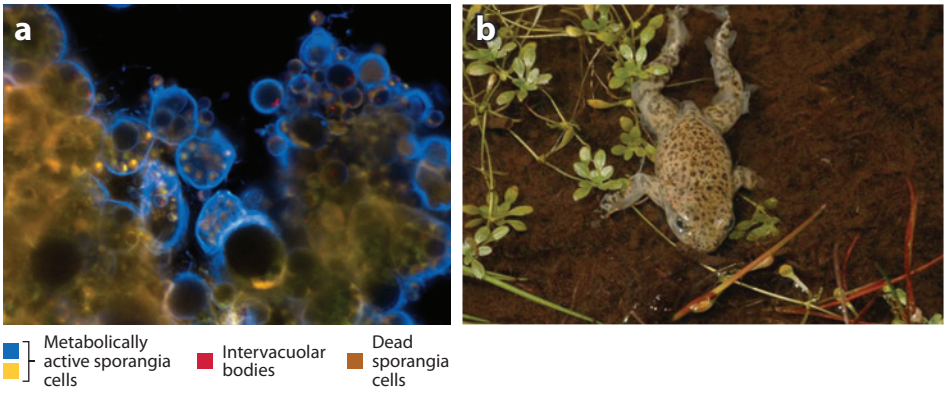

Figure 1

(*a*) Calcofluor White M2R and FUN 1 laser-scanning confocal micrograph of *Batrachochytrium dendrobatidis* in culture. Photo courtesy of Ché Weldon and Marika Gericke. (*b*) *Alytes obstetricans* chytridiomycosis mortality, Pyrennees, Spain.

address these questions. Because there have been several significant reviews on aspects of these questions (28, 29, 91, 104), we provide an overarching synthesis of these findings that take into account the most recent research on *Bd*.

IS *Bd* A NOVEL, SPREADING PATHOGEN OR IS IT WIDELY ENDEMIC?

Much debate has focused on whether *Bd* is a new, emerging pathogen. This is known as the novel pathogen hypothesis (NPH) or the spreading pathogen hypothesis (113). The counterargument is that *Bd* is a widespread endemic commensal, or even symbiont, of amphibians that has become more virulent owing to environmentally driven changes in the host pathogen dynamic. This is known as the endemic pathogen hypothesis (EPH) (91). The NPH receives support from observations that the distribution of *Bd* is patchy at many scales (**http://www.spatialepidemiology.net/bd-maps/**), that epidemic fronts of introduction have been identified in Australia (61) and Central and South America (9, 64, 65), and that infected vector amphibians are detected in the amphibian trade (36) as well as in the environment (26, 40, 122). The EPH is supported by evidence demonstrating that *Bd* was present in global amphibian populations for decades prior to the onset of declines (83, 124); that the occurrence of *Bd* and chytridiomycosis is to an extent determined by environmental variables; and that there are measurable associations between amphibian condition (94), global warming, and the onset of chytridiomycosis (15, 90). Key to resolving the relative contributions of the NPH versus EPH in determining the global occurrence of chytridiomycosis is a detailed phylogenetic analysis of the patterns of genetic diversity harbored in the genome of *Bd*. Using population genetic theory, researchers have recovered epidemiological parameters from genetic data using a suite of analytical phylogenetic techniques that have found extensive use in understanding many important fungal, viral, and bacterial pathogens (32, 37, 50) (**Figure 2**). Recent advances in understanding the epidemiology of *Bd* have stemmed from the sequencing of two *Bd* genomes and from molecular epidemiological work on the global phylogeographic population genetic structure of the fungus. The relationships between genetic, phenotypic, and virulence data for *Bd* integrated with analyses of incidence data utilizing historical amphibian collections and population surveys are leading to insights into the main factors driving contemporary amphibian declines.

Panzootic: an outbreak of infectious disease in animals that involves species worldwide

Chytridiomycota: a paraphyletic basal group of fungi that produce flagellated zoospores

NPH: novel pathogen hypothesis

EPH: endemic pathogen hypothesis

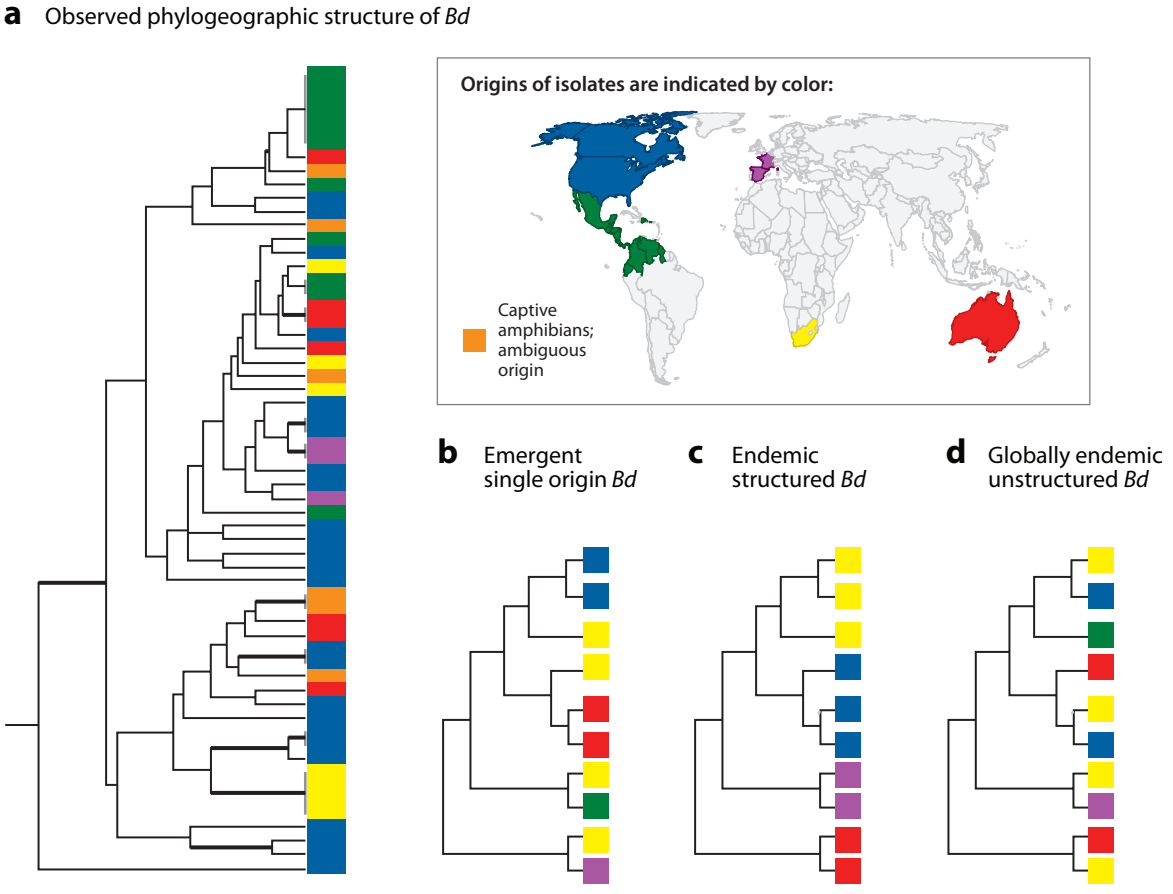

Figure 2

(*a*) Observed phylogeographic structure of *Batrachochytrium dendrobatidis* (*Bd*) with thickened branches indicating bootstrap values of 50% or greater and the spatial origin of the isolates displayed on the map (53). Schematic representations of the different phylogeographic patterns under the novel pathogen hypothesis (*b*) and the endemic pathogen hypothesis (*c-d*).

Bd GENETICS AND GENOMICS POINT TO A SINGLE AND RECENT ORIGIN OF AN ASEXUAL PANZOOTIC LINEAGE

The Joint Genome Institute and the Broad Institute have sequenced the genomes of two isolates of *Bd*, JEL423 from *Phyllomedusa lemur* in Panama and JAM81 from *Rana muscosa* in California, yielding the first molecular toolkit for a chytrid species (34, 105). Assembly of these two genomes revealed a haploid genome assembly size of 23.72 Mb for strain JEL423 and 24.3 Mb for strain JAM81. Although fungi are often haploid, early work on the sequence diversity of *Bd* showed convincingly that the cultured fungus was diploid (76), an observation that has been confirmed in subsequent studies (53, 77). James et al. (53) used multilocus sequence typing (MLST) (70) of 17 sequenced loci in global *Bd* isolates to show that sequence diversity is very low in *Bd*, with no more than two alleles present at a locus. Further confirmation of *Bd*'s low genetic diversity came from sequencing a >11-kb segment of the mitochondrial genome, where no variation was detected (53).

Currently, it is not known whether the *Bd* genome harbors the genes for mating and meiosis, although such genes have been found

MLST: multilocus sequence typing

in the related Zygomycota lineage *Phycomyces blakesleeanus* (51). An answer to this question awaits a detailed analysis of the *Bd* genomes (J.E. Stajich & C.A. Cuomo, unpublished data). All population genetic studies so far have shown that *Bd* demonstrates levels of heterozygosity that are consistent with a predominately asexual mode of reproduction. Of 17 sequenced polymorphic loci, 8 exhibited heterozygote excess. By anchoring the 17 sequenced loci to the genome scaffolds, James et al. (53) showed that levels of heterozygosity were not uniformly distributed across the genome but were significantly reduced on the largest inferred chromosome where loss of heterozygosity (LOH) had occurred. This pattern of LOH is not consistent with sexual reproduction and segregation, but rather with a model of chromosome-specific variation in mitotic (somatic) recombination, a process that is well documented in other fungi including the diploid pathogenic fungus *Candida albicans*, which exhibits vegetative diploidy (80).

This model of asexual LOH driving the diversity of *Bd* isolates is challenged in a study by Morgan et al. (77). Here, nearly 100 isolates of *Bd* were sampled from Sierra Nevada populations of the mountain yellow-legged frogs, *Rana muscosa* and *Rana sierrae*, that were undergoing rapid declines as a result of chytridiomycosis (18). This study showed that, although allelic diversity was low throughout the dataset, within some local populations genotypic diversity was high. In these high diversity populations no new alleles appeared to have been introduced, and no genotypes were shared between different infected populations. Here, it was suggested that local recombination had occurred within introduced lineages infecting particular lakes (77). These findings have two interpretations: Either *Bd* has the potential for outcrossing that is largely unrealized owing to population bottlenecks, causing the loss of complementary mating types, or LOH can occur at variable rates in different populations, generating a spurious signal of sexual recombination. Currently, no appropriate data prove or disprove either hypothesis conclusively.

Broadly speaking, these molecular data are all consistent with a single origin of *Bd* with evidence of local and international spread; these findings lend strong support to the NPH. The finding that *Bd* is diploid, of low sequence diversity, and highly heterozygous suggests that this globalized lineage is the product of a mating between two nonidentical but closely related, heterothallic, parental strains (53). In this case, the question then becomes, Where, and when, did *Bd* arise and who were the parents?

LOH: loss of heterozygosity

MOLECULAR AND HISTORICAL CLUES TO THE ORIGIN OF *Bd* AND GLOBAL VECTORS

Both population genetic approaches and historical surveys have been used in attempts to identify the spatial and temporal point of origin of *Bd*. Currently, the earliest published record of *Bd* is from a specimen of *Xenopus laevis* collected in 1938 from the Western Cape lowlands of South Africa (124), and other studies of historical collections of African amphibians have uncovered similarly early occurrences of *Bd*-infected amphibians from the 1920s to the 1930s (124; A.A. Cunningham, unpublished data) showing that the pathogen had a widespread African distribution in the early half of the twentieth century. Overall, studies of archived amphibians have found the following continental sequence of detections: Africa [1938, *X. laevis* (124)], North America [Quebec 1961, *Rana clamitans* (83)], Australia [1978, *Litoria gracilenta* (113)], South America [Ecuador 1980, *Atelopus bomolochos* (65)], Central America [Mexico 1983, *Rana tarahumarae* (48)], Europe [Spain 1997, *Alytes obstetricans* (16)], Oceania [1999, *Litoria raniformis* (121)], and Southeast Asia [Indonesia, 2007 (58); Japan, 2008 (K. Goka, unpublished data)]. These broad-scale data, while suggestive of a globalization of *Bd* from an African origin, are flawed because (*a*) sampling is biased to regions where amphibian densities are high, and where substantial scientific effort has been focused on monitoring populations or toward what is available in museums and other preserved amphibian archives,

and (*b*) not all global regions have so far been sampled.

Despite this ambiguity about *Bd*'s historical beginnings, compelling data exist on the occurrence of regional invasion and patterns of wave-like spread of *Bd* and chytridiomycosis. Five main systems exist where it appears that spatiotemporal spread of *Bd* has been occurring: western Australia, the Mesoamerican peninsula, the northern cap of South America, the U.S. Sierra Nevada, and the European Pyrenees (M.C. Fisher, S.F. Walker & J. Bosch, unpublished data). In eastern Australia, prospective and retrospective sampling of amphibians has shown that populations were initially *Bd* negative prior to 1978 followed by an expansion north and south from a center in southern Queensland; western Australia was *Bd* negative until mid-1985, whereupon the introduction and spread of disease were detected (9, 113). Mesoamerica has witnessed a rapid wave-like front of expansion from an apparent origin in Monteverde, Costa Rica, southward at estimated rates of between 17 and 43 km per year, and has recently crossed the Panama Canal (65). On the basis of the earliest records of *Bd* in South America, Lips et al. (65) infer two centers of spread based in Venezuela and Ecuador. The epidemic front of chytridiomycosis along the north-south transect of Central America has been predictable enough that researchers have anticipated the arrival of *Bd* in uninfected regions, such as El Copé in Panama, and documented the collapse of the amphibian community upon arrival of the fungus (64).

Attempts to identify the source of the epidemiological sparks that ignited these waves of infection require data on how genetic diversity is partitioned within and between globally sampled isolates of *Bd.* The most comprehensive dataset has been assembled by James et al. (53), who sampled 59 strains of *Bd* from five continents and 31 host species. Overall, little evidence was found for geographic clustering of strains. Strains from North America, Africa, and Australia are equally closely related throughout the dendrogram (**Figure 2**), which is evidence of a recent and rapid panzootic spread of *Bd*. Attempts to identify the original geographic source population of *Bd* were equivocal. Two ancestral centers of spread have been proposed: out of Africa in the *Xenopus* trade (124) and out of America in the North American bullfrog [*Lithobates* (formerly *Rana*) *catesbeianus*] trade (36, 40). Analysis of the equilibrium heterozygosity at the genotyped loci shows that bullfrogs exhibit a higher diversity than *Xenopus* species do, and an isolate of *Bd* from a bullfrog, JEL404, was heterozygous at all polymorphic sites. On the basis of these data, an origin of *Bd* out of Africa is less parsimonious than an origin from the northern United States. However, this finding comes with caveats. First, global sampling of sequenced isolates is still low, with only 7, 11, 7, and 3 isolates genotyped from Africa, South/Central America, Australia, and Europe, respectively. Second, other than southern Africa, areas of the world that are positive for *Bd* but do not appear to be experiencing obvious disease-driven declines have not had *Bd* isolates cultured and genotyped. If *Bd* coevolved with a geographically restricted amphibian species prior to its global spread, then it is likely that this population remains unsampled. For instance, island amphibian communities, such as those found in Japan (K. Goka, unpublished data), are infected by *Bd*; however, their relationship to *Bd* isolates found infecting continental communities is thus far unknown.

The balance of evidence shows that the contemporary distribution of *Bd* is due to the movement of known, and unknown, vectors from a yet unidentified source population in the early half of the twentieth century. However, *Bd* has not established an equilibrium, and spread dynamics are still observable at regional scales.

GLOBALLY MAPPING *Bd* PREVALENCE AND GENETIC DIVERSITY

The rate at which *Bd* has emerged as a significant driver of declines in biodiversity means that new techniques are needed to respond to the crisis. Whereas *Bd* surveillance has burgeoned, efforts to map the spread of the

pathogen are fragmented between regions and research groups. To meet this need, a multiphase project for compiling global *Bd* data and maintaining an updated system for global assessment of the pathogen was initiated, the *Bd* Global Mapping Project. Rapid aggregation, synthesis, and analysis of disease data were assisted by developing a Web-based system, hosted at **http://www.spatialepidemiology.net/bd-maps/**, such that this community project enabled data on the presence or absence of *Bd* and chytridiomycosis to be deposited globally (**Figure 3**). A current snapshot of this research effort shows that from 2449 spatial records, 1168 (48%) discrete sites were infected with *Bd* detected in 387 (50%) of species in 37 (82%) families of frogs, toads, and salamanders. Infection was found in 45 of 78 (58%) of countries sampled on six continents. The distribution of *Bd*, although widespread, was patchy, and several areas exist that contain high amphibian biodiversity but are thus far *Bd* negative. The most notable of these regions is the island of Madagascar, which contains up to 465 species of amphibian (117); the potential for *Bd* to extirpate this unique and megadiverse community has led to calls for a high degree of biosecurity to be implemented (5, 68).

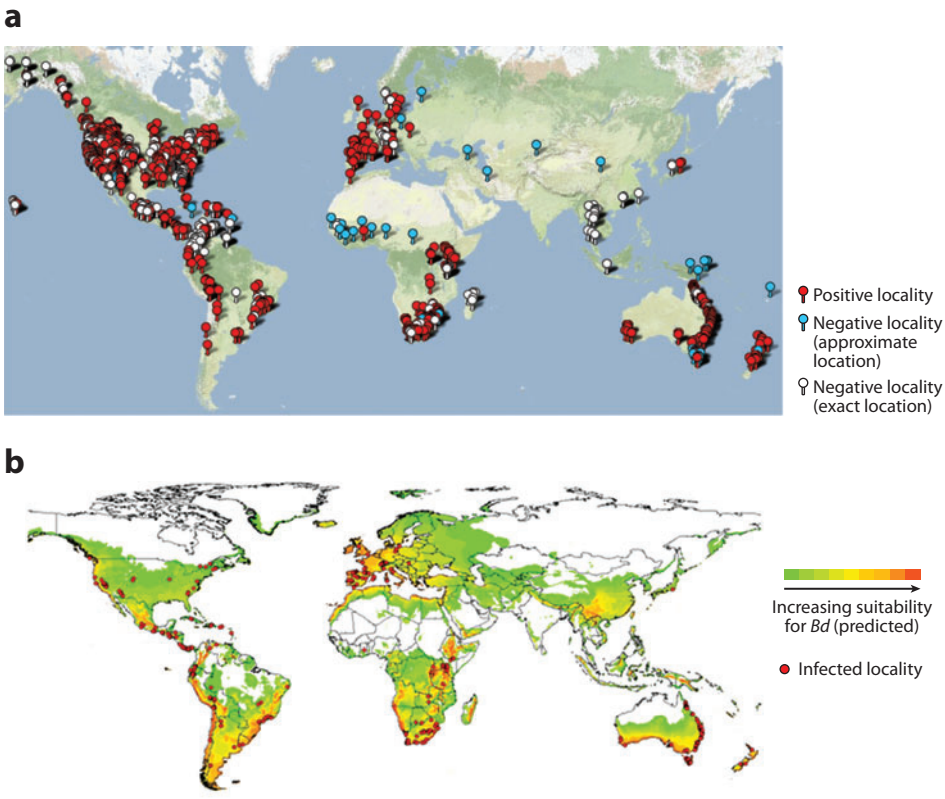

Figure 3

(*a*) Screenshot of the known global distribution of *Batrachochytrium dendrobatidis* (*Bd*) from the Global Mapping project (**http://www.spatialepidemiology.net/bd-maps/**). Points are displayed on a GoogleMaps relief basemap. Bd-Maps was developed by David Aanensen, Imperial College London, in collaboration with Dede Olson and Matthew Fisher. (*b*) Worldwide potential predicted distribution of *Bd* according to the Climate Envelope Model developed by D. Rödder, J. Kielgast, J.B. Schmidtlein, et al. (unpublished data). The infected localities used to run the model are shown as red dots.

The *Bd* mapping project allows MLST genotyping information to be associated with spatial data; the subsequent use of algorithms such as eBURST (**http://eburst.mlst.net/**) to detect clustering and relatedness then provides a means by which patterns of spread of particular invasive *Bd* lineages can be mapped. This allows researchers to pinpoint which regions act as global sources of infection. Further study is then necessary to identify which species act as potential global and regional vectors [as has been done for *L. catesbeianus* (36, 40)]. Such approaches using barcoding techniques to identify sources of *Bd* are starting to be used. For instance, Walker et al. (122) used historical DNA techniques and genotyping to show that infected, reintroduced, *Alytes muletensis* vectored infection to the Mediterranean island of Mallorca. Further developing the global dataset of genotyped *Bd* isolates incorporated into the *Bd* Mapping Project will yield a powerful new tool for identifying how and where disease introductions have occurred.

EVIDENCE OF ONGOING EVOLUTION OF *Bd* AND VARIATION IN VIRULENCE

Despite the apparent rapid spread of *Bd* and the high degree of relatedness between isolates, genotypes differ significantly in their virulence. Three published studies have reported variation in virulence of *Bd* isolates in three species of amphibians, *Litoria caerulea* (8), *Pseudacris triseriata* (96), and *Bufo bufo* (35). Fisher et al. (35) showed that the sporangia of five isolates of *Bd* from Mallorca, all with identical genotypes, were similar in size but differed significantly from those isolates recovered from amphibians in mainland Spain and the United Kingdom. When the virulence of a Mallorcan isolate of *Bd* (TF5a1) and a U.K. isolate of *Bd* (UKTvB) was assayed in *B. bufo* (35) and *A. muletensis* (M.C. Fisher, unpublished data), the Mallorcan strain of *Bd* manifested less than 50% of the virulence observed in the U.K. strain of the pathogen in both host species. Proteomic profiling of a global set of isolates showed that there was significant inter-isolate variation in patterns of protein expression (35). The amount of differentiation among isolates at neutral genetic markers and biological (morphological and proteomic) characters was greatest for morphological traits, suggesting that these characters are under selection and that this is possibly related to local environmental conditions (35). These data are wide-reaching in their implications: If *Bd* generates functional diversity from a genetically depauperate genetic background and thus increases its rate of adaptation to new environments, then *Bd* is more likely to present a shifting target by allowing the pathogen to adapt to new climates, host species, or both. This raises the notion that *Bd* may increase its fitness to new environments or evolve resistance to drugs that are widely used in captivity (44). Therefore, it is likely that *Bd*, like other pathogens, will present a moving target for management strategies.

ENVIRONMENTAL DRIVERS OF CHYTRIDIOMYCOSIS

Although the Global Mapping Project has shown that the distribution of *Bd* is broad (**Figure 3**), reported localities of fatal chytridiomycosis are remarkably scarce. The notable declines of amphibian populations at high altitudes (59, 63, 115, 130) coupled with reports of outbreaks of fatal chytridiomycosis in montane regions (9, 16) have resulted in a tendency to associate localities of fatal chytridiomycosis with high altitudes and cooler temperatures. This is an oversimplification of a complex host-pathogen relationship: Fatal chytridiomycosis close to sea level is not without precedent, e.g., the mountain chicken frog, *Leptodactylus fallax*, on the Caribbean island of Dominica (10, 73). Nevertheless, when species-specific analyses are considered, signatures of spatial, seasonal, and interannual trends in both infection and patterns of disease emerge.

A recent analysis using the outputs from the Global Mapping Project has shown that the presence of *Bd* is significantly associated with environmental variables such as

temperature, annual precipitation, species richness and biome (D. Olson, D.M. Aanensen & M.C. Fisher, unpublished data). From these data it is clear that, although *Bd* is widely prevalent, there are identifiable heterogeneities in the pathogen's distribution that are linked to environmental parameters. To identify those populations of amphibians most at risk from chytridiomycosis, there is a need to integrate assessments of the suitability of the climate for *Bd* (103) with assessments of the relative susceptibility of the species present (12). A precedent for this goal was set by Lötters et al. (68), who have identified areas such as Madagascar, a country in which *Bd* has thus far not been reported, as potentially highly susceptible to the impact of chytridiomycosis. Although the predicted distribution of *Bd* was based on an analysis of climatic variables, the modeling process used (a form of climate envelope model based on maximum entropy) did not itself identify which climatic attributes had the strongest predictive value.

Many aspects of the host-pathogen relationship will be influenced by environmental factors, and will ultimately be expressed as heterogeneities in the presence and prevalence of infection, and by the incidence of chytridiomycosis. One such aspect is the physiological limitations of *Bd* growth in different environments. Using strains of *Bd* isolated from six species of amphibians from various regions across North America, Piotrowski et al. (88) have shown that the survival, growth, and reproduction of *Bd* is highly temperature dependent. Although *Bd* grows and reproduces between 4 and 25°C, it has a growth maximum between 17 and 25°C, and 50% mortality is incurred by cultures subject to 30°C for 8 days (88). How these temperature thresholds are dependent on the *Bd* lineage, and how different lineages may have differing degrees of adaptability to both meteorological (e.g., seasonal) and climatic (e.g., climate change) regimes, has yet to be investigated.

Temporal (seasonal) and spatial studies documenting the prevalence and intensity of *Bd* and localities of disease outbreaks are in general agreement with the hypothesis that *Bd* has a predilection for cooler temperatures (10, 16, 31, 57). However, although *Bd* is known in the tropical Andes at altitudes of 5348 m, where temperature minima fall to −13.5°C and diurnal variability may reach 30°C (111), it has been hypothesized that at extreme altitudes where temperature maxima fall beneath the recognized envelope for optimal *Bd* growth, amphibians may be afforded a refuge from fatal chytridiomycosis (78, 90). Given the vulnerability of montane systems to both recent and projected climate change (23, 79), the future persistence of any such refugia is of key concern.

As explained in the introductory paragraphs of this review, the impact of changing extrinsic factors on an existing host-pathogen relationship is encapsulated by the EPH of Rachowicz et al. (91). The authors suggest that the emergence of pathogenic chytridiomycosis arose from a "change in the immunological, ecological, and/or behavioral parameters of the host or parasite." The necessary field analyses to test this hypothesis are scarce. In the Iberian Peninsula, a comparison of the climate at localities where outbreaks of fatal chytridiomycosis were recorded with climate at localities where *Bd* was present but where no outbreaks had been recorded found a positive association between localities of outbreaks and altitude ($p = 0.0094$), and negative associations between minima air temperatures during August ($p = 0.015$) and mean air temperatures during the warmest quarter ($p = 0.044$) (S.F. Walker, unpublished data). In the Sierra de Guadarrama of Central Spain, a significant increase in the number of days during the period of amphibian metamorphosis, with maximum temperatures exceeding 21–27°C, has been associated with the emergence of fatal chytridiomycosis (15). These data suggest that changing climates can tip the host-pathogen dynamic from an avirulent to a virulent state.

At the macro level, attempts to allocate ecological significance to correlative studies are still in their infancy, due in part to the indiscriminate pooling of different species and the incongruity of scale between climatic data and *Bd*

surveillance data. The frequently cited climate-linked epidemic hypothesis of Pounds et al. (90) arose from analyses that considered the last year of occurrence (LYO) of *Atelopus* species in the mountains of Costa Rica in relation to changes in sea surface and air temperature. The signature of warming that the authors identified was considered critical to the apparent disappearance of approximately 70 species, and the authors hypothesized that the trend of daytime warming and nighttime cooling had created an environment that was more suited to *Bd*. However, no longitudinal data about the *Bd* status of these LYO species was actually presented and evidence that environmental change was causal was only weakly statistically supported (99), presenting difficulties in ascertaining the true cause of these *Atelopus* declines.

The apparent inverse relationship between temperature and virulence has been substantiated by challenge experiments and immunological studies. In vitro, studies on the tropical species *Dendrobates tinctorius* and *Litoria chloris* have shown that *Bd* is most lethal under cool moist conditions (22°C), and infected *L. chloris* could be cleared of infection if maintained at 37°C for prolonged periods (126). The actual relationship between temperature and the ability of a species to resist and/or tolerate an infection is likely to be highly species specific. In vitro work assessing the postmetamorphic survival of infected *Rana muscosa* tadpoles found elevated mortality among animals kept at 17°C (95% mortality) compared with tadpoles kept at 22°C (50% mortality) (4). By contrast, a study on toadlets of *Bufo boreas*, another temperate species, found no difference in mortality among toadlets exposed to *Bd* at 12°C and toadlets exposed at 23°C (22). Toadlets kept on a 5°/30°C diel temperature schedule for 42 days survived (24). Although the mechanism behind any differential mortality may in part be accounted for by the relationship between *Bd* growth rate and infectious burden, when hosts are kept at temperatures considered optimal for *Bd*, as in the study by Andre et al. (4), the more pivotal factor is likely to be the host's ability to respond to infection.

HOST RESPONSE TO EXPOSURE TO *Bd*

It is striking that after a decade of research no clear mechanism for host death due to infection with *Bd* has been published. Chytridiomycosis is notable for an overall lack of disease pathologies: Metamorphosed amphibians infected with *Bd* typically exhibit epidermal hyperplasia and hyperkeratosis, and possibly upregulated skin shedding, but only rarely exhibit any lesions visible to the naked eye (9, 22). Larval anuran amphibians may exhibit visible deformities of keratinized mouthparts (114), and one recent publication described lesions associated with infection with *Bd* on larval caudate amphibians (19). In this last case the lack of a proper, post-mortem examination renders the evidence of signs of disease attributable to *Bd* equivocal. Taken together, pathologies associated with *Bd* infection appear relatively benign when compared with symptoms associated with other amphibian pathogens [e.g., ranavirus (27)], yet experiments have shown repeatedly that *Bd* is the primary cause of death in numerous amphibian hosts (4, 13, 18, 22, 30, 38, 39, 41, 85, 92, 96). *Bd* is hypothesized to produce lethal toxins either before or after infection (13), interfere with water uptake and lead to death due to dehydration (9), or cause sharp osmotic imbalances that are postulated to interfere with water regulation and/or neurological function (118).

A lack of obviously lethal pathology hinders efforts to understand how exposure to or infection with *Bd* can cause death, but some general patterns that emerge from the literature do provide some insight. First, there is extraordinary variation in species-specific responses to exposure to *Bd*. Some species carry sustained infections in the wild with little or no evidence of either mortality or population decline (7, 49, 55, 83, 106, 124), whereas other species are extirpated by the emergence of *Bd* (9, 16, 64, 107). Species-level responses also vary: Some populations of species susceptible to fatal chytridiomycosis may not experience detectable mortality or declines even when harboring infected individuals (18, 97). Second, death associated

with chytridiomycosis appears most common in earlier life-history stages in many species with complex life histories (16, 18, 22, 41). In several species for which death may not be significant in early life-history stages, growth and development costs to larvae due to exposure to *Bd* have been reported (30, 85, 86, 96). Mathematical models also show how greater tolerance of infection by adult frogs may explain species persistence when mortality in earlier life-history stages is near complete (18). Third, lethal chytridiomycosis also occurs at locations outside the proposed optimal environmental envelope for *Bd* growth and reproduction (11, 56, 69, 73, 122). These findings indicate that host tolerance of exposure to and/or infection with *Bd* is governed by intrinsic, as well as extrinsic, factors.

The classical conclusion to draw would be that host immunity is species specific and may improve with age or in older developmental stages. Evidence of host-specific immunity is found in studies of the bioactive properties of antimicrobial peptides released from the skin glands of amphibians (100, 127, 129). For the most part, laboratory-derived measures of the inhibitory ability of Australian frog skin secretions against *Bd* match experimentally derived and species-specific *Bd*-driven mortality schedules (127) as well as patterns of *Bd*-associated declines in the wild (128, 129). However, that an amphibian produces antimicrobial peptides that inhibit *Bd* growth in the laboratory does not always fit with field observations of amphibian decline; antimicrobial peptides of *R. muscosa* are strongly active against *Bd*, yet this species has experienced catastrophic declines due to *Bd* across most of its range (18, 102, 120). In vitro inhibition of *Bd* by antimicrobial peptides is not hampered by cold temperatures (101, 102) that are experienced by hosts at high-altitude sites where *Bd* has often caused population declines and extirpations (15, 65, 78). This suggests that host production of peptides is somehow hampered at colder temperatures, and the development of dermal glands from which these peptides are issued may be prevented through prolonged exposure to colder temperatures (81). The presence of antimicrobial peptides has also been reported for larval anurans (119), and although skin glands responsible for the production of antimicrobial peptides are developed during the larval period, they are present in the dermis before metamorphosis is complete or at metamorphic climax, even in species suffering from lethal chytridiomycosis postmetamorphosis (6, 112). This observation is difficult to resolve with the pattern of increased mortality at or soon after metamorphosis (16, 18, 22, 41).

Mounting an immune response is costly (14), and how a host regulates an immune response may be as important as the ability to mount an immune response (66). Costly upregulation of innate and adaptive responses are subject to trade-offs against one another as well as against other physiological requirements, such as growth, development, and reproduction (109, 123). Trade-offs between immunity and growth, development, and reproduction are reported for amphibians, as are seasonal patterns of immune regulation and experimental alteration of immune function with exposure to cold temperatures (71, 72, 93, 98). Woodhams et al. (127) provide evidence of upregulation of innate immunity in response to infection with or exposure to *Bd*. Recent research with *Silurana* (*Xenopus*) *tropicalis*, a species that is an asymptomatic carrier of infections, has shown that the expression of genes involved in the immune response is temperature dependent (L. Ribas, unpublished data). Here, clearance of *Bd* was not associated with an adaptive immune response, but with the induction of innate immunity including preprocaerulein (PPCP), an antimicrobial peptide precursor. At cold temperatures, *S. tropicalis* lost the ability to mount a PPCP-based reaction, resulting in higher infectious burdens. In the field, temperature-dependent immunity may be forced by seasonal patterns. For example, seasonal surveys of adult red-spotted newts (*Notophthalmus viridescens*) by Raffel et al. (93) showed a lag effect on lymphocyte levels in the spring and a strong seasonal acclimatization of lymphocyte, neutrophil, and eosinophil levels in the autumn. There is a now a clear need for

such field-based immunological studies among *Bd*-infected populations to investigate the importance of the host response in the epidemiological triad (host, agent of infection/pathogen, and the environment) to quantify the influence that changing climates can exert on the ectothermic host response to pathogens such as *Bd*.

Alternatively, geographic variation of responses to pathogens may suggest that host immunity is locally adapted. Immunocompetence is expected to vary in accordance with risk, and host ability to resist or manage infection should correlate positively with parasite diversity or activity (25). Amphibians are commonly adapted to local environments (60, 62, 95, 116), and given that immunity is costly, reduced parasite pressure at high-altitude locations should select for decreased investment in immunity. This effect may be even more pronounced in ectothermic amphibians, as individuals in high-altitude populations may already be operating at physiological limits and may be obligated to develop more quickly than their low-altitude counterparts (75). Bacterial and fungal communities are less diverse with increasing altitude (21, 45), and both the number and activity of aquatic microbes are decreased in alpine water systems (54). If we accept the NPH and that emergence is recent, it is unlikely there has been time for amphibians to have evolved specific immunity to *Bd*. Hosts are therefore forced to confront the pathogen with what defenses are currently available, with the result that populations with fewer defenses would, on average, be more likely to experience the most catastrophic effects of pathogen emergence.

Growth in tadpoles is often inhibited when they are exposed to *Bd* (30, 41, 85, 86). Infection of tadpoles is restricted to keratinized mouthparts, so proliferation of *Bd* in the host is heavily proscribed in larval anurans and is not necessarily an important factor contributing to the accumulation of costs in tadpoles. Phenotypic plasticity during larval development associated with unpredictable environments has been reported for innumerable amphibian species, and decades of experimental data support the existence of potentially lethal costs due to adaptive plasticity (3, 33, 74). We have found that, after exposure to *Bd* during the tadpole stage, mortality in common toads (*B. bufo*) after metamorphosis may be dissociated from infection at time of death (41). This shows that even successful immune responses may be too costly for a host to survive (47) and that the burden of infection may not always be driving mortality (but see Reference 55 for evidence of thresholds of infection associated with mortality in adult frogs). We have also determined that exposure at different times during larval development elicits different growth trajectories (T.W.J. Garner, unpublished data). This raises the interesting question: What exactly is the larval immune response? Because larval amphibians express alternative phenotypes in response to risky environments, it is conceivable that larvae respond to *Bd* as an environmental risk and alter development accordingly, as well as mount more typical immune responses. By doing both, larvae may be paying a cumulative or synergistic cost, with the result that physiological resources become overwhelmed once the additional cost of metamorphosis is imposed. Precedence for this exists in the toxicology literature. Here, tadpoles that die because of exposure to pesticides do so at a greater rate when they are also exposed to the chemical signal of a predator (95).

Ultimately, host responses must be examined in the context of pathogen variation. Broad host range coupled with recent emergence and narrow neutral genetic variation suggest a generalist pathogen, an assumption supported by evidence of inducible plasticity in pathogen growth and development (125). However, the pathogen is evolving, and several studies have described how host response already varies with pathogen isolate. Differences in virulence among isolates could be attributed to evolving host specificity, environmental specialization, or even isolate attenuation in culture of *Bd* (35, 105). Theory predicts that an initially virulent pathogen should become less virulent, unless the pathogen is effective at evading host

immunity or unless both pathogen growth rate and subsequent transmission probability are high (2, 108). Genes associated with immune evasion in bacteria are differentially expressed in *Bd* (105), and exposure of *S. tropicalis* to *Bd* led to detectable downregulation of genes that are components of adaptive immunity (L. Ribas, unpublished data). Therefore, it is likely that a comprehensive understanding of the host response to infection will need to incorporate information on host immunity, as well as *Bd* virulence factors and wider ecological/environmental drivers.

WHAT NEXT? MITIGATING THE PANZOOTIC

The global impact of *Bd* is likely to substantially increase the risk of extinction for many amphibian species if efforts to constrain and mitigate the panzootic are not rapidly undertaken (120). Stemming the progress of infectious diseases in wildlife populations presents unique difficulties and it is likely to prove challenging to mitigate chytridiomycosis in nature. Nevertheless, substantial progress has been made on a number of fronts.

International recognition of the effects of the global trade in amphibians in spreading *Bd* has led the World Organisation for Animal Health (the OIE) to declare *Bd* and ranavirus as notifiable pathogens. This legislation enables countries that are associated with the World Trade Organization to specifically test for, and limit, trade in infected amphibians (82).

Conservation of species that are undergoing rapid *Bd*-driven declines is enabled using ex situ captive-breeding programs. Many of these zoo-led programs are gathered under the umbrella of the Amphibian Ark Program (**http://www.amphibianark.org/**) to coordinate ex situ breeding programs, to disseminate protocols, and to raise awareness about amphibian declines globally. We do not know how successful this program will be; to date the number of species in breeding programs is small and the infrastructure for such a large operation is, to a large degree, absent (43).

Fungicidal protocols have been developed: These include elevated temperature (126), formalin/malachite green (84), and standard veterinary antifungal drugs (42). Good results for clearing infection in captive colonies are leading to proposals to clear infection in natural populations using catch, treat, and release methods using inexpensive, mobile biocontainment laboratories. One of the most exciting new developments in mitigation is the recognition that some bacteria that occur naturally on amphibian skin produce antifungal metabolites (20). This finding may presage a probiotic approach to *Bd* mitigation.

SUMMARY POINTS

1. *Bd* infects an extraordinarily broad diversity of host species and appears to have the widest host range of any known pathogen. This observation is consistent with the argument that *Bd* is a recently evolved generalist emerging pathogen.
2. *Bd* is diploid, contains low sequence diversity, and is highly heterozygous. These factors suggest that the globalized lineage is the product of a mating between two nonidentical but closely related, heterothallic, parental strains.
3. Temporal presence/absence data suggest that *Bd* emerged globally at some point in the early twentieth century. However, molecular epidemiology has so far failed to identify the geographic source of the emergence.

4. Studies show that, despite the apparent rapid spread of *Bd* and the high degree of relatedness between isolates, genotypes differ significantly in their phenotypic and virulence characteristics. There is evidence of ongoing evolution of *Bd*.
5. The distribution of *Bd* and fatal chytridiomycosis is significantly associated with environmental variables, allowing regions that are at risk for disease emergence to be identified by ecological modeling. Similarly, the occurrence of chytridiomycosis is associated with environmental variables, most notably temperature and altitude.
6. Costs due to infection are being identified at the larval, juvenile, and adult stages. Evidence shows that innate immunity is necessary for a successful anti-*Bd* response and that the strength of this immunity is influenced by extrinsic drivers.
7. Mitigating this panzootic is increasingly possible owing to advances in international legislation, ex situ conservation practices, ex situ and in situ antifungal treatment, and connectivity between global scientific communities.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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91. Important review of the principal competing hypotheses, the NPH and EPH, accounting for the emergence of *Bd*.

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Bd-Maps. **http://www.spatialepidemiology.net/bd-maps/**

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