

Species-level correlates of susceptibility to the pathogenic amphibian fungus *Batrachochytrium dendrobatidis* in the United States

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Abstract Disease is often implicated as a factor in population declines of wildlife and plants. Understanding the characteristics that may predispose a species to infection by a particular pathogen can help direct conservation efforts. Recent declines in amphibian populations world-wide are a major conservation issue and may be caused in part by a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd). We used Random Forest, a machine learning approach, to identify species-level characteristics that may be related to susceptibility to Bd. Our results suggest that body size at maturity, aspects of egg laying behavior, taxonomic order and family, and reliance on water are good predictors of documented infection for species in the continental United States. These results suggest that, whereas local-scale environmental variables are important to the spread of Bd, species-level characteristics may also influence susceptibility to Bd. The relationships identified in this study suggest future experimental tests, and may target species for conservation efforts.

Keywords Amphibian · *Batrachochytrium dendrobatidis* · Body size · Infection · Life-history pathogen

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Abbreviations

Bd	<i>Batrachochytrium dendrobatidis</i>
RF	Random forest
OOB	Out of bag

Introduction

Over geological time, five major mass extinction events have been recognized and some believe a sixth major extinction event is underway (Wake and Vredenburg 2008). As part of this phenomenon, members of the class Amphibia are experiencing population declines and extinctions at unprecedented rates (Stuart et al. 2004). Disease has been implicated as a major driver for these events (Daszak et al. 2003). One recently emerged pathogen, the fungus *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis (Longcore et al. 1999), has been associated with worldwide declines and extinctions of amphibian populations (e.g., Lips et al. 2006; Rachowicz et al. 2006; updates on Bd distribution and host species available at <http://www.spatalepidemiology.net>).

Though lethal infection is common for numerous species, there is substantial variation in the effects of Bd. Some species, such as *Rana catesbeiana*, carry asymptomatic infections despite high pathogen loads (e.g., Daszak et al. 2004). Infection severity varies considerably between species of frogs, toads, and salamanders (e.g., Blaustein et al. 2005; Garcia et al. 2006; Padgett-Flohr and Longcore 2007), among species and between larvae and adults of the same species (e.g., Blaustein et al. 2005; Garcia et al. 2006; Smith and Weldon 2007).

Some of this variation may be explained by environmental conditions of host species and their life history stages. Some studies show host mortality events associated with chytridiomycosis during cool seasons (Berger et al. 1998; Bradley et al. 2002). Furthermore, high-elevation species are suspected to be more susceptible to lethal chytridiomycosis compared to lower-elevation species, presumably due to differences in abiotic factors (Berger et al. 1998; Bosch et al. 2001; Rachowicz et al. 2006). However, environmental variation does not explain differential susceptibility to Bd infection between coexisting species in a community that are under similar environmental conditions.

To determine why different species are affected by Bd in different ways, we analyzed the relationships between documented Bd infection (designated as “susceptible”) and key life-history traits of amphibian species. We attempted to identify correlates between species traits and documented positive Bd infection (“susceptibility”) in the absence of locally varying data such as temperature or precipitation. If species characteristics are commonly related to positive Bd infection in the wild, conservation efforts could focus on those species demonstrating these characteristics.

Materials and methods

Data collection

We collected data on all amphibian species in the continental United States that have been tested for infection by Bd. Data on Bd infection were collected from (1) published

literature and (2) data collected by D.H. Olson and M.C. Fisher for the Global Bd Mapping Project (www.spatialepidemiology.net/Bd-maps/). For every species, we recorded whether an individual at any life history stage tested positive for Bd. If an individual tested positive, the species was considered susceptible to Bd infection. If no positive tests were recorded, that species was listed as not susceptible. It is possible that classification of susceptible and non-susceptible species could be biased by false positive or false negative tests for Bd infection. However, these data represent the current state of knowledge on Bd infection across all sampled amphibian species in the United States.

We used published literature and amphibiaweb.org to collect life-history characteristics (Table 1). We limited our searches to amphibians in the continental United States due to the consistency of data available through species accounts in Lannoo (2005), many of which are also available at www.amphibiaweb.org. Data on explosive breeding behavior were collected from www.amphibiaweb.org; Wells (1977, 2007); and Halliday and Tejedo (1995). To ensure consistency among explanatory variables, we standardized our definitions of each life history characteristic (Table 1). In addition, we removed any species with fewer than three representatives of any categorical variable in the analysis.

Predictions

The characteristics we used in our analysis were those that we predicted would be most important in Bd susceptibility and transmission. Because Bd is an aquatic fungus, we predicted that more aquatic species (as determined by breeding habitat and reliance on water) would exhibit a greater tendency toward infection by Bd than less aquatic species. Body size can also influence pathogen exposure and load (Kuris et al. 1980), so we predicted that species obtaining larger body size at maturity would be more likely to test positive than smaller species. Body size at maturity and at metamorphosis were correlated in our data set ($P < 0.001$, Spearman's $\rho = 0.54$). Thus, we only included body size at maturity. Movements between habitats may increase the chances of exposure to Bd; therefore we predicted that species which migrate are more likely to be documented as infected. We also included taxonomic order (caudata or anura) as an additional factor in our analysis to determine whether there was a general difference in infection susceptibility between anurans and caudates, especially given that species within each group may share similar life history traits with each other. Similarly, Bd status may vary due to characteristics shared among species within a taxonomic family. Thus, we included taxonomic family as a predictor variable.

Because Bd is transmitted among amphibians (e.g., Rachowicz and Vredenburg 2004; Rachowicz and Briggs 2007), we predicted that social behavior would play an important role in the spread of Bd. Therefore, we predicted that species that aggregate would display a greater likelihood of being positive for Bd because of prolonged contact with conspecifics. We expected prolonged breeders would have more of an opportunity to contract Bd than those with relatively short breeding periods. Moreover, we predicted that species aggregating for purposes other than breeding might be more likely to be infected with Bd than less social species. For example, we predicted that species huddling for thermoregulation would be more prone to Bd infection than species that do not display this behavior. We predicted that larvae that form schools would be more likely to be infected with Bd than those that do not school. Thus, we predicted that amphibians displaying prolonged breeding or development and those species where individuals aggregate for breeding or thermoregulation would show more occurrences of positive Bd infection than species with relatively short breeding times or species that do not aggregate.

Table 1 Life history variables and their definitions used in collecting data for the Random Forest analysis of species characteristics related to documented *Batrachochytrium dendrobatidis* (Bd) infection of United States amphibians

Variable name	Definition	Range
Size at maturity	The smallest reported snout-vent length of reproductively mature animals. If males and females were listed, we used the smallest size of females	16–115 mm
Oviposition depth	Categorical estimation of average oviposition depth	None = terrestrial reproduction Shallow = oviposition at ≤ 0.5 m Deep = oviposition at > 0.5 m Both = oviposition at both shallow and deep water
Reliance on water	Separates species in which all life stages are terrestrial from those in which at least one stage is dependent on water	Yes = species with at least one life history stage dependent on water No = species that are entirely terrestrial
Length of larval period	A categorical estimator of larval period duration. The longest interval recorded was used if more than one value reported.	None <4 months <8 months <12 months <24 months >24 months
Migratory	If any migrations were listed, the species was considered to migrate	Yes = migrations reported No = no migrations reported
Aggregations	Behavioral aggregation in groups of either kin or non-kin, in larval and older life stages	Yes = aggregations reported No = no aggregations reported
Egg laying mode	Categorical estimation of egg laying mode	Single = eggs individually oviposited Small cluster = 2–100 eggs per cluster, on average Large cluster = > 100 eggs per cluster, on average
Explosive breeding	If breeding occurs in a single burst in the population, the species was considered to be explosively breeding	Yes = explosive breeding reported No = no reported explosive breeding
Order	Taxonomic order	Anura = frogs and toads Caudata = salamanders and newts
Family	Taxonomic family	From amphibiaweb.org
Breeding habitat	Type of water body used for breeding	None = terrestrial breeding Moving = rivers or streams used for breeding Standing = ponds or lakes used for breeding Both = moving and standing water used for breeding

Characteristics of egg laying may influence transmission and embryonic susceptibility to disease (Kiesecker and Blaustein 1997). However, Bd has not been associated with eggs and we predicted that egg laying behavior and depth of oviposition would not be a factor in infection status.

Statistical analysis

Our goal was to generate a model identifying species life history characteristics related to infection status. We used a machine-learning approach called Random Forest (RF; Breiman 2001). RF is a model averaging technique based on classification and regression trees. RF builds a large set of trees (the “forest”; generally >500 trees) from subsets of both the data and the explanatory variables and then combines the trees in the forest to generate an ensemble model of the structure in the data. The classification and regression trees used in RF are non-parametric models that allow for complex non-linear interactions among predictor variables, making these models more appropriate than linear models (i.e., logistic regression) commonly used to explore the relationship between explanatory variables and a binary outcome. RF cannot include phylogenetic trees within the structure of the analysis. We included two taxonomic levels (order and family) to capture some aspects of phylogenetic relatedness within the RF analysis. Moreover, recent empirical work showed that decision tree methods outperformed or were comparable to existing phylogenetic comparative methods, producing consistent predictions for binary response variables (Bielby et al. 2010). We used the Random Forest package (Liaw and Wiener 2002) in R statistical software (<http://cran.r-project.org>) for all analyses.

For each of the classification trees, RF randomly selects two-thirds of the data to build a tree and then predicts the outcome of the remaining one-third of the data (out of bag data) using the tree. This process is repeated n times, where n is specified by the user. Trees in a “forest” are built from random subsets of the variables, resulting in low correlation among the trees in the forest. Two measures of error are reported by Random Forest. A confusion matrix quantifies the number of correctly predicted and incorrectly predicted cases in each class from the test data, while the out of bag (OOB) error is the percentage of samples misclassified from all classes in the test data. In addition, Random Forest reports a measure of variable importance generated through a permutation procedure. Briefly, the permutation procedure is as follows: the out of bag samples are classified using the trees and the number of correct classifications are summed. Each variable is randomly permuted and the data re-run through the tree and the correct classifications are summed. The number of correct classifications in the permuted OOB data are subtracted from the non-permuted OOB data and averaged across all trees in the “forest” (Breiman 2001). Variables with high values of importance have greater influence on classification. Interactions among variables will result in high importance values for all of the interacting variables (Lunetta et al. 2004). Partial plots of variable importance reveal the relationship between variables and the predicted classification.

We used RF to identify variables that are related to infection status by including all variables shown in Table 1 in a model with infection status (susceptible or not susceptible) as the outcome. Because we had relatively low sample sizes ($n = 107$), we built 10,000 trees in the “forest”. We determined that trying two variables at each split minimized overall model error (OOB error; data not shown). We used the `rflmpute` function to estimate missing values using proximities. Data were imputed using 10 iterations of 1,000 trees each.

Results

We compiled Bd infection data for 117 species in the continental United States. Of these 117 species, 63 were documented as infected by Bd at the time of our data collection (January 2009). We eliminated 10 species due to insufficient representation, all due to being the single representative or a part of a pair within a taxonomic family.

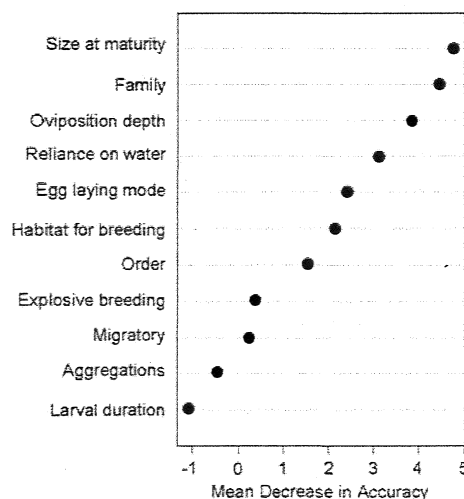
Our statistical approach revealed a relationship between documented infection at the species level and size at reproductive maturity, egg laying behavior and mode, taxonomic order, and relationship with water. Random Forest correctly classified infection susceptibility in 69% of out of bag cases (OOB error = 30.42 %) using the variables in Table 1. Species that tested positive for infection were more often accurately classified than species that tested negative for infection (Table 2). Random Forest revealed a strong relationship between infection status and size at reproductive maturity and family (Fig. 1). In addition, RF suggested a relationship between infection status and oviposition depth, reliance on water, egg laying mode, habitat used for breeding, and taxonomic order (Fig. 1).

Partial plots reveal the relationship between infection status and explanatory variables with high values of variable importance. Species that attain larger body size at maturity were more likely to be documented as susceptible to infection by Bd. In addition, individuals within the families Ranidae and Salamandridae were more likely to be documented as susceptible to infection by Bd, while Plethodontids were least likely to be documented as susceptible. Furthermore, species that lay eggs in aquatic habitats were more likely to be

Table 2 Confusion matrix from the Random Forest analysis of the association of United States amphibian species life history characteristics with *Batrachochytrium dendrobatidis* (Bd) infection status (positive or negative)

Infection class	Classified as		Class error rate
	Negative	Positive	
Negative	30	19	0.39
Positive	14	44	0.24

Fig. 1 Importance of life history variables for infection with *Batrachochytrium dendrobatidis* (Bd) for United States amphibians ($n = 107$ species), measured as the mean decrease in classification accuracy (MDA) when the life history variable is permuted in Random Forest. Higher values of MDA indicate a stronger relationship between the variable and classification of susceptibility to Bd infection at the species level



documented as susceptible to Bd. Of the species that lay eggs in aquatic habitats, generalists (those species that use both deep and shallow habitats for egg laying) were less likely to be susceptible than habitat specialists.

Several other variables had moderate influence on classification of susceptibility to infection. These variables fall into two general categories: biological characteristics of the species (egg laying mode and taxonomic order) and habitat use characteristics (reliance on water and type of aquatic habitat used for breeding). Species that lay eggs in large clusters were more likely to be documented as susceptible to Bd infection, compared with species that lay eggs singly or in small clusters. Salamanders were less likely to be documented as susceptible to Bd than frogs and toads. Species that are reliant on water for some portion of their life cycle were more likely to be documented as susceptible than strictly terrestrial species. Species that use standing water for breeding either opportunistically or obligatorily were more likely to be susceptible to infection, compared to species with terrestrial breeding and species that exclusively use streams and rivers for breeding.

Discussion

Documented infection status at the species-level was successfully classified by our model in .69% of cases, suggesting a relationship between species characteristics and susceptibility to infection by *Batrachochytrium dendrobatidis*. Further, our analysis identified characteristics that may predict infection status at the species level. Although it is clear from previous work that Bd infection in individuals is influenced by environmental factors such as temperature (e.g., Berger et al. 1998), our analyses suggest that some species are more likely to be infected than others due to differences in life history traits.

Size at maturity was the variable with the highest value of importance in our analysis. Amphibians that mature at a larger size may live longer than those that mature at smaller sizes (e.g., LeClair and Lauren 1996; Morrison et al. 2004). This could increase the potential for Bd infection if the risk of non-lethal infection increases with host longevity. Long-lived amphibian hosts have a longer period of time to acquire Bd and could also carry Bd for long periods. Older and larger hosts generally have more kinds of parasites/pathogens and a greater density of particular parasites/pathogens than younger, smaller hosts (Kuris et al. 1980). For example, comparison of parasite faunas of closely related taxa suggests that host species with large bodies have more ectoparasitic lice than do smaller species, presumably due to increased surface area available to support a higher density of lice (Kuris et al. 1980). The surface area of larger amphibians may increase the potential for Bd infection since more keratinized epidermal tissue is available to support Bd infection.

Taxonomic family was an important predictor of documented infection status. Current taxonomic relationships are in flux, particularly for some of the larger families of amphibians (e.g., Ranidae). However, as these taxonomic relationships were originally defined primarily using life-history characteristics rather than phylogenetic analysis, the importance of family as a predictor variable may capture other un-modeled aspects of life history. For example, Ranidae is likely polyphyletic but the species within Ranidae are superficially similar in body shape and habitat use. In addition, family may be correlated with several other variables in our study such as reliance on water and habitat used for breeding.

Characteristics of eggs and egg laying behavior were important in our classification. Species that oviposit in large clusters were more likely to be documented as susceptible to Bd infection, compared with species that lay eggs singly or in small clusters. It is likely

that the relationship we detected is due to a correlation between egg laying behaviors and other, unknown and un-modeled, life history characteristics. For example, those species that lay eggs in large clusters may spend more time in amplexus, resulting in longer contact times between individuals during mating. Bielby et al. (2008) found that among species suffering from declines of enigmatic origin, species with smaller clutch sizes were more at risk of rapid decline. This relationship between clutch size and population decline suggests that number of eggs laid may influence population persistence. Species ovipositing in large clusters may be less susceptible to enigmatic declines, but more susceptible to Bd infection. Of the species which lay eggs in aquatic habitats, generalists that use both deep and shallow habitats for egg laying were less likely to be documented as susceptible than habitat specialists that lay eggs in either deep or shallow water. It is possible that generalist species that oviposit in a variety of habitats and water depths exhibit plasticity in egg laying and other behaviors that allow individuals to avoid areas with sick individuals or where conditions are appropriate for Bd growth.

Aquatic species were more likely reported as susceptible to Bd infection than terrestrial species. In addition, caudates (salamanders) were less susceptible to Bd than anurans. In our analysis, 36% of the salamanders were terrestrial whereas only 1.7% of the anurans were terrestrial. Because Bd is an aquatic pathogen and the anurans in our analysis are more aquatic than salamanders, anurans were more likely to be documented as susceptible to Bd as a group. However, our analysis suggests that aquatic salamander species are more likely to be susceptible to Bd infection than terrestrial salamanders. Similarly, Bielby et al. (2008) found that aquatic anuran species were more likely to have suffered Bd-related population declines. Thus, the relationship we observed is likely driven in part by the difference in documented susceptibility between salamanders and anurans, but also by differences among frogs and toads that use aquatic habitats differently.

We also found that species that breed in ponds and lakes were more likely to be documented as susceptible compared to species that breed in streams. This was unexpected because reports from the tropics suggest that stream dwelling amphibians are especially susceptible to Bd (e.g., Woodhams and Alford 2005; Whiles et al. 2006). These contrasting results may reflect key differences in the dynamics of Bd infection and transmission between tropical and temperate regions. Furthermore, these results may yield clues to the biology of Bd and how it persists in different habitats and in different regions under different ecological regimes, especially in regard to differences in temperature, a key factor influencing the effects of Bd (e.g., Piotrowski et al. 2004; Woodhams et al. 2008).

Several predictions were not supported by our analysis. For example, sociality per se was not a good predictor of Bd incidence. Thus, variables relating to sociality were less important predictors of Bd infection than other variables measured. Intuitively, a highly infectious disease should be more easily transmittable when hosts aggregate. However, more social species may have more highly evolved disease defense mechanisms because of the intense selection pressure of disease (Altizer et al. 2003). Moreover, the important variable for disease transmission may not be sociality per se, but rather host density, or the presence of highly competent individuals or species. Most amphibian species appear to be potential hosts for Bd, suggesting that community composition or interspecific interactions may be important predictors of susceptibility. It is also possible that under certain biotic conditions, Bd is not very contagious.

Our analysis suggests that one avenue of future research should be identifying host life history characteristics that could predict species susceptibility to Bd. Thus, a similar analysis of amphibian species in tropical regions could highlight differences between Bd susceptibility and life history traits of tropical and temperate species. In addition, as

evidence for Bd-related declines is much stronger regions other than North America, similar analyses should be conducted on a region-by-region basis to identify potential correlates of infection outside of the United States. Correlation analyses such as the one presented here are most useful to generate testable hypotheses for experimental work. It is possible that some of the relationships we detected are not due to a relationship between Bd susceptibility and the specific characteristic, but rather to an unknown correlation between the characteristic included in the model and another species-level characteristic that is driving the observed relationship. However, the similarity between some of our results and those of Bielby et al. (2008) suggest important and understudied relationships between species characteristics and susceptibility to infection by Bd. Identifying species life-history characteristics in conjunction with predictive distribution modeling for Bd can highlight those species at high risk for infection by Bd (Ron 2005; Rödder et al. 2009). Species exhibiting life history characteristics highly correlated with positive Bd infection could be targeted for preventative monitoring and disease intervention programs.

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Abstract: Disease is often implicated as a factor in population declines of wildlife and plants. Understanding the characteristics that may predispose a species to infection by a particular pathogen can help direct conservation efforts. Recent declines in amphibian populations world-wide are a major conservation issue and may be caused in part by a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd). We used Random Forest, a machine learning approach, to identify species-level characteristics that may be related to susceptibility to Bd. Our results suggest that body size at maturity, aspects of egg laying behavior, taxonomic order, and reliance on water are good predictors of documented infection for species in the continental United States. These results suggest that, whereas local-scale environmental variables are important to the spread of Bd, species-level characteristics may also influence susceptibility to Bd. The relationships identified in this study suggest future experimental tests, and may target species for conservation efforts.

Response to Reviewers: Responses to reviewer comments:

Reviewer 1:

This reviewer suggested incorporating phylogenetic information in our analysis. As Random Forest does not allow for the inclusion of phylogenetic trees or relatedness, we used family as a proxy for phylogenetic information. We added text to the methods to explain this.

The reviewer asked us to compare our results to a Bielby paper published in 2010 in Biodiversity and Conservation. The paper mentioned is not particularly similar to the current manuscript. We suspect the reviewer meant Bielby et al. 2008 in Conservation Letters. Beyond the finding that aquatic species are more susceptible, the findings including only Bd+ species were not broadly similar, as Bielby et al. used different explanatory variables. However, we added text to the discussion about the Bielby paper to accommodate this request.

We clarified our meaning of "taxonomic order".

This reviewer did not find our supplemental table to be useful. We agree that it is large and unwieldy, so we have left it out of the current version.

Reviewer #2

We clarified our meaning of susceptibility and tried to emphasize in the results and discussion that we were working with documented susceptibility to infection. As we acknowledge in the methods, using the data on documented susceptibility to infection may bias our results, they are the best data available and are useful for identifying potential targets for more research.

This reviewer also suggested phylogenetic analysis, which we dealt with by including family as a proxy for relatedness in the analysis. This is the only method that can incorporate relatedness in a way that is usable in Random Forest. In addition, we cite a source in the manuscript explaining that decision tree methods work as well as phylogenetic analysis for species-level trends.