ECOLOGICAL AND EVOLUTIONARY CONSEQUENCES OF POPULATION CONNECTIVITY IN AN AMPHIBIAN WITH LOCAL ADAPTATION

by

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DEDICATION

To Avery

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ABSTRACT

Worldwide habitat loss and fragmentation remain serious threats to population persistence, as reduced dispersal affects population dynamics and reduced gene flow impacts genetic integrity of populations. While increased isolation of populations and reduction in genetic diversity can negatively impact individual and population fitness, increased isolation may also be beneficial as it can allow populations to reach their adaptive optima. Here, I investigate the causes and consequences of population connectivity using an integrative approach, combining molecular tools, experimental data, field surveys, and geographic information systems data, in the wood frog, *Rana sylvatica*. This species occupies two ecologically divergent habitats (open and closed canopy ponds) over very small spatial scales, where gene flow is likely to play an important role in the divergence of populations. I first assessed the effects of habitat fragmentation on population connectivity of wood frogs by comparing historical and current landscape structure to contemporary genetic structure across 51 populations. Wood frog populations showed rapid neutral genetic divergence following habitat fragmentation. Additionally, I assessed how gene flow affects the local adaptation of populations, using a common-garden experiment to compare trait differences among 16 populations from open- and closed-canopy ponds across a gradient of pond isolation. Overall, wood frog larvae showed similar levels of divergence among open- and closed-canopy ponds at both low and high levels of population connectivity, suggesting that selection is strong enough that divergence can occur despite gene flow. To determine the consequences of the combined effects of selection and gene flow on fitness, I compared population-level fitness correlates across populations ranging from outbred to inbred. Populations with low levels of inbreeding had higher levels of larval survivorship in a common garden experiment and larger population sizes compared to more inbred and outbred populations. The reduced survivorship of outbred populations with the pattern of divergence with gene flow points to disruption of local adaptation as a mechanism for outbreeding depression. Together, these results elucidate the fine balance between strong divergent selection and population connectivity. I discuss the implications for ecology and evolutionary biology, provide suggestions for conservation and land management, and outline areas for future research.

CHAPTER 1

INTRODUCTION

Understanding the consequences of population connectivity has become an increasingly important topic in ecology and evolutionary biology. With the introduction of island biogeography (MacArthur & Wilson 1963) and metapopulation theory in the 1960s (Levins 1969), ecologists began to shift their focus to larger scale processes, studying the consequences of dispersal and gene flow for population regulation (Hanski 1998) and metacommunity processes (Leibold et al. 2004). In addition, rapid human-induced habitat loss and fragmentation have created an urgent need for understanding the role of interpopulation connectivity, making it a central focus of conservation biology (Fazey et al. 2005; Haila 2002).

The investigation of metapopulation-level processes and the consequences of habitat fragmentation require a much larger scale of study than that which is often feasible (Debinski & Holt 2000). However, the recent explosion of molecular tools has provided new opportunities for ecologists to discern patterns of dispersal among populations (Manel et al. 2005), investigate the processes generating these patterns (e.g. Manel et al. 2003; Wade & McCauley 1988), and examine the consequences of population connectivity (e.g. Bijlsma et al. 2000). At the same time, evolutionary biology is becoming increasingly focused on smaller scale processes and patterns, using ecological processes to understand underlying intraspecific genetic structure, such as is evidenced by the burgeoning field of landscape genetics (Manel et al. 2003). The integration of molecular tools into ecological research allows for new opportunities to study the interface between ecology and evolution. Recent research has demonstrated that evolution can occur on ecological timescales (e.g. Losos et al. 1997; Reznick et al. 1997), illustrating the need for further integration of ecological and evolutionary theories.

Using modern molecular techniques, I assessed some of the ecological causes and consequences of changes in connectivity among populations of the wood frog, *Rana sylvatica*, a widespread temperate amphibian. Wood frogs offer an ideal opportunity to study the processes of dispersal and gene flow among populations. Population connectivity appears to play a central role in wood frog population dynamics, as this species shows evidence of metapopulation dynamics, with frequent local extinctions and recolonizations (Skelly et al. 1999). At the same time, population connectivity may have serious negative impacts, since wood frogs utilize two different habitats in which they experience divergent natural selection (Relyea 2002) and thus movement among populations may result in maladaptation of individuals. The consequences of population connectivity for wood frog populations are therefore complex and require detailed investigation. Recent habitat loss and fragmentation across parts of the wood frog's range, allow for investigation of the factors influencing connectivity as well as the consequences of reduced dispersal among populations.

One of the major consequences of habitat loss and fragmentation has been the conversion of the landscape separating populations from one that facilitates dispersal to one that impedes movement among populations. In chapter 2, I investigate the effects of human-induced landscape changes on genetic patterns of connectivity among wood frog populations. By comparing historical and current landscape structure, it is possible to ascertain the rate of population divergence following habitat fragmentation. I discuss the ecological processes that may contribute to population divergence.

Gene flow and population connectivity have often been thought to lead to homogenization of populations and swamping of locally adapted genotypes. However recent research suggests that populations may be able to diverge in the face of gene flow (e.g. Emelianov *et al.* 2004; Jordan *et al.* 2005; Kotlik *et al.* 2008; Larsen *et al.* 2007; Niemiller *et al.* 2008; Nosil *et al.* 2006; Rice & Hostert 1993; Schneider *et al.* 1999; Smith *et al.* 1997), and in fact divergence may even be facilitated by the presence of gene flow (Rieseberg & Burke 2001). In chapter 3, I explore the consequences of gene flow for local adaptation on various traits under divergent selection across open- and closed-canopy wood frog populations. Overall, open- and closed-canopy populations showed similar levels of phenotypic divergence regardless of whether they had high or low connectivity to other populations, although there was variation among traits in the extent to which they showed divergence. These results suggest that selection within these environments is strong such that divergence can occur despite gene flow.

As a result of rapidly changing landscapes and subsequent isolation of populations, researchers have become particularly interested in the consequences of these changes for population persistence and conservation. Increased isolation of populations may lead to higher levels of inbreeding due to smaller population sizes and reduced gene flow. The detrimental consequences of inbreeding depression on individual fitness have been well studied and have been identified in nearly all organisms (e.g. Husband & Schemske 1996; Ralls *et al.* 1988). As a result, outbreeding has been suggested as a strategy for conservation. Although outbreeding often improves fitness through heterosis (hybrid vigor), there can be negative consequences as well when outbreeding disrupts genetic processes, such as local adaptation. In chapter 4 I explore the consequences of inbreeding and outbreeding for fitness correlates across wood frog populations. My results suggest that populations with low levels of inbreeding have increased survivorship and population sizes relative to more inbred and outbred populations and point to important fitness effects of natural levels of outbreeding that may be as relevant as the effects of inbreeding depression.

In the concluding chapter, I integrate the results from the three previous chapters and discuss the implications for ecology and evolutionary biology. I discuss the efficacy of utilizing molecular techniques for investigating ecological questions. In addition, I provide recommendations for conservation using the wood frog as a model system. Lastly, I highlight areas for future research.

While the effects of habitat loss and fragmentation are being seen across virtually all taxa (Fischer & Lindenmayer 2007), the consequences of these human-induced landscape changes are particularly important for amphibians. The worldwide decline and extinction of numerous amphibian species makes amphibians one of the most threatened taxa (Beebee & Griffiths 2005; Stuart et al. 2004), with one out of three species threatened with extinction (Baillie et al. 2004). Habitat loss and fragmentation have been implicated as one of the major causes of these declines (Collins & Storfer 2003). Yet, the effects of these changes for amphibian populations remain understudied relative to other taxa (Cushman 2006; Gardner et al. 2007; McGarigal & Cushman 2002). There is a clear need for further research on the ecological and evolutionary consequences of habitat loss and fragmentation for amphibians.

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CHAPTER 2

DISENTANGLING THE EFFECTS OF HISTORIC VERSUS CONTEMPORARY LANDSCAPE STRUCTURE ON POPULATION GENETIC DIVERGENCE

Abstract

Increasing habitat fragmentation poses an immediate threat to population viability, as gene flow patterns are changed in these altered landscapes. Patterns of genetic divergence can potentially reveal the impact of these shifts in landscape connectivity. However, divergence patterns not only carry the signature of altered contemporary landscapes, but historical ones as well. When considered separately, both recent and historical landscape structure appear to significantly affect connectivity among 51 wood frog (*Rana sylvatica*) populations. However, by controlling for correlations among landscape structure from multiple time periods, we show that patterns of genetic divergence reflect recent landscape structure as opposed to landscape structure prior to European settlement of the region (before 1850s). At the same time, within-population genetic diversities remain high and a genetic signature of population bottlenecks is lacking. Together, these results suggest that metapopulation processes – not drift-induced divergence associated with

strong demographic bottlenecks following habitat loss – underlie the strikingly rapid consequences of temporally shifting landscape structure on these amphibians. We discuss the implications of these results in the context of understanding the role of population demography in the adaptive variation observed in wood frog populations.

Introduction

Landscape connectivity is not only an essential aspect of population dynamics for many species, but it can also have important evolutionary consequences. Heterogeneity in the landscape matrix separating populations can impede or facilitate dispersal (Ricketts 2001) and gene flow, shaping patterns of genetic variation (e.g. Cushman *et al.* 2006; Funk *et al.* 2005; Lowe *et al.* 2006; Spear *et al.* 2005). However, landscape structure can also vary across time, and relatively quickly, as with changes in human land-use practices (Skole & Tucker 1993). This temporal dynamic, in addition to the spatial landscape structure, is becoming increasingly important as anthropogenic impacts have the potential to outpace the ability of organisms to cope with altered landscapes. Yet, the consequences of temporal shifts in landscape connectivity on patterns of gene flow have rarely been considered (except see Keyghobadi *et al.* 2005; Vandergast *et al.* 2007). The implications of these changes are especially important for amphibian populations, which are facing global declines (Stuart *et al.* 2004).

While there is increasing evidence that habitat fragmentation reduces genetic connectivity in disparate taxa (Coulon *et al.* 2006; Cushman *et al.* 2006; Epps *et al.* 2005; Proctor *et al.* 2005; Riley *et al.* 2006; Vandergast *et al.* 2007), the impact of contemporary landscape changes can be difficult to assess, since patterns of genetic differentiation reflect not only recent shifts in landscape structure, but historic patterns as well. It may take tens to thousands of generations to reach equilibrium between genetic drift and gene flow following habitat fragmentation (Crow & Aoki 1984; Varvio *et al.* 1986), making recent landscape changes relatively more difficult to detect. Additionally, historic and contemporary landscape structure may be correlated. By assessing only the effects of contemporary landscapes, we run the risk of incorrectly attributing contemporary genetic patterns to recent landscape changes when in fact the genetic structure reflects more historic processes.

To account for these difficulties, we assessed the impact of changes in landscape structure across time by comparing the contribution of landscape features from three time periods (Figure 2.1), representing pre- and post-European settlement, to genetic connectivity of 51 wood frog (*Rana sylvatica*) populations (Figure 2.2). Genetic structure among wood frog populations is expected to be correlated with landscape structure, because forested habitat is critical for dispersal and foraging of juveniles and adults (Regosin *et al.* 2003). Much older processes are unlikely to play a role in structuring contemporary populations since phylogeographic patterns across the wood frog range indicate that this region was only recolonized during the last 10,000 years following the most recent glacial period (Lee-Yaw, 2008). While amphibians, in general, are highly sensitive to the effects of habitat fragmentation due to their strict habitat requirements (Cushman 2006), based on the recency of the landscape changes across the study site, we expected the genetic structure of wood frog populations to reflect historic as opposed to contemporary landscape patterns.

Methods

Fifty-one ponds were sampled across southeastern Michigan (Figure 2.2); approximately 20 *R. sylvatica* tadpoles were collected from each pond for a total of 1089 individuals. Each pond was sampled by multiple people spread out across the pond to ensure a thorough sample of each population. Since wood frogs are explosive breeders and adults continue to breed in the pond in which they first bred (Berven, 1990), we equate ponds with breeding populations and refer to them as populations throughout the text. The study area is located within a terminal moraine, and is a composite of forest and wetland fragments separated by agricultural and urban areas. The landscape has undergone dramatic transitions with shifting patterns of land-use following European settlement, as documented in county archives of vegetation surveys from 1816-1856 (Michigan Department of Natural Resources) and satellite images from the Michigan DNR for circa 1978 and the National Land Cover Dataset for 2001 (Homer *et al.* 2004). Most of the ponds used in this study are natural woodland ponds or wetlands; however, some wetlands have been created from small dams scattered throughout the region. Ponds ranged in size from approximately 500 – 20,000 m^2. The extent to which each individual pond has remained stable since the mid-1800s is unknown, since wood frog populations from individual ponds frequently go extinct and are recolonized. However, over the period between the two recent time periods used in this study, the number of breeding sites within this region has remained constant (Skelly, 1999), whereas the number of breeding populations has likely declined since post-European settlement due to loss of both wetland and terrestrial habitat.

DNA was extracted from tail clips using the DNeasy tissue kit (QIAGEN). Nine microsatellite loci developed specifically for *R. sylvatica* were analyzed for each individual: loci AAT23 and AAT46 (Newman & Squire 2001), loci C23, C41, D33, D40, and D88 (Julian & King 2003), and loci 1A11and 2B02 (Table 2.1) developed for this study following the protocol of Glenn and Schable (2005). Polymerase chain reaction (PCR) conditions corresponded to those from Newman and Squire (2001) and Julian and King (2003) for the two former sets of microsatellite markers, respectively. For loci 1A11 and 2B02, PCR reactions included 1.0 uL of genomic DNA, 1.0 uL of 10X PCR buffer (Invitrogen), 0.5 uL of 10 uM primer for both the fluorescently labeled forward primer and the reverse primer, 0.3 uL of 50 mM MgCl2, 0.6 uL of 10 mM dNTPs, 0.4 uL of 250 ug/mL BSA, and 0.2 U of *Taq* DNA polymerase (Invitrogen). Reactions were run for 120 s at 94˚, and then 35 cycles of 94˚ for 60 s, 60˚ for 30 s, and 72˚ for 30s, followed by 240 s at 72˚. Individuals were genotyped with ABI PRISM Genetic Analyzer (Applied Biosystems) and GENEMARKER software (Softgenetics).

Tests for genotyping errors and/or null alleles were conducted for each locus with MICROCHECKER v. 2.2.0 (Van Oosterhout *et al.* 2004), and tests for linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) were assessed with GENEPOP v. 3.4; (Raymond & Rousset 1995), where a sequential Bonferroni correction was applied to reduce type I errors (Rice 1989). Genetic diversity within ponds was assessed by calculating Nei's unbiased gene diversity (Nei 1987), the total number of alleles, and the allelic richness (FSTAT: Goudet 1995), as well as the private allelic richness of each population (HP-Rare: Kalinowski 2005). Populations were also assessed for evidence of population bottlenecks using the program BOTTLENECK (Piry *et al.* 1999) with 1000 replications and under the assumption of the Stepwise Mutation Model, since this model has been identified as appropriate for microsatellite loci, instead of the Infinite Alleles Model (Luikart and Cornuet, 1998). Significance was assessed using the Wilcoxon's Test after Bonferroni correction.

Genetic and landscape distances

Pairwise F_{ST} values (Weir & Cockerham 1984; Wright 1951) were calculated among ponds using a weighted analysis of variance (Weir & Cockerham 1984) with GENEPOP v. 3.4 (Raymond & Rousset 1995). Significance of F_{ST} values was assessed after Bonferroni correction. F_{ST} was used as a measure of genetic distance rather than R_{ST}, because F_{ST} has a lower mean squared error than R_{ST} at the level of differentiation observed among ponds (Gaggiotti *et al.* 1999). Permutation tests were carried out using SPAGEDI v.1.2 (Hardy & Vekemans 2002) to confirm that R_{ST} and F_{ST} converge ($p = 0.3991$, based on 20,000 permutations) (Hardy *et al.* 2003).

Two geographic distances were calculated among each pair of ponds, including the Euclidean (straight-line) distance (ED) and the resistance distance (RD: McRae 2006), a distance weighted according to the permeability of the landscape separating populations. The ED between each pair of ponds was calculated using the PATHMATRIX extension (Ray 2005) in ArcView GIS v 3.3 (ESRI). The RD was calculated using CIRCUITSCAPE v 3 (McRae 2006) from 30m resolution friction maps created in ArcGIS v 9.2 (ESRI 2006). Friction maps were generated by coding each pixel of the map as a cost to dispersal based on the type of landscape that it encompassed, with a cost of one assigned to the most permeable habitats and higher values representing less permeable habitats. This method results in correspondingly greater distances between ponds for landscape features incurring a high cost to traverse.

Friction maps were generated for two permanent landscape features – slope and rivers/lakes – and land cover for each of the three time periods (i.e., 1800s, 1978, and 2001; see Figure 2.1), as well as composite friction maps for each of the time periods that included the permanent landscape features (generated using the Map Algebra tool in ArcGIS). Land cover was classified as either *R. sylvatica* habitat (forests, shrubland, and wetlands) or non-habitat (agriculture, urban areas, grasslands, and savannahs) based on habitat use of *R. sylvatica* (Regosin *et al.* 2003) (e.g. Figure 2.1); wood frog habitat was assigned a cost of one, whereas a range of cost values were examined for non-wood frog habitat. Rivers and lakes were included since rivers and lakes do not likely constitute stepping stones to other wetland habitat (wood frogs primarily breed in habitats that lack fish: Hopey & Petranka 1994). Areas not covered by rivers or lakes were correspondingly assigned a cost of one. Slope was calculated based on a 30m resolution digital elevation model (Michigan Department of Natural Resources; Figure 2.2) using the slope function in the ArcGIS data management toolbox, and modeled as a linear function with a cost of one assigned to a slope of zero and a maximum cost assigned to the highest slope possible. Since our ability to detect the effects of landscape distance on genetic differentiation depends on both the landscape features used and the relative costs of each feature, a range of costs were evaluated for each (Perez-Espona *et al.* 2008).

For each of the friction maps, the relationship between genetic distance and landscape distance was evaluated with Mantel tests (Mantel 1967) and partial Mantel tests (Smouse *et al.* 1986) to control for the effects of distance. All analyses were completed using IBDWS v 3 with 10,000 randomizations (Jensen *et al.* 2005). *P* values were calculated in IBDWS using a modified method (Legendre & Legendre 1998) in order to avoid issues with statistical bias and autocorrelation (Bohonak 2002). R values were used to determine the friction map with the highest support for each time period. While not all possible combinations of costs could be evaluated due to computational constraints, a sufficient range of costs was evaluated to reveal a peak in R values for each time period (Table 2.2). Since landscape variables were combined to create a single predictor variable (each friction map), there is no expected inflation of explained variance due to adding additional landscape variables (as in Cushman *et al.* 2006). The relative support of each friction map could thus be evaluated by ranking R values. To test the validity of this approach, we assessed the extent to which adding additional landscape variables affected R values using mirror images of each of the landscape features. Mirror images allowed us to maintain the same amount of information provided in each landscape variable while removing any correlations between genetics and landscape structure. The addition of multiple landscape variables in mirror image did not consistently lead to an inflation of explained variance (Table 2.3), demonstrating that model support can be assessed according to the rank of the model’s respective R-values.

We additionally evaluated whether land cover from each time period remained significant after removing the effects of the other two time periods. Partial Mantel tests were used to control for the effects of time as opposed to distance. To test the robustness of our results, the partial Mantel tests were repeated for all joint friction maps that were significant for both historic and contemporary landscape.

Results

Genetic Structure

There was no consistent evidence of deviations from Hardy-Weinberg equilibrium or linkage disequilibrium within populations across all loci. While there was some evidence of null alleles, there was no consistent pattern across loci or within populations. To test the robustness of our results, the data were reanalyzed after removing the locus with the highest percentage of populations with evidence of null alleles (locus 1A11); the results from these analyses were qualitatively the same (results not shown).

There was a significant amount of genetic structure across the 51 populations (pairwise F_{ST}-values ranged from –0.008 to 0.087), with 392 out of 1275 (30.7%) significant pairwise comparisons of F_{ST} after Bonferroni correction. Genetic diversities within populations were high (Table 2.4), and none of the populations showed significant evidence of a bottleneck after Bonferroni correction. A significant correlation between

Euclidean distance and genetic differentiation indicated a pattern of isolation by distance (Mantel test: $R^2 = 0.187$; $p < 0.0001$).

Effects of Land Cover

For each time period, R values peaked at the same relative costs for each of the landscape features (Rivers/Lakes = 500, Slope = 200, Land Cover = 5, Table 2.2). Friction maps containing all three landscape features provided higher R^{-}values than cost maps containing either one or two landscape factors (Table 2.2). For each of the three time periods, we detected a significant effect of spatial landscape structure on population connectivity among 51 *R. sylvatica* populations, as landscape distances (based on a joint friction map with optimal costs for each landscape feature; Table 2.2) explained a significant amount of the variation in patterns of genetic differentiation among populations, beyond the effects of straight-line geographic distance (partial Mantel tests, controlling for distance: Table 2.5; Figure 2.3).

Since there was support for land cover from each of the three time periods, the effects of each time period independent of the other time periods were also assessed. The results were consistent for all friction maps where both historic and contemporary landscape structure were initially supported (Table 2.6). Historic landscape structure was not significantly correlated with genetic differentiation after removing the effects of land cover from either contemporary land-cover map (Table 2.6), whereas both contemporary time periods were either significant (1978, 2001) or marginally significant (2001) after removing the effects of historic land cover (Table 2.6). Together, these results suggest that contemporary landscape structure explains more of the variance in contemporary genetic structure than does historic landscape structure. There is slightly more support for land cover circa 1978 explaining contemporary genetic structure than circa 2001. However, the lack of significant support for 2001 land cover after removing the effects of 1978, and the marginal support for 1978 after removing the effects of 2001 (Table 2.6), suggest that land cover from both contemporary periods are highly correlated (Figure 2.1).

Discussion

While the effects of land cover from all three time periods on genetic differentiation were initially supported when considered individually (Table 2.5), after controlling for landscape structure from each time period, our results suggest that contemporary patterns of genetic differentiation among wood frog populations reflect recent as opposed to historic landscape structure (Table 2.6). These results demonstrate how the use of multiple time periods can be used to understand the processes contributing to patterns of genetic variation. Even though the substantial human-induced changes to the landscape have been quite recent, the genetic structure nonetheless reflects current landscape structure (after controlling for the influence of the historic landscape configuration on genetic structure). The comparison of multiple time periods thus not only allows for a determination of how genetic structure is affected by the contemporary landscape, but also an assessment of the rate of differentiation following landscape alteration. The small temporal and spatial scales at which the effects of temporally shifting landscape structure are seen highlight the importance of connectivity for amphibian populations.

The differentiation of wood frog populations associated with recent habitat fragmentation (Figure 2.1) has been much more rapid than expected – the genetic consequences having manifested in less than 50 generations. Why would these landscape changes become evident in patterns of neutral genetic divergence so quickly in this species? Two likely demographic scenarios could have enhanced genetic drift, and thereby led to rapid differentiation, among the wood frog populations. Habitat loss and fragmentation might have caused strong bottlenecks, promoting population differentiation. Alternatively, demographic processes, such as metapopulation dynamics, could have enhanced drift-induced divergence through recurrent extinction and recolonization. While metapopulation dynamics theoretically can either increase or decrease genetic differentiation, (depending on the specific modes of colonization, dispersal, and population growth: Pannell & Charlesworth 2000; Slatkin 1977), metapopulation processes tend to increase the variance in reproductive success among populations, thereby enhancing the impact of genetic drift across a wide range of conditions (Giles & Goudet 1997; Whitlock & Barton 1997).

There are several reasons why metapopulation dynamics most likely explain why we observed a significant effect of recent shifts in land-use practices over such a short evolutionary timescale. Genetic diversities remain high within populations (Table 2.4) and there is no evidence for bottlenecks within any of the populations. Moreover, pond-breeding amphibians are often thought to exhibit aspects of metapopulation structure because of their reliance upon discrete aquatic environments for breeding, their high degree of philopatry, and high rates of population turnover (Alford & Richards 1999; Cushman 2006). Although few amphibian populations likely exhibit classic (sensu Levins 1969) metapopulation structure (Smith & Green 2005), many amphibian populations, including the wood frog, show high rates of population turnover (Hecnar & M'Closkey 1996; Skelly *et al.* 1999; Trenham *et al.* 2003; Werner *et al.* 2007), providing the opportunity for extinction and recolonization dynamics to play an important role in the genetic structure of these populations.

The rapid drift-induced differentiation of populations, as measured by the neutral microsatellite markers (i.e., it is highly improbable that the nine markers are linked with selected loci), is especially intriguing in the context of the adaptive phenotypic differences seen among *R. sylvatica* populations (Relyea 2002; Skelly 2004). Wood frog populations show evidence of local adaptation of behavioral, morphological, and life-history traits to opposing selective forces in ponds with varying predator regimes (Relyea 2002). These adaptive differences occur over very small spatial scales (i.e., within the dispersal capabilities of wood frogs: Berven & Grudzien 1990), and it is yet unclear what maintains these phenotypic differences in the face of potentially high levels of gene flow. Our results suggest that metapopulation dynamics may play an important role in contributing to the striking adaptive differences observed over such small spatial scales (e.g. Relyea 2002). Population turnover that increases differentiation of populations over short evolutionary timescales (as opposed to rapid divergence associated with population bottlenecks) could maintain a source of standing genetic variation relevant to adaptive responses among the wood frog populations. Standing genetic variation provides a unique opportunity for selection to operate, as adaptation from standing genetic variation can proceed faster than adaptation from new mutations (Barrett & Schluter 2008). As a result, gene flow due to extinction and recolonization dynamics may instead facilitate the local adaptation of populations (e.g. Morjan & Rieseberg 2004). Future research should focus on comparing species with alternative demographic substructure to fully understand the extent to which metapopulation dynamics contributes to population differentiation.

While numerous studies have shown an effect of population bottlenecks on rates of genetic differentiation (e.g. Baker & Moeed 1987; Bouzat *et al.* 1998; Rowe *et al.* 1998), very few studies have empirically demonstrated that high rates of extinction and recolonization can result in rapid differentiation among populations (e.g. Clegg *et al.* 2002; Knowles & Richards 2005). Furthermore, while metapopulation dynamics have been implicated in cases where genetic differentiation appears to have taken place over very short timescales (Orsini *et al.* 2008), without an assessment of historic landscape structure, past processes may confound interpretations based on the contemporary landscape. Our study provides an important empirical example (see also Giles & Goudet 1997) that complements a growing body of theoretical research (e.g. Pannell & Charlesworth 2000; Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990) on the evolutionary consequences of metapopulation dynamics.

Conclusions

Our results highlight the importance of not only considering spatial heterogeneity in landscape structure, but temporal landscape changes as well. While initially the effects of land cover on contemporary genetic structure was supported for all three time periods, analyses controlling for correlations across time suggest that genetic differentiation reflects recent as opposed to historic land cover. We thus revealed an effect of recent human-induced shifts in landscape structure on patterns of genetic differentiation among wood frog populations, with differentiation having manifest in less than 50 generations. Moreover, the pattern of genetic diversity maintained within populations, suggests a role of metapopulation dynamics in the observed population genetic differentiation. As such, our study provides empirical evidence of the evolutionary consequences of ecological demographic processes, highlighting that such connections are not limited to organisms with short generations (e.g., viruses), but also apply to longer-lived species. Without similar analyses, conservation decisions may be mislead by failing to control for the confounding factors caused by correlations in landscape from different temporal periods, let alone, whether species-specific demographic structures will need to be taken into account in devising conservation strategies.

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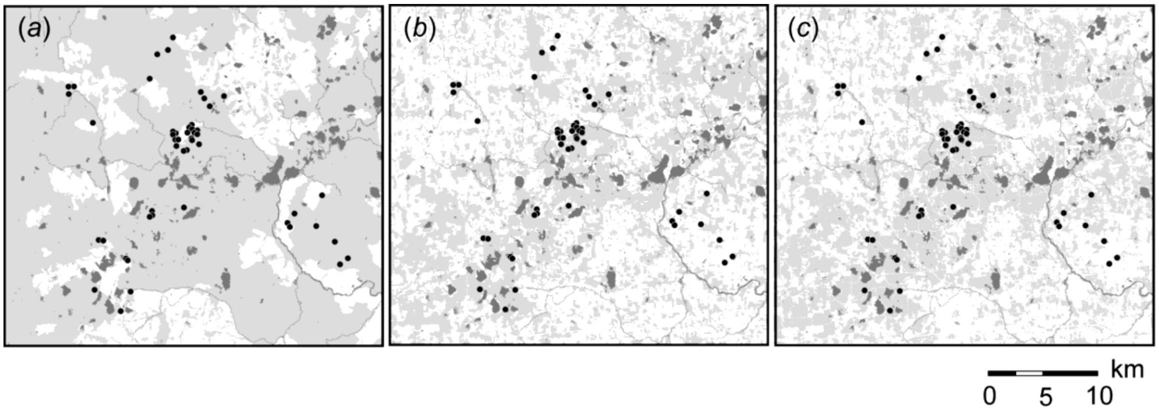

Figure 2.1. Landscape structure of the study site from 1800s to 2001. Maps showing the landscape transitions that have accompanied shifting land-use practices over the last century: (*a*) reconstruction of the area from the 1800s, and aerial photographs of the area from (*b*) 1978 and (*c*) 2001. Areas identified as habitat (shown in light grey) versus non-habitat (shown in white) correspond to forested, shrubland, and wetland areas versus grassland, savannah, agricultural, and urban areas, respectively (Regosin 2003). Rivers and lakes are shown in dark grey, and the sampled populations are represented by black circles.

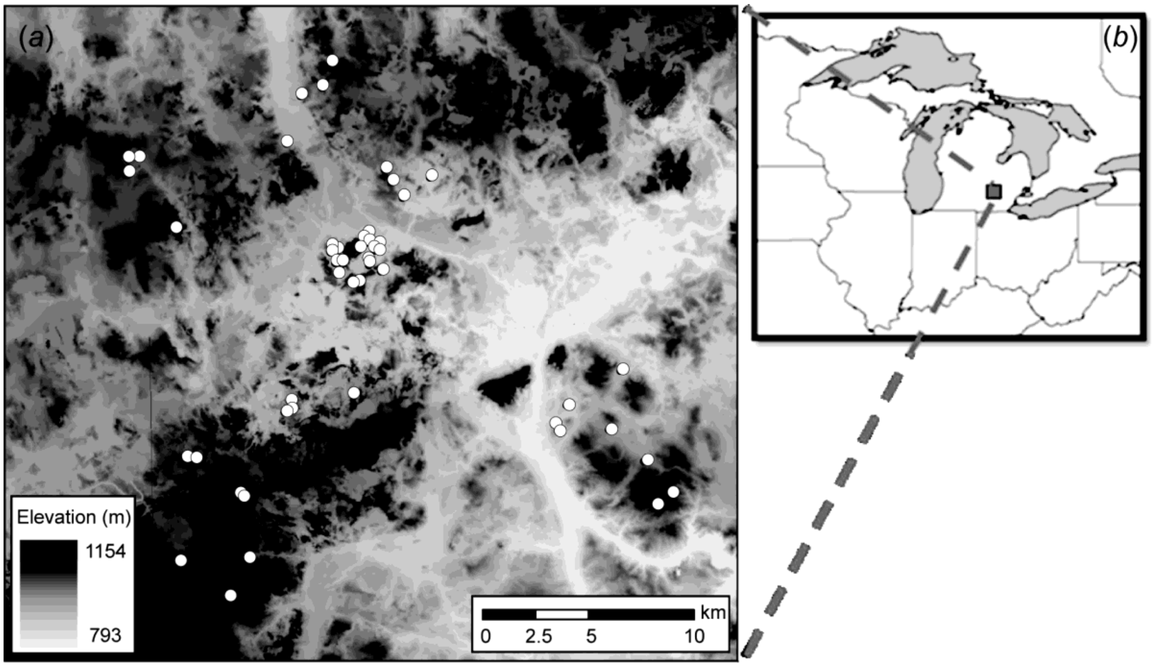

Figure 2.2. Sampled populations. Topographic map of the study area (*a*) from southeastern Michigan, USA (*b*), where sampled ponds are marked with white circles.

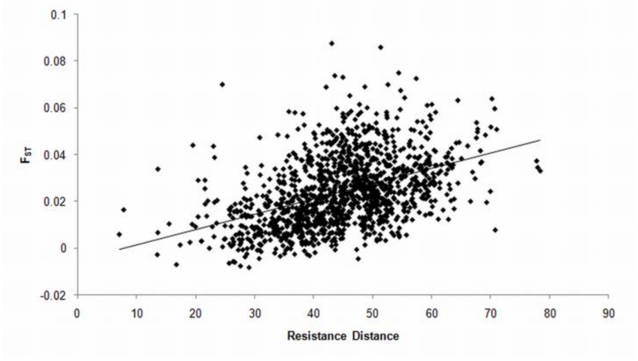

Figure 2.3. Isolation by landscape distance. Pairwise comparisons of genetic differentiation (F_{ST}) as a function of landscape distance (Resistance Distance) (partial Mantel: $R^2 = 0.077$, $p < 0.005$; Table 2.5), based on a model for recent (1978) land cover that also includes rivers/lakes and slope.

Table 2.1. Microsatellite loci. The forward and reverse primers, the repeat motifs, the fragment lengths, and the GenBank accession numbers for the microsatellite loci developed for this study.

Locus		Primer Sequence (5' to 3')	Repeat	PCR Size (bp)	GenBank #
1A11	Forward	AGCCCACCTGGAGTAGGAGT	GT	173-275	GQ422446
	Reverse	TCCTGCCCTGGAAAGTAAAA			
2B02	Forward	GGAACAGTTGGCTTTTGGAA	GT	121-189	GQ422447
	Reverse	TTCAAACCTGCAGTGCCTAA			

Table 2.2. Comparison of support for each time period when varying the costs for different landscape features on the joint friction maps. Landscape features include rivers/lakes (R/L), slope (S), and land cover (LC). Results are from partial Mantel tests that assess the correlation between genetic (F_{ST}) and landscape distance, while controlling for the effects of Euclidean distance. Significant partial Mantel tests are denoted with an asterisk. The results demonstrate support for all three time periods (before controlling for correlations among time periods) since the friction maps with the highest R-values are significant for each time period.

Time Period	**Costs**			**Controlling for Distance**	
	R/L	**S**	**LC**	**R**	***p*** **<**
2001	500	200	5	0.266	0.006*
1978	500	200	5	0.277	0.005*
1800	500	200	5	0.283	0.005*
2001	500	200	50	0.130	0.128
1978	500	200	50	0.206	0.038*
1800	500	200	50	0.276	0.012*
2001	50	50	5	0.211	0.022*
1978	50	50	5	0.250	0.008*
1800	50	50	5	0.272	0.008*
2001	200	50	5	0.234	0.012*
1978	200	50	5	0.269	0.006*
1800	200	50	5	0.283	0.006*
2001	500	500	5	0.263	0.006*
1978	500	500	5	0.268	0.006*
1800	500	500	5	0.272	0.006*
2001	-	-	5	-0.205	0.973
1978	-	-	5	-0.046	0.656
1800	-	-	5	0.202	0.052
2001	-	-	50	-0.220	0.981
1978	-	-	50	-0.033	0.600
1800	-	-	50	0.239	0.029*

2001	200	5	5	0.020	0.386
1978	200	5	5	0.111	0.121
1800	200	5	5	0.237	0.024*
2001	10	5	5	-0.103	0.893
1978	10	5	5	0.021	0.382
1800	10	5	5	0.207	0.044*
2001	10	10	5	-0.039	0.683
1978	10	10	5	0.071	0.220
1800	10	10	5	0.223	0.031*
2001	10	5	10	-0.168	0.955
1978	10	5	10	-0.010	0.521
1800	10	5	10	0.212	0.045*
2001	5	5	5	-0.118	0.923
1978	5	5	5	0.008	0.433
1800	5	5	5	0.203	0.047*
2001	5	10	5	-0.055	0.740
1978	5	10	5	0.058	0.268
1800	5	10	5	0.219	0.033*
2001	5	10	10	-0.146	0.928
1978	5	10	10	0.009	0.435
1800	5	10	10	0.218	0.039*
2001	5	5	10	-0.176	0.958
1978	5	5	10	-0.017	0.555
1800	5	5	10	0.209	0.046*
2001	500	5	5	0.089	0.155
1978	500	5	5	0.155	0.060
1800	500	5	5	0.256	0.017*
2001	500	-	5	0.018	0.414
1978	500	-	5	0.096	0.169
1800	500	-	5	0.243	0.026*
2001	-	200	5	0.237	0.015*
1978	-	200	5	0.249	0.011*
1800	-	200	5	0.261	0.009*
NA	5	-	-	0.030	0.267
NA	10	-	-	0.056	0.159
NA	50	-	-	0.126	0.031*

NA	100	-	-	0.162	0.016*
NA	200	-	-	0.191	0.012*
NA	500	-	-	0.205	0.015*
NA	1000	-	-	0.200	0.018*
NA	-	5	-	0.178	0.020*
NA	-	10	-	0.215	0.012*
NA	-	50	-	0.244	0.011*
NA	-	100	-	0.246	0.012*
NA	-	150	-	0.247	0.012*
NA	-	200	-	0.248	0.015*
NA	-	250	-	0.248	0.012*
NA	-	500	-	0.249	0.012*
NA	200	50	-	0.270	0.003*
NA	500	5	-	0.259	0.001*
NA	500	200	-	0.273	0.007*
NA	500	500	-	0.266	0.006*
NA	5	10	-	0.188	0.020*
NA	10	5	-	0.150	0.030*
NA	10	10	-	0.195	0.016*
NA	5	5	-	0.139	0.030*
NA	200	5	-	0.230	0.003*
NA	50	50	-	0.254	0.006*
1800	-	-	5	0.202	0.052
1800	-	-	10	0.218	0.043*
1800	-	-	50	0.239	0.029*
1800	-	-	100	0.243	0.030*
1978	-	-	5	-0.046	0.656
1978	-	-	10	-0.041	0.633
1978	-	-	50	-0.033	0.600
1978	-	-	100	-0.030	0.585
2001	-	-	5	-0.205	0.973
2001	-	-	10	-0.213	0.978
2001	-	-	50	-0.220	0.981
2001	-	-	100	-0.222	0.984

Table 2.3. Correlations between genetic distance (F_{ST}) and landscape distance from mirror image friction maps, controlling for Euclidean distance. Friction maps were created using the mirror image of each landscape variable (including rivers/lakes (R/L), slope (S), and land cover (LC)) in order to assess whether or not the addition of multiple landscape variables leads to an inherent inflation of explained variance. Mirror images allowed us to maintain the same amount of information provided in each landscape variable while removing any correlations between genetics and landscape structure. Friction maps that include multiple landscape features (i.e., models with non-zero cost values applied to multiple features) do not consistently have higher R-values than individual friction maps, demonstrating that the rank order of the R-values can be used to evaluate model support.

Time Period	**Costs**			**Controlling for Distance**	
	R/L	**S**	**LC**	**R**	***p***
N/A	500	-	-	-0.062	0.714
N/A	-	200	-	-0.055	0.671
N/A	500	200	-	-0.060	0.700
2001	-	-	5	0.093	0.213
1978	-	-	5	0.040	0.356
1800	-	-	5	-0.280	0.996
2001	500	-	5	0.034	0.372
1978	500	-	5	-0.007	0.527
1800	500	-	5	-0.266	0.995
2001	-	200	5	-0.038	0.629
1978	-	200	5	-0.046	0.659
1800	-	200	5	-0.102	0.810
2001	500	200	5	-0.046	0.652
1978	500	200	5	-0.055	0.691
1800	500	200	5	-0.109	0.844

Table 2.4. Genetic diversity within populations. Nei's unbiased genetic diversity (GD; Nei, 1987), number of alleles, allelic richness (AR), and private allelic richness (PAR) for each population. Both AR and PAR were rarified based on the smallest sample size in any population (n = 10). Also shown is the population number (Pop #) as well as the sample size in each population (SS).

Pop #	SS	GD		# of Alleles		AR		PAR	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	21	0.73	0.26	8.67	5.32	6.91	3.59	0.00	0.00
3	21	0.71	0.28	9.33	5.83	6.97	3.84	0.03	0.06
4	24	0.75	0.24	9.78	5.45	7.26	3.50	0.05	0.11
5	21	0.75	0.26	9.44	6.11	7.36	3.82	0.03	0.06
6	22	0.69	0.24	8.78	6.08	6.40	3.64	0.01	0.02
7	20	0.73	0.27	9.00	5.12	7.05	3.65	0.02	0.04
8	25	0.73	0.28	9.44	5.39	7.12	3.59	0.04	0.09
9	23	0.68	0.21	6.44	3.47	5.59	2.69	0.00	0.01
10	20	0.75	0.25	8.33	4.21	6.84	3.02	0.12	0.27
11	21	0.73	0.24	8.78	5.26	6.84	3.60	0.00	0.01
12	20	0.71	0.30	8.56	4.72	6.82	3.45	0.05	0.13
14	20	0.72	0.27	9.11	5.49	6.93	3.47	0.03	0.07
15	21	0.75	0.23	9.33	5.12	7.20	3.58	0.00	0.01
16	20	0.75	0.31	10.11	6.17	7.88	4.15	0.05	0.13
17	20	0.69	0.22	8.44	4.67	6.54	3.30	0.00	0.00
18	10	0.70	0.29	5.78	2.86	5.78	2.86	0.00	0.00
22	20	0.73	0.20	7.89	4.14	6.46	2.89	0.04	0.12
23	21	0.73	0.29	8.56	4.75	6.91	3.56	0.00	0.00
24	21	0.71	0.26	9.11	5.40	6.79	3.50	0.02	0.05
25	21	0.72	0.28	8.89	4.96	6.83	3.32	0.01	0.04
26	21	0.71	0.30	8.78	5.09	6.80	3.62	0.00	0.01
27	20	0.70	0.25	8.33	4.18	6.50	3.05	0.03	0.07
28	20	0.73	0.30	9.44	5.68	7.30	3.81	0.02	0.05
29	21	0.68	0.29	7.78	4.24	6.26	2.97	0.02	0.06
30	23	0.74	0.27	9.33	6.00	7.10	3.82	0.01	0.02
31	21	0.72	0.26	9.33	5.52	7.04	3.65	0.02	0.03
32	22	0.73	0.27	9.67	5.66	7.25	3.68	0.06	0.08

33	22	0.69	0.28	5.78	2.73	5.32	2.43	0.00	0.00
35	20	0.72	0.28	8.33	4.50	6.57	3.06	0.02	0.04
36	21	0.74	0.26	8.67	4.47	6.98	3.26	0.03	0.07
37	22	0.77	0.24	9.67	5.68	7.39	3.46	0.08	0.16
38	21	0.72	0.24	9.11	5.93	7.07	3.98	0.02	0.04
39	20	0.76	0.25	8.89	4.31	7.42	3.38	0.03	0.04
40	21	0.76	0.25	8.56	4.25	7.08	3.09	0.04	0.07
41	21	0.74	0.22	8.11	3.92	6.53	2.88	0.02	0.04
42	21	0.76	0.24	8.78	4.52	7.21	3.28	0.06	0.12
43	22	0.73	0.27	10.44	6.25	7.67	4.03	0.08	0.09
44	21	0.76	0.20	9.22	4.60	7.01	2.83	0.10	0.16
45	24	0.73	0.27	9.89	5.73	7.29	3.61	0.02	0.06
46	21	0.72	0.28	9.89	6.13	7.62	4.20	0.05	0.07
47	25	0.73	0.25	10.22	6.59	7.51	4.13	0.09	0.10
48	22	0.76	0.23	9.11	4.83	7.24	3.38	0.05	0.09
49	28	0.77	0.23	10.22	6.14	7.23	3.14	0.06	0.11
50	24	0.73	0.27	10.44	6.67	7.65	4.07	0.03	0.06
51	23	0.76	0.28	10.89	6.90	7.96	4.16	0.07	0.08
52	24	0.76	0.25	9.00	4.44	7.00	3.01	0.08	0.14
53	23	0.73	0.30	10.00	5.55	7.41	3.79	0.01	0.03
54	20	0.73	0.29	9.67	5.79	7.57	3.96	0.07	0.13
55	21	0.74	0.25	9.89	6.15	7.28	3.91	0.04	0.11
56	21	0.74	0.26	8.89	5.21	7.02	3.63	0.04	0.10
57	22	0.71	0.28	10.00	5.57	7.46	3.93	0.09	0.15
Overall				9.02	0.92	7.00	0.42		

Table 2.5. Effect of landscape structure controlling for distance. Landscape structure from each time period explains a significant amount of the variation in contemporary genetic structure (F_{ST}) after controlling for the effects of Euclidean distance (partial Mantel tests). Results are based on landscape distances from a joint friction map that includes the optimal cost for each landscape feature, including: rivers/lakes (R/L), slope (S), and land cover (LC).

Time Period	**Costs**			**Controlling for Distance**	
	R/L	**S**	**LC**	**R**	***p* <**
2001	500	200	5	0.277	0.005*
1978	500	200	5	0.276	0.012*
1800	500	200	5	0.283	0.005*

Table 2.6. Effect of landscape structure controlling for time. Contemporary land cover is consistently related to genetic differentiation (F_{ST}) after controlling for effects of historical land cover. Partial Mantel results for only the joint friction maps that supported both historic and contemporary landscape structure, with various costs for rivers/lakes (R/L), slope (S), and land cover (LC).

Time Period	**Costs**			**Controlling for Time**					
				2001		**1978**		**1800**	
	R/L	**S**	**LC**	**R**	***p*** **<**	**R**	***p*** **<**	**R**	***p*** **<**
2001	500	200	5	-	-	-0.156	0.907	0.172	0.053
1978	500	200	5	0.187	0.058	-	-	0.218	0.018*
1800	500	200	5	-0.082	0.782	-0.120	0.862	-	-
2001	500	200	50	-	-	0.016	0.446	0.337	0.001*
1978	500	200	50	0.180	0.064	-	-	0.379	0.001*
1800	500	200	50	0.174	0.085	0.178	0.077	-	-
2001	50	50	5	-	-	-0.100	0.804	0.183	0.044*
1978	50	50	5	0.187	0.059	-	-	0.233	0.012*
1800	50	50	5	0.087	0.244	0.062	0.317	-	-
2001	200	50	5	-	-	-0.103	0.808	0.170	0.055
1978	200	50	5	0.183	0.062	-	-	0.218	0.019*
1800	200	50	5	0.089	0.239	0.063	0.311	-	-
2001	500	500	5	-	-	-0.162	0.918	0.169	0.055
1978	500	500	5	0.175	0.070	-	-	0.212	0.019*
1800	500	500	5	-0.134	0.893	-0.175	0.945	-	-

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CHAPTER 3

ADAPTIVE DIVERGENCE WITH GENE FLOW IN WOOD FROG POPULATIONS

Abstract

Gene flow has historically been thought to constrain local adaptation, yet recent research suggests that populations can diverge despite exchanging genes. While evidence for divergence with gene flow is mounting, few studies have accounted for the effects of phenotypic plasticity on differentiation amongst populations. Here I use a common garden experiment to assess the combined effects of gene flow and natural selection on local adaptation of 16 wood frog populations (*Rana sylvatica*), a species known to experience divergent selection pressures in open- and closed-canopy ponds across relatively short geographic distances. Overall, larvae from populations experiencing opposing selective pressures had significant morphological differences, but these differences were of the same magnitude in ponds with both high and low population connectivity. This pattern was apparent even though larvae were raised under common-garden conditions, illustrating the need to control for the effects of plasticity. These results suggest that divergence among these wood frog populations is occurring despite gene flow and that selection within these environments is strong. In addition, there was variation among traits in the extent to which they showed divergence at varying levels of connectivity. The extent of divergence in body depth and tail depth among open- and closed-canopy ponds was similar in both low- and high-connectivity populations, whereas divergence in body length, tail depth, muscle depth and activity showed no effect of canopy type at either level of connectivity, despite the fact that previous studies have demonstrated selection on some of these traits. The results suggest that multiple processes may be occurring within a species such that various traits are impacted differently by the interplay between gene flow and selection.

Introduction

While gene flow has long been known to be a central process influencing the local adaptation of populations, the consequences of this important evolutionary force remain unclear. Gene flow has historically been thought to counteract the effects of selection, preventing local adaptation and leading to the homogenization of populations (Bridle & Vines 2007; Kawecki & Ebert 2004; Lenormand 2002; Slatkin 1985). Yet, recent research suggests that populations may diverge in the absence of strong physical barriers to gene flow (e.g. Emelianov *et al.* 2004; Jordan *et al.* 2005; Kotlik *et al.* 2008; Larsen *et al.* 2007; Niemiller *et al.* 2008; Nosil *et al.* 2006; Rice & Hostert 1993; Schneider *et al.* 1999; Smith *et al.* 1997), and, in fact, gene flow may even facilitate the divergence of populations by providing the genetic variation necessary for selection to act upon (Rieseberg & Burke 2001). This paradox arises in part because each gene differs in the extent to which it is affected by the interplay between gene flow and selection. Genomes are heterogeneous (Wu 2001), with the strength of selection and rate of introgression varying across different genes. As a result, genes coding for phenotypic traits under intense selection pressures may diverge between populations, whereas non-selected genes remain homogeneous (Nosil et al. 2008). Understanding how this interplay between gene flow and selection affects adaptation is crucial, as the extent to which populations can become locally adapted is not only important for understanding the processes of divergence and speciation, but is also necessary for predicting the potential for populations to adapt and cope with human-induced environmental changes following reduced gene flow due to habitat loss and fragmentation.

Divergence with gene flow is often assessed by comparing phenotypic and genetic differentiation among populations of similar and different habitats, where measurements of phenotypic divergence include both the genetic and environmental components of phenotypic variance. However, most traits are plastic under at least some environmental conditions (e.g. Adams & Huntingford 2004; Byars *et al.* 2007; Chapman *et al.* 2000; Jimenez-Ambriz *et al.* 2007; Pfennig & Murphy 2002), limiting any inferences that can be made about the interplay between selection and gene flow on the genome itself. For example, when plasticity is in the adaptive direction (i.e. cogradient variation), we expect similar patterns of phenotypic divergence as those generated by local adaptation. Plasticity may also affect our inferences by changing the relationship between adaptive genetic divergence and gene flow (Crispo 2008). For instance, whereas in some cases plasticity hampers genetic adaptation (e.g., Storfer *et al.* 1999; Storfer & Sih 1998), in other cases plasticity may facilitate local adaptation when there is gene flow, by either allowing individuals to survive in novel habitats (Crispo 2007; Ghalambor *et al.* 2007; Price *et al.* 2003) or by providing novel variation which can later be canalized in the genome (i.e. genetic assimilation; Crispo 2007; Ghalambor *et al.* 2007; Pigliucci & Murren 2003; Price *et al.* 2003; West-Eberhard 2003). It is therefore necessary to take into account the effects of environment on phenotypic differences among populations in order to fully assess the consequences of gene flow for local adaptation.

In this study, I sought to assess the relative effects of gene flow and selection on local adaptation of traits known to exhibit both genetically and environmentally-determined population differences in the wood frog, *Rana sylvatica*. The wood frog offers an excellent opportunity to test how gene flow and selection interact to impact local adaptation. Larval wood frogs inhabit a relatively broad environmental gradient, occupying both open- and closed-canopy ponds (Werner & Glennemeier 1999). Open-canopy ponds have greater resource availability, have higher dissolved oxygen levels, are warmer than closed-canopy ponds (Werner & Glennemeier 1999), and also harbor more invertebrate predators, whereas closed-canopy ponds – due to resource scarcity - have higher levels of intraspecific competition (Werner et al. 2007). As a result, selection pressures in open- versus closed-canopy ponds are strongly divergent. Selection by predators favors individuals that invest in anti-predator defenses, including reduced activity and increased tail development, at the expense of decreased growth rates (Relyea 2000; Relyea 2001a; Relyea 2001b; Relyea & Werner 2000; Van Buskirk 2002; Van Buskirk *et al.* 1997; Van Buskirk & Relyea 1998). In contrast, intense competition (or low resources) favors individuals that maximize growth rates as opposed to anti-predator defenses (Relyea 2002a).

While these selection pressures promote the adaptation of populations to local environmental conditions (Relyea 2002b; Skelly 2004), the alternative habitats are often interspersed across the landscape, such that ponds of opposite canopy type are often separated by distances that are well within the known dispersal capabilities of wood frogs (Berven & Grudzien 1990). As a result, there is a high potential for gene flow to exert a large pressure on patterns of local adaptation across wood frog populations. In fact, at these small geographic scales, there is little evidence of neutral genetic structure among open- and closed-canopy populations (Zellmer et al. unpublished data), suggesting that exchange of migrants does occur among these two habitat types. Although, the evidence for fine-scale local adaptation and the low levels of neutral genetic structure, together, suggest that selection may be strong enough for populations to diverge despite gene flow, no studies have explicitly tested this hypothesis.

To test the extent to which the local adaptation of populations is limited by gene flow, I compared phenotypic divergence of larval wood frog populations from open- and closed-canopy (canopy effect) ponds with high or low connectivity to other populations (connectivity effect) using a common-garden design to control for the environmental component of variation in generating phenotypic differences among populations. If divergence among pond canopy types occurs despite gene flow, then we expect to see phenotypic divergence due to canopy type but not connectivity. In other words, there will be similar levels of phenotypic divergence among open and closed canopy ponds whether they are highly connected or not (Figure 3.1). On the other hand, if gene flow limits divergence, then we expect an interaction effect between canopy and connectivity, with larger phenotypic differences among open and closed canopy ponds when they are relatively more isolated (Figure 3.1). Alternatively, if these populations are not locally adapted to different canopy conditions, then we do not expect to see any phenotypic differences among open and closed canopy ponds, regardless of connectivity (no effect of canopy or connectivity). Lastly, while we do not expect there to be any differences due to connectivity alone, it is possible that populations may also be locally adapted to differences among more isolated and more connected populations independent of any effects of canopy. This pattern would be evidenced by a significant connectivity effect on phenotypic divergence, which would suggest that there are potential environmental differences along a gradient of connectivity that warrant further study.

Since the relationship between gene flow and selection is expected to differ among traits, I measured a number of phenotypic traits including behavioral, morphological, and life historical traits and assessed the effects of connectivity and canopy over all traits as well as on each trait individually. While there is evidence that many of these traits are under selection or correlated with a selected trait (Van Buskirk & Relyea 1998), the phenotypic traits that are expected to show greater levels of divergence with gene flow should be those that are under strong selection and highly heritable, including tail length, growth, and behavior, whereas traits with heritable plasticity should show a greater impact of gene flow on divergence.

Methods

Phenotypic measurements

The common-garden experiment was set-up as a two-by-two factorial design crossing canopy type (open versus closed) with connectivity to other populations (high versus low), with a total of 16 populations (Figure 3.2). The amount of canopy coverage for each pond was confirmed by measuring the percent of light transmission through the canopy during June 16-18 using a fish-eye lens from a camera placed on the surface of the water in the center of each pond. All ponds that were open had less than 50% canopy cover. All ponds that were classified as closed had greater than 65% canopy coverage, except one pond (BBL), which had ~49% coverage. This pond had a number of trees growing throughout it that had recently died (confirmed from satellite images), suggesting that the pond has historically been a closed canopy pond. While the cutoff levels vary slightly from previously published work on open- and closed-canopy wood frog populations (e.g. > or ≤ 75% coverage Werner et al. 2007), the methods used in this study to calculate canopy cover differ from the previous studies. Populations were also characterized as either high or low connectivity to other populations based on the following criteria: distance to nearest pond of opposite canopy type, neutral genetic differentiation from neighboring populations, and topology surrounding the ponds (since increased slope is known to reduce gene flow among wood frog populations (Zellmer & Knowles 2009). Measures of genetic differentiation were based on F_{ST} measures (Weir & Cockerham 1984; Wright 1951) from a previous study (Zellmer & Knowles 2009). The combination of the three genetic and landscape criteria was used to characterize population connectivity as opposed to using F_{ST} estimates alone, because F_{ST} can be influence by processes other than gene flow (Whitlock & McCauley 1999). All populations were at least 1 km apart from one another, but also were within or (in one case) at the maximum dispersal distance of wood frogs (Berven & Grudzien 1990) from at least one pond of the opposite canopy type to ensure that dispersal among populations was possible.

During the 2008 breeding season, ponds were visited routinely to determine breeding chorus locations. The wood frog is an explosive breeder that lays eggs during a 1-2 week period in the spring. Females usually lay their eggs in a single egg mass consisting of approximately 730 eggs (Benard, in prep), and most females from a single breeding chorus lay their egg masses next to one another. Breeding populations are therefore often discrete units. Approximately 100 eggs were collected from each of 10 egg masses from each pond to ensure a broad sampling of the population. For one pond (Cassidy 1), egg masses could not initially be located, and instead approximately 15 amplectant pairs were caught, kept separate, and returned to the lab to breed. Eggs from 10 masses (laid within 24 hours of collection) were kept for this study, and the adults were returned to their pond of origin. Egg masses were later located to confirm that breeding did occur in this pond. All eggs were kept until hatching in outdoor wading pools covered by shade cloth.

Individuals were raised in common-garden mesocosms (1000 L polyethylene cattle watering tanks). Each population was replicated four times across four spatial blocks, for a total of 64 tanks. The mesocosms were set up 16-21 April, 2008. Each tank was filled with aged well water, inoculated with zooplankton and approximately 6 L of filtered pond water to initiate phytoplankton growth, supplemented with approximately 300 g of leaves to serve as a substrate for phytoplankton, and covered with shade cloth to prevent colonization. Each mesocosm was supplemented with approximately 30g of rabbit chow on 24 April.

On 22 April, 420 hatchlings were haphazardly selected from each of the sixteen populations and kept overnight in containers in the lab. Twenty of the hatchlings from each pond were set aside in separate containers for approximately 24 hours to assess effects of handling on mortality at stocking. There was 100% survivorship across all ponds during this period. These twenty hatchlings were then preserved in 10% formalin for morphological measurements. The experiment was initiated on April 23, with 100 hatchlings added to each mesocosm

Behavioral assays

Between 11-14 May, tadpoles were assessed for activity level in either the presence or absence of predatory cue. Ten tadpoles from each tank were split among the two treatments. Five tadpoles from each tank were put into plastic bins with approximately 6 L of water with 0.15 g of rabbit chow and allowed to acclimate for 24 hours before the trials began. Half an hour before the trails began, predatory cue was added to each of the predator treatment bins. Predatory cue was acquired by feeding 1-2 tadpoles to larval dragonflies (*Anax* spp.) in a cup of water. The filtered water in which the feeding took place was then added to tanks. This is a standard method for assessing the non-lethal effects of predators (e.g. Fraker 2008). For the non-cue treatment bins, a similar amount of aged well water was added to the bins instead to maintain a similar amount of disturbance among bins. Bins were then scan surveyed for the number of active tadpoles. Each bin was surveyed ten times approximately every 5 minutes. The average number of active tadpoles across each of the observations was calculated and the data were arcsine square root transformed.

Morphological and life historical measurements

On days 18 and 37, ten tadpoles were removed from each tank and preserved in 10% formalin. All tadpoles collected were photographed, and five morphological measurements were made on each individual, including: body length, tail length, body depth, tail depth, and muscle depth (see Relyea 2000). While geometric morphometric methods provide additional information over linear measurements (Rohlf & Marcus 1993), linear measurements were used because 1) previous studies have identified adaptive differences based on linear measurements in wood frog larvae, and 2) consistency with these previous studies allows for direct tests of hypotheses generated from previous work. Each tadpole was also weighed to determine growth. All morphological measurements were regressed against weight to control for differences due to body size. The experiment was terminated between 11-12 June. All tanks were drained and all surviving individuals were collected, counted, and preserved in 10% formalin.

Analyses

The effects of canopy-type and connectivity on morphological and life historical differences were assessed using MANOVA for both sampling periods. Since the local adaptation of individual traits may be affected differently by gene flow, I also assessed the contribution of canopy-type and connectivity on variation at each trait using ANOVA (Stata v 8.2). For the behavioral assays, the effects of predator treatment, canopy type, and connectivity were also assessed using ANOVA.

Results

Wood frog larvae showed significant variation in morphology and growth due to canopy at both days 18 and 37 (MANOVA: F = 5.11, p =0.0003, Table 3.1; F = 2.62, p = 0.027, Table 3.2). Connectivity did not explain a significant portion of the variation at 18 days (Table 3.1), however, was marginally non-significant at 37 days (F = 2.08, p = 0.071, Table 3.2).

Since the joint effects of gene flow and selection are expected to differ across traits, the analyses were also completed for each trait individually. At 18 days, both tail length and body depth showed significant variation across canopy types (ANOVA: F = 6.1, p = 0.017; F = 9.84, p = 0.003, respectively; Table 3.3), while mass was marginally significant (F = 3.66, p = 0.061; Table 3.3). The remaining traits showed no effect of canopy (Table 3.3). Individuals from open-canopy ponds had larger body depth, shorter tail lengths, and weighed slightly more than those from closed-canopy populations (Figure 3.3). None of the traits showed any significant variation due to connectivity at day 18 (Table 3.3, 3.6). At day 37, only tail length varied significantly across canopy types (F = 6.27, p = 0.015, Table 3.4, 3.6, Figure 3.4). There was also a significant connectivity effect on tail length (F = 4.97, p = 0.030), as well as a significant interaction effect between canopy and connectivity (F = 5.50, p = 0.023, Table 3.4). At closer inspection, the significant interaction effect appears to be due to the fact that low-connectivity, closed-canopy populations had longer tails than any of the other populations (Figure 3.4).

Surprisingly, there was no variation in activity in response to predator treatment or canopy-type, although there was a significant effect of connectivity (F = 6.17, p = 0.014, Table 3.5). Individuals from low connectivity populations had lower activity levels relative to individuals from highly connected ponds (Figure 3.5). There were no significant interaction effects. The lack of an effect of the predator treatment on behavior is surprising since there is ample evidence that larvae of many amphibian species including wood frogs reduce their activity in response to predator cues (e.g. Anholt *et al*. 2000). However, previous research suggests that the response of wood frog larvae to predator cues declines around 300 mg or Gosner Stage 29 (Fraker, personal communication), which was approximately the weight and developmental stage of the larvae used in the behavioral assays. This suggests that the behavioral assays were not an adequate measure of response to predator, although the overall activity levels should still be informative.

Discussion

The presence of fine-scale local adaptation in wood frog populations has been hypothesized to be due to either population isolation or else as a result of very strong selection within ponds (Relyea 2002b). The results presented here suggest that phenotypic differences among open and closed canopy ponds may occur regardless of the level of connectivity among populations. Despite significant variation in morphology among canopy types, there was no effect of connectivity on divergence during either sampling period (Tables 3.1-3.2). Individuals from open-canopy ponds had shorter tails and larger bodies in addition to being overall slightly larger than those from closed-canopy ponds, but these differences were of similar magnitude in both low and high connectivity populations. The traits that showed significant variation are known to be under selection in open- and closed-canopy ponds (Van Buskirk & Relyea 1998) and are heritable (growth and tail length) or correlated with a heritable trait (body depth) (Relyea 2005). The observed patterns of phenotypic variation and the *a priori* predictions as to which traits should show a pattern of divergence among canopy types lends strong support to the hypothesis that selection across these habitats is strongly divergent. Lastly, the results demonstrate the importance of utilizing common-garden experiments for evaluating the joint effects of gene flow and selection independent of the effects of phenotypic plasticity. Under natural settings, a pattern of phenotypic divergence at both high and low levels of population connectivity due to strong selection could easily be incorrectly attributed to plasticity.

The similar levels of phenotypic divergence in both low and high connectivity habitats could be due to divergence despite gene flow or alternatively could suggest that the populations studied did not exhibit levels of connectivity that are necessary for genetic homogenization. However, there are a number of lines of evidence against the latter hypothesis. First, the differences in phenotype occur over very small spatial scales relative to the dispersal distance of the wood frog (Berven & Grudzien 1990), and both contemporary estimates of dispersal plus other genetic evidence demonstrate high levels of gene flow among populations. Each of the populations used in this study are within the average dispersal distance of wood frogs (approximately 1.2 km: Berven & Grudzien

1990) from at least one other pond and are either within or (in one case) just beyond the maximum-recorded dispersal distance (2.53 km: Berven & Grudzien 1990) from at least one other pond of the opposite canopy type. Moreover, mark-recapture research has demonstrated that dispersal among opposite canopy types does occur at this scale (Benard, unpublished data). While dispersal does not always translate into gene flow, these results suggest that gene flow is possible among populations and among canopy types. Moreover, fine-scale analyses of genetic structure due to pond canopy type demonstrate that pond canopy type explains none of the variation in genetic structure among populations (Analysis of Molecular Variance: proportion of variation due to canopy type = -0.06, p = 0.745; Zellmer et al., unpublished data). Together, these results suggest that movement of individuals and gene flow among these populations are both occurring and that any patterns of divergence observed are occurring despite gene flow among populations.

While there is increasing evidence that divergence is possible with gene flow (Emelianov *et al.* 2004; Niemiller *et al.* 2008; Smith *et al.* 1997), most studies assessing the effects of gene flow on local adaptation have found increasing phenotypic divergence with decreasing gene flow or population connectivity (see Rasanen & Hendry 2008 and references therein). However, theory suggests that increasing immigration of maladapted individuals into a population increases the strength of selection within populations (due to a ‘migration load’), and as a result there may be no net change in trait frequencies across time despite immigration (Bolnick & Nosil 2007). This mechanism has been proposed to explain trait means within isolated and connected *Timema* walking-stick populations

(Bolnick & Nosil 2007) and could additionally explain why there is little effect of connectivity on divergence of these wood frog populations. Future research assessing differences in selection differentials and fitness within more isolated and connected populations will be necessary to determine if this mechanism is responsible for the observed patterns of divergence.

Differences among traits

Although overall the results suggested a general pattern of divergence despite gene flow, this pattern varied across different traits. Body depth and tail length differences among open- and closed-canopy ponds were of the same magnitude for both high and low connectivity populations (Figure 3.3), indicating little effect of connectivity on divergence among canopy types. On the other hand, some of the traits showed no variation among canopy types (Tables 3.3-3.4), despite the fact that previous research has demonstrated selection on these traits as a result of some of the differences among pond types (Van Buskirk & Relyea 1998). There are a number of differences among the traits that may explain the varying patterns of phenotypic divergence, including: differences in selection on each trait, the degree of plasticity, the amount of heritability or heritable plasticity, and correlations among traits. While the effects of trait differences were not directly assessed in this study, the results presented here in combination with results from previous studies (e.g. Relyea 2001b; Relyea 2005) provide a number of avenues for future research, particularly in regards to the role of these trait differences in generating variation in the effect of gene flow on local adaptation.

What the variation among traits implies is that multiple processes may be occurring within populations in regards to gene flow and local adaptation. Studies that focus on single traits or single types of traits may incorrectly conclude whether local adaptation is occurring simultaneously with gene flow or if gene flow is constraining local adaptation. These results may furthermore explain why this field has produced such divergent results regarding the role of gene flow in the local adaptation of populations. Although some studies have assessed the impacts of gene flow on different parts of the genome (e.g. Nosil et al. 2008), few studies have focused on the specific differences among traits in the extent to which they diverge in the face of gene flow. Further theoretical models and empirical examples are needed to fully understand how different phenotypic traits respond to gene flow and selection.

Two alternative hypotheses could explain the observed phenotypic differences among canopy types. First, the phenotypic differences could be due to exposure to cues (e.g. predator chemical cues) in the ponds during the approximately 24 hours before eggs were collected. However, recent research suggests that for larval wood frogs, such cues must be associated with actual costs (e.g. chemical cues from depredated conspecifics) in order for predator-related morphologies and behaviors to be induced (Ferrari & Chivers 2009). This situation is not likely in this system since all eggs are laid simultaneously, thus no conspecific larvae would have been present for predators to feed on when eggs were deposited. Similarly, these patterns do not appear to be due to maternal effects, because if maternal effects were responsible, then we would expect higher phenotypic variance among individuals in high rather than low connectivity populations (i.e. traits would be bimodally distributed), since maternal effects should not be affected by gene flow. However, there was no evidence of bimodal distributions in highly connected populations (results not shown). Moreover, hatchling size, an important potential maternal effect, did not differ among canopy types or due to connectivity (Table 3.6). While these results suggest that neither early environmental cues nor maternal effects are responsible for the observed pattern, future research will be needed to assess the relative contribution of these processes to phenotypic differences among populations.

Temporal patterns

Although overall there were similar patterns among the two sampling periods (Tables 1-2), the effects of canopy and connectivity on individual traits differed temporally (Tables 3-4). During the second sampling period, the only trait that showed any significant variation was tail length (Table 4), which was similar in size among all ponds except the low-connectivity, closed-canopy ponds (Figure 3.4). The lack of differences due to canopy type across most of the morphological traits in the second sampling period could be due to the fact that, as the larvae grow, they become less vulnerable to predation by gape-limited invertebrate predators. If this were true, then it would suggest that the effects of canopy type on phenotypic differentiation may not be long lasting and that individuals may be capable of compensating for the differences observed earlier. In addition, it would have implications for how individuals respond to yearly variation in predator populations within ponds. Future research will be necessary to determine whether these differences dissipate over the entire larval period and if there are any lasting effects past metamorphosis.

Effects of Pond Isolation

While the effect of predators on activity (Relyea 2001b) and the heritability of activity levels (Relyea 2005) would predict local adaptation of behavior to canopy type as well, there were no significant differences in behavior among canopy types. A previous study similarly found little difference in behavior among wood frog populations and no differences among canopy types (Relyea 2002b). On the other hand, the experiment uncovered some unexpected results, suggesting that behavioral differences are instead linked to the level of connectivity among populations (Table 3.5; Figure 3.5). Individuals from low-connectivity populations showed decreased activity levels relative to individuals from more connected populations. These behavioral differences parallel differences in morphology at day 37 (Table 3.4), where more isolated populations had significantly longer tails than highly connected populations (Figure 3.3). Selection may be acting along a gradient with gene flow, such that populations with higher levels of gene flow experience opposing selection from more isolated populations. If selection is strong enough across this gradient, it may overpower any of the effects of selection across canopy types. These results open up additional hypotheses regarding the factors generating differences in activity levels across connectivity gradients. Such a pattern could result from differences in predator levels, for example, if predators have reduced or increased ability to disperse to more isolated ponds. Alternatively, differences in abiotic pond conditions could lead to different foraging strategies in isolated versus connected ponds. Numerous abiotic environmental conditions often exist between isolated and connected ponds, particularly when isolation is due to habitat fragmentation (e.g., increased runoff and levels of toxins: Forman & Alexander 1998). Further research will need to be done to determine the cause of these behavioral differences across the connectivity gradient.

Conclusion

Previous research on the effects of gene flow on local adaptation has provided mixed results, with support for gene flow as a constraining force (Bridle & Vines 2007; Kawecki & Ebert 2004; Lenormand 2002; Slatkin 1985) a facilitating force (Rieseberg & Burke 2001), or alternatively having little influence on divergence of populations (Emelianov *et al.* 2004; Niemiller *et al.* 2008; Smith *et al.* 1997). The similar levels of phenotypic divergence among open- and closed- canopy wood frog populations at both low and high population connectivity suggest that divergence may be occurring despite gene flow. This study thus adds to the mounting evidence that selection may often be strong enough to overpower the homogenizing effects of gene flow (Emelianov *et al.* 2004; Niemiller *et al.* 2008; Smith *et al.* 1997). In addition, the patterns of divergence in both high and low connectivity populations demonstrate the importance of using common garden studies to evaluate the joint effects of gene flow and selection independent of the effects of phenotypic plasticity. While overall there was a general pattern of divergence despite gene flow, there were also differences among traits in the extent to which they showed divergence at different levels of connectivity, even for traits that are known to be under selection. These results suggest that the effects of gene flow on divergence can vary among traits, which may help to explain why we see such varied results across studies. Future research should focus on understanding the mechanisms allowing for divergence with gene flow, evaluating the consequences for individual and population fitness, and assessing potential differences in the selective environment along the connectivity gradient.

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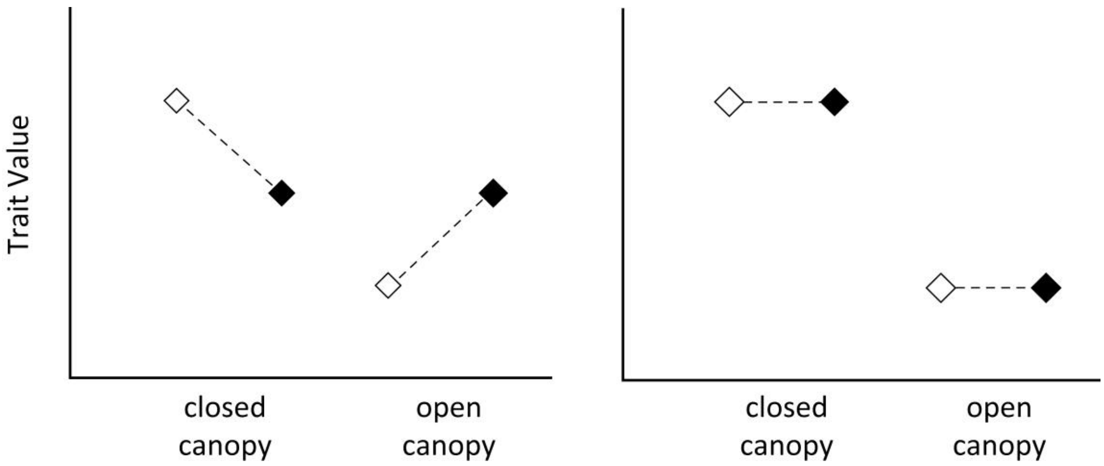

Figure 3.1. Predicted average trait values for open- and closed-canopy populations with either low (open symbols) or high (closed symbols) connectivity with other populations under common-garden conditions. Predictions are shown for either (a) gene flow limiting divergence, with greater phenotypic divergence in high-connectivity populations or (b) divergence despite gene flow, with similar levels of phenotypic divergence in both low- and high-connectivity populations.

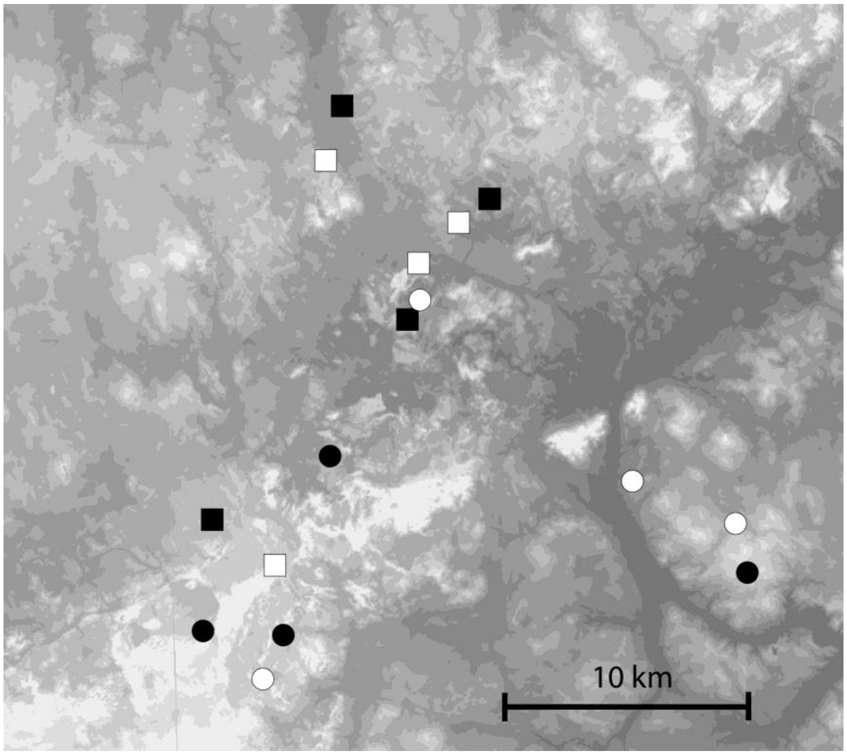

Figure 3.2. Elevation map of the study area. Sampled populations from both open- (white symbols) and closed-canopy ponds (black symbols). Populations are also labeled as connected (squares) or isolated (circles). Elevation ranged from 793m (dark gray) – 1154m (light gray).

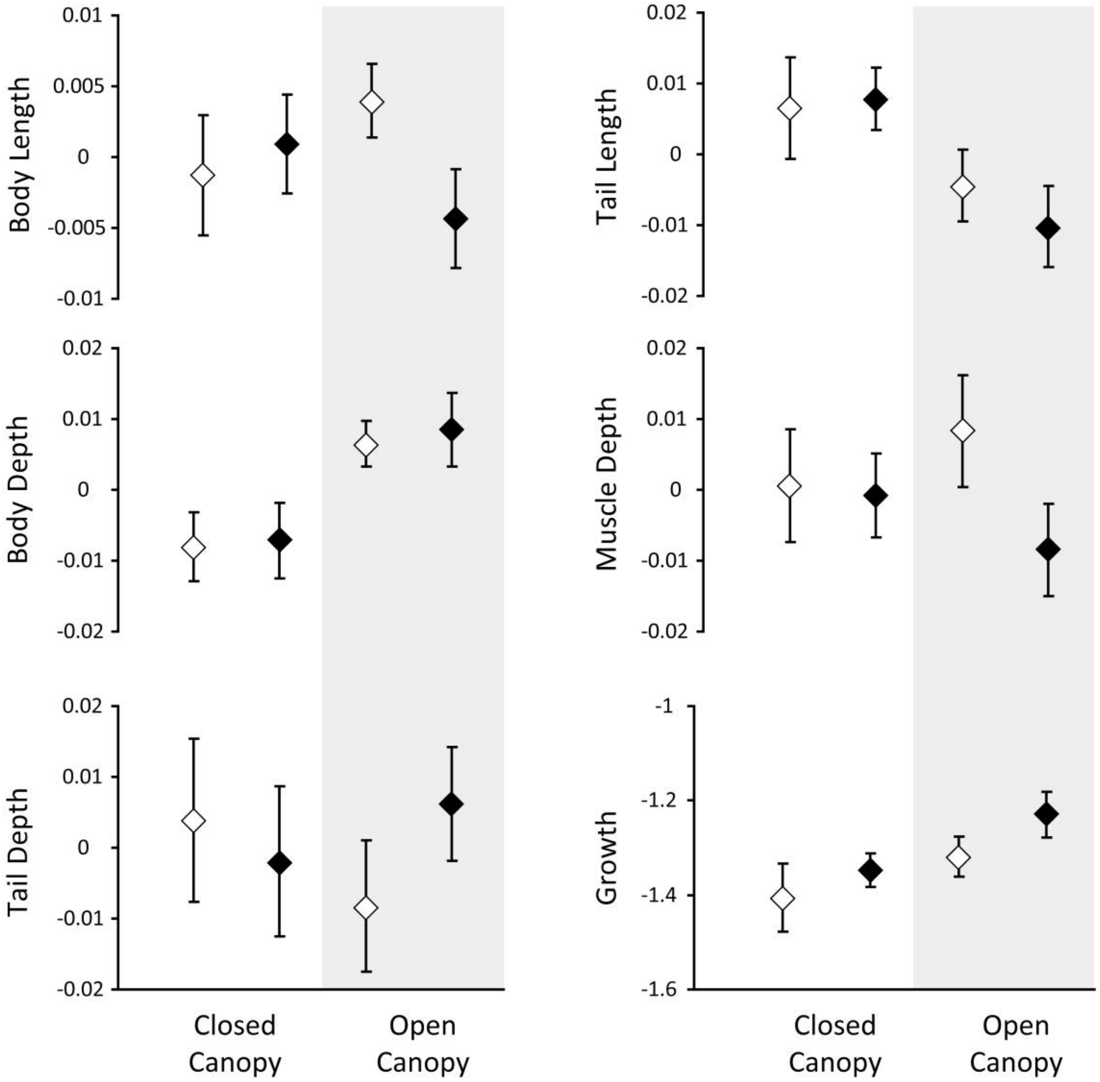

Figure 3.3. Trait differences among canopy types at day 18. Average morphological measurements for open-canopy and closed-canopy ponds separated by connectivity, with isolated (open symbols) and connected (solid symbols) ponds. Bars represent ±1 SE. Morphological measurements are the residual from the regression of ln morphological measurement over ln body weight. Growth is measured as ln body weight.

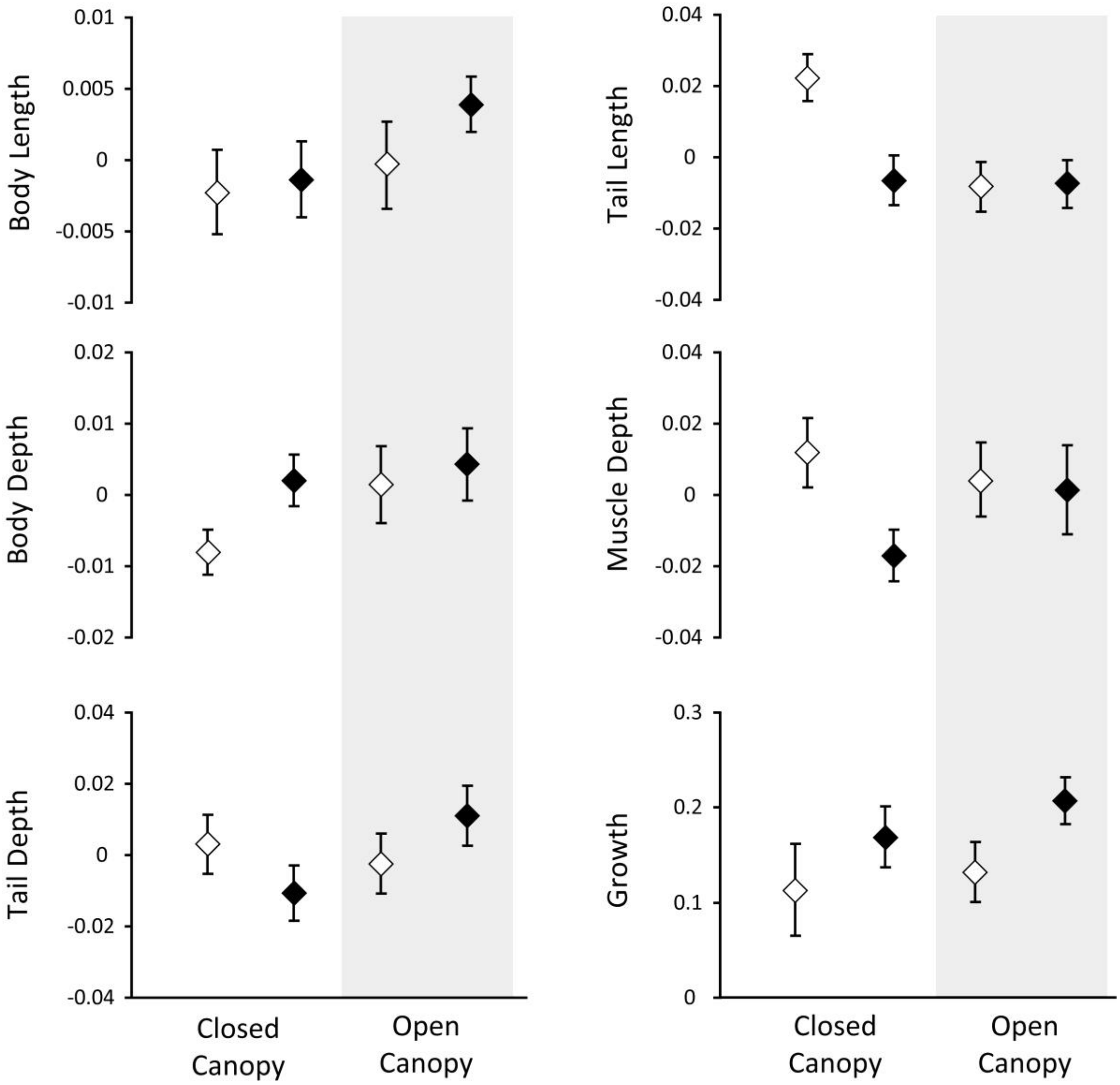

Figure 3.4. Trait differences among canopy types at day 37. Average morphological measurements for open-canopy and closed-canopy ponds separated by connectivity, with isolated (open symbols) and connected (solid symbols) ponds. Bars represent ±1 SE. Morphological measurements are the residual from the regression of ln morphological measurement over ln body weight. Growth is measured as ln body weight.

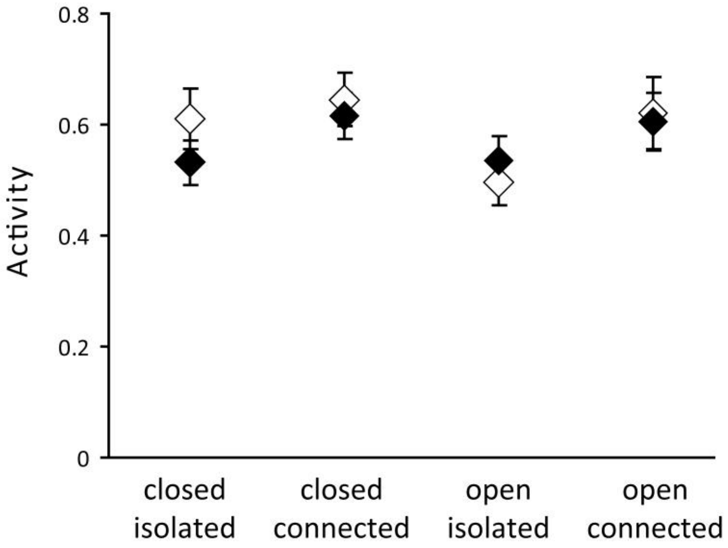

Figure 3.5. Behavioral differences among canopy and connectivity types. Average activity levels for open-canopy and closed-canopy ponds separated by connectivity, in the absence (open symbols) and presence of predator cue (solid symbols). Activity measures are the arcsine square root transformed proportion of larvae swimming per population averaged across ten observations. Bars represent ±1 SE.

Table 3.1. MANOVA results for effects of canopy and connectivity on the five morphological traits and growth combined at day 18. Response variables include all five morphological measures plus growth to day 18. Values are the Wilks' Lambda F statistic and associated *p* values.

Predictor Variable	**df**	***F***	***p***
model	6	3.4	< 0.001*
canopy	1	5.1	< 0.001*
connectivity	1	1.3	0.260
canopy*connectivity	1	1.1	0.357
block	3	4.5	< 0.001*
residual	57		

Table 3.2. MANOVA results for effects of canopy and connectivity on the five morphological traits and growth combined at day 37. Values are the Wilks' Lambda F statistic and associated *p* values.

Predictor Variable	**df**	***F***	***p***
model	6	1.8	< 0.005*
canopy	1	2.6	< 0.027*
connectivity	1	2.1	0.071
canopy*connectivity	1	1.2	0.322
Block	3	1.8	< 0.026*
residual	57		

Table 3.3. ANOVA results for the effects of canopy and connectivity on morphology at day 18. The trait values are residuals from the natural log transformed trait size regressed against the natural log transformed weight of each individual, to control for the effects of body size on trait size. The residuals were then average across ten individuals from each population.

Predictor Variable		**Response Variable**									
		body length		**tail length**		**body depth**		**muscle depth**		**tail depth**	
	df	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
canopy	1	0.0	0.994	6.1	0.017*	9.8	< 0.003*	0.0	0.999	0.1	0.793
connectivity	1	0.9	0.338	0.1	0.707	0.1	0.768	2.5	0.123	0.3	0.569
canopy*connectivity	1	2.8	0.100	0.4	0.549	0.0	0.899	1.7	0.195	1.8	0.185
block	3	5.6	< 0.002*	0.0	0.998	0.3	0.833	11.2	< 0.001*	15.6	< 0.001*
residual	57										

Table 3.4. ANOVA results for the effects of canopy and connectivity on five morphological traits at day 37. The trait values are residuals from the natural log transformed trait size regressed against the natural log transformed weight of each individual, to control for the effects of body size on trait size. The residuals were then average across ten individuals from each population.

Predictor Variable		**Response Variable**									
		body length		**tail length**		**body depth**		**muscle depth**		**tail depth**	
	df	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
canopy	1	1.7	0.196	6.3	< 0.016*	1.9	0.179	0.3	0.575	1.0	0.318
connectivity	1	0.9	0.349	5.0	< 0.030*	2.2	0.141	2.6	0.110	0.0	0.987
canopy*connectivity	1	0.4	0.539	5.5	< 0.023*	0.7	0.405	1.8	0.191	2.8	0.098
block	3	0.5	0.713	4.3	0.008*	1.6	0.207	2.5	0.067	1.8	0.150
residual	57										

Table 3.5. ANOVA results for the effects of canopy type and connectivity on behavior of larvae in both the presence and absence of caged predators. The response variable is the arcsine square root transformed proportion of larvae swimming per population averaged across ten observations.

Predictor Variable	**df**	***F***	***p***
predator	1	0.4	0.508
canopy	1	1.6	0.210
connectivity	1	6.2	< 0.015*
predator*canopy	1	0.9	0.349
predator*connectivity	1	0.0	0.967
canopy*connectivity	1	0.5	0.480
predator*canopy*connectivity	1	0.5	0.469
block	3	9.2	< 0.001*
residual	117		

Table 3.6. ANOVA results for effects of canopy type and level of connectivity on growth of larvae at day 1, day 18, and day 37. Growth is measured as the natural log transformed mass averaged across ten individuals within each tank.

Predictor Variable	**Day 1**			**Day 18**			**Day 37**		
	df	***F***	***p***	**df**	***F***	***p***	**df**	***F***	***p***
canopy	1	0.7	0.436	1	3.7	0.061	1	0.6	0.434
connectivity	1	0.1	0.790	1	1.9	0.171	1	3.3	0.076
canopy*connectivity	1	0.1	0.758	1	0.2	0.663	1	0.1	0.792
block				3	0.3	0.826	3	0.1	0.987
residual	11			57			57		

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CHAPTER 4

CONSEQUENCES OF INBREEDING AND OUTBREEDING FOR POPULATION-LEVEL FITNESS CORRELATES IN AN AMPHIBIAN WITH LOCAL ADAPTATION

Abstract

Inbreeding as a result of habitat loss can have negative fitness effects for individuals, but the consequences of outbreeding are less clear. Outbreeding may have either positive effects through heterosis or negative effects, as in the case of outbreeding depression. I evaluated the effects of inbreeding and outbreeding on population-level fitness correlates in the wood frog, *Rana sylvatica*, a species that shows evidence of adaptation to local environmental conditions at a relatively small spatial scale. The extent of inbreeding was evaluated across 51 populations in relation to the total amount of available habitat around each pond. In addition, population size was measured for 30 populations and larval survivorship was evaluated in the field and in a common-garden experiment across a subset of the populations, ranging from outbred to inbred. While the results corroborate the long-held view that high levels of inbreeding negatively impact populations, the results also suggest that outbreeding can be an important concern and in fact that slight levels of inbreeding can actually be beneficial. Populations with low levels of inbreeding had larger population sizes and increased survivorship in the common garden experiment than more inbred and outbred populations and had increased survivorship in the field relative to more outbred populations. The results point to a potential role of local adaptation as a mechanism of outbreeding depression. This study provides an empirical example of the effects of natural levels of outbreeding on population-level fitness correlates and suggests that in some cases the effects of outbreeding depression may be as ecologically relevant as the effects of inbreeding depression.

Introduction

Rapid worldwide changes to landscape patterns over the last several hundred years have affected connectivity among populations through fragmentation and isolation. Such changes have important consequences for population-level inbreeding and outbreeding, which in turn affect the likelihood of population persistence. The detrimental effects of inbreeding on individual fitness are well documented (e.g. Husband & Schemske 1996; Ralls *et al.* 1988) and have been implicated in the decline and extinction of many species (Frankham 2005). Inbreeding depression is thought to result in reduced fitness because of increased exposure of deleterious alleles and loss of heterozygosity at overdominant loci (Charlesworth & Charlesworth 1987), whereas outbred populations should benefit from the opposite and instead show heterosis or hybrid vigor. Yet, outbreeding can also have negative consequences for fitness as a result of genetic incompatibilities, such as when there is disruption of local adaptation (Templeton 1986), underdominance (Schierup & Christiansen 1996), or epistatic interactions (Waser 1993). Although there are a few well-known examples of the negative effects of outbreeding depression from intentional population crosses (e.g. Brannon 1967; Greig 1979), few examples from natural populations exist (except see: LeBas 2002; Marshall & Spalton 2000) and in general there is a lack of knowledge about the effects of outbreeding relative to what is known about inbreeding (Edmands 2007).

The link between individual and population-level fitness consequences of inbreeding and outbreeding are also little studied. While individuals are known to suffer reduced reproductive fitness as a result of inbreeding depression (Frankham 1995) and outbreeding depression (Edmands 1999; Lynch 1991), reduced individual fitness does not necessarily translate to decreased population growth rates (Lande 1988). One reason for this non-intuitive result is the distinction between hard and soft selection (Wallace 1975) ─ population declines will only occur if selection on inbred individuals leads to density independent mortality (i.e. hard selection). As a result, the effects of inbreeding on population fitness remain a point of contention in the literature, while the effects of outbreeding on population fitness have been little studied.

Here I evaluate inbreeding and outbreeding across a system of wood frog breeding populations and the consequences for population-level fitness correlates. Wood frogs offer an excellent opportunity to assess the interplay between inbreeding and outbreeding and the consequences for population fitness. This species occupies two ecologically divergent habitats, open- and closed-canopy ponds, and these habitats are juxtaposed across the landscape such that gene flow among habitats is possible. At the same time, this species has also faced high levels of habitat loss and fragmentation across parts of its range, and previous work suggests that gene flow among populations is sensitive to this habitat fragmentation (Zellmer & Knowles 2009). As a result, isolation provides an opportunity for populations to reach their adaptive optima and hence have increased population-level fitness. Yet, the increased isolation may instead result in high levels of inbreeding and potential decrease in population fitness.

Using an integrative approach, combining field survey data, a common-garden experiment, geographic information systems, and genetic data, I assessed the combined effects of inbreeding and outbreeding. To characterize the degree of inbreeding and outbreeding across wood frog populations, I first evaluated the effects of habitat loss on the extent to which populations are inbred or outbred by comparing population inbreeding coefficients to the amount of available habitat around each pond. To determine whether inbreeding and outbreeding affect average fitness within populations, I compared estimates of larval survivorship and population sizes in the field to population inbreeding coefficients. In addition, I conducted a common-garden experiment to assess the effects of inbreeding on survivorship through metamorphosis to control for environmental differences among populations, and in particular to control for differences in density.

Methods

Inbreeding

Inbreeding levels were estimated based on multilocus genotypes of approximately 20 larvae for each of 51 populations collected during the spring of 2005 and 2006, using nine microsatellite loci (Zellmer & Knowles 2009). Inbreeding was calculated as the population inbreeding coefficient, F_{IS}, using GENEPOP v.3 (Raymond & Rousset 1995), where values greater than zero indicate more heterozygotes than expected by Hardy-Weinberg Equilibrium (i.e. higher inbreeding) and values less than zero indicate fewer heterozygotes than expected (i.e. higher outbreeding).

Since patterns of inbreeding and outbreeding can be obscured by the presence of null alleles, stutter, and large allele dropout, we assessed the data for the presence of these genotyping issues using MICROCHECKER v. 2.2.0 (Van Oosterhout *et al.* 2004). The genotyping errors can be identified because they leave distinctive allelic patterns that differ from the effects of inbreeding and outbreeding. While both null alleles and inbreeding should lead to a heterozygote deficiency, null alleles should leave a pattern of heterozygote deficiency across many populations for a single locus, whereas inbreeding should lead to heterozygote deficiency across many loci in a few populations. Stuttering is identified by a lack of heterozygotes that differ in a single repeat and also by a relative excess of homozygotes in large allele classes. Large allele dropout is identified by an excess of homozygotes in either extreme of allele classes and when there is homozygote excess and the allelic range is greater than 150 base pairs.

Breeding Population Size

Breeding population sizes were estimated for 30 populations by counting the number of egg masses within ponds. Ponds were visited throughout the breeding season to locate choruses. Following breeding, the number of egg masses at each chorus was counted (by A. Zellmer for 15 ponds and Werner et al. for 13 ponds). Since females lay only one egg mass and typically only one male mates with each female (Halverson *et al.* 2006; Howard & Kluge 1985), the size of the breeding population is expected to be approximately twice the number of egg masses. Since the number of eggs masses per pond can vary widely from year to year, it was necessary to determine whether the results were consistent across years. While we did not have multiyear data for the entire data set, we were able to assess the correlation between the number of egg masses per pond in 2008 to the average number per pond over 12 years (1998-2009) for a subset of the ponds (13 out of 29 ponds, Werner et al. unpublished data). There was a strong correlation between egg masses in 2008 compared to the average (Spearman's Rho: 0.7868, p = 0.0014), suggesting that the results based on egg mass numbers from 2008 are robust to yearly variation.

Pond characteristics

Pond area was estimated for all 29 populations in which egg masses were counted. The pond area was calculated by tracing a polygon around the perimeter of each pond using satellite images from March 2005 (Google Earth, 2005). The area of each polygon was calculated using KML Toolbox (Zonums, 2007). Ponds were also classified as either open or closed canopy by visual estimates of canopy cover as described in Chapter 2.

Landscape classification

The landscape surrounding each pond was classified into habitat, non-habitat and rivers and lakes by calculating percent land cover of each of these cover types within a 200 m (estimated home range size: Porej et al. 2004) radius buffer around each pond using the buffer function in the ArcGIS analysis toolbox. Habitat included forests and wetlands whereas non-habitat included agricultural and urban areas as well as rivers and lakes. The percent of available habitat was normalized through arcsine square root transformation for all parametric analyses.

Survivorship

Larval survivorship was evaluated under both common-garden mesocosm settings (in collaboration with J. Middlemis Maher) as well as in natural ponds (as part of the long-term ecological research survey on the University of Michigan's Edwin S. George Reserve (ESGR) by Werner and colleagues, see below). For the common-garden experiment, individuals from six ponds across a gradient from inbred to outbred were raised in small outdoor tanks in the spring of 2009. Ten egg masses were collected from each of six open-canopy ponds using the same methods described in Chapter 2. Since the presence of environmental stress is thought to interact with inbreeding in its effect on population fitness and extinction risk (Bijlsma *et al.* 1999; Coltman *et al.* 1999; Crnokrak & Roff 1999; Reed *et al.* 2007), larvae were raised under both low- and high-stress conditions, either in the absence or presence of a non-lethal, caged predator, respectively. The experiment was replicated across four spatial blocks for a total of 48 mesocosms (6 populations x 2 treatments x 4 spatial blocks). Each mesocosm was filled with aged well water, inoculated with zooplankton, filtered pond water (to initiate phytoplankton growth), supplemented with approximately 300 g of leaves (to serve as a substrate for phytoplankton), and covered with shade cloth (to prevent colonization). A predator cage consisting of plastic tubing sealed by fine mesh on both ends was inserted into each mesocosm; however, only the mesocosms with the predator treatment contained a predator. Larval dragonflies (*Anax* spp.) were used as the predator, since caged *Anax* are known to have strong effects on behavior, morphology, and survivorship of wood frog larvae (e.g. Anholt *et al.* 2000; Anholt & Werner 1998; Relyea 2000) and because they induce increased stress hormone levels (Middlemis Maher, unpublished data). The predators were fed every three days with conspecific larvae to create the cue that is responsible for these effects (Fraker et al. 2009). In addition, each mesocosm was supplemented with rabbit chow *ad libitum*. Individuals were collected following metamorphosis, and survivorship was calculated as the number of individuals that survived through metamorphosis divided by the number of individuals at the beginning of the experiment.

For the field study, larval survivorship was estimated from demographic data collected by the ESGR survey for 13 ponds (E. Werner, R. Relyea, D. Skelly, K. Yurewicz, and C. Davis unpublished data). Larval survivorship was calculated by subtracting the larval population size in May (prior to metamorphosis) from the estimated number of eggs laid in each pond. The number of eggs per pond was estimated for each pond by multiplying the estimated average number of eggs per egg mass (696 eggs) to the number of egg masses in a pond. This value is close to recent estimates of average numbers of eggs per egg mass, and well within the variance (730 ± 190 S.E., Benard, in prep). The larval population size was estimated by calculating the density of larvae (see Werner et al. 2007 for further details).

Analyses

The effect of habitat loss on inbreeding was evaluated by comparing the population inbreeding coefficient to the amount of forested habitat surrounding each pond at 200m. The relationship was evaluated using Spearman Rank Correlation, since the data were not normally distributed. The most inbred population was identified as an outlier (> 1.5 times the interquartile range and $z = 3.671$), and thus the analyses were repeated without this population. After removing the outlier, the inbreeding coefficient was normally distributed, as confirmed by a skewness and kurtosis test as well as a Shapiro-Wilks' test for normality. Consequently, linear regression was used to evaluate the relationship between inbreeding and habitat loss.

Negative binomial generalized linear models were used to evaluate the relationship between the number of egg masses per pond and inbreeding. Both a linear and nonlinear relationship with inbreeding were evaluated. Pond area and canopy type were also included, since these are likely to influence number of egg masses as well (Werner et al. unpublished data). Log-likelihood calculations were based on the Laplacian approximation. The alternative models were compared using Akaike's Information Criterion (AIC; Akaike 1973) with a second order correction for small sample size (AICc) (Burnham & Anderson 2002). AIC was chosen for model evaluation over QAIC (quasi-AIC) since there was no evidence for overdispersion in the global model (based on the recommendations outlined in Bolker *et al*. 2009). For each model, the difference in AICc from the model with the lowest AICc (ΔAICc) was also calculated to assist in model selection.

To assess the effect of inbreeding on larval survivorship in a common-garden, survivorship was evaluated using a binomial logistic regression model. Both a linear and nonlinear relationship with inbreeding were evaluated. Predator treatment and block were also included in both models. The alternative models were evaluated for significance using Wald χ^2 tests and compared using AICc. For the field survey, linear regression was used to assess the effects of outbreeding since none of the populations sampled had high levels of inbreeding. Normality was confirmed with a skewness and kurtosis test and a Shapiro-Wilks' test. Furthermore, since both open and closed canopy ponds were surveyed and because closed-canopy ponds are known to have lower survivorship than open-canopy ponds (Werner & Glennemeier 1999), the effects of canopy type and outbreeding were evaluated using ANCOVA. All statistical analyses were completed using State Intercooled v. 10.1.

Results

Genotyping Results

There was no evidence of stuttering or large allele dropout in any population for any locus. For seven of the nine loci, there was evidence for null alleles in less than five percent of the populations. Two loci (D88 and 1A11) showed greater evidence for null alleles, but the presence of null alleles was still limited to a fraction of the populations

(12 and 18 percent of populations, respectively). For the locus that showed the most evidence for null alleles (1A11) we removed this locus and reran the analyses. Without locus 1A11, the results were generally the same and in most cases were actually improved over the analyses that included that locus (except for larval survivorship in the field), suggesting that the presence of null alleles in that locus is not responsible for the patterns of inbreeding observed in this study.

Effects of Habitat Loss

Inbreeding was correlated with amount of forest/wetland habitat available, with higher inbreeding in areas with less available habitat (linear regression: R = 0.298, $p = 0.035$; Figure 4.1). Although there was still a negative trend between the population inbreeding coefficient and the amount of available habitat after inclusion of the outlier population, the relationship was not significant (Spearman's rho = -0.190, $p = 0.181$). Most of the highly inbred populations occurred only at the lowest levels of available forest and wetland habitat.

Inbreeding and Population Size

The model with the lowest AICc value included pond area and both inbreeding and inbreeding^2. (Table 4.1). Only one other model, which included pond area and inbreeding^2, had a ΔAICc less than 2 (Table 4.2). The number of eggs increased with increasing pond area and peaked at intermediate values of inbreeding (Figure 4.2).

Common Garden Experiment

The model with the highest support based on AICc included inbreeding, inbreeding^2, block, and an interaction between inbreeding and predator. Survivorship was highest at intermediate inbreeding levels (Figure 4.3). Additionally, inbreeding had a greater effect on survivorship in the no-predator treatment compared to the predator treatment (Figure 4.3). None of the other models had a ΔAICc less than 2; however, all significant models included inbreeding^2.

Field Survey

None of the populations surveyed in the field for survivorship exhibited high levels of inbreeding. I therefore only evaluated the effects of outbreeding on larval survivorship. One pond (Big Island) was removed from the analyses because the cohort went extinct due to non-genetic reasons (i.e. the pond dried before metamorphosis occurred). There was a significant effect of canopy type on survivorship ($F = 8.78$, $p < 0.016$; Figure 4.4). There was also a marginally significant trend between the inbreeding coefficient and survivorship, with lower survivorship in outbred populations ($F = 4.23$, $p < 0.070$; Figure 4.4). The effects of canopy type and inbreeding on larval survivorship were unaffected by inclusion of Big Island.

Discussion

Inbreeding resulting from habitat loss is often cited as a major concern for conservation, and the detrimental effects of inbreeding have been documented in many systems. While the results presented here corroborate this long-held view, the results also suggest that outbreeding can be an important concern and in fact that slight levels of inbreeding can actually be beneficial. Both wood frog population sizes and larval survivorship in the common garden experiment showed a non-linear relationship with inbreeding (models with lowest AICc scores included inbreeding^2; Tables 4.1-4.2). Wood frog populations with low levels of inbreeding had increased population sizes (Figure 4.2) and increased larval survivorship in the common garden experiment (Figure 4.3) relative to more inbred and outbred populations. In addition, populations with low levels of inbreeding showed a trend for increased larval survivorship in the field relative to more outbred populations (Figure 4.4). Interestingly, larval survivorship in the field and the common-garden experiment as well as population size in the field all peaked at approximately the same level of inbreeding (F_{IS} ~ 0.01-0.04). Together, these results suggest that both high levels of inbreeding and outbreeding can negatively impact population-level fitness correlates.

Few populations studied exhibited high levels of inbreeding despite the wide range of habitat loss evaluated (Figure 4.1). Our results suggest that survivorship and population size are reduced in highly inbred populations. The lack of populations that are severely inbred and in patches with little habitat availability of itself suggests that populations may not be viable under such conditions. Previous research has suggested the importance of forested habitat for wood frogs and has demonstrated an absence of wood frog populations below critical thresholds of between 10-30% forest cover at a similar scale, below which few populations are found (Gibbs 1998; Hecnar & M'Closkey 1998; Homan *et al.* 2004). Indeed, the most inbred population that was observed (Earth Art, F_{IS}

= 0.172) went extinct prior to collecting egg data in 2008 (no egg masses were found in 2008). Similarly, high levels of larval mortality were observed in the second most inbred population (Zeeb Powerline, F_{IS} = 0.107) in a previous study (Chapter 3) in comparison to populations with less inbreeding. While it is unclear what mechanisms caused the extinction and the increased mortality, the lack of inbred populations observed and the extinction of the most inbred population suggests that such populations are not viable. Future research will be necessary for addressing the consequences of inbreeding for individual and population fitness of wood frogs.

Mechanisms of outbreeding depression

While outbreeding depression can arise from a variety of mechanisms, including disruption of local adaptation (Templeton 1986), underdominance (Schierup & Christiansen 1996), or epistatic interactions (Waser 1993), the results suggest a role for disruption of local adaptation in reduced population-level survivorship. First, local adaptation plays a key role in generating differences among wood frog populations (Relyea 2002). Yet, gene flow continues among open- and closed-canopy populations (Chapter 2), setting up the potential for disruption of local adaptation. If disruption of local adaptation is responsible for differential survivorship among populations, then we expect to see higher levels of selection in outbred populations, since outbred populations should be incurring a strong migration load (Bolnick & Nosil 2007). In fact, we did observe reduced average survivorship in outbred populations relative to populations with low levels of inbreeding in both the field and in the common-garden experiment. As a result of strong selection on hybrids or migrants, populations may not show any long-term changes in trait means across generations (Bolnick & Nosil 2007). This is confirmed by previous research, which suggests that, at least in some traits, divergence among populations is possible despite the presence of gene flow (Chapter 3). The reduced survivorship in outbred populations in addition to the patterns of divergence with gene flow, suggest that disruption of local adaptation is a primary mechanism driving patterns of outbreeding depression.

The reduced survivorship of outbred populations in the mesocosms however suggests that other genetic mechanisms, such as underdominance or epistatic interactions, also contribute to survivorship differences among populations. Disruption of local adaptation could account for some of the reduced survivorship seen in outbred populations in the common garden experiment, since the mesocosms were designed to replicate open-canopy pond conditions (e.g. predator presence and high sun exposure) and only open-canopy populations were used for this experiment. The reduced survivorship in outbred populations would thus be due to maladaptation to open-canopy pond conditions. This is further supported by the observed reduction in survivorship across all populations in the no-predator treatment (Figure 4.3b), which more closely resembles the conditions experienced in closed-canopy ponds. However, because it is unlikely that we were able to replicate exact open-canopy pond conditions, disruption of local adaptation likely does not explain all of the variance in survivorship among populations in the common garden experiment. Further research will be necessary to determine the relative contributions of these mechanisms to outbreeding depression in wood frogs.

Additionally, the reduction in larval survivorship in both the field and the common-garden experiment could result from maternal effects that may occur as a result of differences among mothers from outbred and inbred populations. Outbreeding is often due to migration among nearby populations. If migration gives a mother a disadvantage relative to resident mothers, such that she has reduced resources to provision to her offspring, then we may see a similar pattern where there is reduced larval survivorship in outbred populations. This may occur in wood frogs since outbred populations are often those that are recently recolonized (linear regression population age (time since last one-year extinction) versus F_{IS}: R = 0.577, $p = 0.019$). However, previous research has failed to find significant differences in hatchling size (an important potential maternal effect) in relation to the inbreeding coefficient (Zellmer, unpublished data). Similarly, variation in numbers of eggs per egg mass among populations could have impacted the estimates of survivorship in the field, particularly if there are fewer eggs per egg mass in outbred populations than more inbred populations. However, this pattern would not have influenced survivorship in the mesocosm, suggesting that the lower survivorship estimates in outbred populations in the field are not due to differences across populations in our ability to estimate survivorship. Further research is required to evaluate the relative contribution of the different mechanisms (genetic, environmental, and maternal effects) driving reduced fitness in outbred wood frog populations.

Empirical evidence for the effects of inbreeding depression far outweigh evidence for outbreeding depression (Edmands 2007), and in fact, traditionally the effects of inbreeding have been thought to be much stronger than those of outbreeding (Lacy et al. 1993; Sheffer et al. 1999). However, more recent research suggests that the consequences of inbreeding and outbreeding may be of similar magnitude (Marshall & Spalton 2000), and outbreeding may even be more detrimental and/or demographically relevant than inbreeding (LeBas 2002). The results presented here demonstrate the importance of considering the fitness consequences of outbreeding and the balance between gene flow and selection. The significant effects of outbreeding may also shed light on the long standing debate regarding the effects of inbreeding on population fitness (Keller *et al*. 2007), which has been partially fueled by the lack of a positive relationship between population heterozygosity and a number of fitness correlates in many species (Britten 1996; Chapman et al. 2009). Such a result may occur if the negative consequences of outbreeding are not considered. Lastly, there are few examples of the fitness consequences of natural levels of outbreeding (except see: LeBas 2002; Marshall & Spalton 2000). These results therefore provide an important empirical example of the effects of outbreeding depression.

Effect of stress environment

The presence of predators affected survivorship and altered the effects of inbreeding on survivorship in the common garden experiment. However, the effects were opposite of that which was expected: lower survivorship was observed in the no-predator treatment.

While we expected predators to represent an increased stress for wood frog larvae, the presence of predators is also known to reduce activity levels of wood frogs (e.g. Anholt *et al.* 2000), which can reduce competition (e.g. Werner & Anholt 1996) and potential alleviate stress. As a result, the no-predator environment may actually represent a higher stress situation due to competition. If this is the case, then the results support the hypothesis that environmental stress exacerbates the effects of inbreeding depression, suggesting that hard selection is operating on inbred individuals. The presence of hard selection is important, because it would suggest a possibility for inbreeding to cause population declines (Keller *et al*. 2007). Future research will be necessary to examine the extent to which these alternative environments correspond to a stressful environment and by what mechanism those differences affect survivorship. Regardless, the results demonstrate that the environmental context is important for the effects of inbreeding, but not outbreeding, on larval survivorship.

Long-term demographic consequences of outbreeding depression

The effect of outbreeding on larval survivorship within populations suggests a potential for outbreeding to have long-term demographic consequences as well, as is suggested by the reduced sizes of outbred populations. However, the causal relationship is difficult to disentangle, since population size may also affect the level of inbreeding. While reduced population sizes can lead to inbreeding, they are not expected to lead to outbreeding. Thus, the relationship between outbreeding and reduced population size seen in the field and in the common garden experiment is likely a consequence – not a cause – of outbreeding. While the effects of outbreeding on population size are only suggestive of reduced population fitness (i.e. population growth rate), stage-structured demographic models of wood frog populations show that larval survivorship contributes to variation in adult female population size and predicts that extinction-risk over a 100-year period can be substantially raised by as little as a 10% decrease in larval survival (Benard et al., unpublished data using Kendall and colleagues' (1999) method for evaluating the fit of stochastic models to time series data). If larval survivorship does translate to reduced population growth rates, then this might suggest the presence of source-sink dynamics in wood frog populations, with outbred populations representing demographic sinks.

Effects of habitat loss

The results suggested a relationship between habitat loss and inbreeding (Figure 4.1). The lack of a strong correlation was partially due to high variance in inbreeding values for populations with the lowest amount of available habitat. This variance could be explained if populations with reduced habitat are strongly affected by stochastic demographic events that result in either inbreeding or outbreeding. How reduced habitat availability can lead to inbreeding is straightforward; reduced population sizes and restricted gene flow lead to loss of heterozygosity. However, when migration events do occur, these same isolated, low-habitat populations are more likely to be swamped by immigrants, resulting in high outbreeding levels. Variance in inbreeding and outbreeding in small populations is especially likely in systems with high levels of gene flow, as in the wood frogs.

Conservation implications

Conservation biologists are often faced with the difficult decision whether to risk the negative effects of outbreeding depression and artificially increase gene flow among populations in order to prevent inbreeding depression. While traditionally the benefits of outbreeding have been thought to far outweigh the negative consequences (Lacy et al. 1993; Sheffer et al. 1999), the results presented here demonstrate that in some cases this might not be true. The results provide an estimate for the optimal level of inbreeding necessary to maintain fitness within wood frog populations. For other species, outbreeding should not be considered as a conservation strategy, unless source population type is taken into account and test crosses among populations are done first. In addition, conservation biologists should manage landscapes such that they preserve natural levels of connectivity among populations, rather than maximizing connectivity. This strategy will assist not only in maintaining optimal levels of inbreeding, but will also protect the genetic integrity of locally adapted ecotypes. This is important for long-term persistence of species in the face of rapid anthropogenic environmental changes, as the increased levels of genetic and phenotypic diversity across the species will allow for evolutionary responses to changing environmental conditions.

Conclusions

Although the negative consequences of inbreeding depression have traditionally been thought to far outweigh the effects of outbreeding depression (Lacy et al. 1993; Sheffer et al. 1999), the results presented here demonstrate that the effects of outbreeding depression in some cases may be highly relevant to population as well. Populations with low levels of inbreeding showed increased larval survivorship in a common garden and increased population sizes relative to more outbred and inbred populations. In addition, populations that were slightly inbred showed a trend for increased larval survivorship in the field relative to more outbred populations. The reduced survivorship may be a result of a disruption of local adaptation, since wood frog populations show evidence of adaptation to open- and closed-canopy ponds over small spatial scales (Relyea 2002). Conservation biologists will need to consider the negative consequences of outbreeding depression when designing strategies for maintaining genetic diversity within populations.

Acknowledgements

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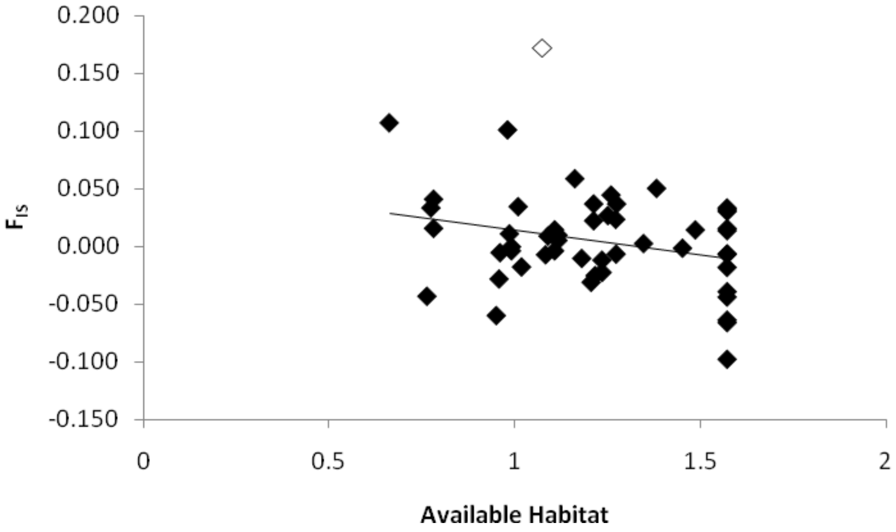

Figure 4.1. Inbreeding in relation to the amount of forest and wetlands available within 200 m of the pond. The correlation was significant with the outlier population (open symbol) removed (linear regression: R = 0.298, *p* = 0.035), but not significant when included (Spearman: p > 0.05). Available habitat is measured as the arcsine square root transformed proportion of forest and wetlands within a 200 m buffer around each pond.

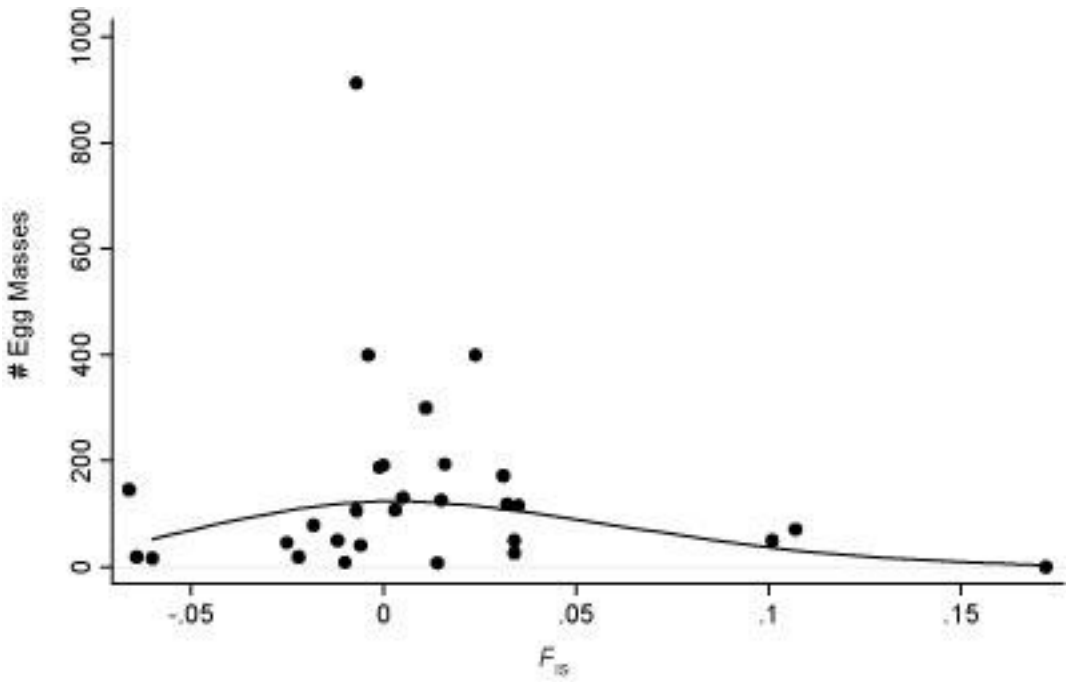

Figure 4.2. The number of egg masses per pond as a function of the population inbreeding coefficient. The line shows the predicted relationship between the number of egg masses and inbreeding from the best-fit model, which included pond area, inbreeding, and inbreeding^2 (lowest AICc score).

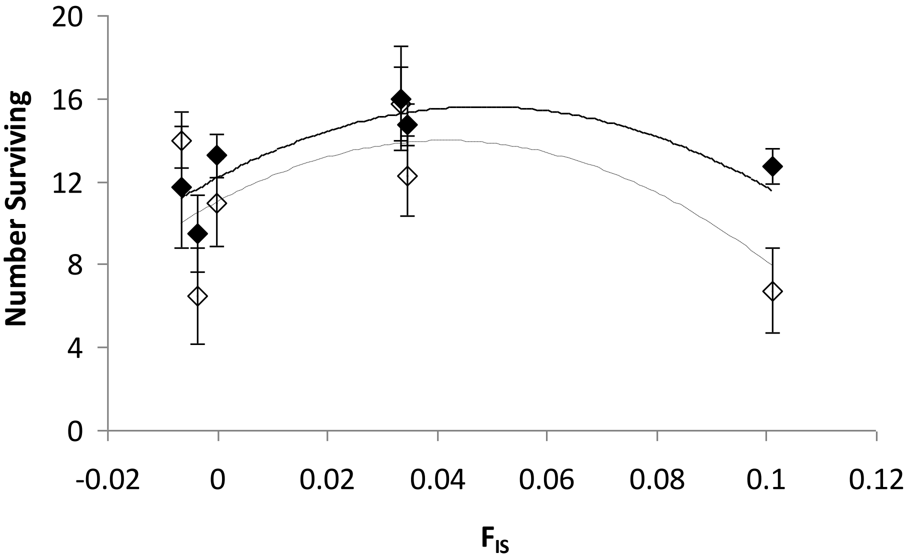

Figure 4.3. Total number of larvae surviving to metamorphosis in the common-garden experiment. Larval survivorship increases with increasing population inbreeding coefficient, from more outbred to more intermediate inbreeding levels. Lines represent predicted values from the best-fit model (lowest AICc), including inbreeding, inbreeding^2, inbreeding by predator interaction, and block. Symbols show average survivorship for six open-canopy populations under two different treatments: high stress, non-lethal predator (solid symbols, solid lines) or low stress, no predator (open symbols, dashed lines). Bars represent ±1 SE.

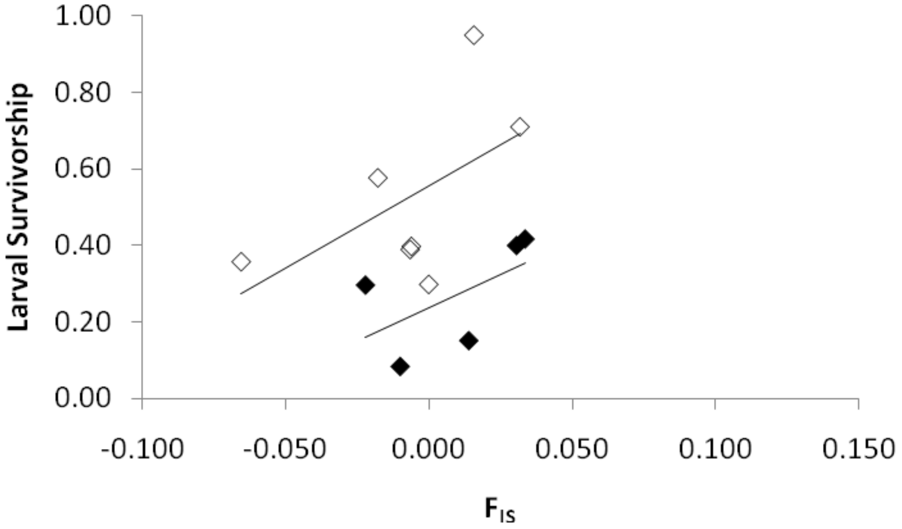

Figure 4.4. Larval survivorship measured in the wild. Larval survivorship increases from outbred to intermediate levels of inbreeding for both open (open symbols) and closed (closed symbols) canopy ponds. None of the ponds sampled showed high levels of inbreeding, so only the effects of outbreeding could be evaluated. The overall model was significant (ANCOVA: F = 5.15, p = 0.032), and there was a significant effect of canopy (F = 8.78, p < 0.016). The relationship between inbreeding and survivorship was of the same magnitude and direction as the results seen in the common garden experiment, but was marginally non-significant (F = 4.23, p < 0.070).

Table 4.1. Results for alternative models of population size (number of egg masses) per pond. Alternative models are ranked according to AICc scores.

Model				**k**	**AICc**	**ΔAICc**
Area		Inb	Inb^2	4	329.9	0.0
Area			Inb^2	3	330.4	0.5
Area	Canopy	Inb	Inb^2	5	332.0	2.2
Area	Canopy		Inb^2	4	332.6	2.7
Area				2	332.6	2.8
Area	Canopy			3	334.6	4.8
Area		Inb		3	335.0	5.2
Area	Canopy	Inb		4	337.3	7.4
	Canopy		Inb^2	3	337.9	8.1
	Canopy	Inb	Inb^2	4	338.5	8.6
			Inb^2	2	339.3	9.4
		Inb	Inb^2	3	340.8	10.9
	Canopy			2	344.0	14.1
	Canopy	Inb		3	345.9	16.0
		Inb		2	348.3	18.5

*Inb: Inbreeding coefficient (F_{IS}), k: Number of parameters in the model.

Table 4.2. Results for alternative models of larval survivorship in the common-garden experiment using negative binomial general linear mixed models. Alternative models are ranked according to AICc scores. Significance of each model was evaluated using Wald χ^2 tests.

Model					**k**	**AICc**	**ΔAICc**	***p***
Inb	Inb^2	Block		Inb*Pred	5	337.8	0.0	<0.0001
Inb	Inb^2	Block	Pred	Inb*Pred	6	340.4	2.6	<0.0001
Inb	Inb^2	Block	Pred		5	340.6	2.8	<0.0001
Inb	Inb^2	Block			4	343.1	5.3	<0.0001
	Inb^2	Block		Inb*Pred	4	353.4	15.6	0.001
	Inb^2	Block	Pred	Inb*Pred	5	355.9	18.1	0.001
Inb		Block		Inb*Pred	4	361.3	23.5	0.078
Inb		Block	Pred	Inb*Pred	5	363.8	26.0	0.078
	Inb^2	Block	Pred		4	368.1	30.3	0.014
	Inb^2	Block			3	370.5	32.7	0.015
Inb		Block	Pred		4	373.8	36.0	0.563
Inb		Block			3	376.1	38.3	0.565

*Inb: Inbreeding coefficient (F_{IS}), Pred: Predator, k: Number of parameters in the model.

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CHAPTER 5

CONCLUSION

Implications

Understanding the ecological and evolutionary consequences of population connectivity remains an important area of research as well as a crucial aspect of conservation biology. Using an integrative approach – combining molecular tools, common-garden experiments, field surveys, and geographic information systems data – I explored the factors affecting population connectivity as well as the genetic and fitness consequences of changes in population connectivity. My research provides evidence that evolution occurs over short time scales and over small distances, and that genetic variation is influenced by ecological processes. Below I review and discuss the implications of results from each chapter separately and then integrate the results to provide the broader conclusions of my dissertation.

My research demonstrated that wood frog populations have naturally high levels of population connectivity, which are rapidly lost following habitat fragmentation (Chapter 2). By controlling for correlations among landscape structure from multiple time periods, I showed that patterns of genetic divergence reflect recent landscape structure as opposed to landscape structure prior to European settlement of the region (pre-1850). Gene flow among populations is limited by a combination of natural and anthropogenic barriers, including topographical relief, inhospitable water bodies (i.e. rivers and lakes), and urban and agricultural development. At the same time, within-population genetic diversity is relatively high with no evidence for population bottlenecks, suggesting that the rapid divergence of populations following habitat fragmentation may be due to metapopulation processes.

As a result of variation in connectivity among populations, I expected adaptive phenotypic divergence to increase in more isolated populations, because they are less likely to receive maladapted immigrants from other ponds. Using a common-garden experiment, I compared morphological, behavioral, and life historical differences among wood frog populations that are due to divergent environmental conditions in open- versus closed-canopy ponds that had either low or high connectivity to other populations. Overall, open- and closed-canopy ponds showed similar levels of divergence whether they had high or low connectivity, although there was variation across traits in the extent to which they showed divergence. (Chapter 3). These results suggest that selection within ponds must be strong such that divergence can occur despite gene flow.

While increased isolation can potentially have negative fitness consequences by exacerbating inbreeding, populations may also benefit from isolation by reducing the negative impacts of outbreeding. Indeed, my results showed evidence that wood frog populations with low levels of inbreeding had increased larval survivorship in a common garden experiment and increased population sizes relative to more outbred and inbred populations. In addition, populations with slight inbreeding showed a trend for increased larval survivorship in the field compared to more outbred populations (Chapter 4). These results suggest that benefits of isolation due to reduced outbreeding among populations exposed to divergent selection may outweigh the detrimental effects of inbreeding across natural levels of population connectivity.

The reduced survivorship seen in outbred populations (Chapter 4) suggests that the phenotypic differences among open- and closed-canopy ponds in areas of high gene flow (Chapter 3) may be maintained by strong selection acting against outbred individuals. This result points to disruption of local adaptation as a mechanism of outbreeding depression. Together, the results suggest that population connectivity in conjunction with high levels of divergent selection may provide a unique opportunity for adaptive evolution. Population connectivity may help maintain high levels of standing genetic variation despite strong selection within populations. High levels of standing genetic variation are thought to provide a greater opportunity for evolution than variation due to mutation alone, since evolution is expected to proceed faster from standing genetic variation (Barrett and Schluter 2008). The combination of gene flow among locally adapted populations with extinction and recolonization dynamics suggests that both evolutionary and ecological processes contribute to evolution. Moreover, these evolutionary changes may then influence ecological processes, as the reduced population sizes in outbred populations suggest a potential for local adaptation to contribute to variation in population growth rates and extinction risk among populations.

The results presented here also demonstrate the efficacy of using modern molecular tools for ecological research. The genetic structure of wood frog populations allowed for assessment of habitat features affecting dispersal as well as ecological population structure (i.e. metapopulation dynamics). Additional genetic tools are becoming available that will allow for even finer-scale appraisal of genetic structure, approaching scales that are more ecologically relevant. Since evolution can occur at small spatial and temporal scales, it is crucial that we continue to integrate ecological and evolutionary theory.

Conservation Implications

Habitat loss and fragmentation are two of the main threats to global biodiversity and have been implicated in the decline and extinction of numerous species across virtually all taxa (Fischer and Lindenmayer 2007). Amphibians, in particular, appear to be highly sensitive to the consequences of habitat fragmentation (Cushman 2006) and as a result have often been used as indicators of environmental health (Collins and Storfer 2003). Using a widespread species, such as the wood frog, to study the effects of habitat fragmentation is ideal, as it allows us to compare pristine versus fragmented populations and also provides an opportunity to do experiments and intensive fine-scale genetic analyses that would not be possible with an endangered, range-limited species. While the wood frog is not endangered, the results presented here demonstrate that the genetic and ecological consequences of habitat fragmentation affect even common species. As a result, these species may be at even more risk, since little attention is paid to them when considering the negative consequences of habitat destruction.

Population connectivity plays an important ecological and evolutionary role across wood frog populations, and this connectivity is sensitive to habitat fragmentation. Since both inbreeding and outbreeding depression can occur, it is necessary to maintain natural levels of population connectivity, especially for organisms like wood frogs that have strong local adaptation. In addition to conserving interpopulation variation, conservation biologists will also need to be aware intrapopulation variation when designing conservation plans. The negative consequences of outbreeding for population-level fitness correlates in wood frogs demonstrates that inbreeding depression should only be managed with outbreeding programs after test crosses between the target and source populations have been completed.

While it may be more efficient to preserve a few large tracts of land, preservation of multiple forest patches connected by corridors may provide a better opportunity to maintain genetic variation across a species. In addition to managing individual landscape features for maintaining population connectivity, my results show that the interaction of different landscape features must be considered for effective land management. For instance, the effect of forest and wetland loss on genetic connectivity of wood frog populations was only seen when both topographical relief and river barriers were considered in concert with habitat structure. While forest preservation should increase connectivity among populations, it may only be beneficial if preserved in appropriate locations where connectivity is feasible, such as in areas with little topographical relief. The presence of interactions among different features to population connectivity illustrates the importance of taking a holistic approach to landscape management in order for success.

Areas for Future Research

Future research will need to continue integrating the fields of ecology and evolutionary biology by using genetic patterns to elucidate ecological processes. One area of ecological research that will particularly benefit from this integration is metapopulation biology. Genetic population structure has been used as evidence of metapopulation dynamics across a variety of systems; however, similar patterns are often marshaled as evidence both for and against metapopulation structure. Researchers will need to focus on utilizing current genetic theory in addition to developing additional theory on the effects of extinction and recolonization dynamics of intra- and inter-population structure to fully make use of molecular methods for testing metapopulation hypotheses. One approach that may improve these techniques is a combination of summary statistics, simulations, and hypothesis testing to compare various models of demographic processes, similar to methods used in statistical phylogeography (e.g. Knowles and Maddison 2002), but for smaller-scale processes. More rigorous hypothesis testing will greatly improve our ability to discern among different ecological process contributing to genetic structure.

Another important area of research that stands to improve from novel molecular techniques is assessment of the interplay between intraspecific gene flow, plasticity, local adaptation, and adaptive plasticity in natural populations. Methods such as expression profiles will allow for assessment of these different processes jointly. A combination of expression profiles developed from both natural field populations and individuals raised under common garden conditions will provide an opportunity to assess how local adaptation, plasticity, and adaptive plasticity vary under different levels of gene flow (e.g. Cheviron et al. 2008). In addition, such methods would allow for further exploration of the consequences of habitat fragmentation on the ability of populations to evolve in response to changing environmental conditions.

Lastly, since outbreeding can have serious detrimental effects for individuals and populations, as suggested by the results presented in Chapter 4, future research should focus specifically on outbreeding depression, particularly with regard to its relative effects compared to inbreeding, and the consequences for local adaptation. Particularly needed are long-term demographic studies for understanding the effects of inbreeding and outbreeding on population dynamics and population viability. In addition, it will be necessary to distinguish among different mechanisms of outbreeding depression and determine their relative effects.

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