The Novel and Endemic Pathogen Hypotheses: Competing Explanations for the Origin of Emerging Infectious Diseases of Wildlife

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Abstract: *Chytridiomycosis, caused by the fungal pathogen* Batrachochytrium dendrobatidis*, is an emerging infectious disease implicated in declines of amphibian populations around the globe. An emerging infectious disease is one that has recently been discovered; has recently increased in incidence, geography, or host range; or is newly evolved. For any given outbreak of an emerging disease, it is therefore possible to state two hypotheses regarding its origin. The novel pathogen hypothesis states that the disease has recently spread into new geographic areas, whereas the endemic pathogen hypothesis suggests that it has been present in the environment but recently has increased in host range or pathogenicity. Distinguishing between these hypotheses is important, because the conservation measures needed to slow or stop the spread of a novel pathogen are likely to differ from those needed to prevent outbreaks of an endemic pathogen. Population genetics may help discriminate among the possible origins of an emerging disease. Current evidence suggests chytridiomycosis may be a novel pathogen being spread worldwide by carriers; until we know how much genetic variation to expect in an endemic strain, however, we cannot yet conclude that* B. dendrobatidis *is a novel pathogen.*

Key Words: amphibian decline, *Batrachochytrium dendrobatidis,* chytridiomycosis, conservation, host-parasite ecology, population genetics

La Hipótesis del Patógeno Incipiente y Endémico: Explicaciones Opuestas del Origen de Enfermedades Infecciosas Emergentes en la Vida Silvestre

Resumen: *La quitridiomicosis, causada por el hongo patógeno* Batrachochytrium dendrobatidis*, es una enfermedad infecciosa emergente implicada en las declinaciones de poblaciones de anfibios en el mundo. Una enfermedad infecciosa emergente es una que ha sido descubierta recientemente; que ha incrementado en su rango de incidencia, geográfico o de huéspedes recientemente; o que ha evolucionado recientemente. Para cualquier brote de una enfermedad emergente es posible enunciar dos hipótesis en relación con su origen. La hipótesis del patógeno incipiente establece que el la enfermedad se ha extendido recientemente hacia áreas geográficas nuevas, mientras que la hipótesis del patógeno endémico sugiere que ha estado presente en el ambiente pero que ha incrementado en el rango de huéspedes o de patogenicidad. Es importante distinguir entre estas dos hipótesis, porque es probable que las medidas de conservación que se requieren para reducir o detener la dispersión de un patógeno incipiente sean diferentes a las requeridas para prevenir brotes de*

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un patógeno endémico. La genética de poblaciones puede ayudar a distinguir entre los posibles orígenes de una enfermedad emergente. La evidencia actual sugiere que la quitridiomicosis puede ser un patógeno incipiente que esta siendo dispersado por transportistas mundialmente; sin embargo, no podemos concluir que B. dendrobatidis *es un patógeno incipiente hasta que no se conozca la variación genética esperada en una cepa endémica.*

Palabras Clave: *Batrachochytrium dendrobatidis,* declinación de anfibios, ecología de huésped-parásito, genética de poblaciones, quitridiomicosis

Introduction

Emerging infectious diseases—those that have recently been discovered; have recently increased in incidence, geography, or host range; or are newly evolved (Daszak et al. 2003)—can be serious threats to the persistence of populations. The abatement of these threats is increasingly seen as a necessity for effective conservation of biodiversity (Daszak et al. 2000; Hudson et al. 2001); most effort on threat abatement to date, however, has been in the field of emerging or reemerging diseases of humans (e.g., CDC 1994).

Emerging infectious diseases originate in two ways. The novel pathogen hypothesis states that the pathogen (or a newly evolved virulent strain of the pathogen) has recently spread into a new geographic area, encountering naïve host individuals or species that are highly susceptible to infection (Alford 2001). The endemic pathogen hypothesis suggests that it has been present in the environment but has entered new host species or increased in pathogenicity because of environmental changes or, possibly, simply escaped previous human notice. Strategies for disease management will differ substantially, depending on which of these hypotheses is correct (CDC 1994). A novel origin suggests a focus on identifying and controlling agents of spread, whereas an endemic origin suggests investigating and managing cofactors, synergies, and context dependence. It is also possible that a disease that is endemic in some species or locations is a novel pathogen in others (e.g., Jancovich et al. 2005). To examine these ideas we use chytridiomycosis, an emerging infectious disease of amphibians, as a model system.

The fungus *Batrachochytrium dendrobatidis*, which causes chytridiomycosis, occurs in more than 100 species of amphibians worldwide (Speare & Berger 2004; Fig. 1a), has been associated with numerous mass mortality events, and is considered the proximal cause of numerous amphibian population declines and species extinctions, primarily in montane areas (e.g., Berger et al. 1998; Lips et al. 2003; Muths et al. 2003). Berger et al. (1998) were the first to report *B. dendrobatidis* infections in Australia and Central America, and Longcore et al. (1999) described *B. dendrobatidis* from captive dendrobatid frogs in the United States. It is now known on five continents (Fig. 1).

B. dendrobatidis has been found on dead frogs in the wild, often at sites with declining populations (e.g., Berger et al. 1999; Muths et al. 2003). Infection occurs when aquatic zoospores penetrate the keratinized skin of animals or mouthparts of tadpoles (Berger et al. 1999; Longcore et al. 1999; Fellers et al. 2001). Although *B. dendrobatidis* kills postmetamorphic individuals of some species, *B. dendrobatidis*-infected individuals of other species appear to suffer no negative health effects (Nichols et al. 2001; Weldon 2002; Daszak et al. 2003).

B. dendrobatidis has no known resting or saprophytic stage; such stages, however, occur in other Chytridiomycetes (Powell 1993). *B. dendrobatidis* grows well in culture, grows on dead frogs and snake skin (both keratin substrates), and thus may grow saprophytically in the wild (Longcore et al. 1999). Zoospores can survive for an extended period in sterilized water (Johnson & Speare 2003), raising the possibility of such survival in the wild.

We evaluated the evidence regarding the origin (novel or endemic) of *B. dendrobatidis*. We discuss research approaches needed to determine whether the pathogen is novel or endemic and the implications of this for investigations of emerging infectious diseases that threaten wildlife populations.

The Novel Pathogen Hypothesis

The spread of novel pathogens has been attributed to translocations of hosts, human encroachment into wilderness areas, and the introduction of exotic species (Daszak et al. 2000, 2003). Because no easy mode of wide-scale dispersal is known for *B. dendrobatidis*, the novel pathogen hypothesis must explain the presence of this highly virulent pathogen in numerous and often remote locations.

Laurance et al. (1996) suggest the novel pathogen hypothesis as an explanation for the decline or disappearance of 14 anuran species in eastern Australia, an area with large protected tracts of land. They propose that an infectious agent spread progressively northward in a wave-like manner, causing rapid die-offs of postmetamorphic frogs. No single agent was isolated at that time, but a highly virulent, waterborne pathogen was suspected and thought to have been introduced via release of aquarium fish into the wild. Similarly, Lips (1999) and Lips

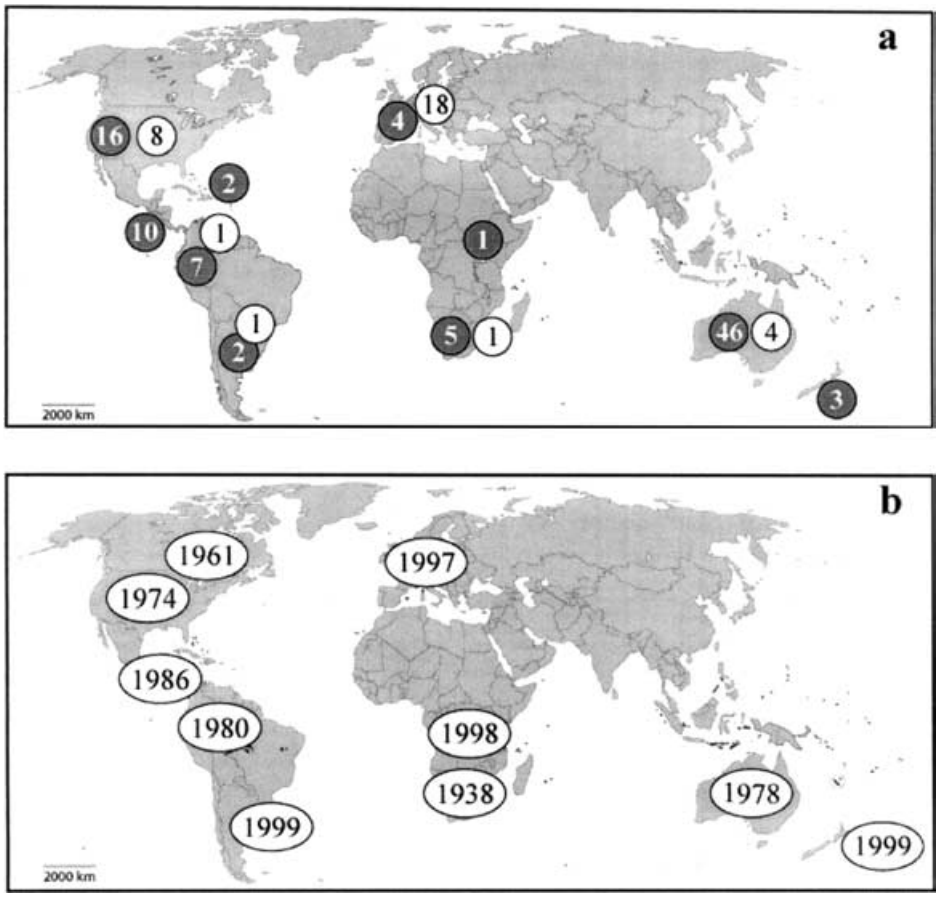

Figure 1. (a) Global distribution of Batrachochytrium dendrobatidis *among wild and captive amphibians (numbers in dark circles are number of species with* B. dendrobatidis *reported from wild populations; those in white circles are the number of species with* B. dendrobatidis *reported from captive populations) and (b) the year of the oldest recorded* B. dendrobatidis *infection among wild and captive amphibians. (See Amphibiaweb [2005] for references.)*

et al. (2004) hypothesize that a virulent, highly contagious pathogen swept from North America in the 1970s through to Central America in the 1990s, causing declines in many families and species of anurans. Lips (1999) suggests that exotic fishes, humans, or animals could have introduced the pathogen. A candidate for the causative agent in these declines was identified when numerous dead postmetamorphic animals collected from eastern Australia and Central America were found to be infected with *B. dendrobatidis* (Berger et al. 1998; Longcore et al. 1999), implicating the newly described disease chytridiomycosis as a cause of mortality.

Amphibians of many species have been transported around the globe for food, medicine, research, education, pets, fish bait, and leather (Jensen & Camp 2003; Mazzoni et al. 2003). Amphibian species that carry *B. dendrobatidis* without showing clinical symptoms could be spreading the fungus by introducing it to sympatric species, which spread it to other native species or vectors, eventually covering the landscape. For example, the American bullfrog (*Rana catesbeiana*) appears resistant to mortality caused by *B. dendrobatidis* and may be a carrier (Daszak et al. 2003; Hanselmann et al. 2004). Bullfrogs have been introduced in many regions of the world in attempts to culture them for human food (Kupferberg 1997). These introductions began in the 1800s (Kupferberg 1997) and have continued at least into the 1990s (Hanselmann et al. 2004). Results of retrospective studies show that the earliest cases of *B. dendrobatidis* primarily date back only to the 1960s–1990s (Fig. 1b); searches of museum specimens, however, may not be comprehensive in many species and locations.

Weldon (2002) and Weldon et al. (2004) propose *Xenopus laevis* as a potential intercontinental carrier based on their discovery that *B. dendrobatidis* has occurred in African populations of *X. laevis* since the 1930s (Fig. 1b) and that this species can asymptomatically carry *B. dendrobatidis*. *X. laevis* were frequently transported intercontinentally during the 1940s–1950s for human pregnancy testing (Hansen 1960). In Australia, where many declines have occurred (Berger et al. 1998), *X. laevis* are restricted to laboratories and are neither widespread nor free ranging. In California introduced *X. laevis* populations exist only in southern California (Tinsley & McCoid 1996), yet major declines in native frogs have been documented mostly farther north (Jennings & Hayes 1994; Davidson et al. 2001). Thus, additional vectors (amphibian or nonamphibian) must be involved in order for the pathogen to have spread from these carriers. Additionally, if *B. dendrobatidis* originated in Africa, the novel pathogen hypothesis does not easily explain the recent outbreaks of chytridiomycosis associated with mortality

in other African anurans sympatric with *X. laevis* (Hopkins & Channing 2003; Weldon & du Preez 2004).

Other potential carriers include aquarium and commercial fish species (e.g., goldfish, mosquitofish, salmonids, and carp) that are transported worldwide (Gillespie & Hero 1999). Experimental and genetic evidence show that the transfer of pathogens between fish and amphibians is possible, as demonstrated by the spread of *Ranavirus* sp. and *Saprolegnia ferax* from fish to amphibians (Mao et al. 1999; Kiesecker et al. 2001*b*) and by the spread of Bohle iridovirus from amphibians to fish (Moody & Owens 1994). The potential for fish to carry *B. dendrobatidis* is an area of current investigation as are other potential carriers (e.g., pollen, insects, birds, humans).

Previous studies suggest that the pattern of sudden mass mortality seen in declines of amphibian populations is typical of epidemics involving an introduced, virulent infectious agent spreading through a naïve population without immunity (Laurance et al. 1996; Daszak et al. 1999). There are other examples of parasites having devastating effects on new host populations (e.g., chestnut blight, HIV). A large number of experiments with viruses, fungi, protozoans, and helminths, however, indicate that pathogens infecting completely novel hosts often initially are less virulent in their new hosts than in their original hosts, to which they are adapted (reviewed by Ebert & Hamilton 1996). Given this range of impacts, the presence or absence of high levels of virulence cannot necessarily be used to determine whether a pathogen is novel.

The Endemic Pathogen Hypothesis

The endemic pathogen hypothesis suggests that the emergence of an infectious disease involves a change in the immunological, ecological, and/or behavioral parameters of the host or parasite, tipping the balance from a relatively benign association toward a more pathogenic relationship. Changes can act through various mechanisms to create an environment more favorable for disease organisms. The susceptibility of hosts may increase because environmental changes create sublethal stresses followed by immunosuppression (Carey 1993). Changes that might affect amphibian immune systems include abiotic stressors, such as changes in temperature, chemical concentration, pH, moisture level, or ultraviolet-B (UV-B) radiation, and biotic stressors such as an increase in host density (e.g., Kiesecker & Blaustein 1995; Carey et al. 1999; Davidson et al. 2001). Immunosuppression could explain the emergence of infectious diseases in amphibian populations in a variety of habitats, including protected, apparently intact ecosystems.

Rollins-Smith et al. (2002) found that antimicrobial peptides kill or inhibit the growth of the amphibian fungal pathogens *B. dendrobatidis* and *Basidiobolus ranarum*. Their results imply that the innate defense system is capable of defending amphibians from these disease-causing organisms but may not be able to do so if the concentration of peptides is lowered or if certain peptides are lacking. Stressors can inhibit antimicrobial peptide production and release from the granular glands of amphibian skin (Simmaco et al. 1997). It is not yet known how the levels of peptides that inhibited fungal pathogen growth in the laboratory (Rollins-Smith et al. 2002) compare with natural concentrations found in amphibians in the wild or with the reduced concentrations that may be induced by stress. Furthermore, the repertoire of antimicrobial peptides is species specific (Conlon & Kim 2000). The role these species-level differences may be playing in the variable impacts that *B. dendrobatidis* is having on different species is unknown but under investigation.

Results of several experiments support the hypothesis that increased susceptibility of amphibians to disease is due to immunosuppression. The water mold *S. ferax* can act synergistically with UV-B radiation so that its impact is enhanced when the embryonic system of disease defense is weakened by the stress of UV radiation (Kiesecker & Blaustein 1995; Kiesecker et al. 2001*a*). Also, lungworm (*Rhabdias ranae*) infection can be more severe in leopard frogs (*Rana pipiens*) exposed to a sublethal dose of pesticides than in those that have not been exposed (Gendron et al. 2003), suggesting that components of the immune system response were significantly suppressed after exposure to the pesticide mixture.

Immunosuppression is hypothesized to be playing a role in the emergence of chytridiomycosis. Opportunistic pathogens, however, which often signify immunosuppression (Carey et al. 2003), have not be found in amphibians infected with *B. dendrobatidis* (Berger et al. 1998). Of 147 wild and captive individual frogs with chytridiomycosis from Australia, only 1 was considered immunosuppressed based on histologic examination of organs involved in immunity, and no significant pathogens other than *B. dendrobatidis* were found based on viral and bacterial culture, electron microscopy, and hematology (Berger et al. 1999). Additionally, amphibians assumed to be immunocompetent are killed following extremely low doses of *B. dendrobatidis* in the laboratory (Berger et al. 1999). Still, it is possible that *B. dendrobatidis* is the first or predominant opportunistic pathogen in an immunosuppressed amphibian and thus cannot yet be ruled out as a mechanism.

Alternatively, changes in the environment may allow a disease to emerge by producing conditions that are more favorable for the reproduction or transmission of a pathogen that was previously rare, including altering the competitive outcome of two or more pathogens or more directly increasing the population growth rate of the pathogen (Carey et al. 1999; Hochberg et al. 2000). In a few generations, interactions between species can change from mutualistic to parasitic because of variation in host density, resource availability, and physical conditions and the influence of additional interacting species

(Bronstein 1994; Herre et al. 1999). For example, eutrophication due to atmospheric nutrient deposition has changed microbial food webs in Sierra Nevada lakes (Sickman et al. 2003), which could make conditions more favorable for a pathogen such as *B. dendrobatidis*, that is widespread in this area (Fellers et al. 2001; Rachowicz & Vredenburg 2004). Eutrophication has also been implicated in altering food webs in U.S. wetlands, which has increased deformity-inducing parasitic trematodes (e.g., *Ribeiroia ondatrae*) by favoring snails that are intermediate hosts of the parasite (Johnson & Chase 2004).

Changing environmental conditions could increase the growth rate or transmission of *B. dendrobatidis*, resulting in an outbreak. Observational evidence supports this hypothesis for the spread of *B. dendrobatidis* in Costa Rica, where global climate change has led to drier conditions in the cloud forests that may have caused amphibians to become crowded in smaller bodies of water and increased the transmission rate of the fungus (Pounds et al. 1999). Environmental change could directly affect the ability of hosts to express behaviors or select microhabitats that normally reduce their susceptibility. Many ectothermic animals, including amphibians, routinely increase their body temperatures by basking in sunlight (Freed 1980; Duellman & Trueb 1986). At least some pathogens of amphibians have thermal optima well below the body temperatures reached by basking animals, and some, such as *B. dendrobatidis*, can be killed by such temperatures in vivo (Woodhams et al. 2003), clearing hosts of infection. Host species that may not normally experience pathogenic effects from these organisms because their normal behavior includes periods of elevated body temperature produced by basking may experience outbreaks when weather conditions prevent the expression of this behavior. Some ectothermic animals also respond specifically to infection by basking or selecting high-temperature microhabitats to produce "behavioral fever" (Parris et al. 2004).

B. dendrobatidis has been found in Australia and the Americas across a broad range of latitudes, longitudes, and elevations (Berger et al. 1998). At many of these sites there is no evidence for large-scale outbreaks of the pathogen and it is unknown how long *B. dendrobatidis* has been present; testing of museum specimens is discovering past cases (Fig. 1b). Ouellet et al. (2005) examined 900 frogs from Canada and the United States. All were museum specimens, and prevalence of *B. dendrobatidis* did not differ significantly between 1960–1969 and 1990–2001. They neither confirmed nor denied *B. dendrobatidis* presence in older specimens but concluded that *B. dendrobatidis* may not be new. Lethal outbreaks, they argue, "appear to have complex causes and may be the result of underlying predisposing factors." These causes and factors need to be identified and tested. A strong association between chytridiomycosis outbreaks, high elevation, and low temperatures has been proposed (Daszak et al. 2003) and supported by determining optimal laboratory temperatures for the pathogen (17–23° C: Piotrowski et al. 2004). The proposed effect of elevation and temperature does not, however, explain the differential impacts seen in sympatric species.

Genetic Evidence for the Novel Pathogen Hypothesis

Population genetics may help discriminate among the possible origins of an emerging disease. A prediction from the novel pathogen hypothesis is that the genotypes of virulent strains of *B. dendrobatidis* from regions where they are exotic should show reduced allelic variation and increased association among loci when compared with genotypes of individuals from the source population. Among animal pathogens this possibility has been proposed for Latin American *Coccidioides posadasii*, which shows reduced allelic variation and increased association among loci when compared with North American *C. posadasii* (Fisher et al. 2001). In addition, the significant relationship between geographic and genetic distance seen in North American *C. posadasii* is lost when Latin American genotypes are included in the analysis (Fisher et al. 2001). This case shows that one genotype of the pathogen has spread into a new area (South America) from an area of endemism (North America).

Alternatively, under the endemic pathogen hypothesis, we would expect congruence between geographic and genetic distance, with strains of the pathogen isolated from outbreaks in different regions showing levels of genetic differentiation related to the likely length of time since genetic exchange between fungal populations. We would also expect that the genotypes of strains isolated from moribund or dead hosts should represent a random sample of the genotypes isolated from healthy hosts or the environment. *Aspergillus fumigatus* provides an example of a fungus that shows no difference between environmental and disease-causing individuals (Debeaupuis et al. 1997; Rosehart et al. 2002), and the salient factor in the occurrence of aspergillosis is host immunosuppression.

A third possibility is that local fungal genotypes could repeatedly evolve to show increased virulence and be responsible for outbreaks. In this case, the genotypes of strains involved in disease outbreaks show reduced allelic variation and increased association among loci because of the selective sweep. Having come from the local population, however, the virulent genotype would not alter the relationship between geographic and genetic distance expected of old, stable populations.

Where pathogenic fungi are the key factor, either from invasion or evolution of a virulent genotype, a full understanding of the history of the epidemic requires information about populations of the fungus that are not involved in virulent outbreaks of disease, either from the geographic area of origin in the case of invasion or the

area of the epidemic in the case of evolved virulence. In both cases, because these strains are not causing disease, they can be difficult to collect. An epidemic of coccidioidomycosis in humans in California in the early 1990s raised suspicions of the emergence of a virulent *C. immitis* clone, but examination of genotypes of cultures of the fungus collected during the epidemic showed no association of loci (Fisher et al. 2000) and no difference from environmental isolates, although environmental isolates were hard to obtain (Greene et al. 2000). Instead, it was proposed that climatic factors, the length of droughts, and the amount of rainfall preceding epidemics could explain epidemics of coccidioidomycosis (Fisher et al. 2000). To carefully apply the population genetic approach, it is important to sample throughout the range of the pathogen to include all possible source populations. Equally important is the characterization of loci that are sufficiently polymorphic to distinguish between source and migrant populations.

The genetic evidence to date for *B. dendrobatidis* points toward the novel pathogen hypothesis; this, however, is not yet conclusive. Morehouse et al. (2003) suggest a recent clonal expansion of *B. dendrobatidis*, based principally on the fact that they found a low level of nucleotide polymorphism among individuals sampled from many geographic areas. Within this low level of polymorphism there was some geographic congruence and some suggestions of long-range geographic transpositions. As the authors note, however, there are two caveats that need to be addressed before it can be concluded definitively that *B. dendrobatidis* is a novel pathogen that has been introduced recently. First, only five polymorphic nucleotide positions were found in the four variable loci, and these polymorphisms may be too few to assess population structure. More polymorphisms (e.g., microsatellites) most likely will be needed to discover population structure (Burt et al. 1996; Fisher et al. 2001). Second, the case for a recent clonal expansion would be more convincing if a source population with elevated genetic variation had been identified. Above we discussed how some anuran species (e.g., *X. laevis*, bullfrogs) that have been introduced widely have been shown to carry *B. dendrobatidis* without causing disease. A more thorough sampling of populations in the native ranges of these species should be a priority for future studies.

Conservation Implications

If genetic evidence shows conclusively that the geographic range of *B. dendrobatidis* has recently expanded, possible carriers and means of transmission should be the focus of future conservation and research efforts. Some sites in which *B. dendrobatidis* is associated with die-offs are in remote and seemingly undisturbed locations such as high-elevation sites in the Sierra Nevada (California), Central America, and northern Australia. If the *B. dendrobatidis* that causes outbreaks leading to die-offs is ultimately demonstrated to be a novel pathogen, the carrier(s) responsible for transportation of this chytrid both among and within continents must be managed.

If more complete genetic evidence suggests that the geographic range of *B. dendrobatidis* has not recently expanded, environmental factors that may have changed the previous relationship between amphibians and this fungus should be investigated. Given the lack of opportunistic pathogens found in many *B. dendrobatidis*-infected animals, immunosuppression is not yet well supported; thus, other mechanisms and effects of environmental changes should be investigated. Statistical models can quantify the association between abiotic and biotic factors and the presence or absence of disease in a given population. Infection experiments can be performed with the factors found to be significant in the statistical models to assess how certain aspects of the habitat influence transmission of the disease. Experiments that vary, for example, amphibian density, temperature, humidity, and nutrient and pesticide load, may help explain how these factors influence the transmission rate directly or indirectly (e.g., through increased contact between host and pathogen, increased abundance of host or pathogen, or changes in host susceptibility). To further investigate the impacts of habitat on disease emergence, genetic analysis can determine whether more than one genotype of *B. dendrobatidis* is present because environmental conditions may influence the outcome of competition between strains. Differences in growth rate between different strains of the fungus can be measured experimentally to assess whether environmental conditions differentially affect the different strains.

Changes to the environment have been implicated in the declines of amphibians worldwide (reviewed in Alford & Richards 1999). Most likely multiple factors are involved and no one factor can explain all amphibian declines (Blaustein & Wake 1995; Kiesecker et al. 2001*a*; Collins & Storfer 2003). Although the available genetic evidence suggests *B. dendrobatidis* is a novel pathogen (Morehouse et al. 2003), one should not rule out the possibility that *B. dendrobatidis* is an old amphibian associate that has emerged recently as a pathogen in some species. We caution that one hypothesis on the origin of the disease may not apply to all species or all areas.

Because strategies for studying and managing the effects of emerging pathogens differ substantially depending on whether the pathogens are novel or endemic, we suggest that discriminating between the novel pathogen and endemic pathogen hypotheses should be an early step in investigations of all emerging pathogens. This is particularly important for pathogens of wildlife, where the fact that a pathogen escaped previous detection is weak evidence for its novelty.

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