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Population of origin and environment interact to determine oomycete infections in spotted salamander populations

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Spatial variation in disease risk in wild populations can depend both on environmental and genetic factors. Understanding the various contributions of each factor requires experimental manipulation of both the environment and genetic composition of populations under natural field conditions. We first examined natural patterns of oomycete composition and infection in the eggs of 13 populations of the spotted salamander *Ambystoma maculatum*. We then performed a fully factorial field transplant of the eggs of six populations to separate the contributions from population of origin and the environment on oomycete resistance in spotted salamanders. Among wild ponds, we found strong variation in oomycete infections in spotted salamander populations and differences in the composition of oomycete communities. In transplant experiments, salamander populations differed in their resistance to oomycete infections via a significant interaction between population of origin and environment. However, not all populations were locally adapted to local conditions. One population was significantly adapted to its home environment, and another one was significantly maladapted. These population effects could originate from differential adaptation of salamander populations to local oomycete communities or environmental conditions that mediate resistance, local adaptation and maladaptation of oomycetes to hosts, or from maternal transmission. Accounting for both environment and population of origin will often be necessary to understand disease dynamics in wild populations.

Disease is a leading cause of extinctions throughout the world (Blaustein et al. 1994, Daszak et al. 2000, Harvell et al. 2002). Amphibians, in particular, are declining globally (Alford and Richards 1999, Houlahan et al. 2000), and disease is thought to play an important role in these declines (Blaustein and Kiesecker 2002, Johnson et al. 2002). Understanding the various mechanisms that determine disease intensity is necessary to understand disease dynamics under natural conditions and protect biological diversity.

Environmental factors often determine disease prevalence and intensity in the wild (Kiesecker and Blaustein 1995, Harvell et al. 2002, Pounds et al. 2006, Ostfeld 2009). However, the local adaptation of hosts or pathogens across the landscape also can influence disease dynamics (Antonovics et al. 2002, Thrall et al. 2002, Laine 2006, Hoeksema and Forde 2008). Differentiating between environmental and genetic contributions to phenotypic variation requires joint manipulation of both factors preferably in a natural setting in order to avoid potential laboratory artifacts (Kawecki and Ebert 2004). Transplant experiments are particularly useful in this regard because they allow the estimation of contributions to phenotypic variation from the genotype, environment, and their interaction and provide direct measurements of local adaptation by estimating fitness differences among populations in home versus foreign environments (Berven 1982, Kawecki and Ebert 2004, Hereford 2009). Despite these many advantages, biologists have seldom evaluated the local adaptation of disease resistance with transplant experiments. Of the 74 transplant studies compiled by Hereford (2009) on a wide range of traits, only two studies evaluated disease resistance. We found only one new study published since then that evaluated differences in the infection rates of oysters from different populations (Carnegie and Burreson 2011). A more robust understanding of the mechanisms determining ecological and evolutionary contributions to disease dynamics requires transplant experiments in diverse systems. Here, we test the relative contributions of population of origin and environmental factors to the resistance of spotted salamander *Ambystoma maculatum* eggs to infections from naturally occurring oomycetes (Saprolegniaceae, Oomycota) in a field transplant experiment.

The oomycete, or water mold, is one of the top three pathogens responsible for global amphibian declines (Blaustein and Kiesecker 2002). Oomycetes occur in freshwater habitats worldwide, where they infect frogs, salamanders, fish and invertebrates (Blaustein et al. 1994, Gomez-Mestre et al. 2006, van West 2006, Ruthig 2009). Despite the widespread threat to amphibians and other organisms throughout the world, no study to date has investigated the

relative contributions of population of origin and environment to oomycete infection risk under field conditions.

Here, we performed field surveys to determine natural infection intensities and prevalence. We evaluated if infection intensity was explained by environmental characteristics, including water temperature, canopy cover, pH, spotted salamander population size and spatial position in the landscape. We also evaluated if oomycete communities differed among ponds by conducting a DNA barcoding study of cryptic oomycete species cultured from natural pond water. We next conducted a fully factorial field transplant experiment where we collected egg masses from each of six populations and transplanted them back into each of the six ponds, including the home pond. We then estimated the relative contributions to infection resistance of population of origin, pond of development, and their interaction. We hypothesized that the simplest explanation for variation in oomycete field infection intensity would involve differences in pond environments that enhance or diminish oomycete populations or infection rates. Alternatively, spotted salamander and oomycete populations might adapt to each other or to the environmental context of disease transmission and resistance in each pond.

Material and methods

Natural history and study site

The spotted salamander is a large terrestrial salamander (up to 22 g) that inhabits the eastern USA and Canada. Each spring, adults migrate from upland habitat into temporary ponds to mate and to lay eggs. Females attach 1–3 egg masses 5–30 cm below the water surface to fallen branches, aquatic vegetation, or directly on pond substrate in shallow ponds. Each egg mass contains on average 81 eggs (n = 240) at our field site. A thick permeable protein-based jelly covers the eggs. Two egg morphs occur depending on the protein in the outer jelly: an opaque white morph and a clear morph (Ruth et al. 1993). Eggs develop into larvae in 4–5 weeks.

We examined the prevalence and intensity of oomycete egg infections in six populations of spotted salamanders that inhabit a set of temporary ponds situated on an isolated forested ridge on Totoket Mountain in Northford, CT (Fig. 1). Hereafter, we use disease prevalence to indicate the percent of egg masses with any infection in a population and disease intensity to indicate the mean percent of eggs infected in each egg mass in a population, including uninfected egg masses. During 12 years of field research on temporary pond amphibians in southern New England, we have observed a striking pattern of variability in oomycete infections among spotted salamander populations. In particular, one population from Lanoue Pond is almost always infected at high intensity, whereas other study populations are much less infected with oomycetes (Urban unpubl.).

Oomycetes are commonly called water molds, but they are unrelated to fungi. Oomycetes release free-swimming zoospores, which can search for hosts (van West 2006). The best known members of this group include some of the most devastating plant pathogens (e.g. the potato famine pathogen, *Phytophthora infestans*, Haas et al. 2009). Other oomycetes, such as members of the family Saprolegniaceae, attack healthy eggs, larvae and adults of amphibians, fish and invertebrates (van West 2006, Kiesecker and Blaustein 1995, Gomez-Mestre et al. 2006, Romansic et al. 2009, Ruthig 2009). Oomycete infections are identified by the cotton-like mycelia that radiate outward in circular patterns from infected hosts (Hatai and Hoshiai 1992, Blaustein et al. 1994, van West 2006). Traditionally, oomycete species were distinguished by the morphology of reproductive structures when viewed with light microscopy. However, more recent molecular phylogenetic studies indicate a history of misidentifications and taxonomic confusion based on oomycete morphology (Johnson et al. 2007, Robideau et al. 2011, Ault et al. 2012, Sandoval-Sierra et al. 2014).

Isolation and identification of oomycetes

We isolated oomycetes in the wild in six ponds in 2011 using standard methods (Laskin and Lechevalier 1978, Kiesecker and Blaustein 1995), which involved placing three 80-ml vials of sterilized hemp seeds in separate locations on the bottom of each study pond during the peak of amphibian egg laying. We collected the vials after one week and placed their contents on sterilized corn meal agar in petri dishes in an incubator set to 22°C. Once we observed the growth of a cotton-like mycelium indicative of oomycetes, we used sterilized forceps to collect the mycelium, washed it with de-ionized water, and froze the sample at −20°C for genetic analysis.

We used DNA barcoding to identify the oomycetes, employing data from the nuclear ribosomal internal transcribed spacer (ITS) 2 region (methods after Fucikova et al. 2013). Several samples were heavily infected with ascomycete fungal pathogens, and the ascomycete ITS amplified along with the target oomycete ITS. Because the ascomycete ITS is ca 200 bp shorter than the oomycete ITS, we separated the two fragments using gel electrophoresis, and isolated the target fragment using band-excision and purification. The PCR product was then cycle-sequenced and the resulting fragments were assembled using Geneious ver. R6 (<www.geneious.com>). We compared the new ITS sequences to publicly available data using the NCBI BLAST tool to confirm their similarity to other published oomycete sequences. Based on the BLAST results, we constructed an alignment containing relevant GenBank sequences, mainly those published in Robideau et al. (2011). The sequences were aligned using Clustal W (Larkin et al. 2007), the alignment was refined manually, and regions of dubious homology were excluded from the analysis. The GTR+ I+ G model of evolution was selected by the software jModeltest2 under the Akaike information criterion (Darriba et al. 2012) and implemented in Garli (Zwickl 2006). In addition to estimating the maximum likelihood tree in Garli, we estimated the statistical support for individual nodes in the tree using nonparametric bootstrapping (100 pseudoreplicates).

Wild disease prevalence and intensity

In 2010 we conducted a disease survey in six ponds used for the transplant experiment to assess wild disease intensity and prevalence. Four weeks after breeding on 21 April 2010, we collected ten wild egg masses in the immediate region

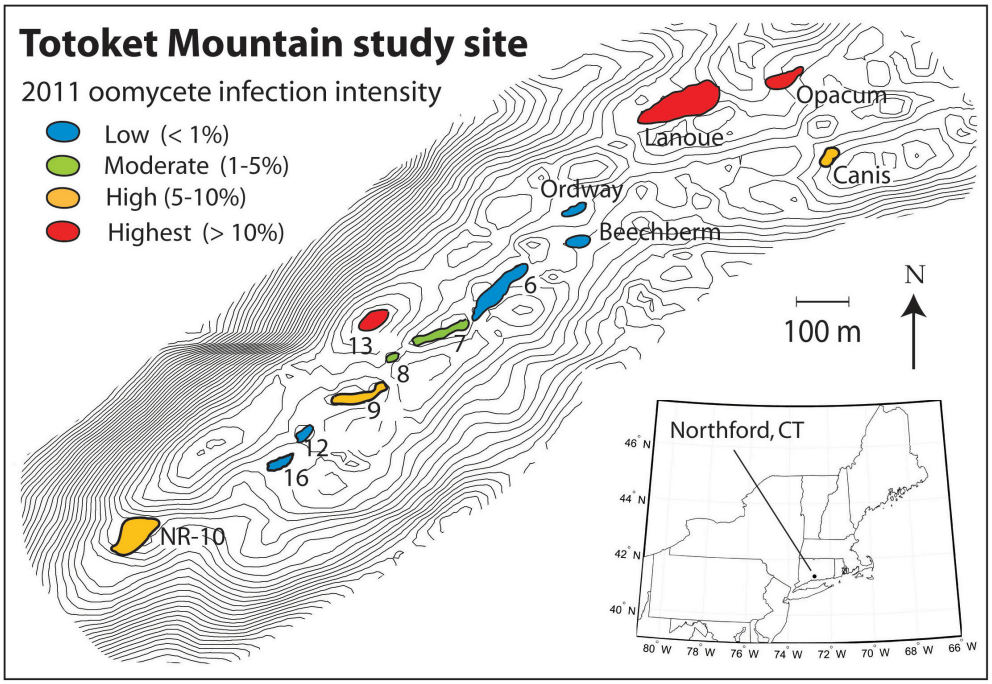

Figure 1. Map of study populations on Totoket Mountain, Northford, CT, USA. Shapes represent approximate pond outlines at maximum water depth. We color-coded ponds based on mean infection intensity in 2011. Lines indicate 2-m contour levels.

surrounding the location of the transplant enclosures. This date corresponds to the same day that transplanted eggs were dissected and evaluated for disease. We conducted a more expansive survey in 2011 for all 13 ponds at the study site in order to evaluate if pond characteristics were associated with infection intensity. In the 2011 survey, we sampled four egg masses four weeks after breeding at each of 1–5 random point locations per pond, with the number of point locations set proportional to pond area by drawing random points from a gridded pond map. In both surveys, each egg mass was dissected with a scalpel into 10-egg sections to allow us to count the number of diseased and healthy eggs. Eggs were characterized as diseased if we observed cotton-like mycelium characteristic of oomycetes (Hatai and Hoshiai 1992, Blaustein et al. 1994, van West 2006). To see if egg morph and egg predation affected field infection risk, we also recorded if egg masses were opaque or clear morphs and if any eggs had been preyed upon by the main egg predator, the caddisfly *Ptilostomis postica* (Rowe et al. 1994).

Environmental characteristics

At each of the 13 study ponds, we collected information on four environmental characteristics in 2011 that we predicted, based on previous literature, would influence oomycete infections: water temperature and pH, canopy cover with its shading effect on ultraviolet radiation, and host population abundance. Past research shows that oomycete growth increases with higher temperatures (Gomez-Mestre et al. 2006, Ruthig 2006). We measured hourly water temperature at a 10 cm depth (the average depth of egg masses) in each pond in 2011 at the pond's maximum depth until egg hatching with loggers. Oomycete composition differs depending on wetland pH (Voronin 2008). We averaged pH at five points in each pond taken in April, May and June with a with pH probe. Ultraviolet light interacts with other factors to promote oomycete infections (Kiesecker and Blaustein 1995). We measured canopy cover as an indirect measure of UV exposure with a spherical crown densiometer and used the average of two observers' measurements at each cardinal direction centered at each egg sampling point and averaged across all survey points to obtain a mean canopy cover for each pond. We also predicted that host egg mass abundance might increase infection intensity in ponds by supporting larger parasite abundances (Green 1999). Egg mass abundances were determined from annual censuses conducted in 2004–2006 and 2009–2010, except for pond NR-10 for which data were only available for 2009 and 2010. We also evaluated if disease intensity was spatially autocorrelated across the landscape using Moran's spatial eigenvector maps to decompose the matrix of distances among ponds at multiple scales (Borcard et al. 2004, Dray et al. 2006, Richardson and Urban 2013). We first selected spatial eigenvectors out of the 13 generated that were retained in the minimum-AIC model for use in the regression with environmental variables. Only the second spatial eigenvector was selected in this way and entered into the dataset of environmental variables.

Transplant experiment

Egg masses were collected within 0–2 days following oviposition based on the frequency of sampling and visual assessment of egg age. Wearing sterile gloves, we collected eggs and transported them to ponds in individual 19-l containers. Eggs from both resident and foreign populations were all treated the same way: removed from native ponds, placed in containers, transported around the study site, and then placed back into rearing containers in the native pond.

All egg masses were visually inspected at the start of the experiment, and any egg masses with signs of disease were not used.

We reared six egg masses from each of six populations in each of the six source ponds (36 egg masses per pond, 216 total egg masses in experiment). We placed one egg mass from each population, including the resident population, into six floating enclosures in each pond. Enclosures were arranged together at the deepest point in each pond and had open tops and screened side openings (1.5 mm) to allow ambient light and water exchange with the environment but exclude egg predators. Egg masses were placed at a mean depth of 10 cm which mimics egg depths in nature. We ended the experiment on 21 April 2010, once eggs neared hatching stage. Egg masses were then dissected and the eggs evaluated for disease in the same manner as for the wild eggs surveys. Surviving embryos were euthanized in MS-222.

Statistical analyses

All statistics were performed in R (ver. 2.10). Differences among wild egg infection rates in 2010 were evaluated in a generalized linear model with quasibinomial errors after detecting overdispersion (Crawley 2007). Regressions between wild infection intensity and environmental variables were estimated using generalized linear mixed-effect models (R: glmer) with binomial errors and with pond identity and point location entered as random effects. We applied a model-averaging approach where we estimated the mean standardized regression coefficients for each factor weighted by the Akaike information criterion for small samples (AIC_c) for the subset of models within four units of the best model (Burnham and Anderson 2002) and found 95% confidence intervals using the model.avg function in the R MuMIn library (ver. 2.15.2).

We used generalized linear mixed-effects models with binomial errors to evaluate infection intensity in the transplant experiment. Fixed factors included transplant pond and population of origin. Random factors included field container nested within population, position within the field container, and an individual-level term that accounts for detected overdispersion (Warton and Hui 2011). We tested each random effect with a likelihood ratio test (Pinheiro and Bates 2000) and simplified the model when random factors were not significant. An analysis of categorical significance is not yet available in glmer models; instead we used log-likelihood ratio tests on maximum likelihood models. We then evaluated individual z-scores and significance values for each coefficient after finding a significant overall effect and using the Holm's procedure to correct for multiple tests.

We first tested for local adaptation across all populations via the contrast between egg survival in sympatric versus allopatric conditions (Blanquart et al. 2013). We used log-ratio tests to compare mixed effects models with and without the sympatric–allopatric comparison with an individual-level term to account for overdispersion of binomial egg survival data. We also evaluated local adaptation as the fitness of the native population in its native pond (W_n) minus the mean fitness of all foreign population grown in the native pond (W_f), divided by the mean fitness of all populations raised in the native pond (Kawecki and Ebert 2004, Hereford 2009):

$$\frac{W_n - \overline{W_f}}{\overline{W}}$$

We measured fitness as proportional egg survival. Positive values indicate that the native population has higher fitness in its native setting than foreign populations whereas negative values indicate that resident populations are maladapted. We bootstrapped values for each population 10 000 times to construct bias and skew-corrected 95th-percentile confidence intervals among mean adaptation values. We then applied the stepwise Holm's procedure to correct for multiple tests.

Data accessibility

Oomycete sequences have been deposited with GenBank with accession numbers: KC758888-KC758895. Field and experimental data have been deposited in Dryad.

Results

Oomycete communities

Oomycetes were isolated from the six study ponds, but we only could obtain usable ITS sequence data from five of the six (Table 1). Three ponds are represented by two independent samples. Eight oomycete ITS sequences were determined and are available in GenBank under accession numbers KC758888–KC758895. Phylogenetic analysis of the ITS data revealed four independent oomycete lineages in the sampled ponds (Fig. 2), most closely corresponding to *Saprolegnia torulosa*, *S. diclina*, *S. turfosa* and *Achlya papillosa*. According to Robideau et al. (2011), *S. turfosa* most likely belongs to the genus *Achlya*. *Saprolegnia torulosa* was prevalent, occurring in three of five ponds, and *S. diclina* was found in two ponds.

Wild infection prevalence and intensity

Across the 13 ponds at the study site in the 2011 survey, the mean infection intensity (mean percent eggs infected per egg mass) averaged 7% (Fig. 3A; range: 0–24%; Table 1), and infection prevalence (percent egg masses infected) averaged 45% (Fig. 3B; range: 0–88%). Infection intensities of wild eggs varied significantly among the 13 ponds (likelihood ratio $(LR)_{df=12} = 57.1$, $p < 0.001$) because the Opacum, B-13 and Lanoue populations were more infected than other populations (Fig. 3A; $p \leq 0.001$). Infection intensities of wild eggs surrounding transplant experiments in 2010 also varied significantly among populations mostly because Lanoue had a higher infection intensity (58%; Fig. 4A; $t_{54} = 5.61$; adjusted $p < 0.001$) than other populations (range: 1–7%). Disease prevalence varied significantly among ponds in 2011 (Fig. 3B; $LR_1 = 13.3$, $p < 0.001$) and 2010 (Fig. 4D; $LR_1 = 37$, $p = 0.053$), although this result was marginal.

Environmental and egg mass characteristics

Disease intensity was significantly higher in ponds with more canopy cover (Fig. 5; $z = 2.84$; $p = 0.004$) and larger spotted

Table 1. Oomycete communities, environmental characteristics and disease intensities of study ponds.

Pond	Oomycetes identified by DNA barcoding	Mean water temperature (C)	Percent canopy cover ± SD	Mean egg mass abundances ± SD*	Field pH	Disease intensity (%) in 2010	Disease intensity (%) in 2011
NR-10	none†	9.8	76.7 ± 7.5	1128 ± 197	5.64	1.2	8.8
B-9	*Saprolegnia torulosa*	8.2	83.3 ± 5.7	150 ± 43	6.48	1.4	5.8
B-6	*Saprolegnia torulosa*	9.5	82.0 ± 8.8	153 ± 72	6.32	2.2	0.1
B-7	*Saprolegnia diclina*	8.4	87.0 ± 0.5	192 ± 36	6.54	3.1	1.1
B-13	*Saprolegnia diclina* *Saprolegnia torulosa*	9.2	90.1 ± 2.5	605 ± 316	6.25	7.3	16.8
Lanoue	*Achlya papillosa* *Saprolegnia [Achlya] turfosa*	8.8	88.7 ± 5.2	670 ± 154	5.92	58.5	16.2

Note. Ponds are ordered by increasing infection intensity in 2010. *Egg mass abundances are from censuses conducted in 2004–2006 and 2009–2010, except for NR-10 for which data was only available for 2009–2010. †Oomycetes were identified visually but DNA barcoding was unsuccessful.

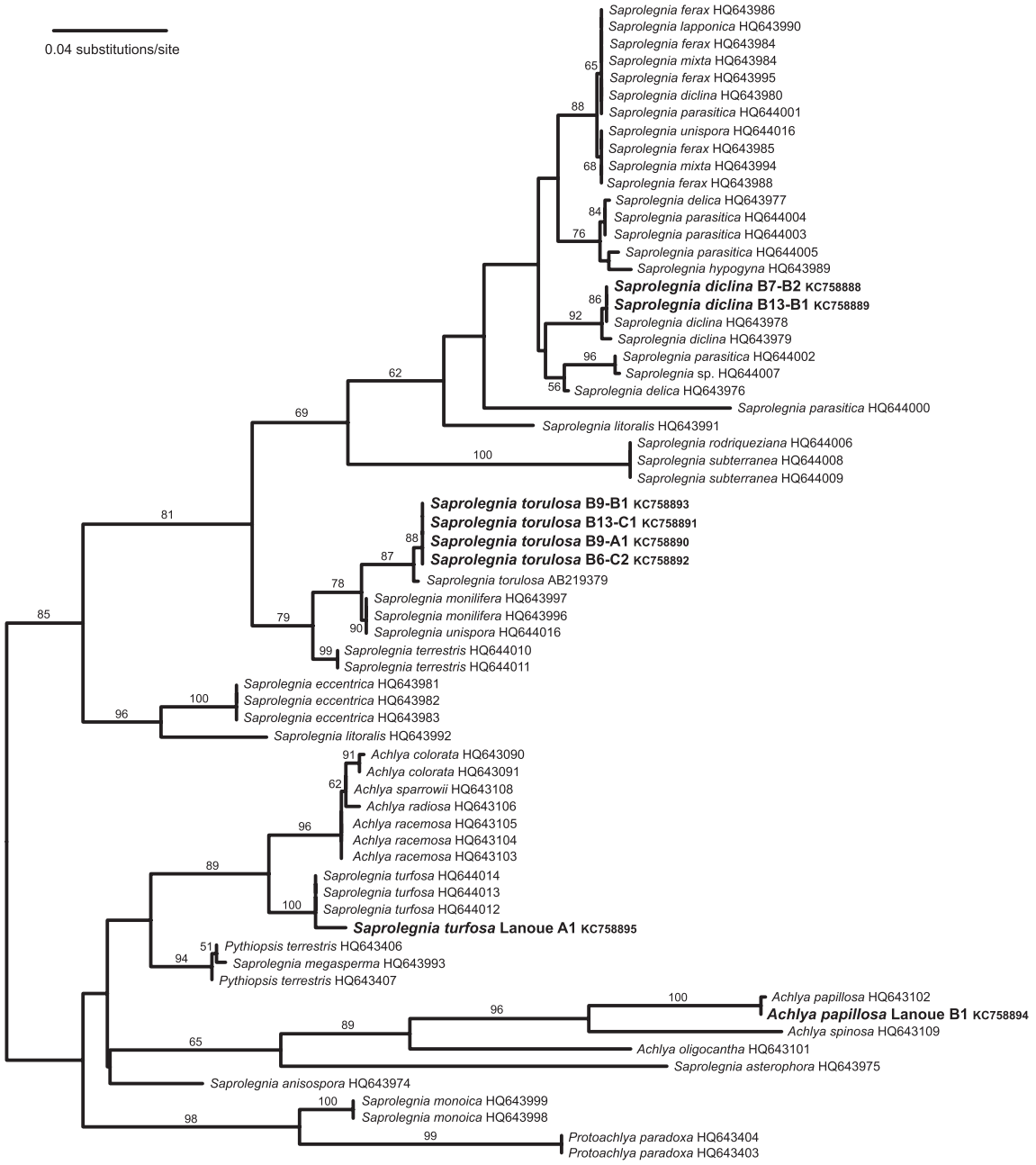

Figure 2. Maximum likelihood tree based on an analysis of the ITS2 data set (662 characters) with bootstrap support values obtained from 100 pseudoreplicates indicating node support. Scale bar represents the number of expected substitutions per site. Tree is unrooted, but oriented using information from Robideau et al. (2011). Names in bold font indicate samples cultured and sequenced in this study. Taxon labels include the pond name followed by the specific sample and GenBank accession numbers.

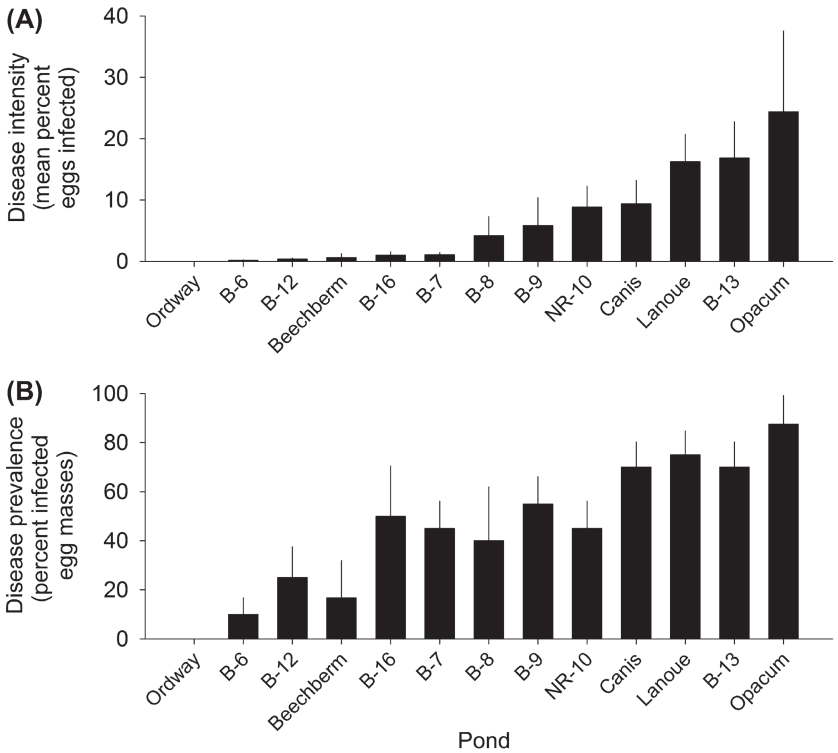

Figure 3. (A) Mean disease infection intensities (percent diseased eggs per mass) and (B) infection prevalence (proportion of egg masses infected) in 2011 among 13 study ponds, arranged in order of increasing infection intensity. Error bars indicate ± 1 SEM.

salamander populations ($z = 2.71$; $p = 0.007$). Higher temperatures and pH were not significantly related to disease intensity ($p > 0.5$). Neither egg morph nor egg predation significantly explained disease intensity in wild eggs ($p > 0.5$). Disease intensity did vary across the landscape ($z = 3.89$; $p < 0.001$). The selected spatial eigenvector indicated a tendency for higher disease intensity in the northeastern and southwestern corners of the study region (Fig. 1: Lanoue, Opacum, Canis, NR-10 and B-13).

Transplant experiment

Population, environment, and the population by environment interaction significantly influenced oomycete infection intensity (Fig. 4B–C; Table 2; $LR_5 = 23.1$, $p \leq 0.001$; $LR_5 = 21.7$, $p \leq 0.001$; $LR_{25} = 41.6$, $p = 0.020$; respectively). Averaged across all environments, eggs from Lanoue were significantly more infected than eggs from other populations ($z = 2.61$; $p = 0.009$). Transplanted populations tended to have lower infection intensities in B-6 and B-7 ($z = -2.73$, $p = 0.006$; $z = -2.67$, $p = 0.008$). Container nested within pond was the only significant random effect ($LR_{21} = 40.72$; $p = 0.006$). Consistent with field results, egg mass morph did not significantly affect disease intensity ($LR_1 = 1.7$; $p = 0.198$). Disease prevalence was high (mean = 67%) and did not vary significantly by population, environment, or their interaction ($p > 0.1$).

Evidence of local adaptation and maladaptation

We did not find an overall signal of local adaptation to local pond conditions across the metapopulation using a contrast between egg survival under sympatric and allopatric conditions ($LR_1 = 1.26$; $p = 0.262$). One salamander population (B-6) showed evidence of adaptation to local conditions because its mean fitness was greater in its home environment than that of foreign populations, and another population (NR-10) was significantly maladapted to its local conditions (Fig. 6, 7). The other four populations (B-9, B-7, B-13 and Lanoue) were not significantly adapted or maladapted to their local environments (Fig. 6, 7).

Discussion

Oomycete infections are a leading cause of declines in amphibians and other aquatic organisms worldwide (Blaustein et al. 1994, Blaustein and Kiesecker 2002). Host populations vary in their infection rates, and most studies have focused on how ecological factors shape this variation. However, recent research suggests a significant evolutionary component. Oomycete infections in moor frogs *Rana arvalis* were affected by a significant population by temperature interaction, an effect of family, and no contribution from maternal effects (Sagvik et al. 2008). This research suggests that some embryonic amphibians possess immune systems that differentially resist oomycete infections, and that this immune function is genetically determined (Sagvik et al. 2008). If oomycete infections are generally determined by interactions between genetic and environmental factors, then natural variation in infection rates might originate from more complex causes than usually suggested.

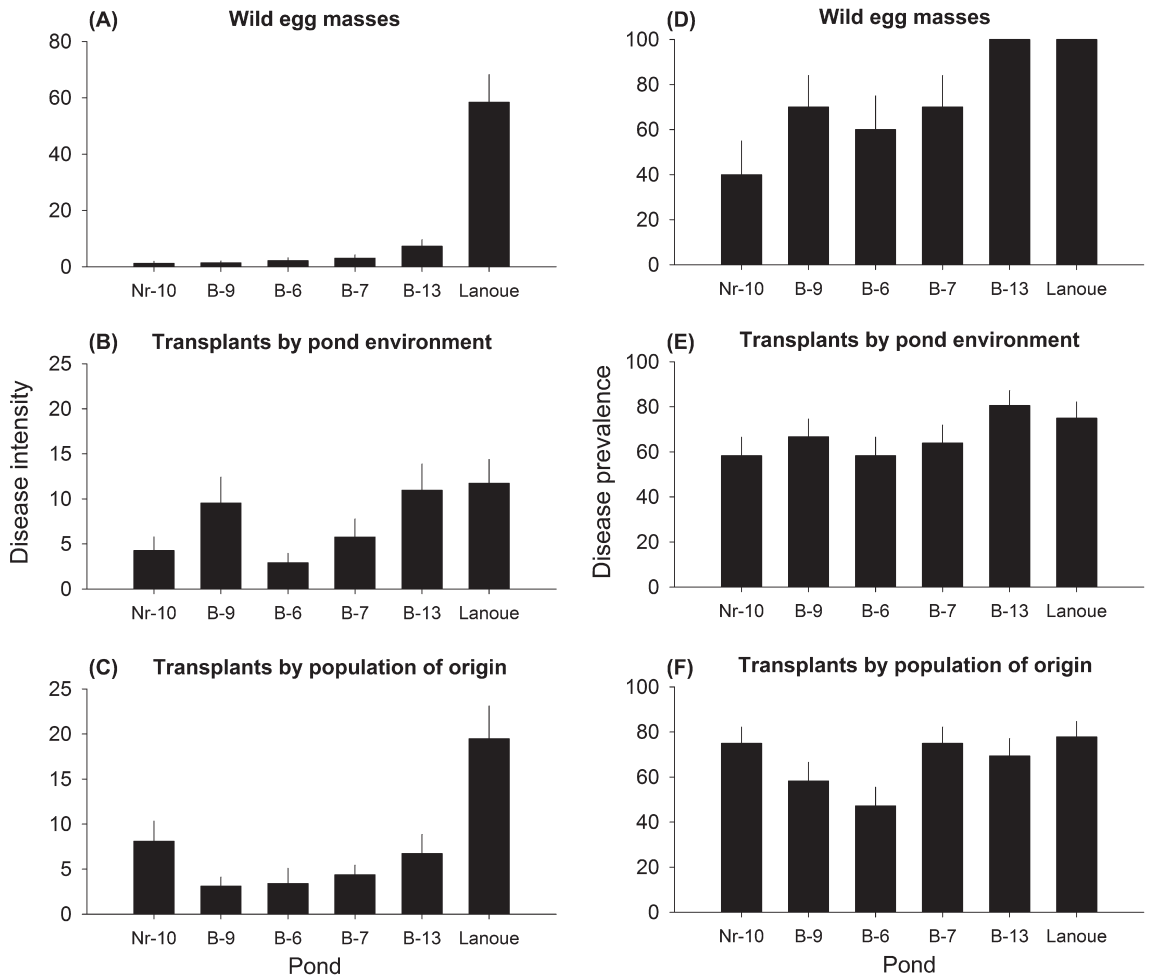

Figure 4. Mean disease infection intensities (A–C) and disease prevalence (D–F) in wild egg masses surrounding transplant experiments in 2010 (A, D), transplanted egg masses by pond environment (B, E), and transplanted egg masses by pond of origin (C, F). Error bars indicate ± 1 SEM.

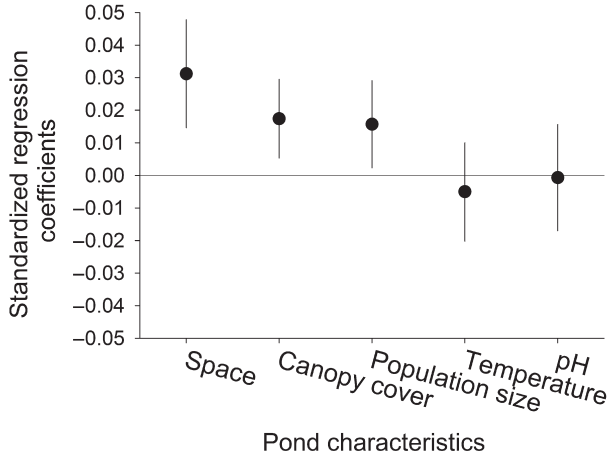

Figure 5. Model-averaged standardized regression coefficients of correlates of wild infection intensity. The relative size of the coefficient indicates its effect on infection intensity; correlates are organized in order of decreasing effect size. Error bars represent the 95% confidence interval of the model-averaged coefficients for models with $\Delta AIC_c < 4$, weighted by their AIC_c- values. Overlap between error bars and zero indicates non-significance.

Wild infection intensities and potential causes

Wild populations varied in their oomycete infections in this study, often by orders of magnitude. Infection intensities of egg masses ranged from 0–58% among ponds, with a mean of 12% in 2010 and 7% in 2011. The mean prevalence of oomycete infections was 45% with a range of 0 to 88%. This disease prevalence is high relative to surveys performed at other sites within northeastern USA. A study in Massachusetts found no field infections (Gomez-Mestre et al. 2006), and a study in New York found that 18% of egg masses

Table 2. Analysis of deviance for egg infection intensity based on population, environment and their interaction.

Factor	Deviance	F	DF	p
Population	848.8	11.4	5,210	< 0.001
Environment	447.2	6.0	5,205	< 0.001
Population × Environment	843.4	2.3	25,180	0.001

Note. We detected strong overdispersion in the initial binomial model (12.2 times), so we used a quasi-binomial model to adjust for this over-dispersion.

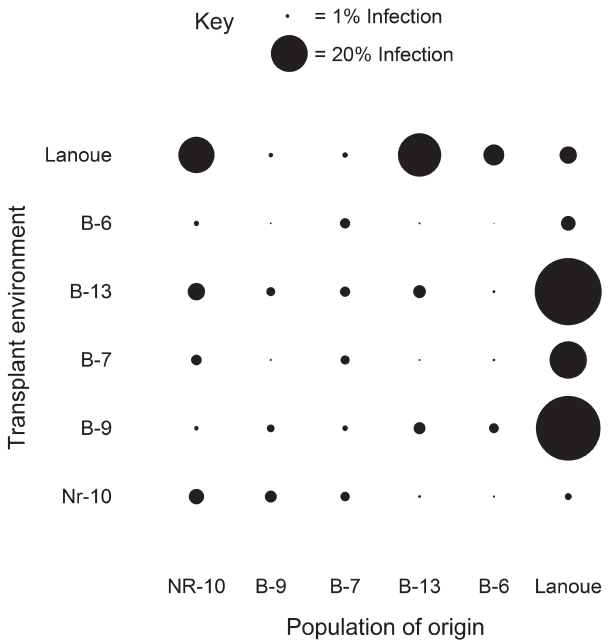

Figure 6. Relative infection intensity indicated by circle area, organized by transplant environment (rows) and population of origin (columns). If populations are locally adapted, then we expect that the lowest infection will occur along the diagonal where the home population is raised in its home pond. The circles in the legend at the top show a 1% and 20% infection intensity for comparison against real data.

were infected (Karraker and Ruthig 2009). One important insight to emerge from this research is that oomycete disease prevalence and intensity varies substantially both among and within regions. Oomycetes are likely to differ in abundance, taxonomic composition, and virulence across space at the same time as salamander populations differ in their susceptibility. Understanding this spatial variation will be critical to understanding the mechanisms underlying disease dynamics in these populations.

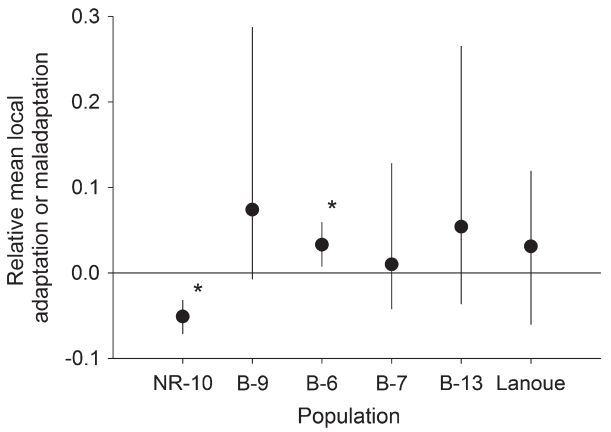

Figure 7. Mean relative local adaptation or maladaptation (± 95% bootstrapped confidence intervals) by population measured as the mean difference between the survival of the resident population versus survival of each of the foreign populations in the home pond. A locally adapted population would be expected to have a positive value – that is higher survival in its home environment. A maladapted population would have negative values. Asterisks denote values significantly different from zero after correcting for multiple tests using the Holm–Bonferroni stepwise method.

We screened ponds for oomycetes using DNA barcoding techniques and found different oomycete assemblages inhabiting different ponds. We found *Achlya papillosa* and *Saprolegnia turfosa*, a taxon that was shown to cluster with *Achlya* (Robideau et al. 2011), in Lanoue where we also recorded some of the highest oomycete infection intensities. *Achlya* caused the highest mortality in (~ 80%) in bullfrog *Lithobates catesbeianus* eggs in Virginia among multiple tested oomycetes (Ruthig and Provost-Javier 2012) and also infected western toad *Bufo boreas* eggs in Washington (Ault et al. 2012). *Achlya papillosa* has been recovered from infected trout *Salmo trutta* eggs in Poland (Czeczuga et al. 2005). Other ponds contained *S. diclina* and *S. torulosa*. *Saprolegnia diclina* infects natterjack toad *Bufo calamita* eggs in Spain, where infections killed up to 84% of tadpoles in 11 days (Fernandez-Beneitez et al. 2008). *Saprolegnia torulosa* infects western toad eggs in Washington state (Ault et al. 2012). We find all of these oomycete species inhabiting ponds within a limited geographic extent of 2 km^2.

On a cautionary note, the taxa and strains of oomycetes collected from one species might not be pathogenic to another species (Ault et al. 2012, Ruthig and Provost-Javier 2012). Also, some oomycetes act as saprobes rather than pathogens in nature (Gomez-Mestre et al. 2006, Ruthig 2008). In our case we used a standard technique for sampling oomycetes that involved collection and propagation on hemp seeds (Laskin and Lechevalier 1978, Kiesecker and Blaustein 1995). Common pathogens of amphibian eggs have been shown to grow readily on hemp seeds, including *Saprolegnia diclina*, *S. ferax* and *Achlya* spp. (Laskin and Lechevalier 1978, Kiesecker et al. 2001, Gomez-Mestre et al. 2006, Touchon et al. 2006, Fernandez-Beneitez et al. 2008, Romansic et al. 2009, Perotti et al. 2013). Also, we found that oomycetes cultured from hemp seeds from natural ponds are pathogenic in a lab experiment. We exposed eggs to hemp seeds with and without field-collected oomycetes in the lab. The oomycete infected hemp seeds caused significantly greater mortality in eggs as compared to eggs exposed to sterile hemp seeds (Supplementary material Appendix 1 Fig. A1), and we observed oomycete infections in dead eggs. However, future work should compare the oomycetes collected using standard media like hemp seeds and those found on amphibian eggs to ensure that collection methods accurately reflect the full community of pathogenic oomycetes in nature.

Canopy cover, salamander population size, and space were significantly associated with oomycete infection intensity. Ponds with the highest canopy cover and largest salamander populations had the highest oomycete infection intensities, including the highly infected Lanoue pond, which is shaded by trees and dense shrubs and hosts a large spotted salamander population. Oomycetes at our field site might be particularly sensitive to UV light. This result contradicts work showing a synergistic interaction between UV-B damage and oomycete infection in frog eggs (Kiesecker and Blaustein 1995). However, UV-B exposure increases with elevation, and our study ponds range from 155–180 m above sea level whereas the ponds in the aforementioned study were above 1000 m. Oomycete species or strains at high altitudes might

be better adapted to UV-B exposure than those found at our low-altitude site. Other environmental factors associated with high canopy cover such as temperature and permanence (Urban 2004) also could play a role. Future experiments that manipulate UV light will be necessary to test this hypothesis. We found spatial autocorrelation of oomycete infection intensity across the landscape, suggesting hot spots of disease in the northeastern and southwestern parts of our study site. These infection hot spots could be associated with unmeasured environmental variation or the localized dispersion of disease agents.

Transplant experiment

Infection intensities were characterized by a significant population by environment interaction. Populations raised in the same environment varied by orders of magnitude in their infection risk although they experienced the same environmental conditions and were exposed to the same oomycetes (Fig. 6). For instance, only 1% of B-6 eggs became infected in B-13 while 38% of Lanoue eggs became infected under the same conditions. As expected, Lanoue was the most infected population. However, the highest infection intensities for Lanoue were not in its native pond, but in foreign ponds. Together, these results indicate that salamander populations are differentially resistant to oomycete infections or environmental conditions.

This population by environment interaction is consistent with a mosaic of local adaptation and maladaptation across the landscape. Whether using the more general criteria of a significant sympatric versus allopatric difference in fitness or using the more specific home versus foreign contrast, we found variable degrees of local adaptation. One salamander population was significantly adapted to local conditions whereas another population was significantly maladapted. Other populations were not significantly adapted or maladapted. One explanation involves a mosaic of evolutionary outcomes, with some salamander populations ahead in a coevolutionary race, and other populations falling behind the rapidly evolving oomycete populations (Lively 1999, Thompson 2005).

Local adaptation of resistance to oomycete communities

Could some salamander populations be adapted to local oomycete communities? Our sampling of ponds revealed that ponds differed in the composition and diversity of oomycete species. For instance, Lanoue had an oomycete community dominated by *Achlya*, which could potentially explain the high disease intensity there and antagonistic selection across ponds. Ponds might also differ in the virulence of oomycete strains. *Saprolegnia ferax* cultivars from *Bufo boreas* populations with high infection rates caused higher infections in the lab than cultivars from populations with lower infection rates, suggesting differences in the virulence of *Saprolegnia* populations (Kiesecker et al. 2001). Ponds also differed in environmental characteristics, any of which might mediate infection transmission and resistance. For instance, if spotted salamanders have higher fitness under local environmental conditions, they might better resist oomycete infections under these specific conditions. Oomycete populations might vary genetically in their ability to infect locally adapted spotted salamander eggs, setting up a mosaic of evolutionary and coevolutionary interactions (Laine 2006, Thompson 2005). We observed both significant local adaptation (B-6) and local maladaptation (NR-10), which is consistent with such a pattern. Alternatively, egg mortality patterns could indicate variation in fitness related to factors unrelated to pathogens. However, natural strains of oomycetes increase mortality under common garden conditions (Supplementary material Appendix 1 Fig. A1), and many of the same species that we identified in ponds have been shown to be pathogenic in other amphibian populations, including *Achlya* spp., *S. torulosa*, and *S. diclina* (Fernandez-Beneitez et al. 2008, Ault et al. 2012, Ruthig and Provost-Javier 2012).

Any of these potential environmental differences could select for local embryonic resistance to infection. This antagonistic selection might lead to locally adapted salamander populations, provided sufficient additive genetic variation exists for resistance (Sagvik et al. 2008) and gene flow is not so great as to swamp local adaptation. In another study, oomycete infections in the moor frog *Rana arvalis* were affected by a significant population by temperature interaction (Sagvik et al. 2008), suggesting that oomycete infections might commonly be determined by genotype by environment interactions, and temperature is an important mediating factor. The spotted salamander populations in the current study have become locally adapted to selection from two predator species (Urban 2007, 2010). Although ponds are located close to each other (often within 100 m), neutral genetic analyses indicates limited gene flow between ponds that differ in selection regime (Richardson and Urban 2013). Thus, strong selection barriers likely exist to decrease effective gene flow between ponds differing in selection and allow for local adaptation to predators and potentially oomycetes as well. Future work will need to separate the effects of different pond environments and oomycete species and genotypes from the environmental term to isolate their individual effects. Exposing each salamander population to separately cultured oomycete species in a common garden would provide an important next step.

Non-genetic influences

We cannot exclude non-genetic influences on the population contribution. In our system, oomycetes could enter the eggs at any point from when the females pick up spermatophores on pond bottoms, fertilize eggs internally, to the hours before we detected freshly laid eggs and moved them into transplant ponds. If infection occurred before relocation, then we would have expected a significant population effect and not the population by environment interaction that we observed. Also, we would expect the same or higher rates of infection in eggs raised in home ponds, but this was not the case in most ponds (Fig. 6). Another potentially important factor is maternal transmission of disease resistance. One study found no significant maternal effects on oomycete resistance (Sagvik et al. 2008). However, other studies suggest that maternal environment can influence the disease resistance of offspring (Grindstaff et al. 2003, Mitchell and Read 2005). Paired mating designs and decadal multi-generational captive

colonies would be necessary to estimate non-genetic contributions in this late-maturing and long-lived salamander.

Conclusions

We found evidence for a significant population by environment interaction in the resistance of spotted salamander eggs to oomycete infection. The pattern suggests either maternal transmission of oomycetes or local adaptation of spotted salamanders to local oomycete communities or environmental conditions. If genetically determined, oomycete resistance would add to the accumulating evidence that spotted salamanders face a mosaic of selection intensities from both predators and disease agents. More generally, if such population by environment interactions commonly occur in host–pathogen dynamics, then standard models of disease transmission will need to incorporate these more complicated eco-evolutionary dynamics in order to provide accurate predictions.

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Supplementary material (available as Appendix oik.01598 at <www.oikosjournal.org/readers/appendix>). Appendix 1

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