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Pearls

The Deadly Chytrid Fungus: A Story of an Emerging Pathogen

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Emerging infectious diseases present a great challenge for the health of both humans and wildlife. The increasing prevalence of drug-resistant fungal pathogens in humans [1] and recent outbreaks of novel fungal pathogens in wildlife populations [2] underscore the need to better understand the origins and mechanisms of fungal pathogenicity. One of the most dramatic examples of fungal impacts on vertebrate populations is the effect of the amphibian disease chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*).

Amphibians around the world are experiencing unprecedented population losses and local extinctions [3]. While there are multiple causes of amphibian declines, many catastrophic die-offs are attributed to *Bd* [4,5]. The chytrid pathogen has been documented in hundreds of amphibian species, and reports of *Bd*'s impact on additional species and in additional geographic regions are accumulating at an alarming rate (e.g., see http://www.spatialepidemiology.net/bd). *Bd* is a microbial, aquatic fungus with distinct life stages. The motile stage, called a zoospore, swims using a flagellum and initiates the colonization of frog skin. Within the host epidermal cells, a zoospore forms a spherical thallus, which matures and produces new zoospores by dividing asexually, renewing the cycle of infection when zoospores are released to the skin surface (Figure 1). *Bd* is considered an emerging pathogen, discovered and described only a decade ago [6,7]. Despite intensive ecological study of *Bd* over the last decade, a number of unanswered questions remain. Here we summarize what has been recently learned about this lethal pathogen.

How Is *Bd* Related to Other Fungi?

Bd is a member of a basal group of fungi, the Chytridiomycota, and is the only known member of its order (the Rhizophydiales) to parasitize vertebrates. *Bd* is phylogenetically distant from any of the other ~1,000 chytrid species [8], and the lack of close relatives capable of parasitizing vertebrates suggests that *Bd* pathogenicity evolved relatively recently. Further, population genetic data on *Bd* isolates collected from different amphibian populations around the world suggest that *Bd* is a recently spread pathogen rather than being endemic with altered relationships with hosts due to environmental change [9,10].

How Has *Bd* Spread around the World So Quickly?

Africa was initially proposed as the geographic origin because the earliest evidence of *Bd* is from skin samples from African clawed frogs (*Xenopus laevis*) collected in 1938. African clawed frogs were traded globally for decades (from the 1930s–1960s) for pregnancy assays in humans [11]. Although based on a small sample size, recent population genetic work shows reduced genetic diversity of isolates from African clawed frogs and, instead, high allelic diversity in North American isolates collected from bullfrogs (*Rana catesbeiana*) [10,12]. Although additional genetic work is needed, these studies suggest that *Bd*'s origin may not be in Africa. Anthropogenic spread of *Bd* is a plausible explanation for at least some introductions [11,13]. Some amphibian species that are traded globally may serve as disease reservoirs because they can carry *Bd* infections without morbidity. A number of mysteries remain about how *Bd* has dispersed to and persisted in remote pristine environments where anthropogenic introduction is unlikely. If *Bd* can survive independently of amphibian hosts, it must use non-amphibian organic materials as nutrient resources. Although *Bd* DNA has been detected in water bodies [14] and on rocks [15], conclusive evidence of *Bd* persistence in the environment is lacking.

How Does *Bd* Kill Frogs?

In infected amphibians, *Bd* is found in the cells of the epidermis and pathological abnormalities include a thickening of the outer layer of skin [6]. Cutaneous fungal infections in other vertebrates are not typically lethal, but amphibian skin is unique because it is physiologically active, tightly regulating the exchange of respiratory gases, water, and electrolytes. Thus, the physiological importance of the skin makes amphibians particularly vulnerable to skin infections. It has been hypothesized that *Bd* disrupts normal regulatory functioning of frog skin, and evidence suggests that electrolyte depletion and osmotic imbalance that occurs in amphibians with severe chytridiomycosis are sufficient to cause mortality [16,17].

What Factors Are Implicated in *Bd* Pathogenicity/Virulence?

The molecular factors influencing *Bd* pathogenicity and virulence have yet to be conclusively identified. Some evidence

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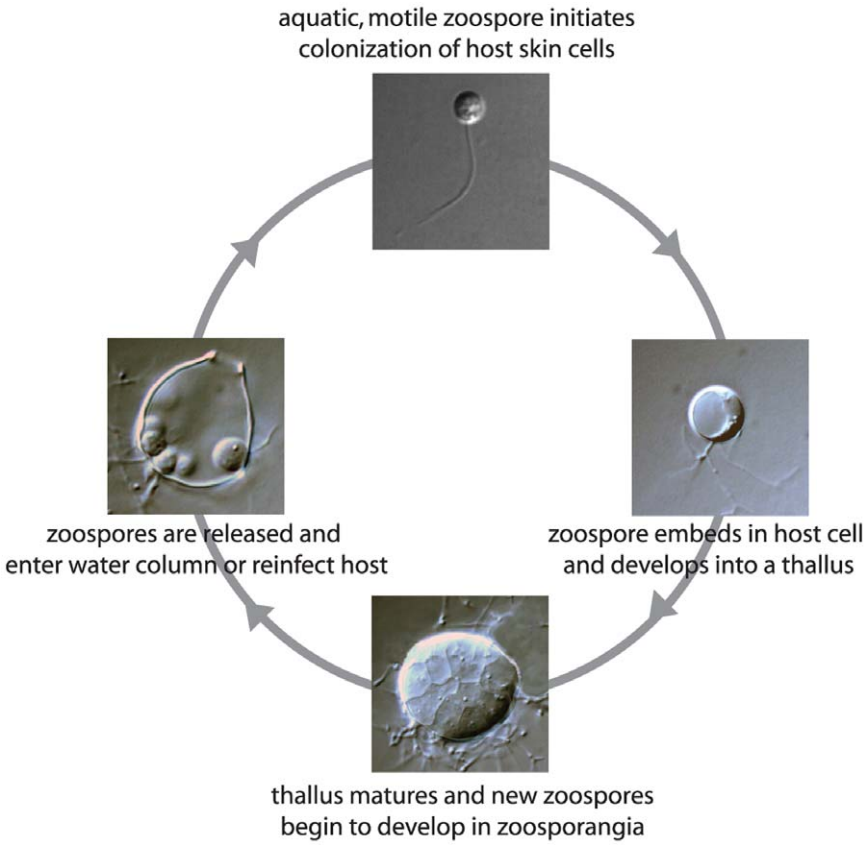

Figure 1. Life cycle of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis*. Images were taken of *Bd* in pure culture grown in 1% tryptone media.
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suggests that *Bd* enzymatic activity directly influences pathogenesis. The initial penetration of *Bd* into amphibian epidermal cells likely requires digestive enzymes. In culture, *Bd* secretes extracellular proteases that degrade casein and gelatin [18,19]. At the molecular level, genomic research into *Bd* is revealing intriguing expression patterns in genes such as those for serine protease and fungalysin metallopeptidase [20], two gene families involved in pathogenesis in other fungal pathogens. Full genomes of two *Bd* isolates have recently been sequenced, providing new resources for the study of molecular mechanisms of pathogenicity [21].

Are There Differences in *Bd* Isolate Virulence?

Several studies have shown variation in virulence among *Bd* isolates. In experimental infections, differences in frog survival have been observed when exposed to different *Bd* isolates (e.g., [22,23]). Initial proteomic work suggests that *Bd* isolates differ in their proteome profiles [23]. However, controlled infection experiments with reciprocal host isolate treatments and paired genomic and proteomic studies are necessary to identify the functional determinants of *Bd* virulence.

Do All Frogs Respond Similarly to *Bd*?

Species, populations, and individuals vary widely in susceptibility to chytridiomycosis. Mortality rates in laboratory infection experiments can range from 0% to 100%, depending on the species (e.g., [22,24]), age of animals [25], and temperature regime [26]. In the wild, some species and populations are extirpated while others, those that survive initial declines, persist with various levels of infection (e.g., [27,28]). While the disease dynamics are undoubtedly influenced by local environmental conditions, particularly temperature, inherent differences in host susceptibility and behavior are also important. Colonization by *Bd* and subsequent disease development may be influenced by host defense mechanisms, such as secretions of antimicrobial peptides [29] or bacterial commensals with anti-fungal properties [30]. Some species-specific behavioral characteristics such as microhabitat selection, basking, aggregating in retreat sites, or association with water bodies may also affect the likelihood of infection and disease [31,32].

How Can We Stem the Tide of *Bd*-Related Declines?

Despite many gaps in our understanding of chytridiomycosis, we are beginning to unravel important elements of this lethal disease and make progress towards amphibian conservation. Multiple conservation strategies have been proposed and are currently being implemented to mitigate the threat of chytridiomycosis. These plans include efforts to limit the spread of the disease, invest in captive breeding programs for highly vulnerable amphibians, and advance basic disease research. Continued research on the biology of both the host and the pathogen is necessary, and efforts to catalog and preserve the *Bd* isolates for ongoing research are particularly important (see http://www.spatialepidemiology.net/bd/ and http://www.bdbank.org/, [33]). The conservation challenges we face with chytridiomycosis—and

other emerging pathogens—are best confronted by increasing our knowledge of disease processes from both host and pathogen perspectives.

References

1. Warnock DW (2006) Fungal diseases: an evolving public health challenge. Med Mycol 44: 697–705.
2. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, et al. (2009) Bat white-nose syndrome: an emerging fungal pathogen? Science 323: 227–227.
3. Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, et al. (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306: 1783–1786.
4. Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, et al. (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc Natl Acad Sci U S A 103: 3165–3170.
5. Schloegel L, Hero JM, Berger L, Speare R, McDonald KR, et al. (2006) The decline of the sharp-snouted day frog (*Taudactylus acutirostris*): the first documented case of extinction by infection in a free-ranging wildlife species? EcoHealth 3: 35–40.
6. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Natl Acad Sci U S A 95: 9031–9036.
7. Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen et sp nov, a chytrid pathogenic to amphibians. Mycologia 91: 219–227.
8. James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, et al. (2006) A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98: 860–871.
9. Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, et al. (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12: 395–403.
10. James TY, Litvintseva AP, Vilgalys R, Morgan JAT, Taylor JW, et al. (2009) Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathog e1000458: doi:10.1371/journal.ppat.1000458.
11. Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R (2004) Origin of the amphibian chytrid fungus. Emerg Infect Dis 10: 2100–2105.
12. Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, Kobayashi A, Inaba S, Mizutani T, Hyatt AD (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Molecular Ecology 18: 4757–4774.
13. Fisher MC, Garner TWJ (2007) The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. Fungal Biol Rev 21: 2–9.
14. Kirshtein JD, Anderson CW, Wood JS, Longcore JE, Voytek MA (2007) Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Dis Aquat Org 77: 11–15.
15. Walker SF, Salas MB, Jenkins D, Garner TWJ, Cunningham AA, et al. (2007) Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. Dis Aquat Org 77: 105–112.
16. Voyles J, Berger L, Young S, Speare R, Webb R, et al. (2007) Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Dis Aquat Org 77: 113–118.
17. Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook, Webb R, Alford RA, Skerratt LF, Speare R (2009) Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326: 582–585.
18. Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96: 9–15.
19. Symonds EP, Trott DJ, Bird PS, Mills P (2008) Growth characteristics and enzyme activity in *Batrachochytrium dendrobatidis* isolates. Mycopathologia 166: 143–147.
20. Rosenblum EB, Stajich JE, Maddox N, Eisen MB (2008) Global gene expression profiles for life stages of the deadly amphibian pathogen *Batrachochytrium dendrobatidis*. Proc Natl Acad Sci U S A 105: 17034–17039.
21. Rosenblum EB, Fisher MC, James TY, Stajich JE, Longcore JE, Gentry LR, Poorten TJ (2009) A molecular perspective: biology of the emerging pathogen *Batrachochytrium dendrobatidis*. Dis Aquat Org;doi: 10.3354/dao02179.
22. Berger L, Marantelli G, Skerratt LL, Speare R (2005) Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Dis Aquat Org 68: 47–50.
23. Fisher MC, Bosch J, Yin Z, Stead DA, Walker J, et al. (2009) Proteomic and phenotypic profiling of the amphibian pathogen *Batrachochytrium dendrobatidis* shows that genotype is linked to virulence. Mol Ecol 18: 415–429.
24. Daszak P, Strieby A, Cunningham AA, Longcore JE, Brown CC, et al. (2004) Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetolog J 14: 201–207.
25. Lamirande EW, Nichols DK (2002) Effects of host age on susceptibility to cutaneous chytridiomycosis in blue-and-yellow poison dart frogs (*Dendrobates tinctorius*). In Proceedings of the Sixth International Symposium on the Pathology of Reptiles and Amphibians, 18–19 April 2001 St. PaulMinnesota. pp 3–13.
26. Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Org 55: 65–67.
27. Retallick RWR, McCallum H, Speare R (2004) Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLoS Biol 2: e351. doi:10.1371/journal.pbio.0020351.
28. Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. Ecology 86: 3149–3159.
29. Woodhams DC, Ardipradja K, Alford RA, Marantelli G, Reinert LK, et al. (2007) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Conserv 10: 409–417.
30. Harris RN, James TY, Lauer A, Simon MA, Patel A (2006) Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of Amphibian species. Ecohealth 3: 53–56.
31. Lips KR, Reeve JD, Witters LR (2003) Ecological traits predicting amphibian population declines in Central America. Conserv Biol 17: 1078–1088.
32. Rowley JJL, Alford RA (2007) Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Dis Aquat Org 77: 1–9.
33. Voyles J, Cashins SD, Rosenblum EB, Pushendorf R (2009) Preserving pathogens for wildlife conservation: a case for action on amphibian declines. Oryx 43: 527–529.

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