Biological Conservation 146 (2012) 213–218

Contents lists available at SciVerse ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Short Communication

Amphibian-killing fungus loses genetic diversity as it spreads across the New World

Guillermo Velo-Antón [a,*], David Rodríguez [a], Anna E. Savage [a], Gabriela Parra-Olea [b], Karen R. Lips [c], Kelly R. Zamudio [a,*]

[a] Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA
[b] Instituto de Biología, Universidad Nacional Autónoma de México, Distrito Federal 04510, Mexico
[c] Department of Biology, University of Maryland, College Park, MD 20740, USA

ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form 29 November 2011
Accepted 4 December 2011
Available online 29 December 2011

Keywords:
Amphibian declines
Batrachochytrium dendrobatidis
Chytridiomycosis
Wildlife disease

ABSTRACT

Emergent infectious diseases are a severe threat to global biodiversity, thus conservation biologists need to understand the emergence, spread, and evolution of pathogens to identify factors driving disease outbreaks. Amphibian chytridiomycosis is a recently emerged amphibian disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) that has led to species extinctions and declines worldwide. The spatio-temporal dynamics of pathogen occurrence and disease outbreaks, and comparative genomic analyses of global *Bd* strains, support the spreading pathogen hypothesis (SPH) with pandemics occurring after introduction of *Bd* into naïve host populations. Here we used population genetics of the amphibian-killing fungus to test for genetic consequences of pathogen spread. Our population genetic analyses are consistent with the spread of *Bd* from North to Central America, based on low genetic diversity, reduction in heterozygosity, and increased allele fixation in *Bd* from recently infected populations. Our findings confirm the spread of *Bd* in the New World, and indicate that future conservation efforts should focus on: (i) functional consequences (such as changes in pathogenicity) of these genetic changes, and (ii) public education and restrictions on wildlife trade to help slow spread of the pathogen at the invasion front.

1. Introduction

Emergent infectious diseases are among the most severe threats to global biodiversity, and because wildlife species can serve as reservoirs, their diseases also have implications for domestic animals and human health (Daszak et al., 2000; National Research Council, 2001). To identify the drivers of disease epidemics, it is critical for disease ecologists, epidemiologists, and conservationists to understand the emergence, spread and evolution of pathogens. In particular, spatial expansion of pathogens can cause rapid species die-offs at the front of advancing epidemic waves (Real et al., 2005; Walsh et al., 2005; Lips et al., 2008); therefore, determining how a pathogen spreads across landscapes, and how it changes during that process, is crucial for predicting and mitigating future species infections in naïve host populations.

Many long-recognized factors such as habitat split, destruction, and fragmentation (Dodd and Smith, 2003; Marsh and Trenham, 2001; Becker et al., 2007), chemical contaminants (Hayes et al., 2002), and global warming (Kiesecker et al., 2001) threaten over 43% of amphibian species with extinction (Stuart et al., 2004). In addition, *Batrachochytrium dendrobatidis* (*Bd*), a recently identified emerging pathogen (Berger et al., 1998; Longcore et al., 1999), causes the amphibian disease chytridiomycosis, and has led to global declines and extinctions of many amphibian species and populations (Berger et al., 1998; Skerratt et al., 2007). The areas of highest projected impact of land-use and climate change on global amphibian species do not coincide with regions most likely to be affected by *Bd* (Hof et al., 2011); in addition, regions with the richest amphibian faunas are disproportionately more affected by one or multiple threat factors than areas with low richness. These results have clear conservation implications, increasing the spatial distribution and interactions of major threats to amphibian persistence, and underscoring the need to better understand how each of these factors impacts amphibian survival.

The occurrence of the *Bd* epizootic in the New World (Rachowicz et al., 2005) is framed by the "spreading pathogen hypothesis" (SPH), which posits disease outbreaks when *Bd* arrives and spreads through naïve populations (Lips et al., 2008). Understanding how this novel spreading pathogen moves and changes over space and time has direct implications for amphibian conservation strategies. Recent genetic and demographic studies confirm the spread of *Bd* at regional (Morgan et al., 2007; Lips et al., 2008; Vredenburg et al., 2010; Cheng et al., 2011) and global (James et al., 2009; Farrer et al., 2011) scales. However, the population genetic

* Corresponding authors. Present address: CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Instituto de Ciências Agrárias de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal (G. Velo-Antón).
E-mail addresses: guillermo.velo@gmail.com (G. Velo-Antón), krz2@cornell.edu (K.R. Zamudio).

0006-3207/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocon.2011.12.003

changes in the invading pathogen along the presumptive epizootic front have not yet been characterized.

Important goals in pathogen genetics are to characterize the extent of genetic variation in disease-causing agents, identify the spatial distribution of variants, and determine how they differ in spread and interactions with hosts (Criscione et al., 2005; Archie et al., 2009; Farrer et al., 2011). Our goal in this study is to test the hypothesis that *Bd* has changed in genetic composition as it spread from North America to Central America. The SPH predicts that *Bd* from more recently infected populations will show genetic similarity to source populations, but with decreased heterozygosity, and increased allele fixation along the invasion front owing to consecutive genetic bottlenecks (Clegg et al., 2002) and/or the progressive loss of heterozygosity (LOH) due to rapid asexual reproduction as populations expand along the wave (James et al., 2009). We analyze samples of *Bd* infecting frog populations in California, Arizona, Mexico and Panama, using nine nuclear loci and population-based sampling (13–40 individuals per population) to infer historical demographic changes in the pathogen as it moved south from North America to Central America, causing extinction and declines of numerous amphibian species (Lips et al., 2004, 2006; Vredenburg et al., 2010; Cheng et al., 2011; Savage et al., 2011).

2. Materials and methods

2.1. Study sites and sample collection

We collected skin swabs from *Bd*-infected amphibian populations in Arizona ($N = 15$), Mexico ($N = 13$) and Panama ($N = 30$) (Table S1) following standardized field protocols (Hyatt et al., 2007). The amphibian hosts sampled included one species from Arizona (*Lithobates yavapaiensis*), four species from Mexico (*Ambystoma mexicanum*, *Craugastor mexicanus*, *Dendrotriton xolocalcae*, and *Hyla eximia*), and four species from Panama (*Atelopus zeteki*, *Colostethus panamensis*, *Lithobates warszewitschii*, and *Silverstoneia nubicola*). We pooled data from our samples with previously published genetic datasets (Morgan et al., 2007; James et al., 2009) to analyze population genetic diversity along a putative epizootic wave that reached Central America from more northern source locations (Wave 1 in Lips et al., 2008).

2.2. DNA extraction and genotyping

We extracted whole genomic DNA from swabs (Hyatt et al., 2007) and used real-time PCR to identify samples with the highest *Bd* concentrations for use as templates (Boyle et al., 2004). We PCR amplified nine previously published polymorphic *Bd* SNP and microsatellite loci: CTSYN1, 8702X2, 8009X2, 8329X2, 8392X2, 9893X2, mb-b13, b7-10c and 6677X2 (Morehouse et al., 2003; Morgan et al., 2007; James et al., 2009; Table S1). Locus 8009X2 was excluded from population genetic diversity analyses because it did not reliably amplify in samples from Panama. Amplicons were purified and sequenced in both directions on an ABI PRISM 3700 DNA Analyzer. We checked electropherograms by eye and scored each for polymorphic SNPs using Sequencher 4.7 (Gene Codes Corporation). Then we converted SNP and microsatellite sequences to biallelic genotypes, and pooled them with previously published samples (Morehouse et al., 2003; Morgan et al., 2007; James et al., 2009).

2.3. Data analyses

Reconstructing patterns of genetic structure in *Bd* has been challenging owing to low genetic variability among populations of the pandemic *Bd* strain and a limited number of available markers (James et al., 2009; Farrer et al., 2011). High frequency of clonal reproduction and the lack of an appropriate outgroup preclude a rigorous phylogenetic analysis with our data. Nonetheless, *Dc* genetic distances visualized as an unrooted neighbor-joining tree provide some indications of genetic similarity among *Bd* strains (Fig. 1). We estimated Dc differences and reconstructed the NJ tree using the program Populations 1.2.31 (Langella, 1999). We analyzed population genetic diversity by pooling newly sampled populations (Arizona, Mexico and Panama; Table S1) with the most genetically similar California populations (Morgan et al., 2007) and nine previously genotyped samples from Panama (James et al., 2009). We calculated genetic diversity (observed, H_O, and expected, H_E, heterozygosities) for the regional samples of *Bd* (Fig. 2A) using GenAlex v. 6.3 (Peakall and Smouse, 2006) and estimated the frequencies of dominant alleles at each locus (Fig. 2C). We tested our hypotheses about genetic diversity along the epizootic wave from North to Central America (Lips et al., 2008) by comparing mean H_O and geographic distances of each sample to the potential *Bd* source (southern Sierra Nevada, California) using Pearson's correlation. We inferred significant pairwise differences in *Ho* among all populations with one-way ANOVA and Tukey HSD pairwise comparison. All statistical analyses were conducted in R v. 2.10.1. Because our regional samples include different numbers of populations (Table 1), we also estimated genetic diversity for California populations independently to assure that regional patterns of genetic diversity are not biased by unequal sampling effort. Finally, we used a Mantel test analysis to evaluate the fit of our data to an IBD model using GenAlex v. 6.3. We estimated pairwise genetic (F_{ST}) and geographic (km) distances and used 9999 matrix permutations to assess significance of correlations (Fig. 2B).

3. Results

The globally distributed panzootic *Bd* strain shows limited geographic structure (Fig. 1) and genealogies are typically unrooted (James et al., 2009; Farrer et al., 2011) precluding their use in direct tests of dispersal pathways. Nonetheless, our genealogy does provide evidence of proximity among our target populations, thus we used the topology to define the best comparative populations for our subsequent population analyses. The newly genotyped samples from Arizona, Mexico, and Panama are interspersed among *Bd* collected in the southern Sierra Nevada of California (one of two *Bd* genetic demes in Sierra Nevada; dark blue; Fig. 1) and more genetically distant from northern Sierra Nevada *Bd* samples (light blue; Morgan et al., 2007). Therefore, we compare our samples to the southern Sierra Nevada populations (Laurel Creek, Hitchcock Lakes, Woods Lake, and Mono Pass; Morgan et al., 2007) in downstream population genetic analyses.

Population-level sampling revealed a reduction in mean heterozygosity (H_O) across populations (one-way ANOVA, $df = 3$, $F = 6.53$, $P = 0.002$) with significant pairwise population differences along the invasion path (Tukey HSD tests; $P < 0.05$: California/Mexico, Arizona/Panama; $P < 0.01$: California/Panama). We also detected an inverse correlation between H_O and distance from the California population (Pearson $r = -0.992$, $P = 0.0039$; Fig. 2A) and increased allele fixation for most genetic markers along the north–south transect (Fig. 2C). The highest and lowest inter-population genetic differentiation, measured as F_{ST}, occurred between the California and Panama populations ($F_{ST} = 0.208$) and Arizona and Mexico ($F_{ST} = 0.042$), respectively. Mantel tests confirmed a significant positive correlation between geographical and genetic distance corroborating isolation by distance among *Bd* populations ($R^2 = 0.4$; $P < 0.001$; Fig. 2B).

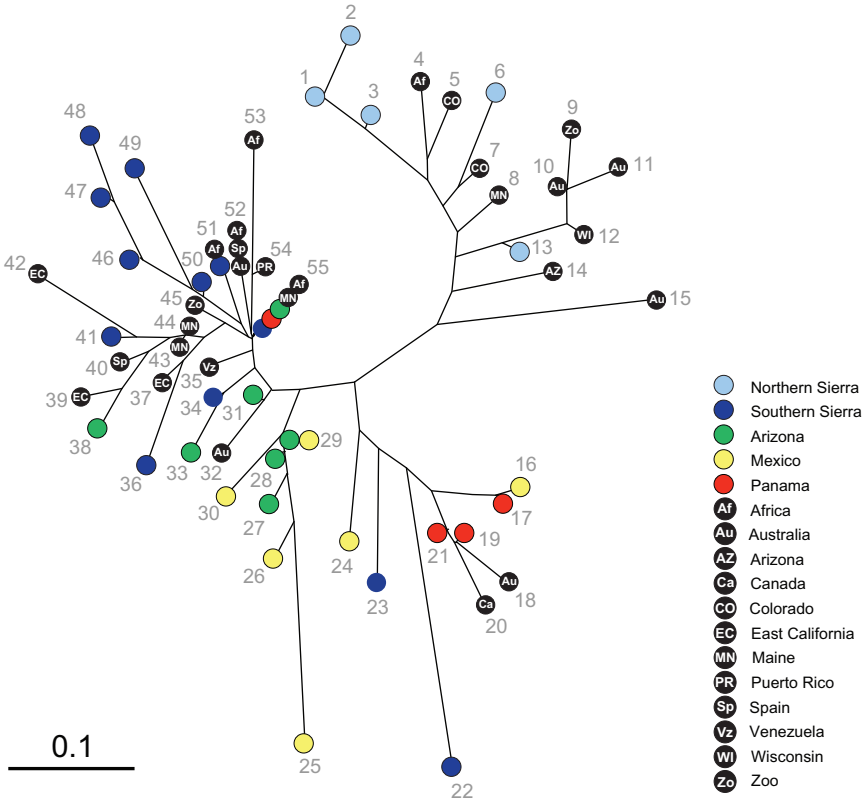

Fig. 1. Neighbor-joining tree of global *Bd* genotypes (Table S1). Blue genotypes correspond to two *Bd* genetic demes in California (Morgan et al., 2007); the newly genotyped samples are most similar to samples from the southern Sierras (dark blue) than the northern Sierras (light blue). Green, yellow and red genotypes correspond to newly genotyped samples from Arizona, Mexico and Panama, respectively. Black dots represent *Bd* genotypes outside our sampling area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

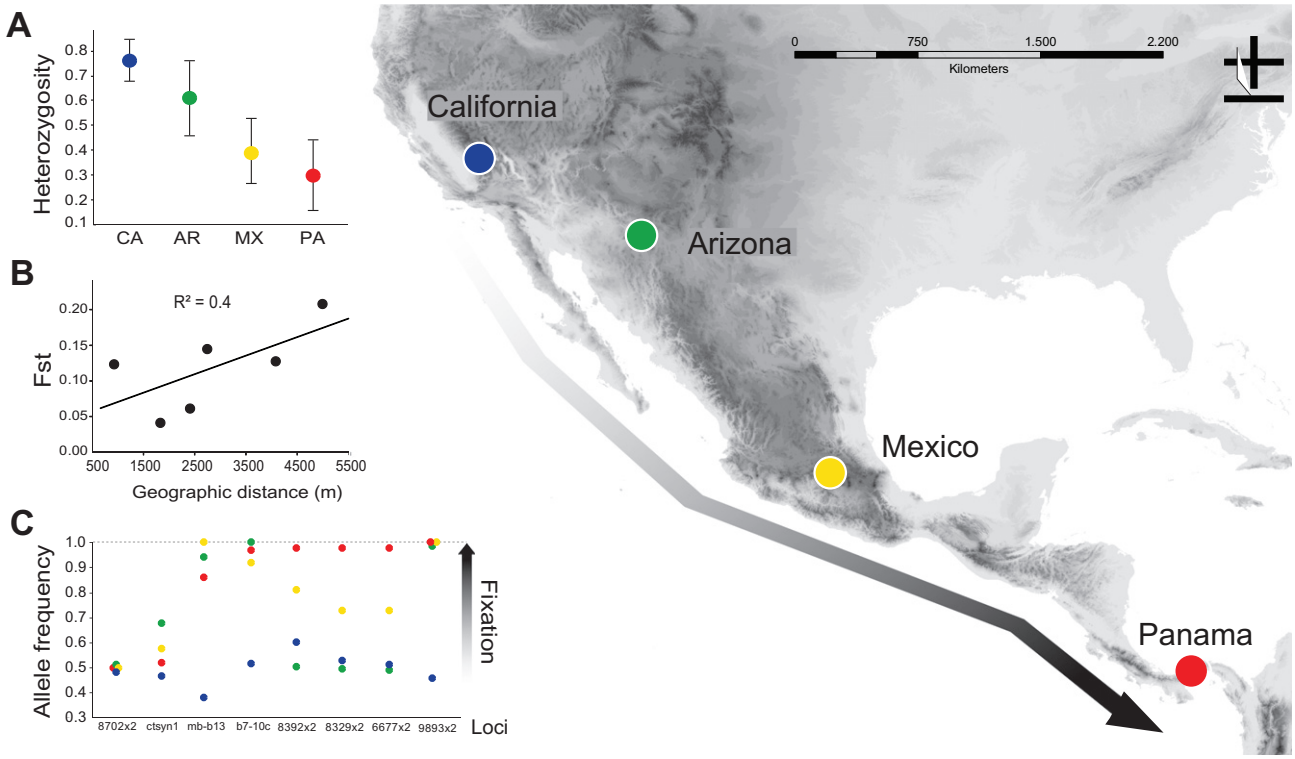

Fig. 2. *Batrachochytrium dendrobatidis* sampling and genetic analyses across New World populations. (A) Observed heterozygosity (H_O; mean and SE). (B) Pairwise Mantel test analysis of F_{ST} and geographic distances ($P < 0.001$). (C) Frequency of dominant alleles at each locus. The arrow at the left of the map indicates the direction of *Bd* spread across North and Central American populations.

4. Discussion

Our results are consistent with the spatial spread of *Bd* from North to Central America, which is correlated with amphibian population declines and species extinctions along its path (Lips et al., 2008; Cheng et al., 2011). We found a continuous reduction in heterozygosity and increased allele fixation in more recently infected populations, both of which are genetic signatures consistent with demographic bottlenecks due to rapid step-wise colonization events (Clegg et al., 2002). Our results are not due to sample size or unequal sampling effort, because we sampled more individuals and host species in Panama, the region with the lowest genetic diversity. Likewise, the spatial genetic trend found in our study is not the result of higher number of populations surveyed in northern populations (Table 1), because California populations show higher genetic diversity even when analyzed independently (Tables 1 and S1). Our results underscore the importance of comparative population genetic approaches; measuring the geographic distribution of genetic variation among *Bd* strains in other regions using larger population sample sizes and more diverse hosts may potentially permit us to test further hypotheses about fine-scale landscape and environmental determinants of pathogen spread, and with sufficient sampling, about the source population of *Bd* strains in the New World (Weldon et al., 2004).

Population genetic analyses of *Bd* have been hampered by the low number of available polymorphic genetic markers for the pathogen (Morgan et al., 2007; James et al., 2009) and by the low number of samples collected from infected populations. The recent publication of 20 globally distributed *Bd* genomes (Farrer et al., 2011) will solve this first challenge and provide abundant markers to further characterize the genetic variation in and among different regions. Our use of field-collected swabs for genotyping *Bd* rather than reliance on cultured strains, allowed for finer-scale spatial genetic analyses from multiple individuals from the same region, but requires extensive field sampling, preferably from wild populations. To obtain sufficient samples in our study, we relied primarily on field-collected samples but had to supplement with animals sold at markets in Mexico to complete the sample for that important point along the invasion pathway. Our Mexican *Bd* samples were primarily collected from Hyla eximia, a species widely distributed in Central Mexico, that is sold at markets in Mexico City. Captive animals carrying non-local *Bd* strains could affect measures of genetic diversity in this region (Goka et al., 2009). However, we found that samples from Mexico were closely related to samples from Arizona and Panama (Fig 1) and showed intermediate genetic diversity compared to those two populations.

Our findings of lower genetic diversity in *Bd* populations at the front of the epizootic wave need to be interpreted in light of the deeper evolutionary history of *Bd*, and its emergence as a hypervirulent pathogen in the last 30 years (James et al., 2009; Farrer et al., 2011). Multilocus sequence typing (James et al., 2009) and whole genome analyses of global strains (Farrer et al., 2011) support the hypothesis of a single hybrid origin of the global panzootic lineage of *Bd* (*Bd*GPL) via ancestral meiosis. That single successful diploid lineage is primarily clonal (James et al., 2009), shows increased virulence compared to other lineages (Farrer et al., 2011), recently expanded on five continents, and is often associated with population declines and local extinctions. Thus far, all New World *Bd* samples that have been characterized belong to the *Bd*GPL (Farrer et al., 2011), although more extensive sampling will be necessary to determine whether more diverse *Bd* strains exist there. The emergence of the *Bd*GPL resulted in a severe genetic bottleneck (down to a single diploid strain), which combined with subsequent loss of heterozygosity (LOH) during mitosis and asexual reproduction, explain its generally low genetic diversity despite its global distribution (Morehouse et al., 2003; James et al., 2009). These findings on the origin of the pathogenic *Bd* predict that samples taken closer to the source populations during a wave-like spread of the clone should have the highest heterozygosity (James et al., 2009) with subsequent loss of genetic diversity along the wave due to the combination of repeated founder events and mitotic recombination in expanding populations over time. This is the pattern we detected in the Central American epizootic wave we tested. The functional consequences of decreased heterozygosity at the leading edge of *Bd* epizootic waves are unknown. The genomic rearrangement that gave rise to the *Bd*GPL increased its virulence or transmissibility from the ancestral state (Farrer et al., 2011). We also know that *Bd*GPL populations vary in their virulence (Farrer et al., 2011), but as of yet these differences have not been correlated with pathogen genetic diversity. In theory, further loss of genetic diversity during pathogen spread, and the absence of frequent outcrossing, could reduce the potential of *Bd* to coevolve with an adapting host population and even result in mutational meltdown (James et al., 2009), although thus far no evidence exists to imply functional changes in pathogen virulence in newly-infected populations.

The spatio-temporal distribution of amphibian declines in the New World show that *Bd* spread from Mexico to Panama quickly (17–43 km/year; Lips et al., 2008), thus enhancing signatures of expansion due to lack of differentiation in newly established populations. This chytrid pathogen was identified only recently (Longcore et al., 1999), thus early population declines that occurred before the discovery of *Bd*, were not linked to this emergent disease. The earliest confirmed *Bd*-induced declines in North America occurred in 1976–1979 in California (Green and Kagarise-Sherman, 2001) and amphibian declines were reported in the l970s and early 1980s in Arizona (Sredl, 2005). In northern Mexico, the earliest detection of *Bd* dates to 1978 and population declines occurred in the early 1980s (Lips et al., 2004; Sredl, 2005). Similarly, population declines occurred in the late l970s and early to mid 1980s in southern Mexico (Lips et al., 2004). In Central America, dates of *Bd*-induced declines are better documented (Berger et al., 1998; Lips,

Table 1
Bd sample sizes, number of amphibian species, and localities surveyed for regional estimates of heterozygosity, and expected, (observed heterozygosity, H_O; expected heterozygosity, H_E) and allele fixation. Samples from southern Sierra populations (in italics; referred to as genetic group 2 in Morgan et al., 2007) were pooled into one population for comparison with our focal populations.

	Sample size	Number of species	Number of localities	H_O (mean ± SE)	H_E (mean ± SE)
Southern California *Woods Lake*	17	1	1	0.750 ± 0.164	0.375 ± 0.082
Southern California *Hitchcock Lakes*	17	1	1	0.846 ± 0.122	0.436 ± 0.062
Southern California *Mono Pass*	20	1	1	0.819 ± 0.118	0.447 ± 0.050
Southern California *Laurel Creek*	4	1	1	–	–
Southern California	58	2	4	0.759 ± 0.086	0.494 ± 0.004
Arizona	15	1	1	0.619 ± 0.170	0.326 ± 0.084
Mexico	13	4	3	0.394 ± 0.137	0.278 ± 0.072
Panama	39	12	2	0.305 ± 0.154	0.176 ± 0.074

1998; Lips et al., 2003, 2008) and show the southward progression of the pathogen; as of 2007, the front of this wave was localized east of the Panama Canal (Woodhams et al., 2008). These qualitative temporal data indicate that in roughly three decades *Bd* spread from California to the Darien of Panama, a rate that could cause the significant loss of genetic diversity we observed over this same region. This southbound *Bd* epizootic wave was recently confirmed using formalin-preserved museum specimens (Chen et al., 2011), confirming a rapid spread from southern Mexico (early 1970s) to Western Guatemala (1980s–1990s) and Costa Rica (1987). Studies of *Bd* population genetics at finer scales, and near the front of the epizootic wave, will contribute to our understanding of transmission dynamics and the evolution of virulence in this spreading pathogen (Archie et al., 2009).

Rapid New World expansion underscores the ability of *Bd* to spread across varied landscapes, from temperate highlands in California (Briggs et al., 2010), to deserts in Arizona and northern Mexico, and tropical montane forests of southern Mexico and Central America. Localized wave-like expansions have occurred in Californiás Sierra Nevada (Vredenburg et al., 2010), in the Pyrenees Mountains, Europe (Walker et al., 2010), and in Australia (Laurance et al., 1996), with high variation in the rate of spread (~700 m/year to ~100 km/year) suggesting the influence of landscape attributes. The *Bd*GPL has clearly expanded at a continental scale but limited data on timing of arrival and outbreaks thus far preclude identifying natural and anthropogenic factors controlling its movement.

Our genetic study corroborates that *Bd* is a novel and spreading pathogen in the New World, and that the population genetic composition of the pathogen is changing during the course of this invasion. The rapid spread of *Bd* in the New World highlights the need for pro-active conservation measures and further research. Given the recent association between invading *Bd* and its hosts, and the strength of selective events at the time of disease outbreaks, characterizing phenotypic and genetic variation within and among amphibian species will elucidate potential mechanisms underlying resistance to chytridiomycosis (O'Brien and Evermann, 1988; Råberg et al., 2007) which may potentially be used in breeding programs and restoration efforts for endangered amphibian species. Controlling or mitigating the devastating effects of *Bd* on amphibian diversity requires collaborative research and multidisciplinary approaches (Skerratt et al., 2009; Woodhams et al., 2011); knowledge of the timing and rate of spread in the New World are important not only for forecasting future effects, but for localizing invasion fronts as targets for action. Recent studies have confirmed variation in virulence among *Bd* strains (Fisher et al., 2009; Farrer et al., 2011) and host genetic variation in *Bd* susceptibility (Savage and Zamudio, 2011). Thus, characterizing functional variation among populations of *Bd* along the invasion wave will provide critical data on how changes in genetic diversity affect evolutionary potential and pathogenicity of the fungus. More generally, conservation efforts of amphibians should focus on curbing pathogen spread, and exploiting potential rapid evolutionary responses in both hosts and pathogens for management (Daszak et al., 2000; Stockwell et al., 2003). Restrictions on wildlife trade and education of tourists and local residents in non-diseased areas could help slow spread of the pathogen at the invasion front.

Acknowledgments

We thank A. Muñoz Alonso, M. Angel Galindo, A. Gata García, and A. Longo Berríos for sample collection and lab assistance. Our work was funded by the Spanish Ministerio de Ciencia e Innovación (G.V.-A.), the U.S. National Science Foundation (D.R., A.E.S, K.R.L, and K.Z.), and Conacyt of Mexico (G.P.-O.).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2011.12.003.

References

Archie, E.A., Luikart, G., Ezenwa, V.O., 2009. Infecting epidemiology with genetics: a new frontier in disease ecology. Trends in Ecology and Evolution 24, 21–30.
Becker, C.G., Fonseca, C.R., Haddad, C.F.B., Batista, R.F., Prado, P.I., 2007. Habitat split and the global decline of amphibians. Science 318, 1775–1777.
Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95, 9031–9036.
Briggs, C.J., Knapp, R.A., Vredenburg, V.T., 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proceedings of the National Academy of Sciences of the United States of America 107, 9695–9700.
Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., Hyatt, A.D., 2004. Diseases of Aquatic Organisms 60, 141–148.
Cheng, T.L., Rovito, S.M., Wake, D.B., Vredenburg, V.T., 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences of the United States of America. doi: 10.1073/pnas.1105538108.
Clegg, S.M., Degnan, S.M., Kikkawa, J., Moritz, C., Estoup, A., Owens, I.P.F., 2002. Genetic consequences of sequential founder events by an island-colonizing bird. Proceedings of the National Academy of Sciences of the United States of America 99, 8127–8132.
Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Molecular ecology 14, 2247–2257.
Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287, 443–449.
Dodd, C.K., Smith, L.L., 2003. Habitat destruction and alteration: historical trends and future prospects for amphibians. In: Semlitsch, R.D. (Ed.), Amphibian Conservation. Smithsonian Institution, Washington, pp. 94–112.
Farrer, R.A., Weinert, L.A., Bielby, J., Garner, T.W.J., Balloux, F., Clare, F., Bosch, J., Cunningham, A.A., Weldon, C., du Preez, L.H., Anderson, L., Kosakovsky Pond, S.L., Shahar-Golan, R., Henk, D.A., Fisher, M.C., 2011. Multiple emergences of genetically diverse amphibian infecting chytrids include a globalized hypervirulent recombinant lineage. Proceedings of the National Academy of Sciences of the United States of America 108, 18732–18736.
Fisher, M.C., Bosch, J., Yin, Z., Stead, D.A., Walker, J., Selway, L., Brown, A.J., Walker, L.A., Gow, N.A., Stajich, J.E., Garner, T.W., 2009. Proteomic and phenotypic profiling of the amphibian pathogen *Batrachochytrium dendrobatidis* shows that genotype is linked to virulence. Molecular Ecology 18, 415–429.
Goka, K., Yokoyama, J., Une, Y., Kuroki, T., Suzuki, K., Nakahara, M., Kobayashi, A., Inaba, S., Mizutani, T., Hyatt, A.D., 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypes, and possible route of entry into Japan. Molecular Ecology 18, 4757–4774.
Green, D.E., Kagarise-Sherman, C., 2001. Diagnostic histological findings in Yosemite toads (*Bufo canorus*) from a die-off in the 1970s. Journal of Herpetology 35, 92–103.
Hayes, T.B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A.A., Vonk, A., 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences of the United States of America 99, 5476–5480.
Hof, C., Araújo, M.B., Jetz, W., Rahbek, C., 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature. doi: 10.1038/nature10650.
Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillot, R., Cambell, R., Marantelli, G., Gleason, F., Colling, A., 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73, 175–192.
James, T.Y., Litvintseva, A.P., Vilgalys, R., Morgan, J.A.T., Taylor, J.W., Fisher, M.C., Berger, L., Weldon, C., du Preez, L., Longcore, J.E., 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathogens 5, e1000458. doi:10.1371/journal.ppat.1000458.
Kiesecker, J.M., Blaustein, A.R., Belden, L.K., 2001. Complex causes of amphibian population declines. Nature 410, 681–684.
Langella, O., 1999. POPULATIONS 1.2.30. CNRS UPR9034.
Laurance, W.F., McDonald, K.R., Speare, R., 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conservation Biology 10, 406–413.
Lips, K.R., 1998. Decline of a tropical amphibian fauna. Conservation Biology 12, 106–117.
Lips, K.R., Green, D.E., Papendick, R., 2003. Chytridiomycosis in wild frogs from southern Costa Rica. Journal of Herpetology 37, 215–218.
Lips, K.R., Mendelson III, J.R., Muñoz-Alonso, A., Canseco-Márquez, L., Mulcahy, D.G., 2004. Amphibian population declines in montane southern Mexico: resurveys of historical localities. Biological Conservation 119, 555–564.

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Pessier, A., Livo, L., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America 103, 3165–3170.

Lips, K.R., Diffendorfer, J., Mendelson III, J.R., Sears, M.W., 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6, 441–454.

Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. *Batrachochytrium dendrobatidis* gen et sp nov, a chytrid pathogenic to amphibians. Mycologia 91, 219–227.

Marsh, D.M., Trenham, P.C., 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15, 40–49.

Morehouse, E.A., James, T.Y., Ganley, A.R.D., Vilgaly, R., Berger, L., Murphy, P.J., Longcore, J.E., 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Molecular Ecology 12, 395–403.

Morgan, J.A.T., Vredenburg, V.T., Rachowicz, L.J., Knapp, R.A., Stice, M.J., Tunstall, T., Bingham, R.E., Parker, J.M., Longcore, J.E., Moritz, C., Briggs, C.J., Taylor, J.W., 2007. Enigmatic amphibian declines and emerging infectious disease: population genetics of the frog killing fungus *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences of the United States of America 104, 1385–1390.

National Research Council 2001. Grand challenges in environmental sciences. National Academies Press, Washington, DC, USA.

O'Brien, S.J., Evermann, J.F., 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends in Ecology and Evolution 3, 254–259.

Peakall, R., Smouse, P.E., 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6, 288–295.

Rachowicz, L.J., Hero, J.M., Alford, R.A., Taylor, J.W., Morgan, J.A.T., Vredenburg, V.T., Collins, J.P., Briggs, C.J., 2005. The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology 19, 1441–1448.

Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318, 812–814.

Real, L.A., Henderson, J.C., Biek, R., Snaman, J., Jack, T.L., Childs, J.E., Stahl, E., Waller, L., Tinline, R., Nadin-Davis, S., 2005. Unifying the spatial population dynamics and molecular evolution of epidemic rabies virus. Proceedings of the National Academy of Sciences of the United States of America 102, 12107–12111.

Savage, A.E., Sredl, M.J., Zamudio, K.R., 2011. Disease dynamics vary spatially and temporally in a North American amphibian. Biological Conservation 144, 1910–1915.

Savage, A.E., Zamudio, K.R., 2011. MHC genotypes associate with resistance to a frog-killing fungus. Proceedings of the National Academy of Sciences of the United States of America 108, 16705–16710.

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B., Kenyon, N., 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4, 125–134.

Skerratt, L.F., Garner, T.W.J., Hyatt, A.D., 2009. Determining causality and controlling disease is based on collaborative research involving multidisciplinary approaches. EcoHealth 6, 331–334.

Sredl, M.J., 2005. Rana yavapaiensis (*Platz and Frost, 1984) Lowland leopard frog*. In: Lannoo, M.J. (Ed.), Amphibian Declines: the Conservation Status of United States Species. University of California Press, Berkeley, CA, pp. 596–599.

Stockwell, C.A., Hendry, A.P., Kinnison, M.T., 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18, 94–101.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783–1786.

Vredenburg, V.T., Knapp, R.A., Tunstall, T.S., Briggs, C.J., 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences of the United States of America 107, 9689–9694.

Walker, S.F., Bosch, J., Gómez, V., Garner, T.W.J., Cunningham, A.A., Schmeller, D.S., Ninyerola, M., Henk, D., Ginestet, C., Christian-Philippe, A., Fisher, M.C., 2010. Factors driving pathogenicity versus prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* and chytridiomycosis in Iberia. Ecology Letters 13, 372–382.

Walsh, P.D., Biek, R., Real, L.A., 2005. Wave-like spread of Ebola Zaire. PLoS Biology 3, e371. doi:10.1371/journal.pbio.0030371.

Weldon, C.L.H., Preez, A., Hyatt, D., Muller, R., Speare, R., 2004. Origin of the amphibian chytrid fungus. Emerging Infectious Diseases 10, 2100–2105.

Woodhams, D.C., Kilburn, V.L., Reinert, L.K., Voyles, J., Medina, D., Ibáñez, R., Hyatt, A.D., Boyle, D.G., Pask, J.D., Green, D.M., Rollins-Smith, L.A., 2008. Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. EcoHealth 5, 268–274.

Woodhams, D.C., Bosch, J., Briggs, C.J., Cashins, S., Davis, L.R., Lauer, A., Muths, E., Puschendorf, R., Schmidt, B.R., Sheafor, B., Voyles, J., 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. Frontiers in Zoology 8, 8.