AMPHIBIAN DISEASE

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

Jamie Voyles,* 1+ Douglas C. Woodhams, 2,3 Veronica Saenz, 4 Allison Q. Byrne, 5 Rachel Perez, 4 Gabriela Rios-Sotelo, 1 Mason J. Ryan, 1,7 Molly C. Bletz, 2 Florence Ann Sobelt, 5 Shawna McLetchie, 6 Laura Reinert, 8 Erica Bree Rosenblum, 5 Louise A. Rollins-Smith, 6 Roberto Ibáñez, 3,9 Julie M. Ray, 10 Edgardo J. Griffith, 11 Heidi Ross, 3 Corinne L. Richards-Zawacki 3,4

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 epidemic 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 of supernatants from anti-Bd skin secretions on (Fig. 1B). Multiple, 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 epidemic 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, zoosporangium sizes, and densities of infectious zoospores. Growth rates did not differ among isolates [mixed linear model (LMM), F1,170 = 1.856, P = 0.117] (fig. S2A), nor did zoosporangium sizes (LMM, F1,17 = 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, F1,120 = 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, F1,150 = 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, F1,72 = 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, F1,143 = 0.838, P = 0.394; 5× concentration, F1,115 = 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 kept frogs to identical doses of three historic isolates, three contemporary isolates, or a sham inoculation (negative control) solution, for a total of seven isolates.

*Corresponding author. Email: jvoyles@unr.edu

1Department of Biology, University of Nevada, Reno, Reno, NV, USA. 2Department of Biology, University of Massachusetts–Boston, Boston, MA, USA. 3Smithsonian Tropical Research Institute, Ancón, Panamá. 4Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA. 5Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA, USA. 6Department of Biology, New Mexico Institute of Mining and Technology, Socorro, NM, USA. 7Arizona Game and Fish Department, Phoenix, AZ, USA. 8Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA. 9Sistema Nacional de Investigación, Panamá, Panamá. 10La Mica Biological Station, El Copé, Panamá. 11Fundación Centro de Conservación de Anfibios, El Valle, Panamá.

1o f31519 (2018) 30 March 2018
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, $P = 0.87$) (Fig. 3C). 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...
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 virescens, and Hyla arborea). 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).

REFERENCES AND NOTES

ACKNOWLEDGMENTS
We thank A. Estrada, M. Forrister, B. Gratwicke, D. Medina, R. Puschendorf, M. Robak, A. Smilanich, the Cruz family, UNR Zoos and Aquariums, NSF (grants DEB-1551488, 1660311, IOS-1603808, 13542421, 1121758, and 1557634), and the NIH (grant 3R01AI083048-05A1) for generous support. The projects were supported by Disney Worldwide Conservation Fund, the Association of Zoos and Aquariums, NSF (grants DEB-1551488, 1660311, IOS-1603808, 12542421, 1121758, and 1557634), and the NIH (grant F203H03245). Author contributions: 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. conceived of the project and designed the experiments. J.V. and L.A.R.-S. analyzed the data and wrote the manuscript. Competing interests: None declared. Data and materials availability: Sequences are available from GenBank (accession no. PRJNA395742). All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.
Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation


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

Use of this article is subject to the Terms of Service

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 2017 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. The title Science is a registered trademark of AAAS.