

AMPHIBIAN DISEASE

Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation

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Infectious diseases rarely end in extinction. Yet the mechanisms that explain how epidemics subside are difficult to pinpoint. We investigated host-pathogen interactions after the emergence of a lethal fungal pathogen in a tropical amphibian assemblage. Some amphibian host species are recovering, but the pathogen is still present and is as pathogenic today as it was almost a decade ago. In addition, some species have defenses that are more effective now than they were before the epidemic. These results suggest that host recoveries are not caused by pathogen attenuation and may be due to shifts in host responses. Our findings provide insights into the mechanisms underlying disease transitions, which are increasingly important to understand in an era of emerging infectious diseases and unprecedented global pandemics.

How do infectious disease outbreaks end? Especially in highly lethal diseases, there is often a shift from an outbreak (epidemic or epizootic phase) to a period when hosts and pathogens coexist (endemic or enzootic phase) (1). Resolving the mechanisms that underpin such disease transitions is challenging because their dynamics hinge on complex and interrelated factors, including the host, the pathogen, and their shared environment (1, 2).

The amphibian disease chytridiomycosis provides a model to investigate the mechanisms underlying epizootic-enzootic transitions. The fungal pathogen that causes chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*), has been linked to population declines in amphibian species around the world (3–5). We investigated a chytridiomycosis epizootic-enzootic transition in a tropical amphibian assemblage by tracking shifts in species detection, community composition, and infection patterns, as well as host resistance and pathogen virulence over time.

More than a decade ago, a series of *Bd*-driven amphibian declines were documented at three sites in Panamá (5–10) (Fig. 1A). These epizootic

events were characterized by increases in *Bd* prevalence with concomitant declines in amphibian host densities (5–10). We conducted long-term field surveys at these three sites (Fig. 1A) (5–10). Within ~5 to 13 years after the epizootic events, populations of some species began to reappear, but *Bd* was still present (11), suggesting a shift to an enzootic phase of disease. We used samples collected before, during, and after the epizootic-enzootic transition and integrated common garden experiments and genomic sequencing to test for temporal shifts in *Bd* pathogenicity. We predicted that the mechanism (s) associated with host recoveries would be a decrease in *Bd* pathogenicity (pathogen attenuation) (table S1), an increase in host resistance, or both.

To test whether the detection of individual species changed over time, we used a binomial generalized linear model to evaluate the presence or absence of 12 riparian species that were driven to critically low levels, or putatively extirpated, by chytridiomycosis (10). We found that nine amphibian species are recovering after a period of no detection during the epizootic phase (Fig. 1B and table S2) (10). Two species, variable harlequin frogs (*Atelopus varius*) and common rocket frogs (*Colostethus panamansis*) (Fig. 1B), provide compelling examples of highly susceptible species that declined (8–10) and subsequently recovered in the same locations (Fig. 1B). We also used species community indices to evaluate changes in the community composition similarity over time. After the epizootic events, the number of detected species increased, and host community composition became more similar to that in the pre-disease reference year (Fig. 1, C to E).

To test for changes in *Bd* infection patterns, we collected 2035 diagnostic samples. We found that *Bd* prevalence has decreased since the epizootic events. Prevalence of *Bd* is now low among

amphibian host species at our three study sites (Fig. 2A), within individual species (such as *C. panamansis*) at a single site (Fig. 2, B and C), and across wet and dry seasons (fig. S1).

To test for changes in *Bd* pathogenicity, we conducted common garden experiments with *Bd* isolates that were collected and cryo-archived from two time points: one in 2004, during epizootic events, and one in 2012–2013, after amphibian communities exhibited signs of recovery (Fig. 1A). *Bd* isolates from these time points are hereafter referred to as “historic” and “contemporary” isolates (table S3). We used identical procedures to cryo-archive and revive all isolates (12). We predicted that *Bd* attenuation would be characterized by measurable changes in *Bd* phenotype, immune evasion, disease outcome in live hosts, and genomic structure (table S1).

To test whether historic and contemporary *Bd* isolates differed in reproductive rate and phenotype, we estimated growth rates, zoospore density, and densities of infectious zoospores. Growth rates did not differ among isolates [linear mixed model (LMM), $F_{4,379} = 1.856$, $P = 0.117$] (fig. S2A), nor did zoospore density (LMM, $F_{1,47} = 0.292$, $P = 0.591$). Moreover, all isolates reached their maximum zoospore densities by days 5 to 6 of growth and produced similar numbers of zoospores (LMM, $F_{1,120} = 1.968$, $P = 0.163$) (fig. S2B).

To test whether historic and contemporary *Bd* isolates differed in their capacities to evade host defense mechanisms, we estimated the differences in growth rates for isolates cultured in the presence of anti-*Bd* skin secretions and in the presence of supernatants from anti-*Bd* cutaneous bacteria (13, 14). The inhibitory effects of skin secretions on *Bd* growth did not differ among historic and contemporary isolates (LMM, $F_{1,80} = 0.029$, $P = 0.865$) (fig. S2C), indicating that all isolates had comparable growth when challenged with anti-*Bd* skin secretions. Similarly, there was no difference in *Bd* growth when isolates were exposed to any of 18 bacterial supernatants (LMM, $F_{17,72} = 0.725$, $P = 0.768$) (table S4).

Bd is known to produce factors that inhibit proliferation and induce apoptosis in B and T lymphocytes (15). We used a lymphocyte viability assay to test the immunotoxicity of supernatants collected from historic and contemporary *Bd* isolates. We used two supernatant concentrations that cause lymphocyte inhibition in other amphibian species (15). The percentages of lymphocyte inhibition did not differ among historic and contemporary isolates, regardless of concentration (2.5× concentration, $F_{1,6,3} = 0.838$, $P = 0.394$; 5× concentration, $F_{1,1,5} = 1.460$, $P = 0.282$) (Fig. 3A and fig. S2D). These results suggest that historic and contemporary *Bd* isolates have similar capacities to evade and inhibit amphibian immunity.

To test for differences in *Bd* pathogenicity in live hosts, we conducted common garden exposure experiments with two frog species, *A. varius* and *Litoria caerulea*. For *A. varius*, we exposed frogs to identical doses of three historic isolates, three contemporary isolates, or a sham inoculation (negative control) solution, for a total of seven

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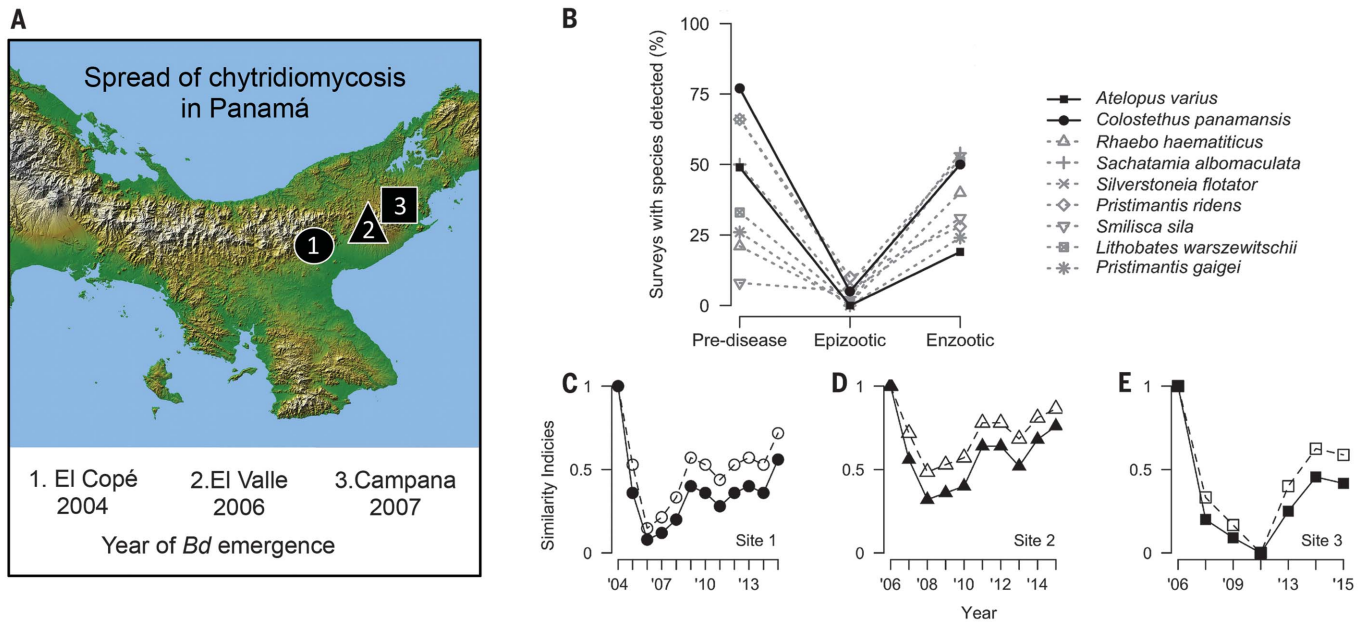


Fig. 1. Amphibian responses to chytridiomycosis outbreaks in Panamá. (A) Map of disease outbreaks in 2004 at El Copé (site 1), 2006 at El Valle (site 2), and 2007 at Altos de Campana (site 3). (B) Proportions of surveys in which amphibian species were detected across three

disease phases. (C to E) Changes in similarity of community composition over time, evaluated with two indices, the Jaccard classic index (closed shapes) and the Sorensen classic index (open shapes). Indices show community similarity relative to the pre-disease year.

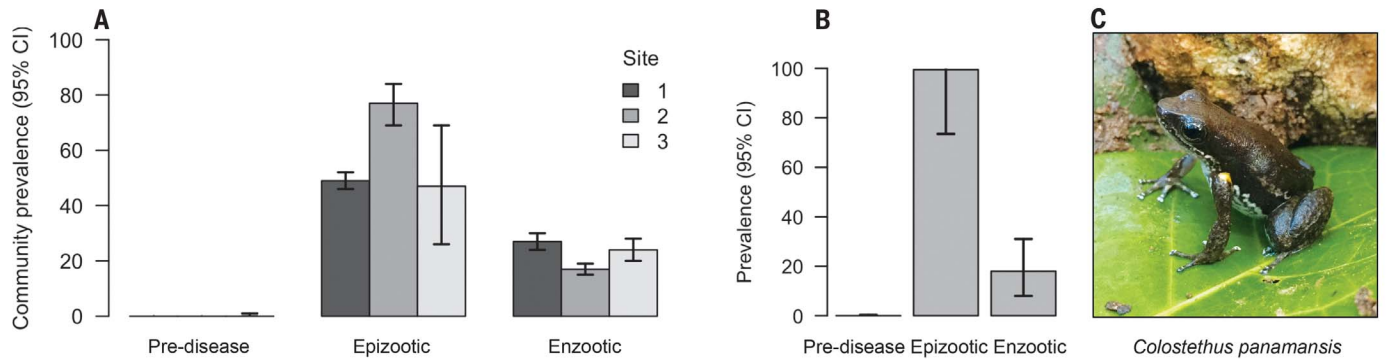


Fig. 2. Prevalence of Bd at three sites and within a single amphibian host species. (A) Community prevalence levels with 95% confidence intervals (CI) for the three study sites in Fig. 1A. Data for the pre-disease

and epizootic phases were obtained from published sources (5–10). (B and C) Prevalence in *C. panamansis* [pictured in (C)] with 95% confidence intervals over three disease phases at site 1.

treatment groups (16). We found no significant differences among the *Bd*-exposed treatment groups in prevalence (100% were infected), infection intensity (LMM, $F_{1,135} = 0.020$, $P = 0.887$) (Fig. 3B), body condition (LMM, $F_{1,207} = 2.625$, $P = 0.107$), or survival (100% mortality within 48 days; Cox regression, $P = 0.331$) (Fig. 3C). We conducted a similar experiment using *L. caerulea* and tested for differences in transmission rates between directly and indirectly exposed frogs (fig. S3). There were no significant differences in infection intensity (LMM, $F_{1,12} = 0.793$, $P = 0.391$) or survival (Mantel-Cox, $P = 0.87$). We also evaluated infection probability over time and found no interaction between exposure methods (direct and indirect) and isolate types (historic and contemporary), indicating that infection probabilities were similar regardless of exposure to

historic or contemporary isolates (LMM, $F_{1,76} = 1.733$, $P = 0.192$).

To investigate genetic diversity and relatedness among *Bd* isolates, we used whole-genome sequencing. All isolates were nested within the global pandemic lineage (17), and there was no phylogenetic substructure or signature of temporal divergence among historic and contemporary isolates (fig. S4). We also evaluated chromosomal copy number variation (CNV) among isolates because gain and loss of chromosomal segments is a proposed mechanism for shifts in *Bd* pathogenicity (17, 18). We found an overall signal of diploidy for all isolates (fig. S5) and no shared patterns of CNV among historic and contemporary *Bd* isolates. Thus, genomic data provided no evidence for differentiation, or shifts in *Bd* pathogenicity, among historic and contemporary *Bd* isolates.

Testing for shifts in host defensive capacities was more challenging because we could not use a common garden approach with live hosts. Instead, we used samples of skin secretions that are indicative of host resistance (13). We compared the *Bd*-inhibitory effects of skin secretions from pre-disease and enzootic populations at different geographic locations (13) and from captive *A. varius* frogs (frogs moved to captive breeding programs before *Bd* emergence and therefore *Bd* naïve) and wild, *Bd*-infected enzootic populations (11). The levels of inhibitory effectiveness differed among species (LMM, $F_{5,109} = 5.501$, $P < 0.001$) and between disease phases ($F_{1,109} = 5.131$, $P = 0.025$) (Fig. 4A). Also, inhibitory effectiveness was greater in samples from wild *A. varius* than in those from frogs in captivity (t test, $t_7 = 44.68$, $P < 0.001$) (Fig. 4B). Though

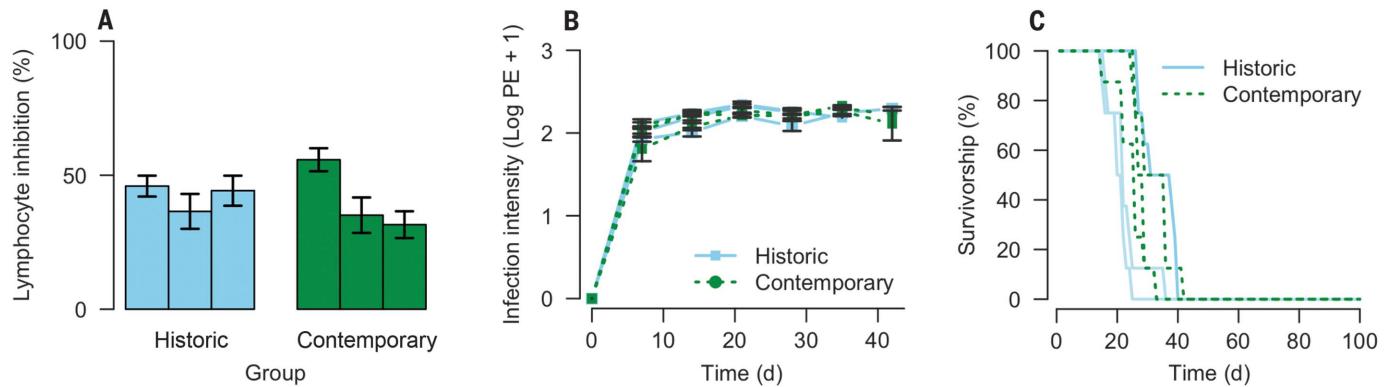


Fig. 3. Comparisons of three historic and three contemporary isolates of *Bd* for immunotoxicity, infection intensity, and survivorship in live hosts. (A) Percentages of lymphocyte inhibition (means \pm SEM) by

historic and contemporary *Bd* supernatants. (B and C) Infection intensity [mean plasmid equivalents (PE) \pm SEM] (B) and survivorship of *A. varius* (C) after exposure to historic and contemporary *Bd* isolates. d, day.

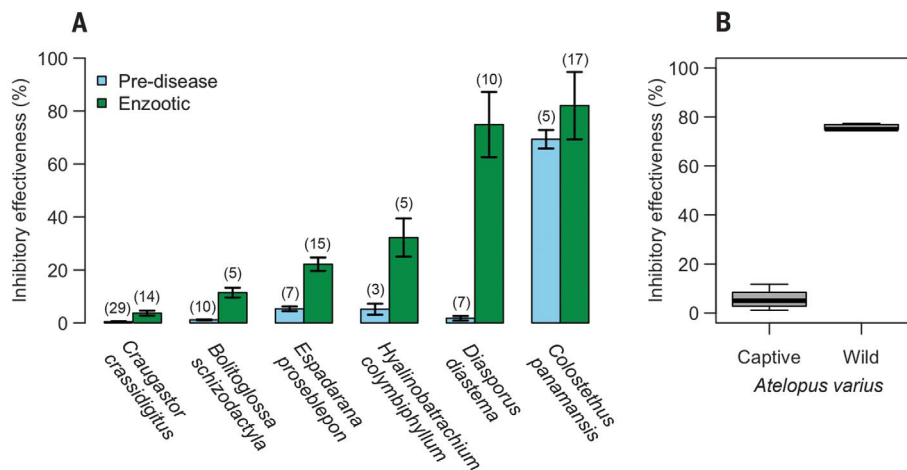


Fig. 4. Comparisons of the levels of effectiveness of skin secretions from amphibian hosts against *Bd*. Percentages of inhibitory effectiveness (means \pm SEM) for skin secretions from six species at the pre-disease and enzootic disease stages (A) and from wild (*Bd*-infected) and captive-bred (*Bd*-naive) *A. varius* populations (B). Numbers in parentheses above the bars are sample sizes for each species.

many host factors warrant investigation, these results support the hypothesis that shifts in host resistance may be contributing to the recovery of some amphibian species.

Overall, our study reveals that some species have recovered in the 11 to 13 years since the initial chytridiomycosis epizootic events at three Panamanian study sites. Although these recoveries represent a subset (~20% at El Copé) of all the species that declined, they nonetheless provide key insights into the mechanisms driving the epizootic-enzootic transition. Specifically, host recoveries are associated with marked shifts in prevalence but no evidence of attenuated pathogenicity in *Bd*. Some recoveries could be explained by upslope dispersal and recolonization of frogs from lowland populations. However, there is currently no evidence that lowland populations are more resistant to *Bd* infection, and this explanation is unlikely for montane endemics that lack low-elevation source populations (such as *Agalychnis lemur*, *Hyalinobatrachium*

vivivittatum, and *Hyloscirtus colymba*). An additional plausible mechanism is that some species persisted at low numbers and subsequently increased in abundance, possibly because of evolution in host defenses.

Our results, along with those from studies of other highly virulent disease systems (19, 20), suggest that some disease dynamics may be driven largely by host factors (e.g., standing genetic variation or the capacity to rapidly evolve effective pathogen resistance), particularly when infectious agents remain highly pathogenic. Such insights are increasingly important for understanding spatiotemporal shifts in host-pathogen interactions, especially in this extraordinary time of emerging infectious diseases that threaten plant, animal, and human health (1, 21).

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ACKNOWLEDGMENTS

We thank A. Estrada, M. Forriester, B. Gratwicke, D. Medina, R. Puschendorf, M. Robak, A. Smlanich, the Cruz family, UNR (University of Nevada, Reno) Evol-Doers, the Maryland Zoo, and the Smithsonian Tropical Research Institute. **Funding:** This study was supported by Disney Worldwide Conservation Fund, the Association of Zoos and Aquariums, NSF (grants DEB-1551488, 1660311, IOS-1603808, 13542421, 1121758, and 1557634), and the NIH (grant P20GM103451). **Author contributions:** J.V., C.L.R.-Z., E.B.R., and L.A.R.-S. conceived of the project and designed the experiments. J.V., D.C.W., V.S., A.Q.B., R.P., G.R.-S., M.J.R., M.C.B., F.A.S., S.M., L.R., L.A.R.-S., R.L., J.M.R., E.J.G., H.R., and C.L.R.-Z. performed field and experimental work. J.V., D.C.W., M.J.R., E.B.R., L.A.R.-S., and C.L.R.-Z. analyzed the data and wrote the manuscript. **Competing interests:** None declared. **Data and materials availability:** Sequences are available from National Center for Biotechnology Information BioProject (accession no. PRJNA395742). All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/359/6383/1517/suppl/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
References (22–25)

30 July 2017; accepted 1 March 2018
10.1126/science.aao4806

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Science **359** (6383), 1517-1519.
DOI: 10.1126/science.aao4806

Resistance is not futile

The fungal disease chytridiomycosis has wreaked havoc on amphibians worldwide. The disease is caused by the organism *Batrachochytrium dendrobatidis* and was first identified in the late 1990s. Voyles *et al.* revisited protected areas in Panama where catastrophic amphibian losses were recorded a decade ago (see the Perspective by Collins). Although disease theory predicts that epidemics should result in reduced pathogenicity, they found no evidence for such a reduction. Despite this, the amphibian community is displaying signs of recovery—including some species presumed extinct after the outbreak. Increased host resistance may be responsible for this recovery.

Science, this issue p. 1517; see also p. 1458

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