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Differences in sensitivity to the fungal pathogen *Batrachochytrium dendrobatidis* among amphibian populations

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Abstract: *Contributing to the worldwide biodiversity crisis are emerging infectious diseases, which can lead to extirpations and extinctions of hosts. For example, the infectious fungal pathogen* Batrachochytrium dendrobatidis *(Bd) is associated with worldwide amphibian population declines and extinctions. Sensitivity to Bd varies with species, season, and life stage. However, there is little information on whether sensitivity to Bd differs among populations, which is essential for understanding Bd-infection dynamics and for formulating conservation strategies. We experimentally investigated intraspecific differences in host sensitivity to Bd across 10 populations of wood frogs (*Lithobates sylvaticus*) raised from eggs to metamorphosis. We exposed the post-metamorphic wood frogs to Bd and monitored survival for 30 days under controlled laboratory conditions. Populations differed in overall survival and mortality rate. Infection load also differed among populations but was not correlated with population differences in risk of mortality. Such population-level variation in sensitivity to Bd may result in reservoir populations that may be a source for the transmission of Bd to other sensitive populations or species. Alternatively, remnant populations that are less sensitive to Bd could serve as sources for recolonization after epidemic events.*

Keywords: amphibian declines, chytridiomycosis, emerging infectious disease, *Lithobates sylvaticus*, reservoir populations

Diferencias en la Sensibilidad al Patógeno Micótico *Batrachochytrium dendrobatidis* entre las Poblaciones de Anfibios

Resumen: *Las enfermedades infecciosas emergentes están contribuyendo a la crisis mundial de biodiversidad, lo que puede llevar a la extirpación y extinción de los hospederos. Por ejemplo, el patógeno micótico infeccioso* Batrachochytrium dendrobatidis *(Bd) está asociado con la extinción y declinación mundial de poblaciones de anfibios. La sensibilidad a Bd varía con las especies, la temporada y la etapa de vida. Sin embargo, existe poca información sobre si la sensibilidad a Bd varía entre las poblaciones, lo cual es esencial para entender las dinámicas de infección del hongo y para formular estrategias de conservación. Investigamos de manera experimental las diferencias en la sensibilidad a Bd entre diez poblaciones de ranas de bosque (*Lithobates sylvaticus*), criadas desde huevos hasta la metamorfosis. Expusimos a las ranas post-metamorfosis a Bd y monitoreamos su supervivencia bajo condiciones controladas de laboratorio durante 30 días. Las poblaciones difirieron en la supervivencia general y en la tasa de mortalidad. La carga de infección también difirió entre las poblaciones pero no estuvo correlacionada con las diferencias poblacionales en el riesgo de*

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muerte. Dicho nivel poblacional de variación en la sensibilidad al hongo puede resultar en poblaciones de reserva que pueden ser una fuente de transmisión de Bd a otras poblaciones o especies sensibles. De manera alterna, las poblaciones remanentes que son menos sensibles al hongo podrían funcionar como fuentes para la recolonización después de un evento epidémico.

Palabras Clave: declinación de anfibios, enfermedades infecciosas emergentes, *Lithobates sylvaticus*, poblaciones de reserva, quitridiomicosis

Introduction

Infectious disease is one of the principle threats to global biodiversity (Daszak et al. 2000; Fisher et al. 2012; McCallum 2012) and is increasing in both number and impact (Jones et al. 2008). Most pathogens infect multiple host species (Woolhouse et al. 2001), and one way a disease can emerge is through the transmission from a reservoir host species to a sensitive species (Engering et al. 2013). Reservoir hosts can harbor and transmit the pathogen without succumbing to disease, potentially maintaining the long-term persistence of a disease across a landscape (Schmidt & Ostfeld 2001; Ostfeld & Keesing 2012) and driving sensitive populations to extinction (Keesing et al. 2010; McCallum 2012).

Differences in infection susceptibility to emerging infectious diseases has been studied extensively at the species level (Haydon et al. 2002; Power & Mitchell 2004; Hughes & Macdonald 2013), but little is known about variability in susceptibility at the population level. Our aim was to investigate differences in infection susceptibility and disease sensitivity to an emerging disease at the population level under controlled experimental conditions in populations of an amphibian host species. Because amphibians are undergoing worldwide population declines and disease is one major contributing factor in these declines (Stuart et al. 2004), amphibians are an ideal model system to examine population differences in disease susceptibility.

The infectious chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis (Berger et al. 1998; Longcore et al. 1999), has been associated with numerous amphibian population extirpations and species extinctions (e.g., Stuart et al. 2004; Lips et al. 2006; Vredenburg et al. 2010). Several experimental studies have shown differences in how host species respond to Bd infection (Blaustein et al. 2005; Garcia et al. 2006; Gahl et al. 2011; Searle et al. 2011; Van Rooij et al. 2012; Gervasi et al. 2013*a*), and field studies have revealed differences in chytridiomycosis sensitivity within a species across a landscape of environmental gradients (Kriger et al. 2007; Van Sluys & Hero 2009; Savage et al. 2011). At smaller spatial scales, field studies suggest differences in how Bd is manifested at pond level (Briggs et al. 2005; Brem & Lips 2008; Briggs et al. 2010). However, there is little information on population-level differences in Bd sensitivity. Wood frogs (*Lithobates sylvaticus*) are an excellent species to examine population-level differences in Bd susceptibility. Wood frogs infected with Bd have been observed in the field (Chatfield et al. 2009; Davidson & Chambers 2011), and exposure to Bd can cause mortality in the laboratory (Searle et al. 2011). Furthermore, this species exhibits strong site fidelity and has a limited home range (Bellis 1965; Vasconcelos & Calhoun 2004), which allows identification of genetically distinct populations (Relyea 2002; Squire & Newman 2002; Cothran et al. 2013; Hua et al. 2013). As synchronous breeders, wood frog eggs from different populations can be collected at approximately the same time across a landscape. This oviposition behavior allowed us to collect wood frog eggs of approximately the same age from different populations for our experiment. We raised these individuals to metamorphosis under common-garden conditions and exposed the recently metamorphic frogs to Bd in a controlled laboratory experiment to test the hypothesis that wood frog populations differ in their sensitivity to Bd infection.

Methods

Husbandry

We acquired Wood frogs as eggs to ensure individuals in this study were not previously exposed to Bd. Eggs were collected from 10 populations in northwestern Pennsylvania (U.S.A.) that were 4–80 km apart (Table 1). The pathogen is endemic in the region from which we collected eggs (Groner & Relyea 2010). We are unaware of any published evidence of Bd-infected wood frogs in the region despite monitoring attempts (Glenney et al. 2010; Groner & Relyea 2010). However, it is unknown if any of the populations we collected had previously been exposed to Bd. We collected 10 egg masses from each population; eggs consisted of early-stage embryos (Gosner 1960). The eggs were collected from 4 to 11 April 2011. Immediately after collection, eggs were transported to the University of Pittsburgh, where they were placed in 100-L outdoor pools filled with 90 L of aged well water.

To ensure all eggs hatched at approximately the same time, eggs from populations collected on 11 April (locations: Bowl, Log, Road, and Reed) were held indoors at approximately 20° C in 14-L plastic containers containing 10 L of aged well water, while eggs from the other

Table 1. Information on the populations of wood frog used in the study of heterogeneity of sensitivity to chytridiomycosis.

Population	*Location*	*Date collected in 2011*	*Sample size*
Blackjack	41 39.934 N, 80 30.762 W	4 April	48
Bowl	41 55.625 N, 79 48.234 W	11 April	38
Graveyard	41 41.062 N, 80 02.837 W	5 April	40
Log	41 58.147 N, 79 35.922 W	11 April	37
Mallard	41 41.518 N, 80 30.046 W	4 April	26
Reed	41 58.801 N, 79 58.093 W	4 April	52
Relyea	41 37.341 N, 80 27.261 W	6 April	34
Road	41 53.078 N, 79 36.320 W	11 April	24
Square	41 50.486 N, 80 14.402 W	7 April	32
Turkey Track	41 37.823 N, 79 54.769 W	4 April	36

populations remained outdoors. Once hatched, free-swimming tadpoles were transferred to outdoor pools. Across all 10 populations, all eggs hatched within 48 hours between 24 and 25 April. During the period, when some populations were housed indoors and some populations outdoors (i.e., 11 to 24 April), the outdoor average daily minimum and maximum temperature was 4° and 13° C, respectively.

After all 10 populations had free-swimming tadpoles, we moved them into 100-L outdoor mesocosms. Each mesocosm contained 90 L well water, 1 L pond water, 5 g ground alfalfa, and 100 g dried oak leaves (*Quercus* spp.). We let these mesocosms sit for 15 d to allow the community to develop. We randomly assigned three mesocosms to each population and stocked each mesocosm with 25 tadpoles. Each mesocosm was covered with 60% shade cloth to exclude predators and prevent the escape of the wood frogs as they metamorphosed.

Upon metamorphosis, individuals were transferred to 1-L containers where they were kept until tail absorption (Gosner 45). Recently metamorphic frogs were fed crickets ad libitum before being shipped overnight to Oregon State University (OSU), Corvallis, Oregon (U.S.A.).

Upon arrival at OSU, frogs were transferred to 40-L glass terraria. Terraria were housed in a temperature-controlled room (14 °C) with a 12:12 hour photoperiod, and frogs were allowed to acclimate for 48 hrs. At the start of the experiment, we measured the mass and snout vent length (SVL) of each frog and placed single individuals into 14×1 cm Petri dishes with 10 mL of dechlorinated water, where they were housed for the duration of the experiment. Each Petri dish had a lid with three 4-mm holes to provide air circulation. Over the 30-day experiment, we changed the water in the Petri dishes every 7 days and individuals were fed 4, 1-week old crickets twice per week.

Bd Exposure

Half of the individuals from each population were randomly selected to be either in the Bd-exposed or unexposed treatments. Individual frogs were exposed to Bd strain JEL 274, which was originally isolated from a western toad (*Anaxyrus boreas*) in Colorado (U.S.A.) (Annis et al. 2004). This strain was selected because it was putatively an equally novel strain for each of the ten populations as well as having been deemed one of the more virulent strains associated with major amphibian populations declines (Rosenblum et al. 2013). The pathogen was grown in pure culture on 1% tryptone agar in 10-cm diameter Petri dishes. The Petri dishes were inoculated with liquid culture 8 to 16 days prior to the start of the experiment and incubated at 22 °C. To harvest the zoospores, each plate was flushed with 15 mL of 22 °C dechlorinated water and remained undisturbed for 5 minutes. The plates were scraped with a rubber spatula and the inoculum from each plate was then pooled in a beaker. The number of moving zoospores was determined using a hemocytometer, and then the solution was diluted to a concentration of 1.03×10^4 zoospores/mL.

Individuals in the Bd-exposed treatment were exposed to 15 mL of inoculum (1.55×10^5 total zoospores) poured directly on their dorsal surface. When added to the 10 mL of water already in the Petri dishes, this additional volume of liquid brought the total volume to 25 mL, which covered the bottom of the Petri dish with a thin film and kept the individuals in constant contact with the water covering the bottom. Control individuals were exposed to 15 mL of inoculum solution lacking the Bd culture (made from 1% tryptone sterile agar plates following the same methods), which we added to the 10 mL of water already in the Petri dishes.

All individuals were observed daily for 30 days following the inoculations. Animals that died during the experiment were preserved in 95% ethanol. Individuals that survived until the end of the experiment (i.e., day 30) were euthanized in a 2% solution of MS-222 and then preserved in 95% ethanol.

We used quantitative polymerase chain reaction (qPCR) to determine Bd-infection prevalence and to quantify Bd-infection load. Following standard protocols (Searle et al. 2010; Gervasi et al. 2013*b*), we randomly selected 6 Bd-exposed individuals per population. This subsampling examined those individuals that died prior

to the end of the experiment to investigate differences in infection load at the time of death as opposed to potential differences in infection loads among individuals that survived the 30-day experiment. Additionally, we quantified Bd-infection status in three randomly sampled unexposed individuals from the control treatment from each population.

To sample individuals for Bd, we used a sterile, fine-tipped, dry swab (Medical Wire and Equipment, Corsham, Wiltshire, England) and swabbed the right ventral surface of individual frogs 10 times, including the feet, legs, and drink patch. We placed each swab into a sterile screw-capped vial. We extracted the DNA by adding 60 μL of Prepman Ultra (Applied Biosystems, Carlsbad, California), heating the vial for 10 minutes at 100 °C, cooling the vials for 2 minutes, and then extracting the supernatant. We diluted supernatant to a 10% solution and then performed the qPCR. We conducted qPCR using an ABI PRISM 7500 sequencer (Applied Biosystems) according to the methods of Boyle et al. (2004). All samples were run in triplicate and averaged. If a sample tested positive for Bd-DNA in only 1 or 2 replicates, we reanalyzed the sample. If a second analysis was required, we re-swabbed the individual on its left side and analyzed the sample from the second swabbing. An individual was considered Bd-positive if all 3 samples (run once) or 4 out of 6 samples (run twice) were positive.

Statistical Analyses

We performed statistical analyses in TIBCO Spotfire S+ version 8.1 for Windows. We used a Cox proportional hazards (CPH) model, which allows one to compare the survival of two or more groups (Cox 1972) and provides a hazard ratio (HR) to quantitatively compare the relative survival of groups.

We began with a CPH model to compare the effect of Bd exposure (Bd-exposed versus unexposed), population, and mass on wood frog survival. This analysis allowed us to determine the strength of the effect of exposure to Bd among populations and detect a Bd-exposure-by-population interaction because the survival of non-exposed individuals was high across all populations, whereas the survival of exposed individuals differed among populations. As a result, we used a subsequent CPH model to examine the effect of population and mass for individuals that were exposed to Bd. This analysis allowed us to determine the strength of the effect of population identity given Bd exposure and to explicitly test the hypothesis that populations of wood frog differed in their response to Bd exposure. For each of the two CPH analyses, models with all possible combinations of sets of explanatory variables were compared and the model with the largest likelihood ratio (LR) was selected as the most parsimonious (Parmar & Machin 1995). To compare survival among the ten populations when exposed to Bd, we performed a Bonferroni adjustment to maintain an $\alpha = 0.05$ (Gotelli 2012).

We also examined whether individuals exposed to Bd had population-level differences in mass at the start of the experiment. To test for differences in mass, we performed a 1-way analysis of variance (ANOVA) followed by a Tukey-Kramer procedure due to unequal sample size among populations. Additionally, to determine the strength of the relationship between the masses of the 10 populations and the HR of each population we used a simple linear regression (SLR).

A Fisher's exact test was used to test for differences in infection prevalence. To test for difference in infection loads among populations, we began by log transforming the infection loads obtained by qPCR (log-mean genome equivalents per individual + 1), which was necessary to successfully normalize the data. We then analyzed the effects of population and mass on log-transformed infection loads with an ANCOVA. We then examined the relationship between mass and log-transformed infection loads and the relationship between frog mass and frog length (SVL) with SLR.

Table 2. Candidate Cox proportional hazards models of survival of frogs exposed to *Batrachochytrium dendrobatidis* (Bd) among the 10 populations studied in increasing order of likelihood ratio.

*Model**	*Likelihood ratio (LR)*	*df*	*p*
Bd-exposed and unexposed individuals			
M	15.4	1	< 0.001
P	18.4	9	< 0.031
P+M	30.9	10	< 0.001
Bd	220	1	< 0.001
Bd+M	252	2	< 0.001
Bd*M	253	3	< 0.001
Bd+P	279	10	< 0.001
Bd*M+P	303	12	< 0.001
Bd+M+P	303	11	< 0.001
Bd-exposed individuals only			
M	29.6	1	< 0.001
P	59.7	9	< 0.001
P+M	82.6	10	< 0.001

Key: M, mass measured at the start of the experiment (mg); P, population.

Results

Survival of unexposed wood frogs was 96%, whereas survival of Bd-exposed wood frogs was 27%. The selected CPH model that tested whether Bd-exposure treatment affected wood frog survival (Table 2) contained main effects of all the explanatory variables: Bd-exposure, population, and mass (LR = 303, df = 11). A model including the main effects of all explanatory variables as well

Table 3. Summary information for 10 populations of wood frogs used in the study of heterogeneity of sensitivity to chytridiomycosis in decreasing order of hazard ratio when comparing survival between the Bd-exposed and unexposed control treatments.

Population	*Mean body size (SE)*	*Mean body size 95% CI*	*Median survival time (days)*	*Hazard ratio (95% CI)*
Graveyard	0.450 (0.010)	0.430, 0.470	4.5	66.4 (8.21, 537)
Bowl	0.348 (0.012)	0.324, 0.371	4	59.4 (7.46, 473)
Turkey Track	0.446 (0.010)	0.424, 0.467	4	59.1 (7.39, 472)
Mallard	0.429 (0.014)	0.400, 0.459	5	31.2 (3.54, 274)
Blackjack	0.446 (0.010)	0.423, 0.468	5	27.6 (5.73, 133)
Log	0.405 (0.009)	0.387, 0.423	27	21.5 (2.68, 173)
Road	0.407 (0.019)	0.366, 0.446	7	21.3 (2.58, 176)
Reed	0.427 (0.011)	0.404, 0.450	*	20.3 (2.59, 160)
Square	0.428 (0.010)	0.408, 0.449	6	20.2 (2.54, 160)
Relyea	0.531 (0.014)	0.502, 0.560	*	10.9 (1.33, 88)

**Individuals in the Bd-exposed treatment for the Reed and Relyea populations never reached 50% mortality.*

as an interaction between Bd exposure and mass was considered but rejected because it explained results no better and was less parsimonious. Across all populations, Bd exposure increased the risk of mortality by a factor of 56.9 (95% CI 26.2, 123.8, df = 11, LR = 303, $p < 0.001$) relative to unexposed animals, yet among populations there was a 6-fold difference in hazard ratios, which ranged from 10.9 to 66.4 (Table 3). Additionally, frogs of a smaller mass had a 41% increase in the risk of mortality after exposure to Bd (CI 22.1%, 61.7%, df = 11, LR = 303, $p < 0.001$) for each 0.05 g decrease in mass from the mean mass (0.43 g SE 0.006) of individuals in the Bd-exposure treatment. However, when investigating each population individually, mass was a significant predictor of mortality in only three populations: Blackjack (df = 2, LR = 36.5, $p < 0.001$), Mallard (df = 2, LR = 21.4, $p = 0.017$), and Reed (df = 2, LR = 19, $p = 0.044$), increasing the risk of mortality by factors of 2.03 (95% CI 1.34, 3.08), 1.87 (95% CI 1.12, 3.13), and 1.57 (95% CI 1.01, 2.44), respectively.

For the CPH model that examined the effect of population and frog mass only in the presence of Bd, the largest likelihood ratio was obtained when population and mass were both included as explanatory variables (LR = 82.6, df = 10). Of the 45 pairwise comparisons among the 10 populations, 6 differed in survival after Bonferroni correction. Two populations (Graveyard and Turkey Track) had high mortality and associated larger HR values; each differed significantly from several other populations with low mortality and smaller HR values (Fig. 1, Table 4). Survival of individuals from the Graveyard population differed from both the Log and Reed populations and had an increased mortality risk by a factor of 4.9 and 6.1, respectively. Survival of individuals from the Turkey Track population differed from the Square, Log, Relyea, and Reed populations; mortality risk increased by a factor of 4.2, 5.7, 6.5, and 7.4, respectively.

Frog mass differed (ANOVA, df = 9, $p < 0.001$) among populations (Table 3). However, population differences in mean mass were not correlated with the population hazard ratios (SLR, df = 8, adjusted $R^2 = 0.008$, $p = 0.330$), suggesting that mean mass of the population was not related to population-level differences in risk of mortality.

All of the 30 individuals subsampled from the control treatment tested negative for Bd infection. Further, 59 of 60 individuals subsampled from the Bd-exposed treatment tested positive for Bd infection. We found no differences in infection prevalence (Fisher's exact test, $p = 1$), but infection load differed (ANCOVA, $F_{9,39} = 2.66$, $p = 0.016$) across populations (Fig. 2; Supporting Information) after accounting for mass and ranged from 0.5 to 450 Bd genomic equivalents. Additionally, infection load was positively correlated with frog mass (SLR, df = 57, adjusted $R^2 = 0.099$, $p = 0.009$); an increase in the median infection load by a factor of 16.8 (95% CI .378, 733) was associated with each 0.1 g increase in body mass. Additionally, there was a mass-by-population interaction in infection load (ANCOVA, $F_{9,39} = 2.48$, $p = 0.024$) and frog mass was positively correlated with frog length (SLR, df = 365, adjusted $R^2 = 0.584$, $p < 0.001$); frog mass increased 0.058 g for every millimeter increase in frog length (95% CI 0.052, 0.063).

Discussion

The negative effect of exposure to Bd was not consistent across the 10 populations (Table 3). Two populations with high levels of mortality (Graveyard and Turkey Track) differed in survival from several other populations, whereas two other populations with high levels of survival (Reed and Relyea) composed nearly half of all Bd-exposed individuals that survived the 30-day experiment.

Heterogeneity of host responses to a pathogen can result in complex host-parasite dynamics (Dobson 2004; Metcalf et al. 2013; Streicker et al. 2013) at both the species and population levels. A population that is less sensitive to Bd infection may better survive exposure

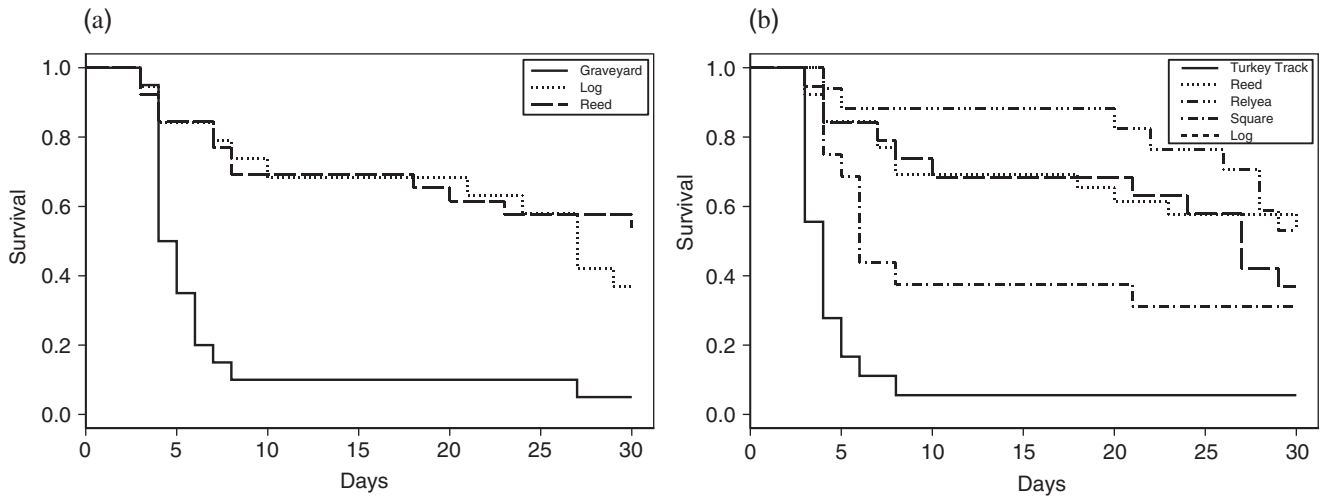

Figure 1. Kaplan–Meier survival curves comparing (a) the proportion survival of wood frogs in the Bd-exposed treatment for the Graveyard, Log, and Reed populations and (b) the proportion survival in the Bd-exposed treatment for the Turkey Track, Square, Log, Relyea, and Reed populations over the 30-day experiment.

Table 4. Summary information for the 6 wood frog population contrasts that differed in survival after exposure to *Batrachochytrium dendrobatidis* (Bd) and after a Bonferroni-adjustment to maintain an $\alpha = 0.05$.

Populations compared	*Hazard ratio (95% CI)*	*df*	*Likelihood ratio (LR)*	*p*
Graveyard : Log	4.9 (2.16, 11.0)	2	66.4	< 0.001
Graveyard : Reed	6.1 (2.71, 137)	2	59.4	< 0.001
Turkey Track : Square	4.2 (1.87, 9.52)	2	59.1	< 0.001
Turkey Track : Log	5.7 (2.49, 13.1)	2	31.2	< 0.001
Turkey Track : Relyea	6.5 (2.41, 17.5)	2	27.6	< 0.001
Turkey Track : Reed	7.4 (3.25, 16.9)	2	21.5	< 0.001

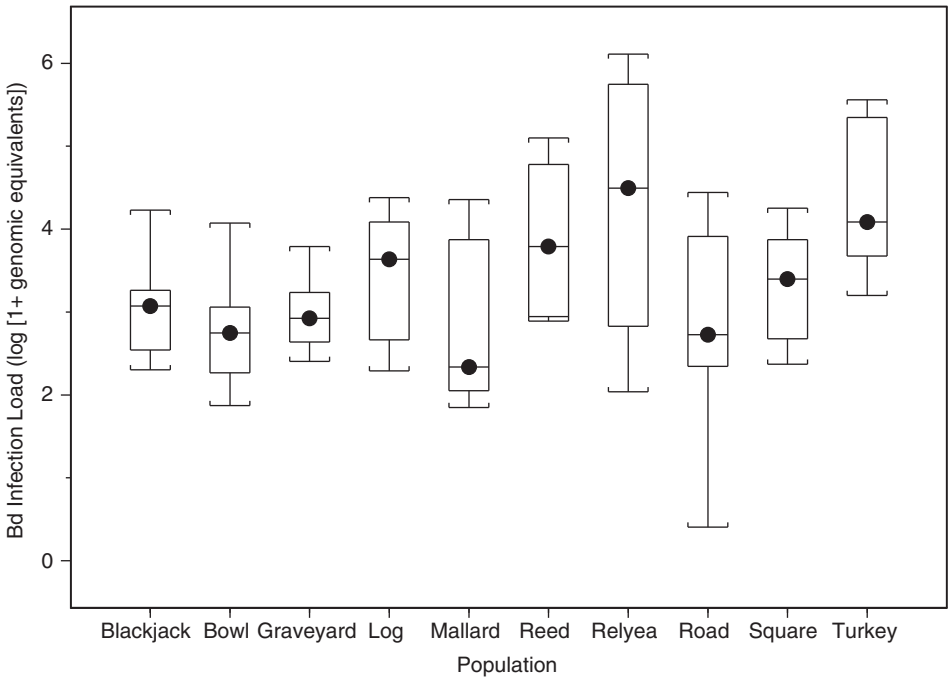

Figure 2. The Batrachochytrium dendrobatidis *(Bd) infection load in the 10 populations of wood frog used to investigate the variation in sensitivity to chytridiomycosis (boxes, median and interquartile range; whiskers, 2 most extreme data points within 1.5 x IQR from the edge of the box). Summary statistics, including 95% CI for the mean infection load for each population, are in Supporting Information.*

to the pathogen and act as reservoir population, which would allow the disease to persist (Haydon et al. 2002; Mitchell et al. 2008). A reservoir population made up of individuals with elevated infection tolerance may produce and shed more infectious propagules into the environment, or alternatively maintain normal health and behavior, leading to a longer period of shedding propagules or a higher host-host contact rate. Either of these possibilities may allow persistence of chytridiomycosis in the ecosystem, potentially increasing the risk to sympatric species less tolerant to Bd (Venesky et al. 2012) as well as other nearby wood frog populations.

Alternatively, populations made up of individuals that are less sensitive to Bd infection could remain as remnants in the face of epidemics and serve as a source for recolonization after epidemic events. Such individuals would be instrumental in species persistence and potentially could be used in conservation efforts (Venesky et al. 2012; Scheele et al. 2014). Several layers of complexity may exist when considering such scenarios (Longo et al. 2014; Vander Wal et al. 2014). However, it would be important to determine the processes that contribute to heterogeneity for conservation efforts (Streicker et al. 2013).

Our results suggest a decoupling of infection load, frog mass, and population-level survival patterns in wood frogs. Infection load was positively correlated with mass (Fig. 2), yet mass was positively correlated with overall survival of frogs in the Bd-exposed treatment and differences in population mean mass were not correlated with population hazard ratios. Whereas we observed population-level differences in survival (Fig. 1), neither mass nor infection load accounted for these differences.

When data across all populations were pooled, we observed a greater proportion of mortality and a faster rate of mortality in smaller individuals within the Bd-exposed treatment. These results are similar to those reported by others (Carey et al. 2006; Searle et al. 2011; Tobler & Schmidt 2011). However, when we investigated the populations individually, we found this same relationship in only three of ten populations (Blackjack, Mallard, and Reed). Further, of the populations that differed in survival (Table 4), in only one of six contrasts (Turkey Track – Relyea) did the populations also differ in mass.

Infection load measured at death differed among the ten populations but did not explain population-level differences in survival. Of the six population comparisons that differed in survival (Table 4), only two also differed in pathogen load (Turkey Track – Relyea and Turkey Track – Square). Of those two comparisons, pathogen load and survival were positively associated for one comparison (Turkey Track – Relyea) and negatively associated for the other (Turkey Track – Square). The remaining four comparisons had similar levels of infection load as measured at death despite differences in population survival, suggesting that these wood frog populations differed in their ability to tolerate infection loads of a given magnitude. With near 100% mortality in the Bd-exposed treatment, neither the Graveyard nor Turkey Track populations tolerated similar infection loads that resulted in lower levels and rates of mortality in the Log or Reed populations.

Despite uniform sample sizes across all ten populations when individual tadpoles were moved to the outdoor mesocosms, we initiated the laboratory experiment with unequal sample sizes based on the number of animals that successfully metamorphosed in each group (Table 1). There, however, were no clear trends between the losses prior to the start of the laboratory experiment and any explanatory or response variable investigated.

In a study of chytridiomycosis in post-metamorphic common midwife toads (*Alytes obstetricans*), Tobler and Schmidt (2011) investigated survival across three populations under controlled conditions. Using individuals collected as 1-year-old larvae that tested positive for Bd infection, they too found that populations differed in their response to Bd exposure. However in our study, all individuals were raised from eggs to metamorphosis under similar conditions and housed in the same laboratory under identical environmental conditions. Thus, the differences in mortality we observed can be explained by intrinsic biology (e.g., genetic differences) of individuals among the populations; neither abiotic and biotic environmental differences among ponds nor differences in host density are necessary to explain observed population-level differences in survival in the presence of chytridiomycosis. To our knowledge, this is the first study to empirically show population-level differences in chytridiomycosis-related survival experimentally under identical environmental conditions with individuals previously unexposed to the pathogen.

Our study was designed to test the hypothesis that wood frog populations differ in their response to exposure to Bd. However, our study does not reveal the mechanism or mechanisms responsible for population-level differences in sensitivity to chytridiomycosis. Differences in anti-microbial peptides (Rollins-Smith & Conlon 2005; Woodhams et al. 2006), skin microbiota (Harris et al. 2009; Lam et al. 2009), MHC genotypes (May et al. 2011; Savage & Zamudio 2011), or behavior (Rowley & Alford 2007; Venesky et al. 2011; Hossack et al. 2013) may all influence sensitivity to Bd and may all vary across populations. Our experiment was performed on animals collected as eggs and raised under identical conditions; thus, our results strongly suggest one of the above-mentioned mechanisms or some other genetic component is affecting the sensitivity of individuals to chytridiomycosis in this species.

Our experiment demonstrates that there is heterogeneity in wood frog sensitivity to Bd infection at the population level. Furthermore, because this experiment was performed under controlled environmental conditions, the observed population-level differences in survival

after exposure to the pathogen can be credited to intrinsic biological factors of the host populations rather than to environmental differences between the locations from where the populations were collected.

Whereas population-level heterogeneity in sensitivity may result in reservoir populations acting to maintain or amplify the pathogen across the landscape, there is, however, another side to this coin. After an epidemic episode such heterogeneity could allow for the recovery (Newell et al. 2013) or emigration and recolonization of areas that previously were inhabited by members of a host metapopulation. Moreover, individuals of populations surviving such an episode could be used in conservation efforts and captive breeding programs with the intention of reintroduction or translocation of individuals to areas where the species had been extirpated. However, the sensitivity of a population to infection is complex at both the individual and population levels. Heterogeneity in sensitivity could be due to within-host differences in resistance or tolerance or within-population differences in host behavior, in addition to being context dependent. Once surviving individuals are released into the field, these causes could be acted upon by natural selection in complex ways that might negate the artificial selection performed in captivity. Similarly, a lack of sensitivity observed under controlled conditions might not equate to persistence in the dynamic natural environment.

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Supporting Information

A table reporting infection load summary statistics among the 10 populations of wood frog (Appendix S1) is available online. Authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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