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# Effects of habitat fragmentation and water quality on wood frog population genetic structure in vernal pools

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# EFFECTS OF HABITAT FRAGMENTATION AND WATER QUALITY ON WOOD FROG POPULATION GENETIC STRUCTURE IN VERNAL POOLS

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ABSTRACT

EFFECTS OF HABITAT FRAGMENTATION AND WATER QUALITY ON WOOD FROG POPULATION GENETIC STRUCTURE IN VERNAL POOLS

By

Charlotte Gabrielsen

University of New Hampshire, September, 2011

Habitat fragmentation associated with suburbanization can have negative consequences on population persistence through the reduction of dispersal and concomitant gene flow. Using nine polymorphic microsatellite loci, I assessed the effects of forest fragmentation and water quality on the genetic structure of a vernal pool-breeding amphibian, the wood frog (*Lithobates sylvaticus*), across 20 ponds in an unfragmented, forested landscape and 45 ponds in a fragmented landscape. Analyses were performed at the broad-scale of the study area and at a fine-scale, with spatially independent clusters of ponds selected within each landscape. Bayesian clustering approaches and AMOVA identified little population structure at the scale of the study area. At the fine-scale analysis, however, BARRIER and maps of genetic divergence identified barriers associated primarily with roads and suburban development in the fragmented landscape, and with large bodies of water and elevation in the unfragmented landscape. Tests of isolation by distance and Mantel tests of road effects and genetic differentiation ($F_{ST}$) were significant in only one of the three clusters in the fragmented landscape. Spatial autocorrelation and calculation of mean parent-offspring dispersal distances indicated restricted dispersal in a different cluster of pools in the fragmented

landscape. Lastly, using the program GESTE, I tested the potential effects of water quality and hydroperiod on the observed genetic patterns and found that pH had a significant effect on structuring in the fragmented landscape, with higher pH resulting in increased genetic differentiation. The results of this study indicate that wood frog populations are well connected across the landscape of southeastern New Hampshire. However, I detected fine-scale population structuring associated with roads and other fragmenting features of suburbanization. Though inconsistent over clusters, this suggests that increased fragmentation might negatively impact wood frog populations in the future.

# CHAPTER 1

# INTRODUCTION

## Habitat Fragmentation and the Connectivity of Animal Populations

Habitat fragmentation, or the reduction of contiguous landscape into discontinuous, sometimes isolated, patches (Lord and Norton 1990), is an issue of primary conservation concern due to its potential to limit the dispersal of animal populations (*reviewed in* Cushman 2006). Among the causes of habitat fragmentation, human development is one of the most prevalent. Throughout the northeastern United States, the amount of forested land has been in decline as a result of anthropogenic disturbances of the landscape, including residential and urban development, agricultural land use, forestry management, and road development. Concurrently, the rate at which fragmentation occurs is accelerating as the human population grows. Since 1950, New Hampshire's population has doubled and is projected to rise to nearly 1.6 million by 2020, making New Hampshire one of the fastest growing states in the northeast (Sundquist and Stevens 1999). Although New Hampshire is currently the second most forested state in the nation, forested land in New Hampshire is in steady decline; between 1983 and 1997, net New Hampshire forestland decreased at a rate of 0.2% per year, with the percentage of forested area declining from 87% to 84% (Thorne and Sundquist 2001). Predictive modeling suggests that the extent of New Hampshire forests will decline to 80% cover by 2020 (Thorne and Sundquist 2001). The greatest reductions are anticipated to occur in the southeastern areas of the state (Thorne and Sundquist 2001), where this study was conducted. As forest cover decreases and suburban development increases, an

increasingly fragmented (patchy) landscape will result.

Metapopulation dynamics may arise between populations existing in patchy distributions across the landscape. Metapopulations are comprised of several local populations that interact via migration and gene flow (Hanski and Gaggiotti 2004). Within the context of a metapopulation, local populations may experience extinctions, but the metapopulation persists in the landscape due to successive recolonization of these populations (Levins 1969, Gilpin and Hanski 1991; Hanski and Simberloff 1997). When metapopulation dynamics play a role in long-term viability, conservation efforts limited to the protection of individual populations may be ineffective over the long term if connectivity among populations is not maintained (Compton et al. 2007). One potential source of loss of connectivity is fragmentation, which can lead to the patchy distribution of suitable habitat across the landscape and the populations therein. If fragmentation disrupts dispersal, recolonization may not be possible. Resulting variations in gene flow can influence the genetic structure of populations. In cases where populations become increasingly fragmented, such as in suburbanized areas, genetic diversity can decrease and populations can become differentiated (Hitchings and Beebee 1997; Rothermal 2004).

Roads are one source of fragmentation with the potential to alter metapopulation dynamics. Their diverse and systemic effects on many aspects of both terrestrial and aquatic ecosystems (Balkenhol and Waits 2009) contribute a suite of ecological effects such as road-kill, dispersal impairment, and contamination from runoff (Fahrig et al. 1995; Vos and Chardon 1998; Trombulak and Frissell 2000; Gibbs and Shriver 2005; Karakker et al. 2008; Balkenhol and Waits 2009). Roads can lead to an immediate loss of

suitable habitat, both in the actual area they cover and in the proximate areas that can be affected by road-zone effects. They can also present barriers to individual movements, either through behavioral avoidance, or by presenting a physical obstacle (Balkenhol and Waits 2009). All of these effects may reduce connectivity as spatially critical habitat patches (e.g. "stepping stones") become unoccupied due to increased local mortality or reduced recolonization (Trombulak and Frissell 2000).

When connectivity is impaired by fragmentation and roads, animal populations may experience negative effects. For amphibians, population connectivity is predominantly affected through juvenile dispersal (Madison 1997; Preisser et al. 2001; Guerry and Hunter 2002; Rothermel, 2004; Cushman 2006). While the least mobile species might be expected to be the most impacted by fragmentation, species with greater dispersal ability may come into more frequent contact with barriers to dispersal and thus be more heavily impacted by fragmentation effects than less vagile species (Gibbs 1998; Carr and Fahrig 2001; Newcomb Homan et al. 2004). Decreases in abundance due to mortality during dispersal (Vos and Chardon 1998; Carr and Fahrig 2001) could consequentially lower colonization rates by reducing the number of individuals exchanged between populations.

## Using Landscape Genetics to Study Habitat Fragmentation

Landscape genetics provides powerful tools to characterize animal dispersal patterns and to elucidate the impacts of habitat fragmentation. Landscape genetics combines the research areas of population genetics, landscape ecology, and spatial statistics, with the goal of characterizing the effect of the landscape on genetic variation in animal populations (Balkenhol et al. 2009; Manel et al. 2003; Storfer et al. 2007). This approach relates aspects of the physical landscape to population structure and gene flow

to provide insight into the relationships among them (Balkenhol et al. 2009; Holderegger and Wager 2008).

Dispersal can be estimated indirectly through a genetic analysis in which rates of gene flow are inferred from measures of genetic differentiation between populations (*reviewed in* Epperson 2005). These inferences can help to make predictions about connectivity, a major focus of landscape genetics. Recently, a statistical approach has been developed in which the individual serves as the unit of analysis, rather than the entire population, as is the case for demographic studies (Rousset 2000). This individual-based approach enables the direct identification of individual migrants, permitting measurement of current dispersal patterns on a local geographic scale, and allowing a clearer understanding of dispersal ability (Manel et al. 2003).

Landscape genetics studies may employ microsatellite analyses in conjunction with various statistical approaches (Manel et al. 2003; Balkenhol et al. 2009; Storfer et al. 2009) to characterize genetic structure. From microsatellite data, population genetic metrics, including allelic diversity and richness, heterozygosity, and the inbreeding coefficient, can be used to assess genetic diversity of populations. Population-based genetic distance metrics, including $F_{ST}$ (Wright 1951), and individual-based Bayesian clustering approaches can be conducted to measure connectivity among populations and with respect to landscape features (Balloux and Lugon-Moulin 2002; Jehle and Arntzen 2002; Beaumont and Rannala, 2004; Selkoe and Toonen 2006; Bos et al. 2008).

In addition to assessing connectivity, another major aim of landscape genetics research is identifying potential barriers to gene flow. While natural landscape features may affect gene flow on a small scale, manmade structures, including roads (*reviewed in*

Balkenhol and Waits 2009), may impede genetic connectivity to an even greater degree at this same scale. Genetic software (i.e. BARRIER; Manni et al. 2004) can utilize genetic data to identify genetic barriers.

Once a spatial genetic pattern is identified, it is possible to test for correlation with landscape or environmental variables, a third principal aim of landscape genetics (Manel et al. 2003; Foll and Gaggiotti 2006; Faubet and Gaggiotti 2008). Several statistical methods exist to make these assessments, including: Mantel and partial Mantel tests, stepwise regression analysis (Kleinbaum and Kupper 1978), and a hierarchical Bayesian method employed in the software GESTE (Foll and Gaggiotti 2006). Using these methods, the environmental variables that are most likely to influence the observed genetic patterns can be identified. This comprehensive approach of landscape genetics and its aims of assessing connectivity, identifying barriers to gene flow, and relating environmental variables to genetic structure, provide a useful lens for assessing the impacts of fragmentation on landscapes undergoing rapid suburban development.

## Importance of Connectivity to Vernal Pool Breeding Amphibians

Issues of landscape connectivity are germane to the patchy forests of southeastern New Hampshire, where abundant small, shallow ponds exist. These ponds, known as vernal pools, typically occur in discrete patches within a matrix of upland forest habitat and are characterized by a lack of fish and annual or semi-annual periods of dryness (Semlitsch and Bodie 1998). Vernal pools are important landscape components, providing significant biological, hydrologic, and ecosystem functions (Lichko and Calhoun 2003). However, as a consequence of their small size, lack of hydrologic connection to permanent water bodies, and predominantly private ownership, vernal

pools receive minimal regulatory protection at both federal and state levels (Gibbs 1993; Semlitsch and Bodie 1998; Snodgrass et al. 2000; Lichko and Calhoun 2003; Calhoun et. al. 2005). The New Hampshire State Wildlife Action Plan assessed conservation priorities and declared vernal pools at risk, identifying human development and transportation infrastructure among the top five risk factors (Chapter 3; New Hampshire Fish and Game Department 2006). Where wetland management regulations do exist, they often include initiatives to protect wetlands, yet afford only limited protection to surrounding terrestrial habitats (Semlitsch and Bodie 1998).

Despite the generally limited protection they receive, vernal pools provide critical habitat for many aquatic and semi-aquatic vertebrates and invertebrates. The majority of amphibian species throughout the United States, and 16 of 23 amphibian species in New England, breed either exclusively or facultatively in vernal pools (DeGraaf and Yamasaki 2001). Many utilize vernal pools seasonally for breeding and larval development, but are fully terrestrial during the rest of the year (Regosin et al. 2003; Porej et al. 2004; Rittenhouse and Semlitsch 2007). For these species, pond occupancy and population persistence are strongly influenced by the landscape features of the surrounding terrestrial habitat (Marsh and Trenham 2001). The wood frog (*Lithobates sylvaticus*) is one such species.

The wood frog is one of the most widely distributed amphibians in North America; its range extends from above the Arctic Circle to the southern United States (Heatwole 1961; Conant and Collins 1998). Wood frogs have a complex life cycle that includes aquatic larval and terrestrial juvenile and adult stages, making both aquatic and terrestrial habitats critical for population persistence (Baldwin et al. 2006). Adult wood

frogs spend summer months in moist woodlands, forested swamps, or bogs (Bellis 1965). In the fall, frogs leave the pools and overwinter in the surrounding upland habitat. In the early spring, adult wood frogs emerge from hibernation and return to nearby vernal pools to breed (Bellis 1965). By breeding in early spring, wood frogs increase their offspring's chances of metamorphosing before pools dry. Following larval development in the pools and metamorphosis, juveniles will migrate to the upland terrestrial habitat. Some fraction of these juveniles will disperse, permanently leaving the vicinity of their natal pools. Given this widespread movement and dependence on multiple habitat types, conserving wood frogs requires a landscape perspective.

Historically, wood frogs and other pond-breeding amphibians have been characterized as having strong site fidelity, low vagility, and metapopulation structure (Sinsch 1990; Blaustein et al. 1994; Marsh and Trenham 2001). Accordingly, several genetic studies have shown many species of amphibians to be highly substructured (*reviewed in* Shaffer et al. 2000). Most studies, however, have been conducted on a large geographical scale that is not relevant to metapopulation dynamics. As a result, these studies identified isolation by distance as a factor that contributes to the high degree of genetic differentiation reported (Shaffer et al. 2000; Ficetola et al. 2004).

Determining local population genetic differentiation instead requires an approach at a fine scale perspective relevant to amphibian dispersal. Studies using this approach have discovered species-specific differences, with some species exhibiting significant genetic structuring on a fine scale (e.g., *Bufo calamite*, Rowe et al. 2000; *Bufo bufo*, Brede and Beebee 2004), and other species exhibiting high rates of gene flow at short geographic distances, such as the wood frog, *Lithobates sylvaticus* (Newman and Squire

2001). Dispersal distances of up to 2.5 km have been reported for the wood frog, with the average dispersal distance being recorded at 1000 m from natal pools (Gill 1978; Berven and Grudzien 1990; Semlitsch 1998; Lichko and Calhoun 2003). These results suggest that many amphibians may be more vagile and less philopatric than previously believed (Marsh and Trenham 2001; Petranka et al. 2004; Smith and Green 2005; Petranka and Holbrook 2006).

It has been suggested that some amphibians may exhibit stepping stone dispersal, provided that ponds are distributed continuously in the landscape (Berven and Grudzien 1990, Newman and Squire 2001). In this case, movements between ponds may take place at such high rates that local pond populations do not develop a significant degree of demographic independence and consequentially lack metapopulation structure (Smith and Green 2005). Populations isolated by distances over 10 km in range are more likely to exhibit metapopulation structure than less isolated populations (Smith and Green 2005). *Lithobates sylvaticus* may undergo dispersal up to 10 km, but this long distance dispersal is rare and is likely to have little effect on population structure. Smith and Green (2005) stated that at a local scale of ≤10 km, individuals dispersed over a distance and at a rate that made even isolated populations connected to the whole, suggesting that for at least some amphibians, metapopulation structure in the classic sense might not exist.

## Other Environmental Influences on Population Structure

In studying the effects of fragmentation on genetic structure, other factors besides landscape features might warrant consideration. In particular, it may be insightful to study the effect of environmental quality where populations reside. For amphibians, a relevant consideration is the effect of water quality on genetic structure. Because pool-

breeding amphibians are intimately tied to an aquatic environment, the quality of the water in which they live can affect their growth, development, and survival. In an extreme case, reduced survival could lead to local extinctions. Amphibian species may exhibit differing degrees of sensitivity to water quality and pollutants (Marco et al. 1999; Griffis-Kyle and Ritchie 2007). For example, wood frogs have been shown to be particularly sensitive to the water quality effects of urbanization (Snodgrass et al. 2008), making it relevant to evaluate the effects of water quality on their population processes and dynamics.

Road chemical runoff has the potential to influence ecosystem health through water quality degradation (Semlitsch 2000; Andrews et al. 2008; Balkenhol and Waits 2009). The severity of this degradation depends on a combination of natural landscape features, such as: geology, topography, and soils; climate and atmosphere contributions; and human activities related to land use and management. For example, many wetlands in suburban areas receive stormwater runoff from impervious surfaces, which may contain a wide range of pollutants such as heavy metals, phosphorus, fertilizers, pesticides, suspended solids, hydrocarbons, and salts (Paul and Meyer 2001).

Due to the shallow depth and small size that is characteristic of vernal pools, pollutants can accumulate in these water bodies at high concentrations. However, some ponds may be more prone to water quality degradation than others. Ponds in close proximity to impervious surfaces are more likely to receive run-off containing chemicals that may threaten the fitness of resident populations (Forman and Alexander 1998). Some pollutants in road-adjacent wetlands are present in concentrations well above toxic thresholds (Turtle 2000; Sanzo and Hecnar 2006; Karraker et al. 2008).

A major component of road runoff in the Northeast is salt, which is commonly used in road maintenance for winter de-icing (Karraker et al. 2008). Road salt may be present in high quantities on roads within the Northeast, and has been reported to greatly increase the conductivity and chloride concentration of run-off into adjacent wetland habitats (Karraker et al. 2008). Elevated chloride concentrations affect amphibians by lowering their survivorship and impacting their growth and development rates (Sanzo and Hecnar 2006). Collins and Russell (2009) determined that wood frogs did not occupy pools with high chloride concentrations (150-175 mg/L $Cl^-$) and established, through acute toxicity tests, that wood frogs are among the most chloride-sensitive pool-breeding amphibians.

In addition to elevated chloride concentrations, low pH can result in toxic effects on amphibians (Pough 1977; Sadinski and Dunson 1992; Horne and Dunson 1994). Vernal pools are susceptible to acidification due to their low alkalinity and buffering capacity (Horne and Dunson 1994). The reduction in recruitment associated with acidification of ponds can affect both the local distribution and abundance of amphibians (Pough 1977). Horne and Dunson (1994) found that wood frogs were negatively impacted by low pH. At a pH of 4.5, the negative effects included increased time to metamorphosis and decreased survival and wet body mass at metamorphosis.

Similarly, wood frogs are negatively impacted by high dissolved organic carbon (DOC; Horne and Dunson 1994). DOC may have an acutely toxic effect on amphibian breeding in temporary ponds. Horne and Dunson (1994) showed that a high concentration of DOC slowed the developmental process in wood frog larvae. This delayed development could result in poor recruitment during dry years, if ponds dry before the

larvae are mature enough to metamorphose and disperse into the surrounding upland habitat (Sadinski and Dunson 1992; Horne and Dunson 1994). In this way, hydroperiod is also a critical determinant of wood frog abundance due to its potential to limit development and survival of embryos (Babbitt et al. 2003; Veysey et al. 2011).

It is possible that short hydroperiod, high chloride concentrations, low pH, high DOC, or any interactions between them could limit wood frog breeding success in vernal ponds, reducing the number of wood frogs that might disperse and recolonize adjacent populations. If dispersal were interrupted, ponds might become increasingly structured, as gene flow decreased among ponds in the landscape. If productivity in a pond was so low as to result in local extinction, the pond could experience a founder effect, where it is recolonized by a small group of individuals from another population causing population differentiation and greater genetic structure.

## Objectives

In this study, I used a landscape genetics approach to determine the effects of habitat fragmentation on wood frog (*Lithobates sylvaticus*) population structure. I compared patterns of genetic connectivity between vernal pools in a continuously forested, unfragmented landscape and a fragmented landscape characterized by moderate levels of suburban development and roads. Specifically, my objectives were to:

1. Characterize the genetic structure of wood frog populations in the two landscapes;
2. Determine the effect of roads on genetic connectivity of wood frog populations;
3. Determine the effect of environmental factors, including water quality and hydroperiod, on wood frog genetic structure.

I hypothesized and predicted that:

1. Habitat fragmentation will decrease the genetic connectivity of wood frogs in the fragmented landscape relative to that in the unfragmented landscape.
2. Roads will act as barriers to dispersal to wood frogs, thereby decreasing genetic connectivity among populations separated by roads.
3. Hydroperiod, chloride concentration, pH, and dissolved organic carbon (DOC) are important water quality parameters that could influence genetic structuring of wood frog populations through their effects on population growth and persistence. I predicted that shorter hydroperiod, increased chloride concentration, low pH, and high DOC will negatively impact the growth, development, and survival of wood frog embryos, potentially resulting in local extinctions and increased genetic differentiation due to a founder effect.

# CHAPTER 2

# METHODS

## Study Area

This study was conducted in Rockingham and Strafford counties in southeastern New Hampshire. Small (<1 ha) wetlands with National Wetland Inventory (NWI) classifications of PUBF, PFO1/4E, or PSS1E (i.e., vernal pools) were selected using NWI maps and aerial photographs, and locations were verified in the field. Identified wetlands were entered into a geographic information system (GIS) as a data layer to conduct spatial analyses.

Spatial scale is important to consider in landscape genetics studies, as different scales can influence the resulting patterns that are detected (Storfer et al. 2007; Schwartz and McKelvey 2009; Anderson et al. 2010; Segelbacher et al. 2010). Accordingly, selecting an appropriate sampling scheme is critical. Ideally, a sampling scheme should capture appropriate spatial and genetic variability in the landscape. This can be accomplished by sampling populations at relatively small distances from one another across a large geographic area (Storfer et al. 2007, Schwartz and McKelvey 2009). To this end, I conducted this study at two different scales: 1) broad-scale, encompassing the entire study area in southeastern New Hampshire, and; 2) fine-scale, in which separate clusters of vernal pools were analyzed independently.

I selected vernal pool clusters in each of two different landscapes: one unfragmented, forested landscape, located in Pawtuckaway State Park, Raymond, NH;

and one fragmented landscape, characterized by moderate levels of suburban development and roads in the towns of Lee, Nottingham, and Barrington, NH (Fig. 1). From GIS analysis of the NH Public Roads and New Hampshire Land Cover Assessment (2001) layers from NH GRANIT, I characterized each cluster within the two landscapes based on the relative proportions of roads, forest cover, development, agriculture, and land use for public utilities and quarries, as a fraction of total land area (Table 1). I used this comparative approach, with clusters in both a fragmented landscape and an unfragmented landscape as a reference, to facilitate detection of the impacts of fragmentation on wood frog populations.

Two clusters of vernal pools were selected in the unfragmented landscape. These clusters, hereafter designated 36 and 39, are situated on the east and west sides of Pawtuckaway State Park, and are separated by a 2 km tract containing South Mountain (elevation 908') and large tracts of swampy marsh at the lower elevations. No wood frog egg masses were found during survey of this intervening region. The majority of Pawtuckaway State Park is characterized as hemlock-hardwood-pine forest, consisting primarily of hemlock (*Tsuga canadensis)*, second-growth mixed hardwoods, and white pine (*Pinus strobus*). Though there are access roads for visitors' use, the interior of the park has no roads and the landscape is free of development and cleared land.

Additionally, three clusters, hereafter designated 16, 18, and 29, were selected in the fragmented landscape. The ponds within these clusters are separated by several anthropogenic features, such as major roads, including: New Hampshire State divided highways Rte 4, Rte 125, and Rte 9; local paved and dirt roads; agricultural fields; and housing developments.

Ponds within the designated clusters were selected in a manner that would facilitate hypothesis-testing about the effects of geographic and man-made barriers. I attempted to sample ponds continuously throughout each cluster to ensure that relationships between adjacent ponds could be detected in the analyses, and to minimize the possibility of overlooking stepping stone ponds, which could facilitate wood frog dispersal. Continuous sampling is important for making inferences about dispersal distances and influences of landscape features on spatial genetic structure. Due to the significance of this sampling scheme for my study, I sampled wood frogs from any wetlands under the NWI classification that I encountered with egg masses; this resulted in the inclusion in my study of two roadside ditches and three man-made ponds that failed to meet a strict vernal pool designation.

This sampling procedure resulted in a total of 65 ponds distributed in 5 clusters comprised of 9 to 18 ponds each (Fig. 1). Forty-five ponds were distributed throughout three clusters in the fragmented landscape (Cluster 16: N=11; Cluster 18: N=18; and Cluster 29: N=16), and 20 ponds were distributed across two clusters in the unfragmented landscape (Cluster 36: N=11; Cluster 39: N=9). Ponds were separated from one another by distances ranging from approximately 100 m to 6 km. The varied distances between ponds were selected because wood frogs have exhibited dispersal distances ranging between 60 m (Regosin et al. 2003) and 2.5 km (Berven and Grudzien 1990). Evidence even exists for high rates of dispersal between ponds separated by 10 km or less (Smith and Green 2005). By incorporating a range of distances between the ponds, I could test for the effect of distance on dispersal ability and genetic structure.

Within each cluster, the largest pair-wise distance between ponds was 6 km (cluster 29), 4.5 km (clusters 16 and 18), 3.5 km (cluster 36), and 2.5 km (cluster 39). The clusters within each landscape were separated by distances of 2 to 4 km, and the unfragmented and fragmented landscapes themselves were separated by a distance of 8 km.

## Sampling Methods

In April and May of 2005 and 2008-2010, vernal pools were surveyed for wood frog egg masses and samples were collected for genetic analyses. At each pond, samples were collected from a target of twenty to thirty different spawn clumps. One embryo was sampled per spawn clump to ensure that maximum genetic variation was sampled at each site. Sample sizes from ponds across the study area ranged from 15-30 egg masses. A total of 1,489 wood frog embryos were collected from the 65 ponds.

Embryos were stored individually in sterile 1.5 ml vials in 95% ethanol for preservation prior to DNA extraction. In addition, I conducted egg mass counts subsequent to wood frog breeding events. I visited ponds twice to account for differences in egg-deposition time, and used the maximum observed egg-mass count from each pool for analyses. Egg mass counts provide a relatively accurate estimate of the number of breeding females (Crouch and Paton 2001). Given a 1:1 sex ratio, census adult population size can be estimated (Brede and Beebee 2004), subsequently providing an estimate of population density.

Hydroperiod and water quality parameters, including temperature, pH, conductivity, dissolved oxygen, and DOC, were measured at time of sampling and again every two weeks from June to October. Temperature, pH, conductivity, and dissolved

oxygen were measured using a water meter (YSI 556 MPS; YSI Incorporated, Yellow Springs, OH). I determined pond hydroperiod by monitoring water levels every two weeks from the first date in which wood frog eggs were observed until the drying date. The hydroperiod score given to each pond is based on 2-week increments, such that every unit increase in the hydroperiod score equals a 2-week increase in hydroperiod. A score of 20 was given to ponds that retained water for the duration of the season. In addition, grab samples were collected at time of sampling and were analyzed for major nutrients, anions, cations, and organic matter (including DOC) at the UNH Water Quality Analysis Laboratory.

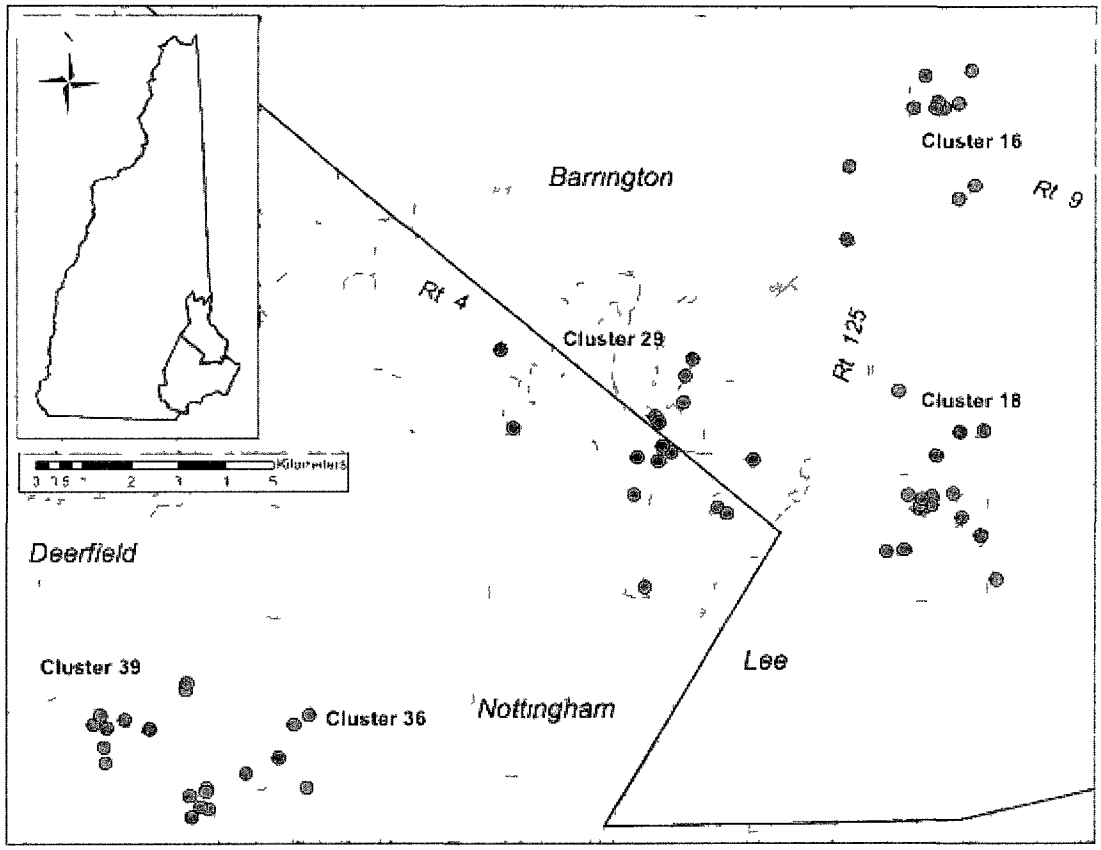


Figure 1. Locations of five clusters of 65 vernal pools within Rockingham and Strafford counties in southeastern New Hampshire where wood frogs were sampled

Table 1. Land cover characterization for the five vernal pool clusters in the study area.

| Landscape Type | Cluster | Proportion of Coverage | | | | |
|---|---|---|---|---|---|---|
| | | Roads | Forest Cover | Development | Agriculture | Powerlines & Quarries |
| Fragmented | 16 | 0.05 | 0.75 | 0.20 | 0.01 | 0.02 |
| | 18 | 0.03 | 0.68 | 0.14 | 0.07 | 0.10 |
| | 29 | 0.04 | 0.81 | 0.09 | 0.01 | 0.09 |
| Unfragmented | 36 | 0.01 | 0.79 | 0.04 | 0.00 | 0.00 |
| | 39 | 0.01 | 0.98 | 0.01 | 0.00 | 0.00 |

## DNA Extraction and Microsatellite Analysis

I extracted DNA from whole wood frog embryos using the DNeasy® Tissue Kit (Qiagen, Valencia, CA). DNA was amplified by polymerase chain reaction (PCR) at nine polymorphic tetranucleotide loci, using species-specific primers in two multiplexes: one multiplex containing primers *RsyC52, RsyC83, RsyD32*, and *RsyD55*, and the other multiplex containing primers *RsyC11, RsyC41, RsyC63, RsyD20*, and *RsyD77* (Julian and King 2003; Appendix A).

Microsatellite loci were amplified in 15 μl polymerase chain reactions consisting of 3 μl of eluted genomic DNA, 0.2-0.3 μM of each primer (fluorescently-labeled with HEX, NED, or FAM), 2.1 μM $MgCl_2$, 1X GoTaq Flexi PCR buffer (Promega Corp., Madison, WI, U.S), 0.2 mg/ml bovine serum albumin (BSA), 0.2 mM of deoxyribonucleotides (dNTPs), and 0.75 units of GoTaq Flexi DNA polymerase (Promega). Polymerase chain reactions were conducted in a Eppendorf Thermocycler under the following conditions: initial denaturing step of 4 min at 94 °C; 30 cycles of 30 s at 94 °C, 45 sec at 58 °C, and 72 °C for 1 min; and a final extension for 5 min at 72 °C.

Amplified products were electrophoresed using an automated DNA sequencer (ABI

3130 genetic analyzer, Applied Biosystems, Foster City, CA), from which genotype data were collected and genotypes scored using PEAKSCANNER software (Applied Biosystems). Positive controls were used in conjunction with the software program Allelogram (Morin et al. 2009) to standardize allele calls across electrophoretic runs. Alleles were binned manually based on the normalized raw scores generated by Allelogram.

I used the software program MICROCHECKER (van Oosterhout et al. 2004) to check the data set for potential scoring errors, presence of null alleles, and large-allele dropout. In addition, I used the computer program FreeNA (Chapuis and Estoup 2007) to estimate null allele frequencies for each locus for each population using the Expectation Maximization (EM) algorithm of Dempster et al. (1977). MICROCHECKER identified null alleles in two of the nine genotyped microsatellite loci. FreeNa indicated that null alleles occurred at a frequency of 7.6% at locus *RsyD55* and 14.1% at locus *RsyC63*. Locus *RsyC63* was dropped from the dataset, as its frequency exceeded 10%, our *a priori* determined threshold for null allele inclusion. To confirm that the inclusion of locus *RsyD55* would not impact the results, I used a Mantel test in GenAlEx (Peakall and Smouse 1995) to compare the genetic distance matrices with and without *RsyD55* and found almost no difference between the datasets ($R^2$=0.98, P<0.01). All statistical analyses are thus based on genetic data from eight microsatellite loci, including *RsyD55*.

Measures of genetic diversity, including mean number of alleles, allelic richness, $F_{IS}$ and observed and expected heterozygosities ($H_O$ and $H_E$) were calculated in the program GDA (Lewis and Zaykin 2001) for each locus and population sampled. Locus-specific $F_{ST}$ values were calculated in GENEPOP version 4.0.10 (Raymond and Rousset

1995). Using GENEPOP, I also tested the data for deviations from Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium (LD) within each population at each locus. Tests employed the Markov chain Monte Carlo (MCMC) method and significance testing was performed using 10,000 iterations and 10,000 batches prior to analysis. I adjusted for the number of simultaneous tests using Bonferroni corrections, to maintain an overall significance value of 0.05.

**Broad-scale Population Genetic Structure**

To characterize genetic differentiation among ponds, I calculated pair-wise $F_{ST}$ values in FSTAT (Goudet et al. 1995). Significance testing was performed using 1,000 permutations, with a Bonferroni adjustment for multiple tests ($\alpha = 0.05$). To test for genetic differences among populations that were sampled in two separate years (Ponds 16, 16C, 16E, 16H, 16I, 18O, 29N, 36K, and 39E), I compared pair-wise $F_{ST}$ values for the same pond across years. As no significant differences in $F_{ST}$ values were observed when comparing the same sites across years, multi-year data for these ponds were combined.

I used the Bayesian clustering method implemented in STRUCTURE 2.3 (Pritchard et al. 2000) to characterize the genetic structure of the entire study area, across both the fragmented and unfragmented landscapes, without defining populations *a priori*. Using this approach, individuals from a population are partitioned into a number of clusters or subpopulations (K) based on their genetic similarity. I conducted five independent runs for each K between one and 50 using the LocPrior model (Hubisz et al 2009) with admixture, with a burn-in period of 500,000 replications followed by 100,000 Markov chain Monte Carlo steps. I assumed that there would be ponds within the

landscape with similar structure; therefore, I did not run K=1-65 (the true number of populations), as this would overburden the analysis.

I also used the program TESS version 2.3 (François et al. 2006) to determine the number of subpopulations in my study area. TESS is similar to STRUCTURE in that it implements a Bayesian clustering algorithm for spatial population genetic analyses without assuming *a priori* designation of population units. However, unlike STRUCTURE, TESS uses geographic coordinates of the sampling locations in its model (Chen et al. 2007). I used TESS to determine the number of clusters at two scales: the entire study area and each pre-defined cluster within the study area. Running TESS on the individual clusters allowed me to test for fine-scale clustering on a local geographic scale. For the analysis of the entire study area, I ran five independent runs for each K between two (the lowest number of populations TESS will test) and 25, and for the analysis of each cluster, I conducted 10 independent runs for each K between two and 10. For both scales, I used both the admixture and no admixture models with a burn-in period of 50,000 steps followed by a total of 100,000 sweeps.

I tested for hierarchical population structure with an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) using the software ARLEQUIN (Excoffier et al. 2005). This approach apportions the variation in the genetic data into among-group and within-group components, allowing for hypothesis tests of population structuring at different hierarchical levels. Ponds were placed into several different hypothesized population groupings based on their spatial arrangement with respect to my sampling scheme and hypothesized road barriers. Population groupings were based on pre-defined cluster assignments, as well as groupings of ponds separated by roads, in a number of different

arrangements around New Hampshire state highways Rte 4, Rte 125, and Rte 9. Groupings included: 1) the two landscape types; 2) the five predefined clusters; 3) ponds in the fragmented clusters, east and west of Rte 125; 4) ponds in the fragmented clusters, north of Rte 9, between Rte 4 and Rte 9, and south of Rte 4; 5) ponds in cluster 18, north and south of Rte 4; 6) ponds in cluster 29, north and south of Rte 4; and 7) ponds in both clusters 18 and 29, north and south of Rte 4.

**Effect of Geographic Distance and Roads on Genetic Structure**

To determine the effect of geographic distance on genetic structure, I tested for isolation by distance (IBD) effects within each cluster by comparing matrices of geographic distance and genetic distance (linearized $F_{ST}$) using a Mantel test with 1,000 permutations in GenAlEx 6.1. I also used Mantel tests to identify potential correlations of genetic distance with road barriers in the three fragmented clusters. To quantify the barrier effect of roads, I mapped the NH Public Roads layer from NH GRANIT within the study area in GIS. I assigned roads values of 1-6, according to their road class, reflecting their hypothesized barrier effect (resistance to dispersal): 1= "no roads" was assumed to have the lowest resistance to frog movement and was scored a one; 2= "dirt, non-public, and private roads"; 3= "local dirt roads"; 4= "local paved roads"; 5= "major and minor collector," a low to moderate-capacity road, and; 6 = "principle arterial," a high-capacity urban road, representing the highest resistance to frog movement. A matrix of additive road barrier effects was constructed for all pair-wise comparisons of ponds, following a least-cost path (LCP) model (Adriaensen et al. 2003). I performed partial Mantel tests in R version 2.5.0 (R Development Core Team 2007) using the Vegan package (Oksanen et al. 2006), to determine the relationship of $F_{ST}$ and roads, while

controlling for the effect of distance. Significance was assessed with 1000 permutations.

To evaluate spatial genetic structure and potential fragmentation effects on wood frog dispersal, I conducted analyses on a fine-scale using spatial autocorrelation. This approach allows for the detection of finer scale patterns that might not be evident through analyses conducted at a broader scale. Spatial autocorrelation tests the significance of the correlation between geographic distance and genetic distance (relatedness) for pairs of individuals at defined distance classes, and thereby evaluates the degree to which related individuals tend to be clustered together or evenly dispersed (Smouse and Peakall 1999). The autocorrelogram shows the distance to which significant positive spatial genetic structure occurs. Individuals found within distances smaller than the significant positive correlation share a higher proportion of genes, and individuals more distant than this threshold are regarded as genetically independent. This method has been used to detect fragmentation effects on dispersal in a number of species in both animal and plant populations (Smouse et al. 2008; Walker et al. 2008; De-Lucas et al. 2009; Mora et al. 2010). I performed spatial autocorrelation for each cluster of ponds in each landscape using GenAlEx 6.1. Eight even distance classes of 250 m were used, from 0 to 2000 m within each cluster, and 999 permutations were run for each test.

I used the computer program SPAGeDi (Hardy and Vekemans 2002) to calculate individual mean parent-offspring dispersal distances within each cluster of ponds. Dispersal distances can be inferred from patterns of population genetic structure by fitting a model of IBD, which regresses pair-wise linearized genetic distances (Rousset's "a;" Rousset 2000) onto log-transformed geographic distances among populations (Wright 1943). The slope of this regression ($b$) allows estimation of the standard deviation of the

distribution of dispersal distance ($\sigma$) using the relationship $b= 1/4D\pi\sigma^2$, where $D$ represents the effective density of reproducing individuals in the population (Broquet and Petit 2009). I calculated the density of each cluster by dividing the population sizes totaled across ponds within the cluster (represented by the number of egg masses multiplied by two, assuming a 1:1 sex ratio; Brede and Beebee 2004) by the cluster area, to achieve an estimate of the number of wood frogs per square meter. I calculated the area of each cluster in GIS by drawing a polygon with straight lines between the outermost populations to encompass each respective cluster. Any discrepancies in the literature regarding the assumption of a 1:1 sex ratio for wood frogs can be accounted for by variation in sex ratio during any one breeding season. Thus, across the whole population, a 1:1 sex ratio represents a reasonable approximation (Berven 1990).

With SPAGeDi, I also computed the *Sp* statistic, which is a measure of the strength of spatial genetic structure (Vekemans and Hardy 2004). $Sp= -b/(1-F1)$, where $-b$ is the slope of the regression of individual genetic distance (relatedness) on log-transformed distance, and $F_1$ is the pair-wise relationship coefficient at the first distance class. Relationship coefficients were calculated via two methods: Queller and Goodnight (1989) and Wang (2002).

## Detection of Barriers to Dispersal

To evaluate potential effects of landscape features on genetic variation, I used the single species genetic divergence tool in the Landscape Genetics Toolbox in ArcGIS (Vandergast et al. 2010). This approach creates a genetic landscape based on pair-wise population genetic divergence, enabling visualization of the distribution of genetic diversity across geographic space. Areas of high and low divergence can be compared to

underlying landscape features. Maps of genetic divergence were created for each cluster within each of the two landscapes.

I then used the program BARRIER (Manni et al. 2004) to identify potential barriers to gene flow by identifying genetic discontinuities between pairs of ponds in relation to landscape features. Geographic coordinates were provided for each pond and were connected by Delauney triangulation (Brassel and Reif 1979) using a pair-wise $F_{ST}$ genetic matrix. Monmomier's maximum distance algorithm (Monmomier 1973) was then applied by starting at the edge with the largest associated pair-wise genetic distance in the triangulation and extending the barrier across subsequent adjacent edges associated with the largest genetic difference, until the edge of the network was reached, at which point a new barrier could be started at the next greatest genetic distance. Accordingly, putative genetic barriers were identified across each of the geographical landscapes. I conducted the analysis using the eight single locus $F_{ST}$ matrices and the multilocus matrix. To avoid spurious results from one or a few loci, only barriers supported by more than half of the loci were considered. These barriers were overlaid on a GIS layer in relation to anthropogenic landscape features such as roads, agriculture, and development.

**Effect of Water Quality and Hydroperiod on Genetic Structure**

To test for the effects of water quality parameters and hydroperiod on wood frog genetic structure, I used regression-based approaches with pond-specific $F_{ST}$s calculated in the program BIMr (Faubet and Gaggiotti 2008) as the dependent variable. Pond-specific $F_{ST}$s represent an average level of genetic divergence of each pond relative to the others. I calculated pond-specific $F_{ST}$s on the scale of each cluster of ponds in an effort to restrict the analyses to the relevant scale of local interactions.

I performed linear regressions to model the general relationship between pond-specific $F_{ST}$s and several water quality variables previously linked with wood frog productivity and survival (Pough and Wilson 1977; Babbitt et al. 2003); these regressions were conducted separately for ponds in the fragmented landscape and ponds in the unfragmented landscape. The independent variables in these analyses were hydroperiod, pH, conductivity, and dissolved organic carbon (DOC). In addition, I performed stepwise multiple regression analysis (Kleinbaum and Kupper 1978) in JMP version 9.0.1 (SAS Institute Inc., Cary, NC) to test the influence of the water quality variables on genetic structure. To test for differences in water quality effects in the unfragmented and fragmented landscapes, I performed separate analyses for the two landscapes. The dependent variable was pond-specific $F_{ST}$ for each pond and the independent variables were hydroperiod, pH, conductivity, DOC, and population size, as estimated by egg mass counts conducted at time of sampling. Step-wise regression was performed with the threshold to enter the model set at p=0.25. In addition, all possible models were ranked according to their AIC values.

A generalized linear model approach based on a hierarchical Bayesian method implemented in the program GESTE (Foll and Gaggiotti 2006) was used to assess the relative importance of both the environmental parameters and landscape features on the pond-specific estimates of $F_{ST}$. Posterior probabilities were estimated from different alternative models, each including a different set of environmental variables. I conducted analyses in GESTE by landscape type to test the hypothesis that ponds in the fragmented landscape are affected differently by alterations to their surrounding environment. I first ran the models with only the water quality parameters as the non-genetic factors. I then

ran another analysis that included the water quality parameter(s) from the highest probability model, in addition to parameters used to represent road effects, geographic distance, and estimated population size. To obtain a population specific parameter to represent road effects, I calculated the mean least cost path (LCP) road cost for each pond as the average of pair-wise LCP road costs from each pond to every other pond within each cluster (Lada et al. 2008). The same method was used to calculate mean geographic distance.

# CHAPTER 3

# RESULTS

## Descriptive Statistics

Of the 1,489 individuals genotyped, 6 individuals (0.4%) had missing data, and these lacked data for no more than 2 loci. By population, mean number of alleles ranged from 9.8 to 13.4 and allelic richness ranged from 9.3 to 11.1 (Table 2). Population observed and expected heterozygosities ranged from 0.716 to 0.880, $F_{IS}$ ranged from -0.038 to 0.161, and pond-specific $F_{ST}$s ranged from 0.007 to 0.028 (Table 2). Individual loci were variably polymorphic, with the mean number of alleles for each locus ranging from 10-35. Locus-specific $F_{ST}$s ranged from 0.005 to 0.01, locus-specific $F_{IS}$ ranged from -0.002 to 0.15, and mean observed and expected heterozygosities ranged from 0.632 to 0.942 (Table 3). There were deviations from Hardy-Weinberg in 6.7% of the tests performed, but none of these deviations were significant after Bonferonni correction (adjusted P value <0.000096). Linked loci were observed in 4.2% of the tests, and only 0.16% of these tests were significant following Bonferonni correlation (adjusted P value <0.000027).

Table 2. Genetic diversity of wood frogs from 65 populations in southeastern New Hampshire. N is the number of individuals sampled per population. Observed ($H_O$) and expected ($H_E$) heterozygosities, $F_{IS}$, number of alleles, and allelic richness for each population are averaged across 10 microsatellite loci. Pond-specific $F_{ST}$ values were calculated in the program BIMr.

| Pond | N | $H_O$ | $H_E$ | $F_{IS}$ | Pond specific $F_{ST}$ | # of alleles | Allelic richness |
|---|---|---|---|---|---|---|---|
| 16 | 26 | 0.788 | 0.853 | 0.077 | 0.007 | 13.0 | 10.9 |
| 16C | 27 | 0.838 | 0.847 | 0.010 | 0.014 | 12.6 | 10.4 |
| 16E | 17 | 0.838 | 0.849 | 0.013 | 0.020 | 9.9 | 9.5 |
| 16H | 26 | 0.817 | 0.843 | 0.031 | 0.010 | 12.0 | 10.4 |
| 16I | 23 | 0.761 | 0.846 | 0.102 | 0.010 | 12.0 | 10.5 |
| 16L | 22 | 0.835 | 0.854 | 0.022 | 0.010 | 12.1 | 10.7 |
| 16K2 | 25 | 0.813 | 0.856 | 0.052 | 0.008 | 12.8 | 10.6 |
| 16L10 | 18 | 0.855 | 0.848 | -0.009 | 0.013 | 12.5 | 11.0 |
| 16L4 | 29 | 0.845 | 0.849 | 0.004 | 0.012 | 12.1 | 9.8 |
| 16L6 | 19 | 0.824 | 0.852 | 0.034 | 0.009 | 13.0 | 10.4 |
| 16M2 | 20 | 0.775 | 0.814 | 0.050 | 0.019 | 9.8 | 10.4 |
| 18A | 19 | 0.875 | 0.849 | -0.032 | 0.012 | 12.4 | 10.6 |
| 18G | 20 | 0.799 | 0.843 | 0.054 | 0.014 | 11.8 | 10.2 |
| 18N | 20 | 0.793 | 0.843 | 0.061 | 0.012 | 11.6 | 10.8 |
| 18O | 25 | 0.783 | 0.847 | 0.078 | 0.014 | 11.4 | 9.8 |
| 18C2 | 21 | 0.825 | 0.833 | 0.010 | 0.018 | 12.0 | 10.5 |
| 18J | 18 | 0.820 | 0.855 | 0.042 | 0.009 | 12.3 | 9.6 |
| 18K | 25 | 0.835 | 0.845 | 0.012 | 0.013 | 11.6 | 10.7 |
| 18L | 25 | 0.860 | 0.860 | 0.000 | 0.007 | 12.5 | 10.2 |
| 18M | 25 | 0.839 | 0.840 | 0.001 | 0.010 | 11.5 | 10.6 |
| 18P | 25 | 0.822 | 0.859 | 0.044 | 0.009 | 12.5 | 9.9 |
| 18B2 | 25 | 0.875 | 0.877 | 0.002 | 0.009 | 12.5 | 10.9 |
| 18B3 | 24 | 0.780 | 0.844 | 0.077 | 0.015 | 11.3 | 9.9 |
| 18B5 | 26 | 0.800 | 0.843 | 0.052 | 0.013 | 11.0 | 10.4 |
| 18Focal2 | 25 | 0.828 | 0.857 | 0.035 | 0.009 | 12.4 | 9.8 |
| 18J2 | 25 | 0.869 | 0.838 | -0.038 | 0.021 | 10.5 | 10.2 |
| 18V | 19 | 0.808 | 0.829 | 0.025 | 0.013 | 10.5 | 10.0 |
| 18Z | 20 | 0.706 | 0.839 | 0.161 | 0.028 | 10.1 | 10.1 |
| 18Z2 | 28 | 0.833 | 0.873 | 0.047 | 0.009 | 12.3 | 9.5 |
| 29F | 25 | 0.750 | 0.857 | 0.128 | 0.016 | 11.4 | 9.8 |
| 29G | 24 | 0.809 | 0.865 | 0.066 | 0.012 | 11.6 | 11.0 |
| 29N | 27 | 0.794 | 0.845 | 0.062 | 0.017 | 11.1 | 10.0 |
| 29Q | 25 | 0.825 | 0.864 | 0.047 | 0.011 | 11.9 | 10.7 |
| 29A3 | 25 | 0.880 | 0.871 | -0.011 | 0.007 | 13.3 | 10.2 |

Table 1, con't.

| Pond | N | $H_O$ | $H_E$ | $F_{IS}$ | Pond specific $F_{ST}$ | # of alleles | Allelic richness |
|---|---|---|---|---|---|---|---|
| 29J | 24 | 0.857 | 0.862 | 0.006 | 0.008 | 12.3 | 10.5 |
| 29M | 25 | 0.807 | 0.832 | 0.030 | 0.009 | 12.0 | 10.7 |
| 29N3 | 30 | 0.798 | 0.865 | 0.080 | 0.012 | 11.9 | 10.5 |
| 29N5 | 25 | 0.869 | 0.852 | -0.021 | 0.011 | 11.8 | 10.6 |
| 29O2 | 25 | 0.788 | 0.819 | 0.039 | 0.022 | 10.3 | 10.4 |
| 29S | 27 | 0.810 | 0.837 | 0.033 | 0.010 | 12.0 | 10.7 |
| 29S2 | 15 | 0.818 | 0.858 | 0.048 | 0.011 | 11.9 | 9.8 |
| 29W5 | 21 | 0.869 | 0.874 | 0.006 | 0.016 | 11.6 | 11.0 |
| 29W6 | 25 | 0.774 | 0.836 | 0.076 | 0.017 | 11.8 | 9.9 |
| 29X2 | 20 | 0.799 | 0.844 | 0.055 | 0.007 | 12.9 | 10.1 |
| 29X5 | 24 | 0.781 | 0.855 | 0.089 | 0.012 | 11.9 | 10.7 |
| 36J | 21 | 0.845 | 0.833 | -0.015 | 0.014 | 11.4 | 9.6 |
| 36G | 15 | 0.810 | 0.845 | 0.043 | 0.012 | 11.6 | 10.5 |
| 36K | 20 | 0.750 | 0.822 | 0.090 | 0.016 | 10.1 | 9.3 |
| 36H | 24 | 0.795 | 0.863 | 0.080 | 0.009 | 12.8 | 10.7 |
| 36B2 | 24 | 0.759 | 0.830 | 0.088 | 0.012 | 11.5 | 11.1 |
| 36C2 | 23 | 0.825 | 0.851 | 0.031 | 0.011 | 11.9 | 10.7 |
| 36L | 24 | 0.829 | 0.851 | 0.027 | 0.016 | 10.8 | 10.3 |
| 37 | 21 | 0.825 | 0.844 | 0.024 | 0.018 | 11.1 | 10.6 |
| 38 | 21 | 0.835 | 0.838 | 0.004 | 0.020 | 11.4 | 10.5 |
| 38E | 20 | 0.805 | 0.839 | 0.041 | 0.018 | 11.4 | 9.3 |
| 36I | 21 | 0.789 | 0.834 | 0.054 | 0.019 | 11.1 | 10.5 |
| 39E | 24 | 0.818 | 0.837 | 0.023 | 0.009 | 13.4 | 10.5 |
| 39F | 20 | 0.792 | 0.850 | 0.070 | 0.021 | 12.1 | 10.6 |
| 39N | 26 | 0.835 | 0.865 | 0.036 | 0.014 | 12.4 | 9.8 |
| 39O | 28 | 0.850 | 0.855 | 0.006 | 0.016 | 11.8 | 10.6 |
| 39Q | 24 | 0.792 | 0.804 | 0.016 | 0.010 | 12.5 | 10.3 |
| 39R | 19 | 0.845 | 0.850 | 0.006 | 0.018 | 12.9 | 10.2 |
| 39 | 16 | 0.875 | 0.849 | -0.032 | 0.011 | 11.4 | 11.1 |
| 39S | 22 | 0.801 | 0.829 | 0.034 | 0.020 | 11.0 | 9.6 |
| 39T | 22 | 0.818 | 0.837 | 0.023 | 0.015 | 11.5 | 10.2 |
| **Total** | **1489** | | | | | | |

Table 3. Per locus mean number of alleles, observed ($H_O$) and expected ($H_E$) heterozygosities, $F_{IS}$, and $F_{ST}$ estimates averaged over all 65 wood frog populations.

| Locus | Mean alleles | $H_O$ | $H_E$ | $F_{IS}$ | $F_{ST}$ |
|---|---|---|---|---|---|
| *RsyC83* | 10 | 0.633 | 0.632 | -0.002 | 0.013 |
| *RsyD32* | 19 | 0.887 | 0.901 | 0.016 | 0.011 |
| *RsyC52* | 35 | 0.861 | 0.894 | 0.037 | 0.010 |
| *RsyD55* | 26 | 0.770 | 0.911 | 0.155 | 0.011 |
| *RsyC41* | 15 | 0.737 | 0.749 | 0.016 | 0.007 |
| *RsyD77* | 29 | 0.920 | 0.942 | 0.023 | 0.007 |
| *RsyC11* | 26 | 0.890 | 0.912 | 0.025 | 0.005 |
| *RsyD20* | 22 | 0.837 | 0.903 | 0.073 | 0.010 |

## Population Genetic Structure

Across the entire study area, 56 of 2025 pair-wise comparisons of $F_{ST}$ were significant. Within the clusters, several pairs of ponds were significantly differentiated by pair-wise population $F_{ST}$ estimates. Within the fragmented landscape, ponds in cluster 16 showed no significant genetic differentiation (0 of 55 pair-wise comparisons; Appendix B-1). In cluster 18, 16 of 153 pair-wise comparisons were significant, with 11 of the comparisons associated with pond 18Z, and two of the comparisons associated with pond 18C2 (Appendix B-2). Cluster 29 had four significant pair-wise comparisons of a total of 120 (Appendix B-3). Within the unfragmented landscape, cluster 36 showed no significant genetic differentiation (0 of 55 pair-wise comparisons; Appendix B-4); however, 8 of 36 pair-wise comparisons in cluster 39 were significant, with pond 39F showing significant differentiation from all but two of the other ponds and pond 39R exhibiting significant differentiation from two adjacent ponds in the cluster (Appendix B-5).

The program STRUCTURE detected no significant population structure within the study area and suggested K=1 as the most probable number of populations, with an average logarithm probability of the data lnPr (X|K)= -75321 (Fig. 2). Similarly, TESS clustering results did not detect any significant population structuring across the entire study area or within any of the clusters. At both scales, TESS analyses identified K=2 (the lowest number of clusters TESS can detect) as the most likely number of populations, suggesting that ponds are fairly well connected across the study area.

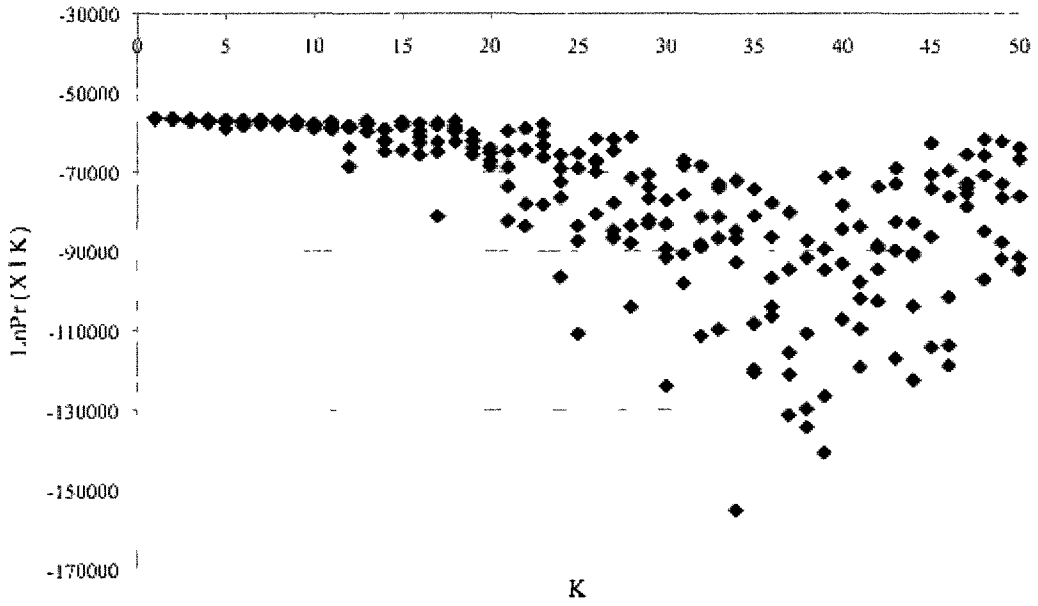


Figure 2. Number of populations (K) and their associated LnPr (X|K) from five independent runs for K=1-50, as calculated by STRUCTURE. The probability was maximized for K=1.

I conducted hierarchical analyses of molecular variance (AMOVAs) to detect the potential effect of roads on population genetic structure and to test if the clusters were differentiated. In all population groupings performed, the percentage of variation among the groups was exceedingly small, constituting less than a fraction of a percent in all cases (values range from 0.07 to 0.14%; Table 3). Though the values are highly significant ($p<0.001$), the small percentage of among group variation suggests that the groupings do not explain genetic differentiation or barrier effects among populations in the study area.

Table 4. Analysis of molecular variance (AMOVA) for 65 wood frog populations. Samples were partitioned according to their defined cluster designations and their proximity and separation by major roads. *P* values are given in parentheses below each estimate of percentage of variation.

| | | | Percentage of Variation | | |
|---|---|---|---|---|---|
| Ponds analyzed | Number of Groups | Population Groupings | Among Groups | Among populations within groups | Within populations |
| All ponds | 2 | Continuous<br>Fragmented | 0.09<br>(<0.001) | 0.94<br>(<0.001) | 98.97<br>(<0.001) |
| All ponds | 5 | Cluster 16<br>Cluster 18<br>Cluster 29<br>Cluster 36<br>Cluster 39 | 0.14<br>(<0.001) | 0.87<br>(<0.001) | 98.99<br>(<0.001) |
| Fragmented ponds | 2 | East of Rte 125<br>West of Rte 125 | 0.1<br>(<0.001) | 0.81<br>(<0.001) | 98.09<br>(<0.001) |
| Fragmented ponds | 3 | North of Rte 9<br>Btwn Rte 4 & Rte 9<br>South of Rte 4 | 0.09<br>(<0.001) | 0.8<br>(<0.001) | 99.11<br>(<0.001) |
| Cluster 18 ponds | 2 | North of Rte 4<br>South of Rte 4 | 0.18<br>(<0.001) | 0.78<br>(<0.001) | 99.04<br>(0.07) |
| Cluster 29 ponds | 2 | North of Rte 4<br>South of Rte 4 | 0.07<br>(<0.001) | 0.81<br>(<0.001) | 99.12<br>(0.003) |
| Cluster 18 and 29 ponds | 2 | North of Rte 4<br>South of Rte 4 | 0.08<br>(<0.001) | 0.82<br>(<0.001) | 99.11<br>(0.002) |

## Effect of Geographic Distance and Roads on Genetic Structure

Mantel tests indicated no effect of geographic distance on genetic distance over the entire study area ($R=0.007$; $P<0.05$). When Mantel tests were conducted by cluster, two of the three fragmented clusters (cluster 16 and cluster 18) showed patterns of isolation by distance (IBD), with genetic differentiation positively correlated with geographic distance, though this correlation was only significant for cluster 16 (Fig. 3). No IBD effect was observed in either of the unfragmented clusters (Fig. 4).

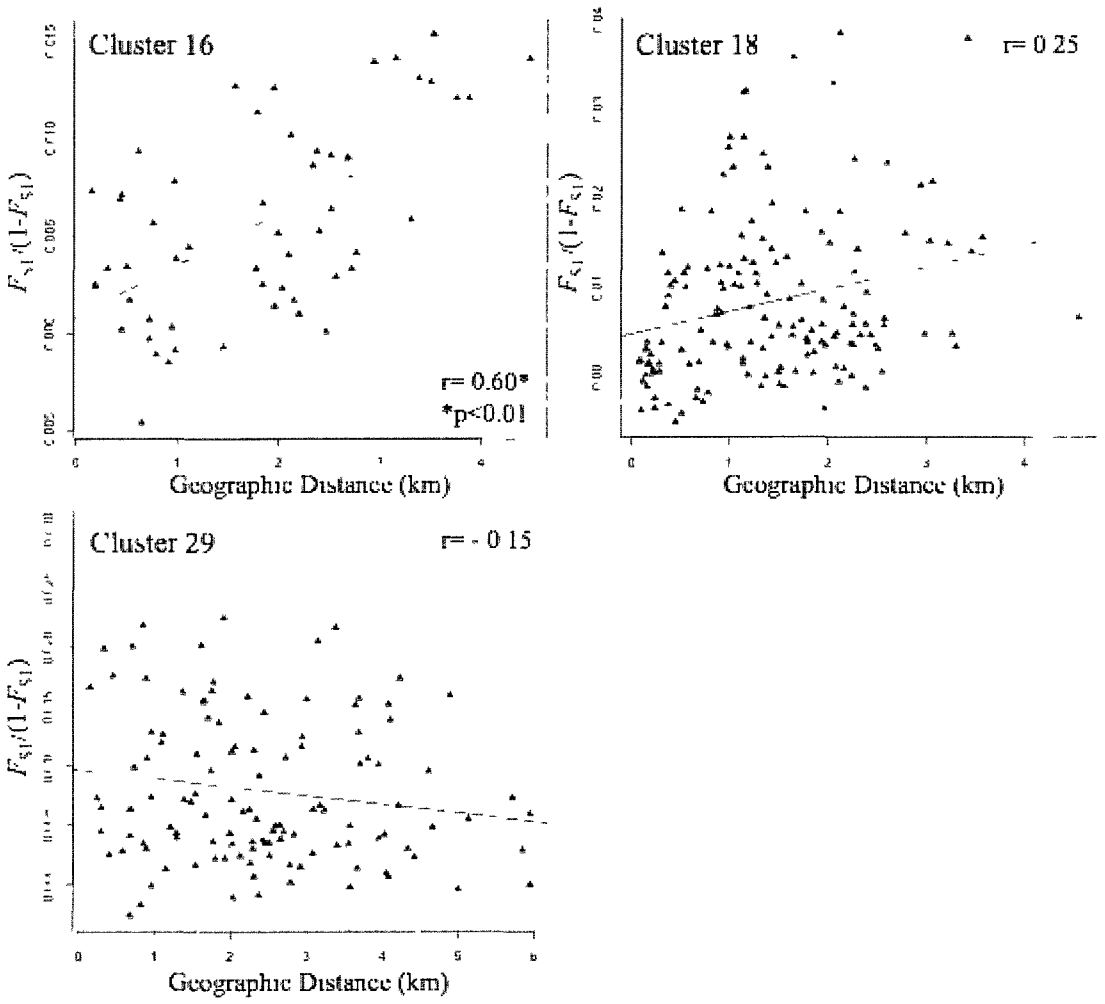


Figure 3. Relationship of genetic distance and geographic distance in wood frogs within ponds located in the three fragmented clusters. Significance by a Mantel test is indicated with an asterisk (*).

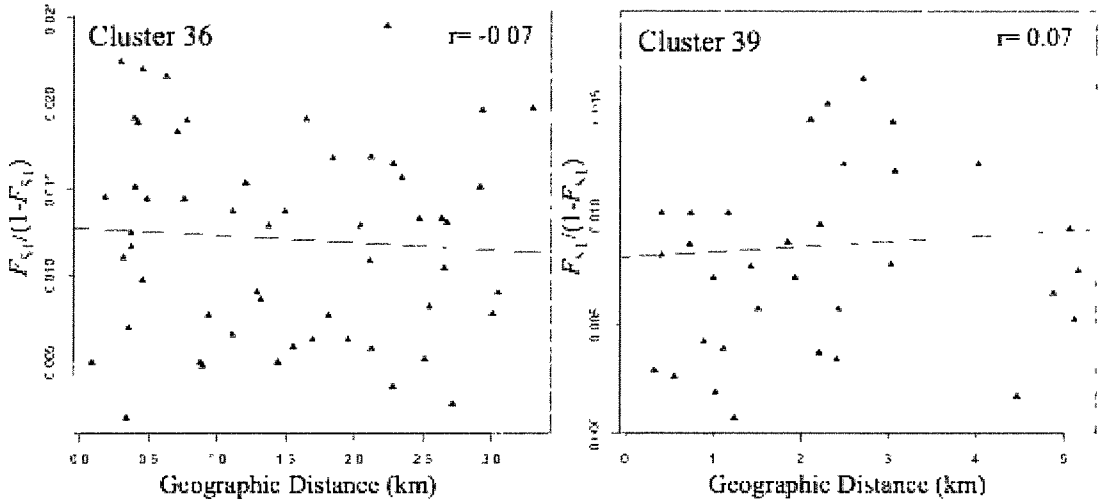


Figure 4. Relationship of genetic distance with geographic distance in wood frogs within ponds located in the two clusters in the unfragmented landscape. Neither cluster showed a significant isolation by distance effect.

Cluster 16 was the only fragmented cluster for which a Mantel test showed significant correlation of genetic distance (linearized $F_{ST}$) and roads (R=0.7406; p<0.01; Fig. 5). This correlation was significant after controlling for distance with a partial Mantel test (R=0.55; p<0.01; Table 4). A small positive, but nonsignificant, effect was also apparent in Cluster 18.

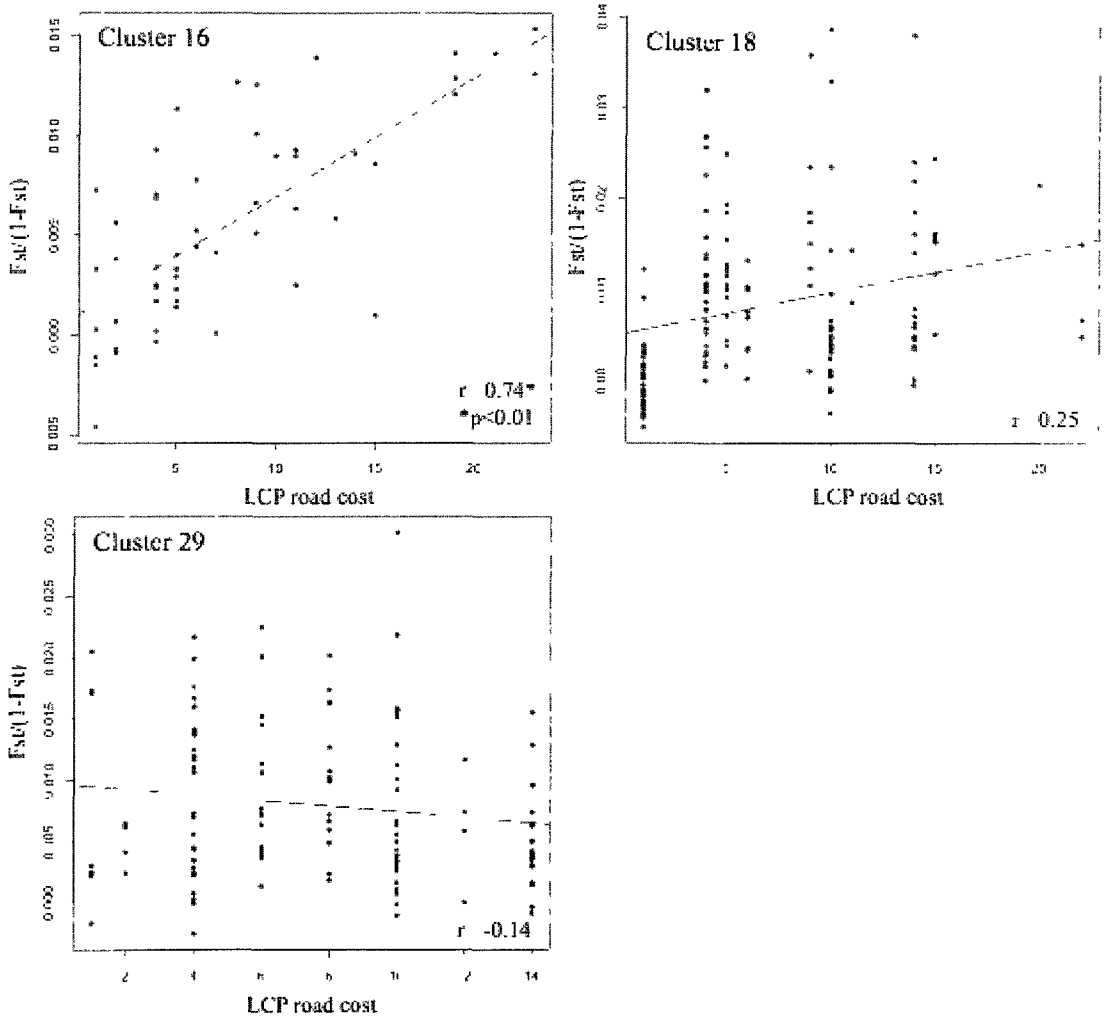


Figure 5. Relationship of genetic distance and road barriers, as calculated by the least-cost path (LCP) road cost between pairs on ponds within the three clusters in the fragmented landscape. Significance by a Mantel test is indicated with an asterisk (*).

Table 5. Mantel tests of the relationships between genetic and geographical distance, and genetic distance and road effects, while controlling for geographic distance (partial Mantel) for each cluster of wood frog ponds. Significant correlations are indicated in bold and p-values are given in parenthesis under each correlation coefficient. Road effects were only tested on the fragmented clusters.

| | | Mantel Test | | Partial Mantel Test |
|---|---|---|---|---|
| Landscape | Cluster | Isolation by distance | LCP road cost | LCP road cost, controlling for distance |
| Fragmented | 16 | **0.60** (0.008) | **0.74** (0.001) | **0.55** (0.012) |
| | 18 | 0.25 (0.107) | 0.25 (0.093) | 0.07 (0.326) |
| | 29 | -0.15 (0.795) | -0.14 (0.937) | -0.09 (0.819) |
| Unfragmented | 36 | -0.07 (0.656) | NA | NA |
| | 39 | 0.07 (0.339) | NA | NA |

Four of the clusters, Cluster 16 and 29 in the fragmented landscape and Cluster 36 and 39 in the unfragmented landscape, exhibited similar patterns of spatial autocorrelation. The autocorrelations began above the 95% confidence intervals and then dropped below the horizontal axis before the first distance class. Positive spatial genetic structure was only detected at the zero distance class, which represents relatedness of frogs within the same pond. The extent of the spatial genetic structure in these clusters, as measured by the x-intercept, ranged from 168 m to 246 m (Fig. 6 and 7).

Cluster 18 in the fragmented landscape exhibited a different pattern of spatial structure, having positive spatial structure up to 500 m and an x-intercept of 837 m, which was higher than that of the other clusters (Fig. 7).

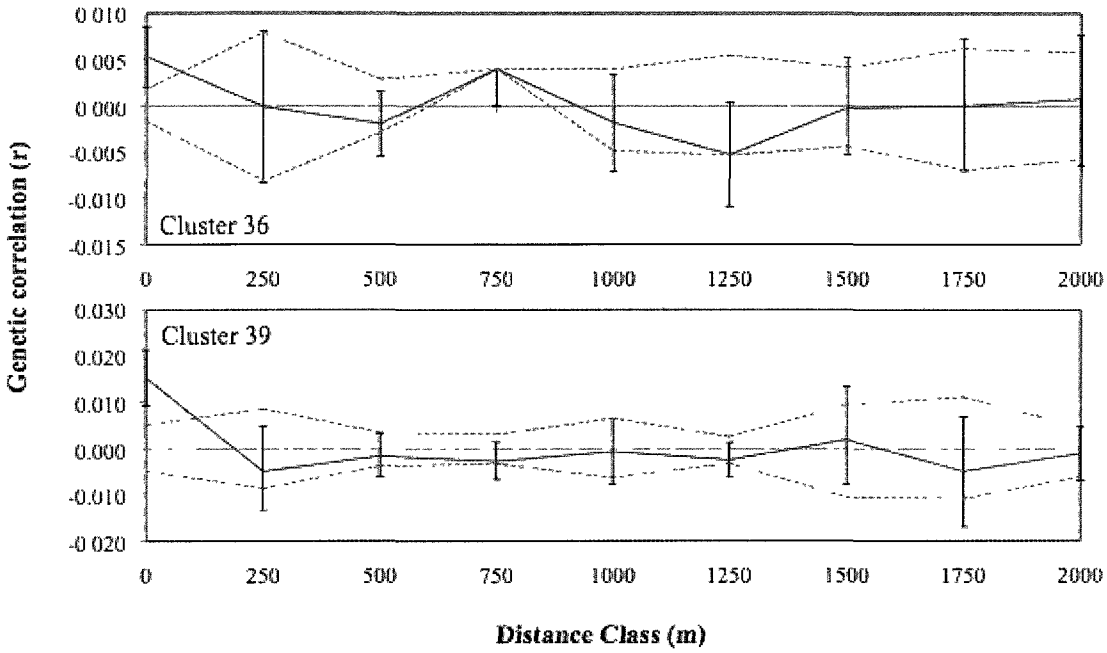


Figure 6. Correlograms of the genetic correlation coefficient ($r$) as a function of distance in the two clusters in the unfragmented landscape. Distance classes were set to 250 m. The extent of the spatial genetic structure, as measured by the x intercept, is 245 m and 188 m for Cluster 36 and 39, respectively. Null hypothesis of a random distribution of individuals is bounded by the 95% confidence intervals (dashed lines). Error bars for mean $r$ at each distance class were determined by bootstrapping.

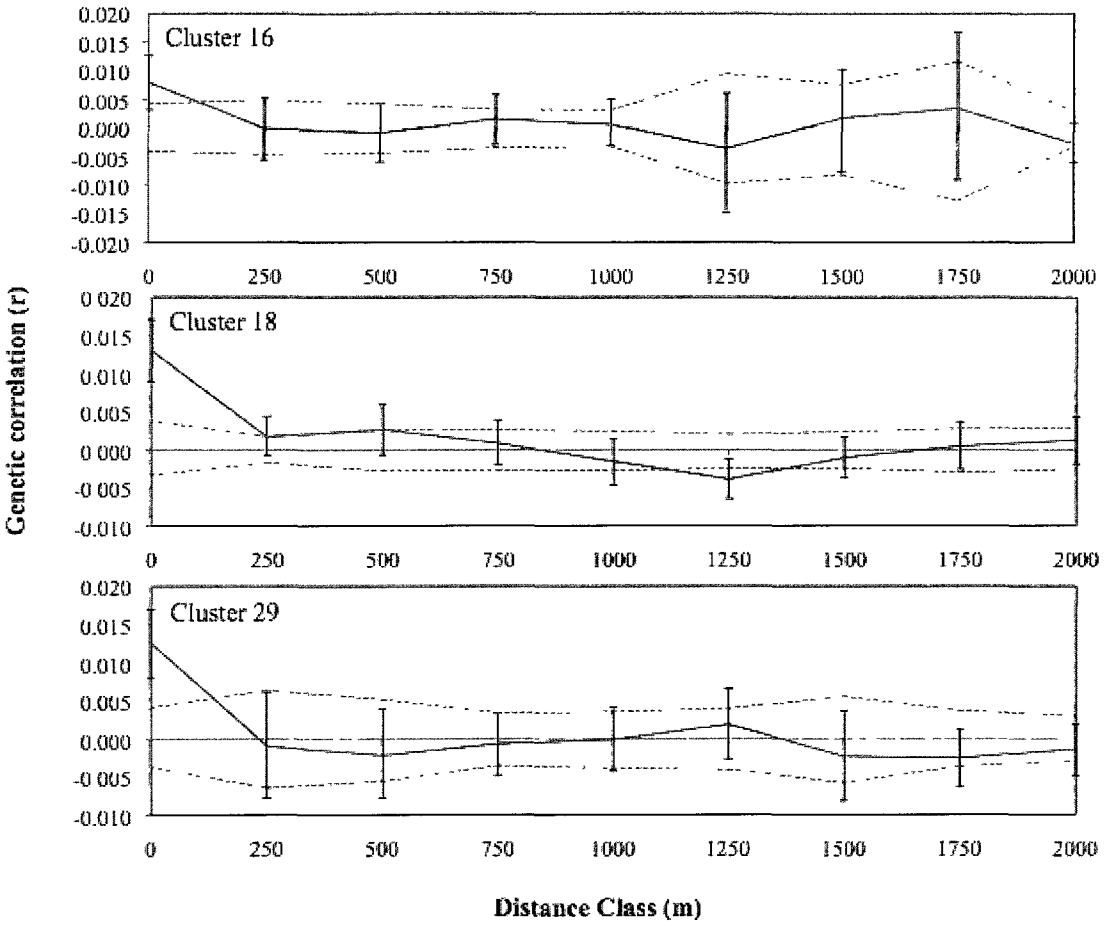


Figure 7. Correlograms of the genetic correlation coefficient ($r$) as a function of distance in the three clusters in the fragmented landscape. Distance classes were set to 250 m. The extent of the spatial genetic structure, as measured by the x intercept, is 246 m, 837 m, and 168 m for Cluster 16, 18, and 29, respectively. Null hypothesis of a random distribution of individuals is bounded by the 95% confidence intervals (dashed lines). Error bars for mean $r$ at each distance class were determined by bootstrapping.

Dispersal distances estimated in SPAGEDI ranged from 166 m to 453 m (Table 5). Wood frogs in cluster 18 had a reduced dispersal distance relative to the other two fragmented clusters, but this dispersal distance was very similar to that of the unfragmented clusters. Values of the level of spatial genetic structure (*Sp*) were low, with few values above 0.001 (Table 5). *Sp* was an order of magnitude higher in cluster 18, which coincides with the pattern of spatial autocorrelation observed; however, values of *Sp* in cluster 39 were similar, even though no spatial structure was observed in the correlogram.

Table 6. Mean parent-offspring dispersal distances calculated with the program SPAGeDi for each of the five clusters of wood frog ponds. Values of *Sp*, indicative of the level of spatial genetic structure, were calculated using relationship coefficients derived via two different methods: Queller and Goodnight (1989) and Wang (2002).

| | | | *Sp* | |
|---|---|---|---|---|
| Landscape | Cluster | Dispersal Distance | Queller & Goodnight | Wang |
| Fragmented | 16 | 421 m | 0.0011 | 0.0003 |
| | 18 | 177 m | 0.0037 | 0.0054 |
| | 29 | 453 m | 0.0002 | 0.0007 |
| Unfragmented | 36 | 166 m | 0.0010 | 0.0027 |
| | 39 | 187 m | 0.0022 | 0.0060 |

## Detection of Barriers to Dispersal

Genetic divergence maps were used to identify landscape features associated with hot spots of genetic divergence. In the fragmented landscape, areas of genetic divergence in the clusters (indicated by warmer colors) were associated primarily with roads, as well as development and agriculture (Fig. 8, 9, 10). In the unfragmented landscape, areas of high divergence coincided primarily with large bodies of water and high elevation (Fig. 11 and 12). In cluster 36, the park's access road also appears to be located in an area of greater genetic divergence.

Results from BARRIER analyses were used to identify barriers to gene flow within the clusters of ponds in each landscape. Barriers supported by more than half of the loci were overlaid on the genetic divergence maps to provide a comparison between the two congruent methods. Within the three fragmented clusters, barriers appeared to coincide largely with suburban development, roads, and agriculture. The first order barrier in cluster 16 was supported by 7 out of 8 loci and was associated with division occurring north and south of Rte 9, as well as with the effect of the subdivision in the southwestern portion of the cluster (Fig. 8). The second order barrier, supported by 6 of 8 loci, coincided with a road subdividing a housing development, which formed a barrier between adjacent ponds separated by less than 200 m. Seven out of 8 loci supported a third order barrier that corresponds to an unmaintained dirt road (Old Green Hill Road), assigned a road cost of four, that separates one pond, 16H, from the rest of the ponds in the cluster. In cluster 18, the first order barrier (supported by 6 out of 8 loci) was associated with Rte 155 running north and south and Steppingstone Road (a local paved road), assigned a cost of four, running northwest (Fig. 9). The second order barrier

(supported by 7 out of 8 loci) appears to isolate pond 18C2, with barriers associated with a housing development and two roads. The third order barrier (supported by 6 of 8 loci) separates populations north and south of Rte 4. Lastly, for cluster 29, all three barriers were supported by 5 of the 8 loci and seem to be roughly correlated with Rte 4 and the two roads running perpendicular to Rte 4 (Hall Rd to the North and Smoke St. to the south; Fig. 10). It is important to note that while there do appear to be barriers associated with Rte 4 in Cluster 29, the barriers do not correspond perfectly with the road. For example, there are two ponds separated by only 200 m that exist on opposite sides of Rte 4 that have neither a hotspot nor a barrier detected between them.

Barriers identified in the unfragmented clusters were associated primarily with large bodies of water and high elevation. In cluster 36, both the first and second order barriers were supported by 6 of the 8 loci and segregated ponds found on the opposite side of a ridge approximately 600 feet tall. The third order barrier (also supported by 6 of 8 loci) corresponds to a large body of water (Fig. 11). In cluster 39, all barriers were supported by 7 of the 8 loci. It is not clear if the first order barrier in cluster 39 is associated with any particular landscape feature, although a small dirt visitor access road does separate the ponds on either side of the barrier. Similarly, the landscape surrounding the pond encompassed by the second order barrier is not composed of any obvious features that might be expected to isolate that pond. In addition, the pond is not separated by a large geographic distance from any neighboring pond. Lastly, the third order barrier appeared most closely associated with a large body of water (Fig. 12).

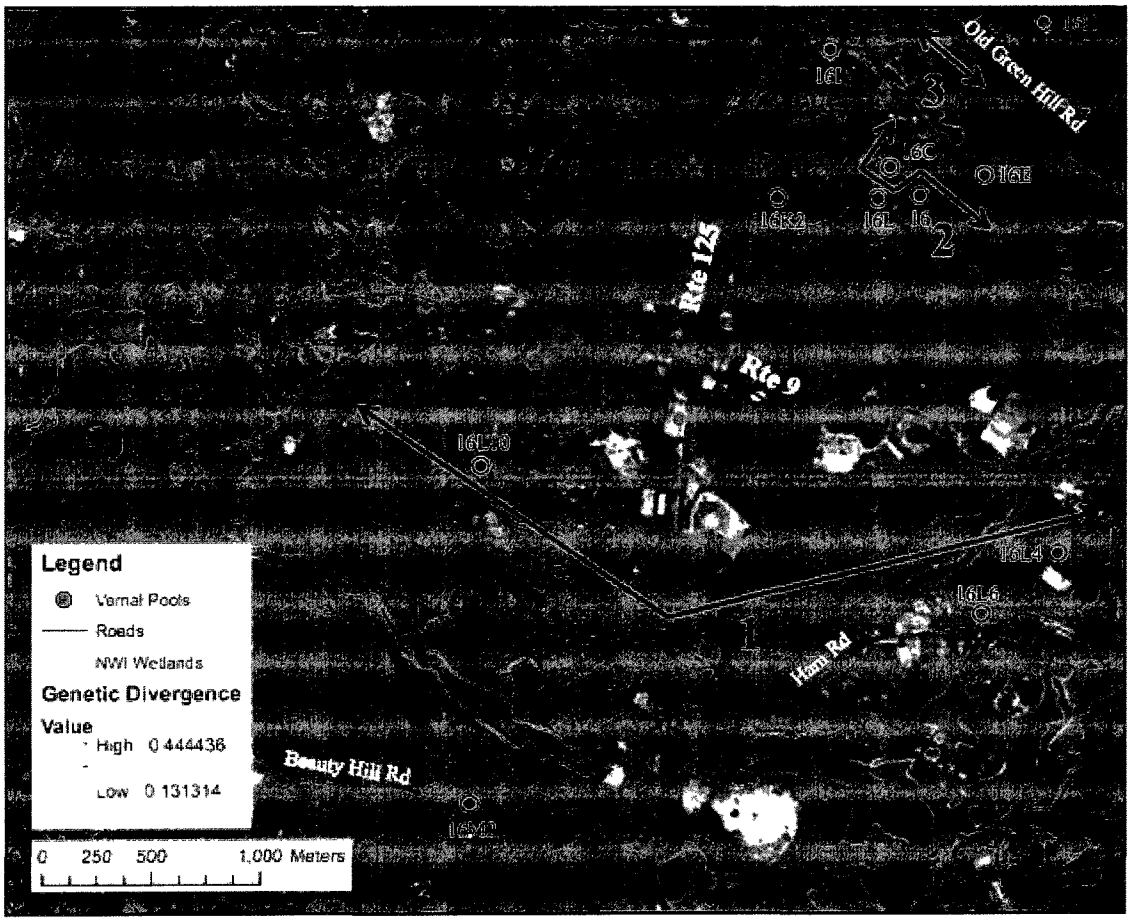


Figure 8. Map of genetic divergence in Cluster 16 within the fragmented landscape. Divergence is represented by colors red to green, with warmer colors representing areas of elevated genetic divergence. Overlaid are first, second, and third order genetic barriers (labeled 1-3).

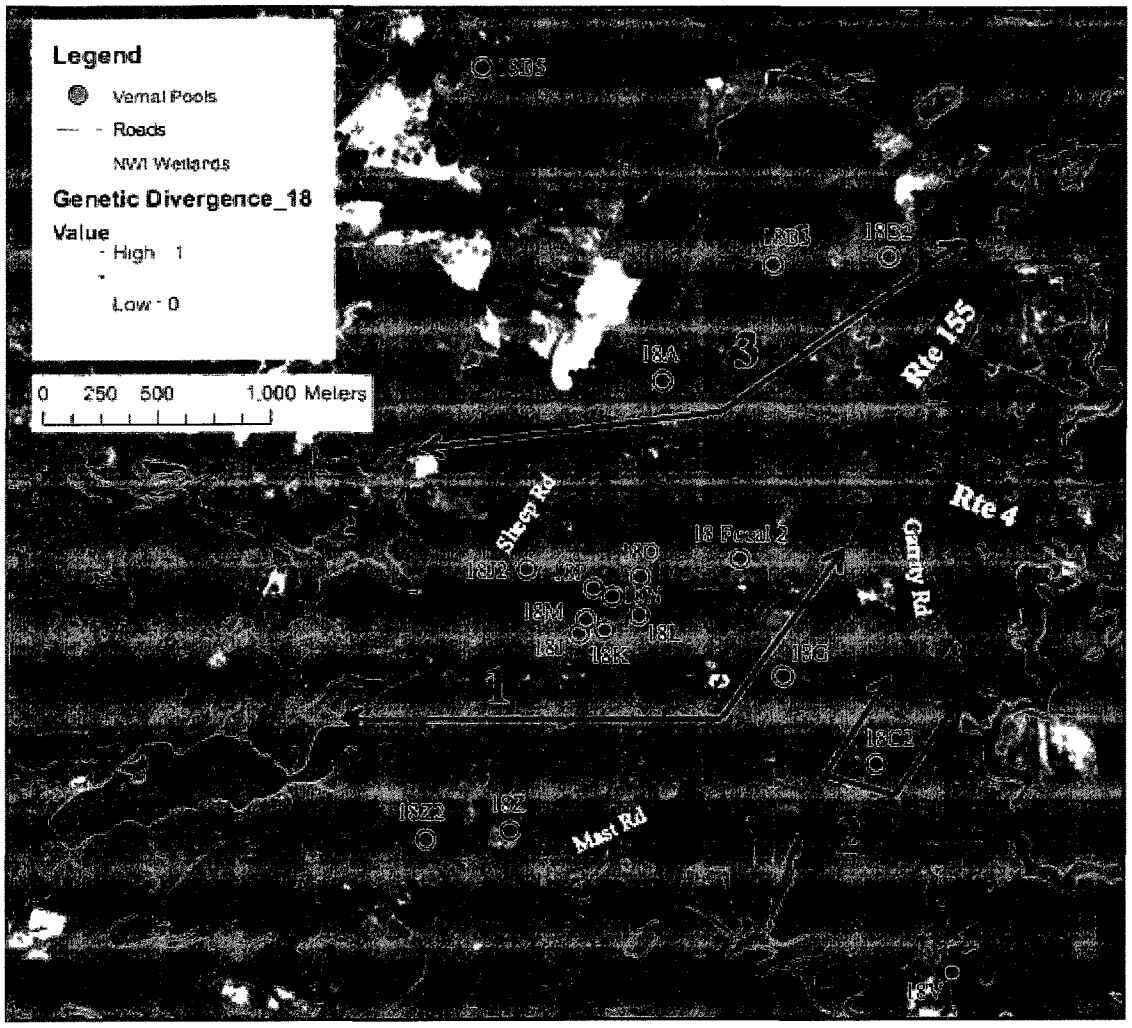


Figure 9. Map of genetic divergence in Cluster 18 within the fragmented landscape. Divergence is represented by colors red to green, with warmer colors representing areas of elevated genetic divergence. Overlaid are first, second, and third order genetic barriers (labeled 1-3).

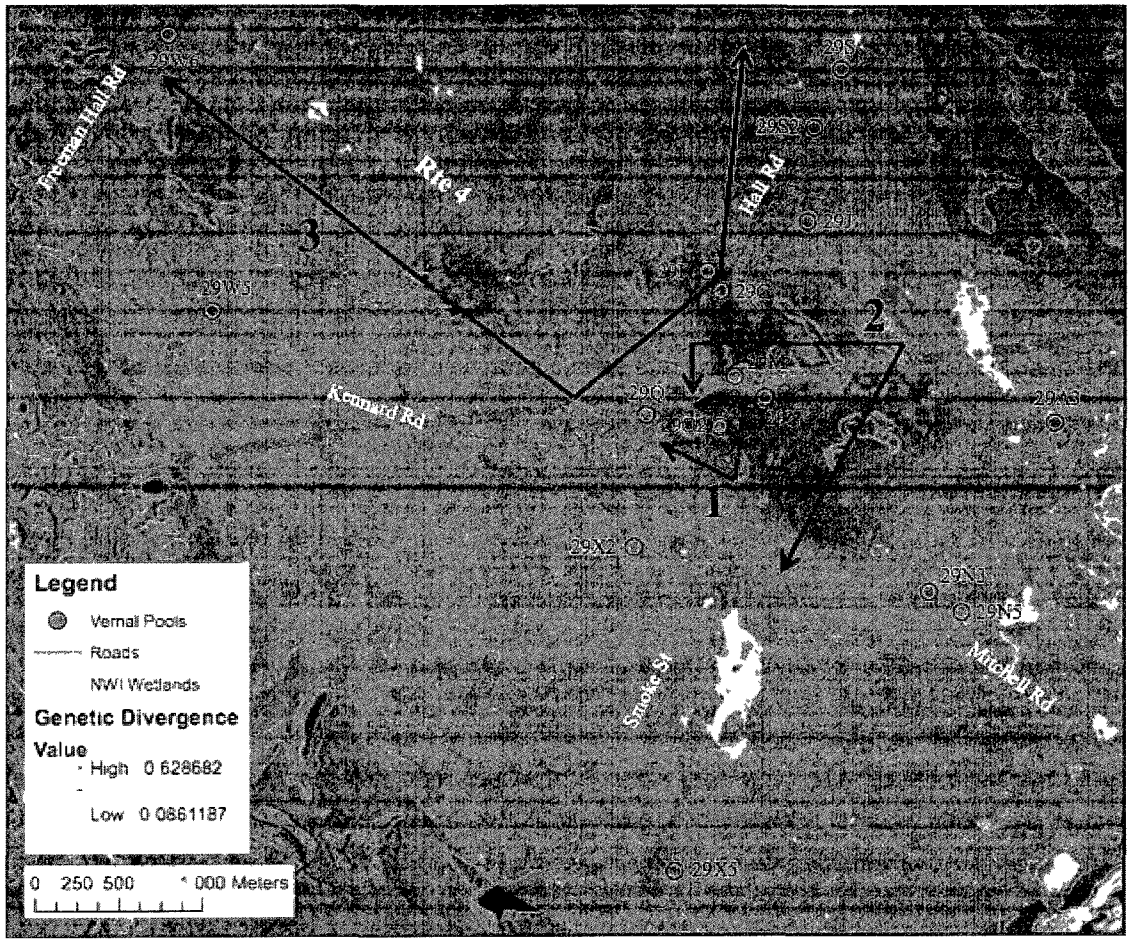


Figure 10. Map of genetic divergence in Cluster 29 within the fragmented landscape. Divergence is represented by colors red to green, with warmer colors representing areas of elevated genetic divergence. Overlaid are first, second, and third order genetic barriers (labeled 1-3).

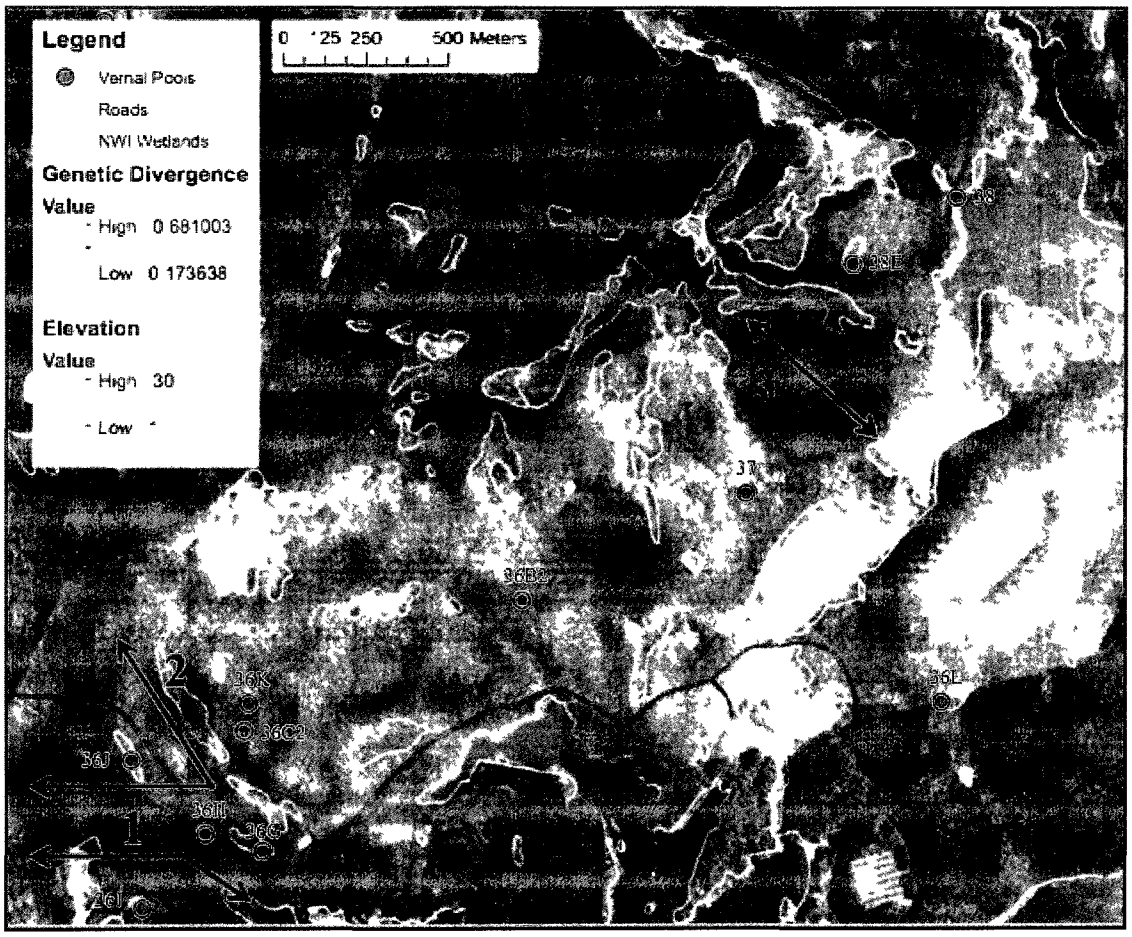


Figure 11. Map of genetic divergence in Cluster 36 within the unfragmented landscape. Divergence is represented by colors red to green, with warmer colors representing areas of elevated genetic divergence. Elevation is depicted by a shade gradient of dark to light, with darker shades indicative of higher elevation. Overlaid are first, second, and third order genetic barriers (labeled 1-3).

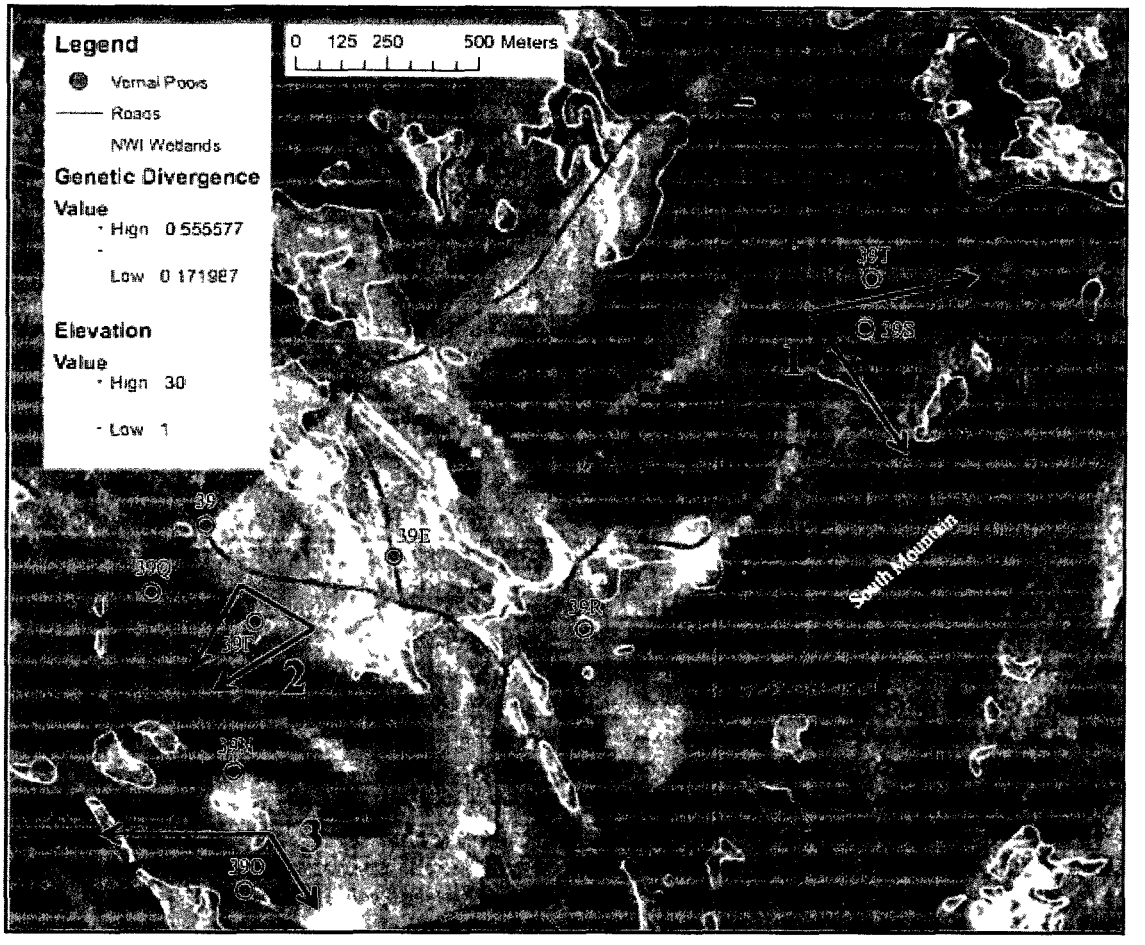


Figure 12. Map of genetic divergence in Cluster 39 within the unfragmented landscape. Divergence is represented by colors red to green, with warmer colors representing areas of elevated genetic divergence. Elevation is depicted by a shade gradient of dark to light, with darker shades indicative of higher elevation. Overlaid are first, second, and third order genetic barriers (labeled 1-3).

## Effect of Water Quality and Hydroperiod on Genetic Structure

Results of linear regression showed little to no correlation of the water quality variables with pond-specific $F_{ST}$, with values of $r$ ranging from -0.009 to 0.197 in the unfragmented landscape and 0.035 to 0.216 in the fragmented landscape (Appendix C). Stepwise regression analyses showed similarly non-significant results for both landscapes with $R^2$ ranging from 0.0001 to 0.09 and AICc ranging from -503.5 to -496.6. None of the environmental parameters in the stepwise regression analysis met the threshold requirement of p=0.25 to enter into the model.

GESTE analysis of ponds in the unfragmented landscape yielded the constant model as the highest probability model, suggesting that the environmental factors are not correlated with any pattern in genetic structure. However, the highest probability model resulting from the analysis of ponds in the fragmented landscape was that containing the constant and pH, with a posterior probability of 0.73 (Table 6). Within the relatively small geographic area, the variability of pH was high among ponds in the landscape, with values of pH ranging from 3.43 to 7.21 (Appendix D). All other factors and interactions between them were not retained by the Bayesian analysis.

Table 7. Summary of results of GESTE analyses of environmental and landscape variables on population genetic structure, showing the highest probability model selected for each analysis and the associated posterior probabilities.

| Landscape Type | Analysis | Highest probability model | Posterior probability |
|---|---|---|---|
| Fragmented | Water factors [hydroperiod, pH, conductivity, DOC] | Constant, pH | 0.656 |
| | pH + road effect + geographic distance + population size | Constant, pH | 0.727 |
| Unfragmented | Water factors [hydroperiod, pH, conductivity, DOC] | Constant | 0.601 |
| | Geographic distance + population size | Constant | 0.531 |

# CHAPTER 4

# DISCUSSION

## Population Structure

I found high connectivity and little genetic structuring among wood frog (*Lithobates sylvaticus*) populations across the scope of the study area. Pair-wise values of $F_{ST}$ were low (ranging from 0.000 to 0.037), neither of the two Bayesian clustering methods showed evidence of structuring, and the majority of the variation in the AMOVAs was accounted for by the within populations component, all of which suggest high levels of gene flow. These results are consistent with previous studies of wood frog populations (Newman and Squire 2001; Squire and Newman 2002; Crosby et al. 2009) that found similarly high levels of connectivity at similar scales.

Environmental features, historical processes, and life histories may all, to some extent, shape the genetic structure of populations (Balloux and Lugon-Moulin 2002). The lack of substantial structuring in this area of southeastern New Hampshire is likely a result of the short amount of time since the onset of major suburbanization in the area and high rates of dispersal among the study ponds. Anthropogenic fragmentation in this region may be too recent to allow detection of genetic differentiation. A time lag may exist between the processes responsible for the formation of spatial genetic structure and the observed spatial genetic structure itself (Anderson et al. 2010).

Numerous studies (Gill 1978; Berven and Grudzien 1990; Sjogren 1991; Sinsch 1992; Hecnar and M'Closkey 1996; Driscoll 1997; Skelly et al. 1999; Marsh and

Trenham 2001) have reported that amphibians exist as metapopulations, due largely to the assumption of limited dispersal ability, strong site fidelity, and spatially discrete breeding habitat of pond-breeding amphibians (Sinsch 1990; Blaustein et al. 1994; Alford and Richards 1999; Marsh and Trenham 2001). Smith and Green (2005) argue that not all amphibian populations exist as metapopulations, as they may not meet the assumptions required for the existence of a metapopulation effect, as defined by Hanski et al. (1991), particularly the assumption of limited dispersal. Due to the high rates of dispersal and connectivity observed, wood frogs in this study do not appear to be structured into metapopulations. However, due to differences in habitat quality, individual pond characteristics, and the generally high rate of population turnover in wood frogs, recolonization and extinction dynamics are still likely to be a prominent process occurring across ponds in this landscape (Hecnar and M'Closkey 1996; Alford and Richards 1999; Werner et al. 2007).

Despite the high degree of variability in the structure and dynamics of amphibian metapopulations (Newman and Squire 2001; Marsh and Trenham 2001; Jehle et al. 2005; Johansson et al. 2005), it is important to consider the effects of interpond movement on gene flow and resulting population structure. The effects of metapopulation dynamics (local extinctions and recolonizations) on population structure depend largely on the number and origin of founders that recolonize populations (Slatkin 1977; Pannell and Charlesworth 2000; Allendorf and Luikart 2007). Genetic differentiation of local populations may be enhanced if populations are founded by a few individuals, so that genetic differentiation is enhanced by bottleneck, in what is known as a founder effect (Wright 1940; Mayr 1942). In contrast, extinctions and recolonizations may homogenize

gene flow and reduce genetic differentiation if populations are founded by several individuals drawn from different populations (Slatkin 1987).

Vernal pools are abundant throughout southeastern New Hampshire (New Hampshire Wildlife Action Plan 2006). As such, ponds likely serve as stepping stones across the landscape and may facilitate dispersal, allowing adequate movement of wood frogs between breeding ponds in the landscape to prevent any large-scale genetic structuring for this species. High genetic connectivity in this area may further be attributed to the life history characteristics of a wood frog. Wood frogs are highly vagile and exhibit volatile population dynamics, with high rates of population turnover (Berven 1990; Hecnar and M'Closkey 1996; Alford and Richards 1999; Skelly et al. 1999; Werner et al. 2007). The lack of genetic structure may be a result of the homogenization of populations through recolonization by individuals from multiple adjacent ponds.

In an attempt to characterize the genetic structure of wood frogs, it is important to address the issue of scale. Within the study area, an area spanning approximately 25 km, wood frogs exhibited high levels of connectivity, with values of genetic differentiation ($F_{ST}$) among ponds ranging from 0.0001 to 0.0345, and an overall $F_{ST}$ value of 0.009. This low level of structuring could be a result of the scale of the study area, which might be too small to account for the variability that could affect wood frogs in the landscape. Higher genetic differentiation might occur across a greater spatial scale. To identify these potential scale effects, I determined the amount of genetic differentiation between the population of wood frogs in this study and a population of wood frogs in east-central Maine, just east of Milford, ME (northeast of Bangor and Orono), genotyped at the same 8 microsatellite loci (S. Coster, A. Kovach, K. Babbitt; unpublished data). Significant

genetic differentiation ($F_{ST}$=0.08) was found between these two populations, separated by a distance of 340 km. Though this genetic differentiation is an order of magnitude higher than within the current study, it still represents a relatively low level of differentiation, suggesting that wood frogs are well connected in the landscape, even over a large geographic distance. This might be indicative of extensive gene flow between local populations (ponds) in the past leading to extensive admixture (Zamudio and Wieczorek 2007).

Though ponds appeared to be highly connected at the broad-scale of the study area, fine-scale analyses revealed that not all ponds are equally connected throughout the landscape. Though evidence of some fine-scale spatial genetic structure was apparent, I did not find a clear, consistent pattern to the structure. There was a large degree of variability in the genetic differentiation among ponds, and pair-wise $F_{ST}$ values ranged from 0.00 to 0.037. This level of differentiation was significant for only a few pairs of ponds within each of the clusters; however, many pair-wise comparisons ranged between 0.01 and 0.03 that were not statistically significantly differentiated. These values are substantial, even if not statistically significant, and could prove meaningful in evaluating the potential causes of discontinuities across the landscape.

No single factor best explains the largest or most significant pair-wise differences in the study. Sometimes these differences occurred between adjacent ponds at small geographic distances, suggesting that habitat heterogeneity, landscape features, pond-specific characteristics, population processes, or any combination thereof, may be responsible for genetic structuring in these landscapes.

The length of time that the pond held water seemed to be an important factor in structuring populations at the fine-scale. Within the study area, wood frogs were sampled from ponds with a wide range of hydroperiod scores (5.36 to 20). Across this range, I found wood frogs breeding in roadside ditches as well as semipermanent and permament ponds. Woods frogs are known to breed opportunistically at less optimal sites, which can vary greatly across years in the production of emerging metamorphs, thereby preventing the development of stable populations. Unstable population dynamics may explain high differentiation of a few of the ponds in this study. Pond 18Z within the fragmented landscape exhibited significant genetic differentiation from 11 of the 18 ponds in its cluster, with significant pair-wise $F_{ST}$s ranging from 0.016 to 0.037, and the highest pond-specific $F_{ST}$ across all populations, at a value of 0.028. 18Z is a deep (~10 foot deep), man-made pond in the middle of a mowed lawn on private property. The pond is stocked with fish and snapping turtles (personal observation and communication with land owner, 2010), common predators of amphibian eggs (Egan and Paton 2004). While the presence of predators should render the pond unsuitable for amphibian survival and development and therefore unstable for population persistence, opportunistic breeding occurs. Frequent recolonizations of the pond by a few founders may promote genetic differentiation from frogs in nearby ponds with good quality breeding habitat.

Additionally, Pond 18J2, a roadside ditch also in cluster 18, had high values of differentiation (0.010-0.032) and may also represent an unstable population undergoing the same process of recolonization as described above. In this case, however, the genetic differentiation may be a result of short hydroperiod relative to adjacent ponds, which could reduce larval survival if the pond dried up before wood frogs metamorphosed.

Short hydroperiod is likely the cause of genetic differentiation of pond 29O2 in cluster 29 ($F_{ST}$ ranging from 0.011 – 0.029), as well, as no significant barriers to dispersal exist between 29O2 and the ponds directly adjacent to it, with which the pond showed significant differentiation. 29O2 is a small vernal pool, immediately adjacent to the road, supplied with water from a culvert. With a short hydroperiod and high conductivity (Appendix D), population turnover may be high, making the pond more genetically distinct from ponds in its immediate proximity.

Isolation may also cause population structuring as a result of decreased immigration and colonization rates (MacArthur and Wilson 1963; Hanski 1991), sometimes due to unsuitable habitat between extant and extinct groups (Sjogren 1991; Blaustein et al. 1994). This might be the explanation for high levels of population differentiation observed in pond 18C2 ($F_{STS}$ ranging from 0.012 – 0.035). 18C2, though not geographically isolated from other ponds, is isolated by the potentially unsuitable habitat surrounding it. The pond is located in the bottom of a gravel pit. Due to the stringent moisture requirements of amphibians, the lack of canopy cover at this site may increase the risk of desiccation in dispersing individuals.

Lastly, landscape features can contribute to structuring either by impeding dispersal or facilitating movement across the landscape. In cluster 39 in the unfragmented landscape, genetic differentiation was most pronounced for pond 39F, which had significant pair-wise $F_{STS}$ ranging from 0.004 to 0.010. 39F is situated between a ridge and a dirt access road; both features could be responsible for the observed structuring, although the ridge is more likely to restrict movement of amphibians in this area.

## Effects of Habitat Fragmentation and Other Landscape Influences

Population structure and dispersal between amphibian populations is influenced by various natural and anthropogenic landscape features including roads (Fahrig et al. 1995; Vos and Chardon 1998; Trombulak and Frissell 2000; Gibbs and Shriver 2005; Balkenhol and Waits 2009), large-scale development, elevation (Funk et al. 2005; Spear et al. 2005; Giordano et al. 2007), and large bodies of water (Lampert et al. 2003). All these factors have the potential to reduce the connectivity of populations by reducing the permeability of the landscape to amphibians (*reviewed in* Semlitsch 2000 and Marsh and Trenham 2001), resulting in dispersal barriers.

Through a comparative approach, I found evidence of isolation by distance, road effects, and reduced mean parent-offspring dispersal distance among some clusters of ponds in the fragmented landscape, while clusters in the unfragmented landscape did not exhibit these effects. These findings suggest that habitat fragmentation in the developed suburban landscape of this study area has measurable, although not yet substantial, effects on the population dynamics and structure of wood frogs.

Habitat fragmentation can impede dispersal, potentially resulting in greater population structuring in areas of high anthropogenic influence. The vast network of roads increases landscape fragmentation, resulting in small, isolated habitat patches (Forman and Alexander 1998). In combination, road-induced habitat loss, barrier effects, and mortality can result in increased extinction risk for populations (Findlay and Bourdages 2000; Trombulak and Frissell 2000; Gibbs and Shriver 2005). These impacts of fragmentation have been exhibited by several animal populations, including amphibians (Vos et al. 2001; Spear et al. 2005; Clark et al. 2008). Roads in close

proximity to vernal pools have been found to impact amphibians negatively, primarily through the reduction of abundance and disruption of movement (Gibbs 1998). The genetic impacts of population fragmentation may range from insignificant to severe, depending on the characteristics of the resulting population structure and dispersal among fragments.

Although this study detected effects of distance and roads on genetic differentiation, the combination of analyses used to determine these effects did not always produce consistent or predictable results across the separate pond clusters. Spatial clustering of ponds using AMOVA did not indicate that roads were the primary force structuring the ponds within clusters or across the fragmented landscape as a whole. The influence of roads was detected, however, by analyses of roads at the fine-scale, utilizing fine-scale landscape genetic approaches including isolation by distance, Mantel tests, and spatial autocorrelation.

Typically, isolation by distance (IBD) effects are expected in populations in unfragmented landscapes, where levels of gene flow tend to decrease with increasing geographic distances, resulting in increasing genetic differentiation among individuals (Wright 1943). IBD may also be pronounced among populations of species with short dispersal distances (or low vagility) (Broquet et al. 2006). In fragmented landscapes, barriers to gene flow can disrupt IBD patterns, resulting in no correlation with geographic distance and gene flow, if the correlation is instead associated with the barrier (Coulon et al. 2004; Broquet et al. 2006). Alternatively, fragmentation can produce IBD effects through restricted dispersal in an otherwise unfragmented population that would not exhibit IBD at the same spatial scale in the absence of fragmentation (Smouse et al. 2008;

Walker et al. 2008). These IBD effects have been detected in studies of amphibians in landscapes fragmented by roads and other anthropogenic features (Hitchings and Beebee 1997; Stevens et al. 2006).

In this study, IBD was not detected in the unfragmented landscape. The lack of IBD in the unfragmented clusters may be indicative of high levels of gene flow among populations, over the relatively short distance sampled within the clusters. In the fragmented landscape, clusters 16 and 18 exhibited patterns of IBD and road effects. This pattern is consistent with restricted dispersal caused by roads, which would manifest in an IBD effect. The lack of IBD or road effects in cluster 29 suggests that gene flow in this cluster is adequate to prevent any genetic differentiation over the sampled distance, despite the presence of major roads in this cluster. I hypothesized that this result may be a result of cluster 29 having the greatest proportion of forest cover and the smallest proportion of development of the fragmented clusters (Table 1).

If so, this suggests that rather than characterizing the clusters as either fragmented or unfragmented, it might be more appropriate to view them as points along a continuum of fragmentation. Applying this logic to the fragmented clusters supports this view, as correlations of genetic distance with geographic distance become stronger as the proportion of development within the clusters increases. However, there are likely several additional factors at play in influencing the observed structure within the clusters.

Though roads have some observable effect on genetic structure within clusters in the fragmented landscape, roads do not appear to have a significant effect on structuring of wood frogs across the study area overall, as evidenced by the relatively low $F_{ST}$ across all populations and the lack of hierarchical structuring due to roads on the scale of the

study area. There may be such a high abundance of wood frogs that even if roads have a negative impact on some proportion of dispersing individuals, gene flow remains high, provided that the majority of individuals make it across roads safely to other ponds in the landscape. If a single migrant per generation is sufficient to prevent significant genetic differentiation (Mills and Allendorf 1996), only ponds that do not exchange regular dispersers will diverge genetically. Vucetich and Waite (2000) suggested that for some populations, >10 immigrants are required per generation to avoid a substantial loss of genetic diversity, while other populations require >20 immigrants per generation, though these estimates require fluctuations in population size to be taken into account. Given the widespread distribution and high abundance of wood frogs, as well as the general wetness and high proportion of forest cover in the landscape, I felt it was likely that this rate of immigration would be obtained, thereby enhancing connectivity.

The time frame over which fragmentation occurs is important to consider, as genetic effects in populations may not be observed for several generations following the creation of significant barriers. Some studies using $F_{ST}$ have found long lag time for detection of the genetic effects of landscape change using $F_{ST}$ (Holzhauer et al. 2006; Keyghobadi et al. 2005). Landguth et al. (2010) suggest that the lag time for the signal of a barrier to become established is approximately 200 generations using $F_{ST}$. However, they also suggest that for species with relatively large dispersal distances, the lag time may be significantly shorter, on the order of 15 generations, which would allow early detection of population fragmentation. Previous studies have demonstrated that roads can exhibit rapid effects on population structure, even over a short time period for species with both short and long generation times (Keller et al. 2005; Riley et al. 2006; Clark et

al. 2010).

In this study, the largest roads within the study area (Rte 4, Rte 125, and Rte 9) were constructed in the early 1920s to the mid-1930s (NH DOT Highway Design Records). Assuming a generation time of two to three years for wood frogs (Berven 1982; Redmer and Trauth 2005), I estimated that approximately 25 to 45 generations have passed since major road construction. Assuming a relatively high rate of dispersal, there has been sufficient time for a signal for wood frog populations to develop significant genetic differentiation (Landguth et al. 2010). This suggests that roads do not act as absolute barriers to gene flow.

Understanding dispersal is important for shaping regional patterns of genetic variation (Driscoll 1998; Shaffer et al. 2000). Amphibians as a group have limited dispersal, as a consequence of their physiology and behavior, and, in particular, their strong dependence on moisture (Sinsch 1990, Blaustein et al. 1994). Nevertheless, the maximum dispersal distance reported for wood frogs is 2.5 km (Berven and Grudzien 1990). In this study, mean dispersal distance calculated from each cluster ranged from 166 m to 453 m.

I expected wood frog dispersal distances to differ between the fragmented and unfragmented landscapes. Specifically, I expected wood frog dispersal to be reduced in the fragmented clusters as a result of greater resistance to movement across barriers formed by roads and other anthropogenic features. This was true for one of the clusters in the fragmented landscape (cluster 18), where the dispersal distance was estimated at 177 m, a value less than half of the distances calculated in clusters 16 and 29. It is important to note that though road effects were significant in cluster 16, dispersal distance in this

cluster showed no evidence of being restricted.

The two clusters in the unfragmented landscape had small dispersal distances, similar to the restricted dispersal distance of cluster 18 in the fragmented cluster, which is inconsistent with the trend expected for wood frog dispersal in an unfragmented landscape free of dispersal barriers.

Dispersal distance is notoriously difficult to estimate regardless of the method used, including this one. Accordingly, there are several things to keep in mind with these results. First, dispersal distance is inferred from the regression of the correlation between geographic and genetic distance (known as isolation by distance or IBD), however this is assuming that IBD exists in the system. From the Mantel tests described previously, IBD was significant in only one cluster (cluster 16), and so this calculation of the mean parent-offspring dispersal distance violated the assumption of IBD in all other clusters.

Second, the density estimate required for this calculation is crude, due to failure to sample all ponds or to faulty assumptions of wood frog sex ratio. Though I attempted to sample continuously throughout each cluster, it is possible that I could have failed to sample some ponds in the two fragmented clusters with large calculated dispersal distances, for reasons including inaccessible privately owned land or outdated NWI map data. If ponds were missed, abundance would be underestimated, thereby leading to overestimation of dispersal distance.

Additionally, while studies of anurans typically assume a sex ratio of 1:1 to estimate population size (using egg mass counts as a proxy for the number of breeding females), this number does not take into account the number of juveniles in the landscape, nor is it specific to wood frogs. The sex-ratio of wood frogs is not known with

certainty, and some researchers have suggested that there is substantial variability in the sex ratios observed in wood frog populations; some studies of wood frogs have found a sex ratio 2:1 (Howard and Kluge 1985; Berven and Grudzien 1990; Crouch and Paton 2000; Stevens and Paszkowski 2004), while others have exhibited sex ratios of approximately 1:1, as documented by Crouch and Paton (2000). These discrepancies in sex ratio could impact the calculated dispersal distances, as density of wood frogs is a required parameter for the analysis (Table 7). Regardless of the sex ratio used to calculate dispersal distance, the pattern across pond clusters remains the same, which is of primary interest for this study. As a result of varied sex ratios and the possibility of sampling error, the dispersal distances reported herein should be regarded as relative values rather than actual values, and are offered to provide insight into the processes affecting movement of individuals within the two separate landscape types.

Regardless of the sex ratio used to calculate dispersal distances, all of the values reported are much lower than the maximum dispersal distance of 2.5 km reported for wood frogs. However, it's important to note that this method calculates the mean parent-offspring dispersal distance, and not the maximum dispersal distance. It could be that, for wood frogs in this study area, the average parent-offspring dispersal distance is small, ranging between 100 and 200 m, as estimated in SPAGeDi, but there may be some rare long distance dispersal events (potentially much longer, at distances of one to a few kilometers) that could account for the lack of structure observed. Wet forested habitat is still abundant in NH, which could allow for these long-distance dispersal events to occur. Additionally, vernal pools are abundant in this landscape, and so, wood frogs might not have to disperse as far, which could also explain the small dispersal distances calculated.

Table 8. Mean parent-offspring dispersal distances calculated with the program SPAGeDi for each of the five clusters of wood frog ponds using a range of sex ratios of males to females to estimate population density. Sex ratio of 0:1 is representative of the egg mass count, or the number of breeding females only.

| | | Dispersal Distance (m) | | | |
|---|---|---|---|---|---|
| Landscape | Cluster | 0:1 | 1:1 | 2:1 | 3:1 |
| Fragmented | 16 | 595 | 421 | 343 | 297 |
| | 18 | 251 | 177 | 145 | 125 |
| | 29 | 691 | 453 | 370 | 320 |
| Unfragmented | 36 | 235 | 166 | 135 | 117 |
| | 39 | 264 | 187 | 153 | 132 |

In the fragmented landscape, barriers and areas of high genetic divergence were associated primarily with roads and development, while elevation and large bodies of water corresponded with barriers and genetic divergence in the unfragmented landscape. However, calculated barriers and genetic hot spots did not always correspond perfectly to these structuring features, suggesting that there may be other factors responsible for generating differentiation between populations in the landscape.

For instance, in cluster 16, the most pronounced genetic hot spot and first order barrier occurs between 16M2 and 16L10, two ponds in the southwestern portion of the cluster, separated by 1.5 km. I expected hot spots and barriers to be associated with roads, particularly in this cluster, given the significant effect of roads detected by the Mantel and partial Mantel test. No major roads separate these ponds; however, the hot spot appears to be associated with a large housing development, which could act as a significant dispersal barrier for wood frogs for a variety reasons, including: lack of moisture from reduced canopy cover and large lawns, pesticides and chemicals associated with lawn care, and degraded habitat. No hot spot or barrier was present between 16L10 and 16K2, which is unexpected, since the two ponds are separated by a large distance (1.8 km) and both Rte 125 and Rte 9. Similarly, no hot spot or barrier was detected between 16M2 and 16L6, ponds separated by Rte 125 and a distance of 2.6 km. The second order barrier in cluster 16 is interesting, as it separates two ponds on either side of a road in a housing development, with pond 16C on the interior and pond 16 outside the development. The fact that a barrier was detected between these two adjacent ponds is important to note, but the results also show that the barrier separates 16C from 16L, an adjacent pond also located within the interior of the development, suggesting that roads

might not be the best factor to explain the barrier. Other existing influences may affect gene flow between these ponds.

Similarly inconsistent effects were detected in cluster 29, where I predicted that New Hampshire Rte 4, a divided highway (15-20 m in width, depending on the location), would be the greatest barrier to dispersal, therefore causing the greatest level of genetic divergence between ponds on either side of the road. There was a signal of genetic divergence associated with Rte 4, with the most pronounced divergence present at the intersection of Rte 4, and two local paved roads: Hall Rd, to the north, and Smoke St to the south. Additionally, some barriers did separate the populations north and south of Rte 4. However, no barrier was detected between two nearby ponds on either side of Rte 4 (29M and 29N), separated by only 170 m. Interestingly, a culvert runs under Rte 4, which may serve as a connection between the two ponds.

There is evidence to suggest that culverts are used by a range of vertebrates (Hunt et al. 1987) and may serve as effective corridors for amphibians. Culverts may facilitate dispersal by providing a moist conduit that mitigates the risks ordinarily associated with crossing roads (Yanes et al. 1995; Jackson and Griffin 2000; Dodd et al. 2004; Patrick et al. 2010). Some studies have suggested that culvert characteristics, such as the length of the culvert, may influence whether they are utilized by amphibians (Lesbarreres et al. 2004; Woltz et al. 2008). The culvert connecting the two ponds in cluster 29 spans a section of Rte 4 that is approximately 20 m in width. Dodd et al. (2004) found widespread use of 44 m long culverts by amphibians, suggesting that the same use of the culvert in my study area should be possible. Even if only a small proportion of individuals utilize the culvert for dispersal, the reduction of mortality from road effects

may be enough to promote connectivity across ponds separated by Rte 4 in this location.

Within the unfragmented landscape, I expected barriers to be associated with natural landscape features such as elevation and large bodies of water, as previous research has shown these features to structure amphibian populations. Funk et al. (2005) found significantly reduced gene flow between Columbia spotted frog populations (*Rana luteiventris*) situated on either side of mountain ridges and between low-and high-elevation populations, despite close geographic proximity. Major water bodies have also been found to create resistance to gene flow. Lampert et al. (2003) found that rivers served as effective barriers to Túngara frogs (*Physalaemus pustulosus*). Though no rivers exist in the current study area, large ponds, lakes, and streams are present which are also believed to present high resistance to amphibian dispersal (Compton et al. 2007).

Clusters in the unfragmented landscape showed some consistency in the barrier effect of elevation and large bodies of water; however, there were some cases where large bodies of water seemed to have no effect on genetic structure. In cluster 36, hot-spots of divergence and the second and third order barriers were associated with large bodies of water. In cluster 39, however, the barrier effects were isolating, separating single ponds from the rest of the populations in the cluster. No hot spots or barriers were present across the greatest distance in the cluster, a distance that was separated by a mountain and large water bodies. Given these unclear barrier associations, it is difficult to pinpoint any single factor responsible for driving population genetic differentiation in this area.

## Effects of Water Quality and Hydroperiod

Negative effects on wood frog survival and abundance have been associated with water quality changes resulting from fragmentation (Pough and Wilson 1977; Sadinski and Dunson 1992; Horne and Dunson 1994; Sanzo and Hecnar 2006; Karraker et al. 2008; Collins and Russell 2009). Additionally, fragmentation can alter the hydrology of vernal pools, with potential decreases in the hydroperiod, which can have a large effect on the ability of wood frogs to produce metamorphs for dispersal (Pechmann et al. 1989; Babbitt et al. 2003).

In the current study, wood frog genetic structure in the unfragmented landscape was not significantly influenced by any of the water quality parameters tested. In the fragmented landscape, however, pH was identified by the GESTE analysis as a factor influencing the genetic differentiation of ponds. The mean σ2 value associated with the pH model was moderate at a value of 0.36. This parameter represents the deviation from the regression, and a low value is indicative of a good fit between the model and the $F_{ST}$ values. In addition, the posterior probability for the null model was relatively low (0.12), further suggesting the validity of the model with pH; however, I have reason to believe that this result is spurious, and suggest that the supposed effect of pH is the product of a separate influence.

The positive value of alpha (α), the vector of the regression coefficient corresponding to the factor (Foll and Gaggiotti 2006), suggests a positive relationship between pH and $F_{ST}$, whereby higher pH is related to higher $F_{ST}$, or greater genetic differentiation. This result contradicts my prediction that low pH would influence genetic structuring, due to its negative effect on amphibian survival and abundance. Negative

effects of low pH, including increased time to metamorphosis and decreased survival and body mass at metamorphosis, have been reported for several species of amphibians (Horne and Dunson 1995). However, differential sensitivity to pH has been shown, with wood frogs exhibiting high tolerance to low pH. Several studies have stated that low pH generally does not limit the breeding success of wood frogs under field conditions (Freda and Dunson 1986; Sadinski and Dunson 1992). In controlled experiments, wood frog embryos have been shown to tolerate pH of 4.2 (Freda et al. 1991). Pierce et al. (1984) even reported that reduced hatching success, development, and survival of wood frogs to metamorphosis was only significantly altered at pH 3.75 and lower.

The pH of precipitation in New Hampshire is typically within the range of 4.0 and 4.8 (New Hampshire Department of Environmental Sciences, 2008). The majority of ponds in the study had values of pH within this range or lower. Twenty-seven of 65 ponds in the study area had values of pH greater than 5.0. Of these 27 ponds, five had a pH above 6.0, with some ponds approaching neutrality. Ponds with values of pH this high are indicative of ponds with groundwater seepage and had long hydroperiods, with several ponds holding water for the duration of the survey. These were deep ponds with little vegetation, and at least three of the ponds had fish present, causing me to believe that these ponds may serve solely as opportunistic breeding sites for wood frogs. Due to the high risk of predation, these ponds may not be used for breeding each year, and high population turnover could result in greater genetic distinctiveness, if extinction-recolonization events produce transient differentiation through founder events. Therefore, the result that high pH can lead to greater population structure is more likely representative of the association of high pH with permanent ponds. Given these results, it

is surprising that hydroperiod was not a significant factor in generating population differentiation.

I hypothesized that one potential reason for my failure to detect an effect of hydroperiod was the method I used to score hydroperiod across ponds. In my analyses, ponds that held water for the duration of survey received a score of 20. I hypothesized that assigning this value to "permanent" pools might not make biological sense from a wood frog's perspective, since it might be more important to consider the hydroperiod relative to the time the wood frogs spend in the pool prior to metamorphosis, rather than throughout the entire season. To devise an alternative score for these permanent ponds, I examined data from a Maine survey of wood frogs, and noted that the majority of wood frog metamorphs left the vernal pools by mid-August (J. Veysey and K.Babbitt; unpublished data). Under my scoring method, the approximate hydroperiod score associated with a mid-August date is 12. Thus, I performed a post hoc investigation with linear regression of pond-specific $F_{ST}$ and hydroperiod, using a hydroperiod score of 12, instead of 20, for ponds that retained water for the duration of the study. Using this new value, however, I still found no correlation between hydroperiod and genetic differentiation ($R^2$=0.009; p=0.44), which indicates that the initial lack of correlation was not due to a scoring artifact. Hydroperiod is not influential regardless of scoring system. The correlation of high pH with increased genetic differentiation may instead be related to an indirect influence of some other unmeasured factor or combination of factors characteristic of long hydroperiod ponds, rather than the direct influence of pH on wood frogs. If so, the designated hydroperiod score of 20 could be used to represent these factors.

The lack of correlation between genetic differentiation and the other water quality parameters, conductivity and DOC, may be attributed to the relatively moderate values of both in the ponds within the current study area. Karraker et al (2008) demonstrated significant effects of conductivity on wood frog development and survival at levels of 3000 µS, a value far exceeding that found in any of the ponds in my study (mean=117 µS; maximum=1231 µS; Appendix D). Similarly, levels of DOC were not within the level found to have a negative effect on wood frogs. Horne and Dunson (1995) showed that wood frogs required more time to develop and showed lower survival in water containing DOC of 30 mg/L. Average DOC in ponds in the current study area was 8.51 mg/L and the maximum DOC recorded was 26.02 mg/L (Appendix D). Though these values were too low to show any effect on wood frog genetic differentiation over all the ponds together, some ponds did exhibit values approaching those associated with negative effects. If acidification and road run-off increases with increased development and suburbanization, more ponds in the landscape may begin to have values in these upper ranges, warranting concern for amphibian populations inhabiting these ponds. At this point, however, water quality does not appear to be negatively impacting wood frogs or the processes required for population persistence.

# CHAPTER 5

## CONCLUSION

The primary objective of this study was to characterize the genetic structure of wood frog populations in two different landscapes of southeastern New Hampshire: an unfragmented landscape, and a fragmented landscape characterized by roads and moderate levels of suburban development. Results of the study suggest that wood frogs are well connected across the scope of the study area. However, by using a fine-scale approach, evidence of genetic structuring was found within the separate clusters of vernal pools in each of the two landscapes. In the unfragmented landscape, I detected no effects of fragmentation in the clusters. However, I did find evidence of isolation by distance, road effects, and reduced mean parent-offspring dispersal distance among some clusters of ponds in the fragmented landscape. Since greater levels of these effects appear related to higher levels of development, it may be more useful to view the two landscapes on a continuum of fragmentation rather than as two distinct types.

In the fragmented landscape, barriers and areas of high genetic divergence were associated primarily with roads and development, while in the unfragmented landscape, elevation and large bodies of water corresponded with barriers and genetic divergence. Barriers did not always correspond to the hypothesized structuring features, however, which could suggest that there may be other factors responsible for the observed patterns of genetic differentiation.

Overall, water quality did not appear to have any effect on genetic differentiation of

wood frogs, although a potential effect of pH on genetic differentiation was identified for ponds in the fragmented landscape. While this result suggests that higher pH is related to higher differentiation, it may instead be related to a separate, indirect influence on pH or another unmeasured factor, through a correlation with pond permanence.

The degree and duration of fragmentation in southeastern New Hampshire is probably not currently great enough to create genetic effects across the scope of the study area. New Hampshire's landscape is not yet grossly fragmented, and vernal pools are abundant in high densities within the southeastern portion of the state. For a species with high abundance and dispersal ability, such as the wood frog, the presence of any detectable structure in the landscape could indicate the beginning of fragmentation effects on the population. In this study, such structure was supported by the association of genetic differentiation and barriers with roads and anthropogenic features that characterize suburban fragmentation. As a result, those seeking to conserve wood frog populations should recognize the potential effects of suburbanization on wood frog population connectivity, although the effects are not yet sufficiently severe to warrant broad-scale conservation measures. Since both landscapes in this study afforded adequate connectivity to facilitate wood frog dispersal, the actual amount of conservation required to maintain gene flow remains unknown. Determining this amount presents an important challenge to future studies.

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# APPENDICES

## APPENDIX A. MICROSATTELITES AND MULTIPLEX INFORMATION

Descriptive information on the nine wood frog microsatellite markers used and the multiplex to which each locus belonged.

| Locus | Repeat motif | Primer sequences (5' – 3') | Size range (bp) | Multiplex |
|---|---|---|---|---|
| *RsyC52* | $(TACA)_{17}$ | F: 5'-CCATACAACCGTGATTACAAAAG-3'<br>R: 5'-ATATACCACCCTTCCAGAGATG-3' | 125-210 | A |
| *RsyC83* | $(TACA)_{10}$ | F: 5'-TGCATTGTTTTACTTATGTGTGAAG-3'<br>R: 5'-TTACCTGGTCTTGTAATGGGAC-3' | 95-135 | A |
| *RsyD32* | $(TAGA)_{11}$ | F: 5'-GGACACACAATTCCTTGGTTC-3'<br>R: 5'-GAGGAGATTTCCAAAACAATCC-3' | 160-230 | A |
| *RsyD55* | $(TAGA)_{25}$ | F: 5'-GAGTTGGGACTCCTGAATAGAG-3'<br>R: 5'-AGTCTTTGCTTTGTAAATTGGC-3' | 150-250 | A |
| *RsyC11* | $(TACA)_{9}$ | F: 5'-TTACTTTCAGTTTCAAAAGGCAG-3'<br>R: 5'-TACACAGTGCTTCACAAGTTCC-3' | 105-185 | B |
| *RsyC41* | $(TACA)_{8}$ | F: 5'-GTCAAAACACAGATGCACAATC-3'<br>R: 5'-ACAAAACAGGAATCGGTCATAC-3' | 80-135 | B |
| *RsyC63* | $(TACA)_{12}$ | F: 5'-CAGAAAATTGCCGAAAAGG-3'<br>R: 5'-TGGGCTTAAGAAACAAAAGAAC-3' | 125-255 | B |
| *RsyD20* | $(TAGA)_{18}$ | F: 5'-GTTACTGTGGAGGTGATGTCTG-3<br>R: 5'-TTCTATATCAAGCACCCATCTG-3' | 200-280 | B |
| *RsyD77* | $(TAGA)_{15}$ | F: 5'-GCATAGTGACATGTTTCACCC-3'<br>R: 5'-GTAAGAGAAGGTTCACCTCAGC-3' | 165-225 | B |

## APPENDIX B. PAIRWISE $F_{ST}$ VALUES BY CLUSTER

**Appendix B-1.** Pair-wise $F_{ST}$ values for 11 wood frog populations in vernal pools in Cluster 16 within the fragmented landscape. No pair-wise $F_{ST}$ values were significant following standard Bonferonni correction ($p<0.0009$).

| | 16 | 16C | 16E | 16H | 16I | 16L | 16K2 | 16L10 | 16L4 | 16L6 | 16M2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | — | | | | | | | | | | |
| 16C | 0.0025 | — | | | | | | | | | |
| 16E | 0.0033 | 0.0068 | — | | | | | | | | |
| 16H | -0.0009 | 0.0077 | 0.0056 | — | | | | | | | |
| 16I | -0.0011 | 0.0092 | -0.0015 | 0.0038 | — | | | | | | |
| 16L | 0.0024 | 0.0072 | 0.0034 | 0.0044 | -0.0003 | — | | | | | |
| 16K2 | -0.0046 | 0.0017 | 0.0003 | -0.0007 | 0.0007 | 0.0002 | — | | | | |
| 16L10 | 0.0092 | 0.0085 | 0.0089 | 0.0058 | 0.0063 | 0.0010 | 0.0025 | — | | | |
| 16L4 | 0.0033 | 0.0124 | 0.0112 | 0.0001 | 0.0029 | 0.0066 | 0.0040 | 0.0089 | — | | |
| 16L6 | 0.0014 | 0.0100 | 0.0023 | 0.0041 | 0.0033 | 0.0051 | 0.0017 | 0.0052 | 0.0070 | — | |
| 16M2 | 0.0127 | 0.0151 | 0.0119 | 0.0139 | 0.0119 | 0.0129 | 0.0139 | 0.0125 | 0.0137 | 0.0090 | — |

**Appendix B-2.** Pair-wise $F_{ST}$ values for 18 wood frog populations in vernal pools in Cluster 18 within the fragmented landscape. Bold indicates significance following standard Bonferonni correction ($p<0.0003$).

| | 18A | 18G | 18N | 18O | 18C2 | 18J | 18K | 18L | 18M | 18P | 18B2 | 18B3 | 18B5 | 18Foc2 | 18J2 | 18V | 18Z | 18Z2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18A | — | | | | | | | | | | | | | | | | | |
| 18G | 0 0141 | — | | | | | | | | | | | | | | | | |
| 18N | 0 0033 | 0 0038 | — | | | | | | | | | | | | | | | |
| 18O | 0 0075 | 0 0119 | 0 0031 | — | | | | | | | | | | | | | | |
| 18C2 | 0 0085 | 0 0121 | 0 0091 | 0 0152 | — | | | | | | | | | | | | | |
| 18J | 0 0002 | 0 0104 | 0 0004 | -0 0031 | 0 0044 | — | | | | | | | | | | | | |
| 18K | **0.0130** | 0 0181 | 0 0039 | 0 0006 | **0.0243** | -0 0005 | — | | | | | | | | | | | |
| 18L | 0 0102 | 0 0016 | 0 0002 | 0 0016 | 0 0126 | 0 0015 | 0 0014 | — | | | | | | | | | | |
| 18M | 0 0099 | 0 0123 | -0 0011 | 0 0038 | 0 0190 | 0 0017 | -0 0038 | -0 0024 | — | | | | | | | | | |
| 18P | 0 0036 | 0 0071 | 0 0018 | 0 0008 | 0 0125 | 0 0006 | 0 0025 | -0 0035 | 0 0031 | — | | | | | | | | |
| 18B2 | 0 0020 | 0 0159 | 0 0058 | 0 0024 | 0 0238 | 0 0009 | 0 0011 | 0 0039 | 0 0049 | -0 0036 | — | | | | | | | |
| 18B3 | 0 0052 | 0 0051 | **0.0046** | -0 0009 | 0 0116 | 0 0004 | 0 0038 | 0 0006 | 0 0042 | 0 0055 | 0 0030 | — | | | | | | |
| 18B5 | 0 0086 | 0 0150 | 0 0047 | 0 0059 | 0 0154 | 0 0057 | 0 0065 | 0 0006 | 0 0031 | -0 0012 | 0 0035 | -0 0011 | — | | | | | |
| 18Foc2 | 0 0069 | 0 0099 | 0 0014 | -0 0051 | 0 0114 | -0 0018 | -0 0014 | -0 0041 | -0 0028 | -0 0024 | 0 0005 | -0 0011 | 0 0035 | — | | | | |
| 18J2 | 0 0229 | 0 0229 | 0 0171 | 0 0101 | 0 0183 | 0 0181 | 0 0106 | 0 0115 | 0 0078 | 0 0137 | 0 0181 | 0 0063 | 0 0036 | 0 0097 | — | | | |
| 18V | **0.0210** | 0 0010 | 0 0058 | 0 0001 | 0 0122 | 0 0038 | 0 0078 | -0 0005 | 0 0070 | 0 0047 | 0 0147 | 0 0048 | 0 0066 | 0 0045 | 0 0234 | — | | |
| 18Z | **0.0371** | 0 0229 | **0.0309** | 0 0114 | **0.0345** | **0.0221** | **0.0250** | 0 0155 | **0.0261** | **0.0261** | **0.0214** | **0.0158** | **0.0365** | 0 0132 | **0.0310** | 0 0318 | — | |
| 18Z2 | 0 0141 | 0 0103 | 0 0065 | 0 0057 | 0 0148 | 0 0015 | 0 0038 | 0 0000 | 0 0077 | 0 0031 | 0 0034 | 0 0048 | 0 0138 | 0 0027 | 0 0103 | 0 0094 | 0 0090 | — |

**Appendix B-3.** Pair-wise $F_{ST}$ values for 16 wood frog populations in vernal pools in Cluster 29 within the fragmented landscape. Bold indicates significance following standard Bonferonni correction (p<0.0004).

| | 29F | 29G | 29N | 29Q | 29A3 | 29J | 29M | 29N3 | 29N5 | 29O2 | 29S | 29S2 | 29W5 | 29W6 | 29X2 | 29X5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29F | — | | | | | | | | | | | | | | | |
| 29G | 0 0164 | — | | | | | | | | | | | | | | |
| 29N | **0.0214** | 0 0197 | — | | | | | | | | | | | | | |
| 29Q | 0 0073 | 0 0030 | 0 0098 | — | | | | | | | | | | | | |
| 29A3 | 0 0036 | 0 0061 | 0 0161 | 0 0035 | — | | | | | | | | | | | |
| 29J | 0 0063 | 0 0041 | 0 0125 | 0 0016 | 0 0022 | — | | | | | | | | | | |
| 29M | 0 0034 | 0 0171 | 0 0071 | 0 0036 | 0 0024 | 0 0064 | — | | | | | | | | | |
| 29N3 | -0 009 | 0 0156 | 0 0109 | 0 0034 | 0 0043 | 0 0091 | 0 0038 | — | | | | | | | | |
| 29N5 | 0 0049 | 0 0143 | 0 0168 | 0 0018 | 0 0039 | 0 0045 | 0 0042 | 0 0072 | — | | | | | | | |
| 29O2 | 0 0127 | **0.0293** | 0 0195 | **0.0173** | 0 0111 | 0 0160 | 0 0152 | 0 0198 | 0 0135 | — | | | | | | |
| 29S | 0 0069 | 0 0076 | 0 0115 | 0 0037 | 0 0024 | -0 0001 | -0 0026 | 0 0062 | 0 0033 | 0 0112 | — | | | | | |
| 29S2 | 0 0118 | 0 0013 | 0 0058 | -0 0011 | 0 0007 | 0 0028 | 0 0044 | 0 0015 | 0 0063 | **0.0020** | 0 0025 | — | | | | |
| 29W5 | 0 0154 | 0 0026 | 0 0212 | 0 0044 | 0 0055 | 0 0149 | 0 0155 | 0 0048 | 0 0157 | 0 0201 | 0 0150 | 0 0105 | — | | | |
| 29W6 | 0 0049 | 0 0127 | 0 0171 | 0 0101 | 0 0029 | 0 0042 | 0 0039 | 0 0073 | 0 0059 | 0 0137 | 0 0007 | 0 0100 | 0 0139 | — | | |
| 29X2 | 0 0095 | 0 0153 | 0 0048 | -0 0017 | 0 0050 | 0 0063 | 0 0035 | 0 0022 | 0 0071 | 0 0105 | 0 0066 | 0 0016 | 0 0115 | 0 0066 | — | |
| 29X5 | 0 0014 | 0 0034 | 0 0123 | 0 0002 | -0 0002 | 0 0010 | 0 0030 | 0 0030 | 0 0055 | 0 0106 | -0 0004 | 0 0095 | 0 0023 | 0 0000 | 0 0043 | — |

**Appendix B-4.** Pair-wise $F_{ST}$ values for 11 wood frog populations in vernal pools in Cluster 36 within the unfragmented landscape. No pair-wise $F_{ST}$ values were significant following standard Bonferonni correction ($p<0.0009$).

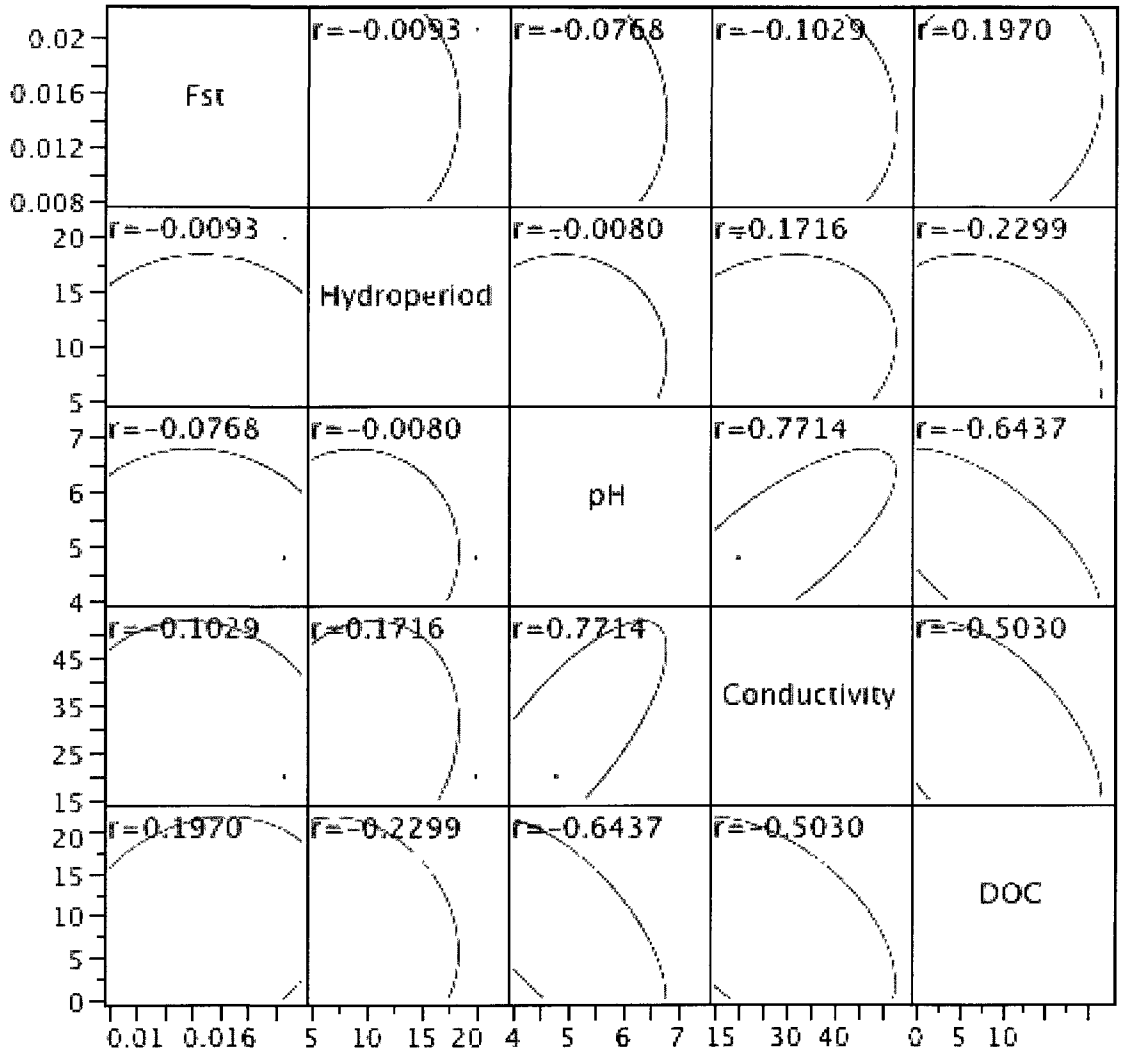
| | 36J | 36G | 36K | 36H | 36B2 | 36C2 | 36L | 37 | 38 | 38E | 36I |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 36J | — | | | | | | | | | | |
| 36G | 0.0142 | — | | | | | | | | | |
| 36K | 0.0188 | 0.0096 | — | | | | | | | | |
| 36H | 0.0109 | 0.0143 | 0.0185 | — | | | | | | | |
| 36B2 | 0.0089 | 0.0065 | 0.0047 | 0.0151 | — | | | | | | |
| 36C2 | 0.0069 | 0.0123 | 0.0049 | 0.0017 | 0.0076 | — | | | | | |
| 36L | 0.0131 | 0.0166 | 0.0107 | 0.0035 | 0.0085 | 0.0057 | — | | | | |
| 37 | 0.0127 | 0.0165 | 0.0187 | 0.0062 | 0.0142 | 0.0062 | 0.0049 | — | | | |
| 38 | 0.0089 | 0.0149 | 0.0103 | 0.0077 | 0.0076 | 0.0025 | 0.0058 | 0.0135 | — | | |
| 38E | 0.0129 | 0.0081 | 0.0162 | 0.0131 | 0.0049 | 0.0154 | 0.0127 | 0.0186 | 0.0115 | — | |
| 36I | 0.0215 | 0.0149 | 0.0180 | 0.0219 | 0.0135 | 0.0211 | 0.0051 | 0.0239 | 0.0193 | 0.0192 | — |

**Appendix B-5.** Pair-wise $F_{ST}$ values for 9 wood frog populations in vernal pools in Cluster 39 within the unfragmented landscape. Bold indicates significance following standard Bonferonni correction ($p<0.0014$).

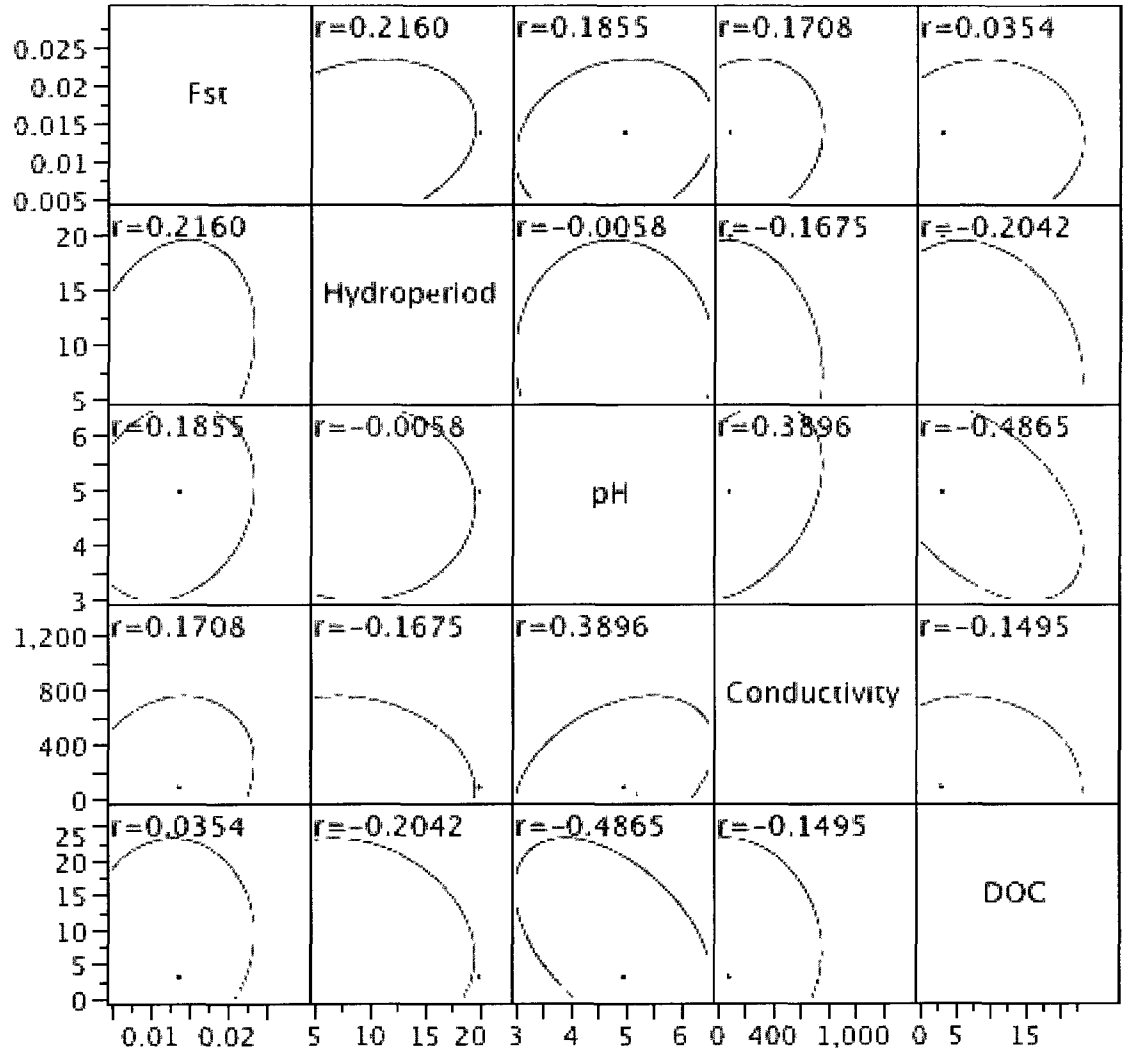
| | 39E | 39F | 39N | 39O | 39Q | 39R | 39 | 39S | 39T |
|---|---|---|---|---|---|---|---|---|---|
| 39E | — | | | | | | | | |
| 39F | **0.0081** | — | | | | | | | |
| 39N | 0.0086 | **0.0100** | — | | | | | | |
| 39O | 0.0071 | **0.0100** | 0.0029 | — | | | | | |
| 39Q | 0.0095 | 0.0149 | 0.0160 | 0.0141 | — | | | | |
| 39R | 0.0026 | **0.0042** | 0.0019 | **0.0100** | **0.0183** | — | | | |
| 39 | 0.0017 | 0.0064 | 0.0093 | 0.0074 | 0.0052 | 0.0122 | — | | |
| 39S | 0.0076 | **0.0087** | 0.0142 | 0.0167 | 0.0122 | 0.0039 | 0.0119 | — | |
| 39T | 0.0057 | **0.0071** | 0.0037 | 0.0034 | 0.0057 | 0.0007 | 0.0077 | 0.0169 | — |

APPENDIX C. CORRELATIONS OF $F_{ST}$ AND WATER PARAMETERS

**Appendix C-1.** Scatterplot matrix of $F_{ST}$ with water quality parameters from ponds sampled in the unfragmented landscape.



**Appendix C-2.** Scatterplot matrix of $F_{ST}$ with water quality parameters from ponds sampled in the fragmented landscape.



## APPENDIX D ENVIRONMENTAL FACTORS

Pond-specific values for environmental factors including hydroperiod score, pH, conductivity, dissolved organic carbon (DOC), population size, geographic isolation, and road cost Geographic isolation was the average pair-wise geographic distance from each pond to every other pond in the cluster Similarly, road cost was the average of pair-wise LCP road costs from each pond to every other pond within each cluster

| Pond | Hydroperiod | pH | Conductivity (µS/cm^3) | DOC (mg C/L) | Population Size | Geographic isolation | Road cost |
|---|---|---|---|---|---|---|---|
| 16 | 8 36 | 4 52 | 158 | 14 03 | 19 | 1 27 | 5 30 |
| 16C | 7 36 | 4 21 | 119 | 5 76 | 30 | 1 28 | 7 90 |
| 16E | 5 36 | 3 88 | 33 | 24 71 | 16 | 1 41 | 5 30 |
| 16H | 7 36 | 4 24 | 16 | 11 72 | 175 | 1 93 | 6 80 |
| 16I | 6 43 | 4 14 | 35 | 22 13 | 16 | 1 64 | 5 40 |
| 16K2 | 8 36 | 3 77 | 66 | 9 67 | 65 | 1 26 | 7 90 |
| 16L | 5 36 | 3 97 | 30 | 6 67 | 50 | 1 40 | 5 40 |
| 16L10 | 5 36 | 5 14 | 359 | 2 79 | 25 | 2 39 | 11 10 |
| 16L4 | 5 36 | 4 21 | 27 | 15 75 | 125 | 2 06 | 7 10 |
| 16L6 | 10 79 | 4 89 | 294 | 11 93 | 60 | 2 11 | 6 90 |
| 16M2 | 10 79 | 5 76 | 836 | 2 83 | 15 | 3 27 | 17 70 |
| 18 Focal2 | 6 43 | 5 48 | 96 | 2 65 | 75 | 1 38 | 7 76 |
| 18A | 5 36 | 5 22 | 28 | 6 48 | 21 | 1 24 | 7 82 |
| 18B2 | 10 79 | 3 95 | 35 | 6 48 | 16 | 0 93 | 4 65 |
| 18B3 | 20 | 4 97 | 82 | 3 07 | 14 | 0 95 | 4 65 |
| 18B5 | 20 | 3 85 | 10 | 6 21 | 50 | 1 63 | 7 82 |
| 18C2 | 9 5 | 4 66 | 32 | 8 63 | 100 | 0 99 | 4 65 |
| 18G | 5 36 | 5 94 | 162 | 4 25 | 30 | 0 96 | 4 65 |
| 18J | 9 5 | 5 23 | 52 | 4 8 | 150 | 0 94 | 4 65 |
| 18J2 | 10 79 | 6 19 | 91 | 4 64 | 100 | 0 96 | 4 65 |
| 18K | 6 43 | 5 05 | 38 | 5 41 | 35 | 0 96 | 4 65 |
| 18L | 9 5 | 4 3 | 35 | 9 82 | 23 | 2 06 | 10 59 |
| 18M | 10 79 | 5 01 | 43 | 8 69 | 80 | 1 82 | 10 59 |
| 18N | 9 5 | 3 54 | 37 | 4 69 | 70 | 2 67 | 10 59 |
| 18O | 6 43 | 4 78 | 39 | 7 63 | 48 | 1 10 | 4 65 |
| 18P | 10 79 | 5 02 | 50 | 7 12 | 30 | 1 12 | 7 29 |
| 18V | 6 43 | 3 96 | 39 | 26 02 | 16 | 2 42 | 14 71 |
| 18Z | 20 | 6 14 | 58 | 3 82 | 85 | 1 60 | 6 88 |
| 18Z2 | 20 | 5 32 | 20 | 7 29 | 150 | 1 81 | 6 88 |
| 29A3 | 8 36 | 4 34 | 27 | 4 03 | 30 | 1 73 | 9 20 |
| 29F | 5 36 | 6 08 | 309 | 5 37 | 25 | 1 68 | 7 67 |

## Appendix D cont...

| Pond | Hydroperiod | pH | Conductivity (µS/cm^3) | DOC (mg C/L) | Population Size | Geographic isolation | Road cost |
|---|---|---|---|---|---|---|---|
| 29G | 5 36 | 5 13 | 1231 | 10 63 | 25 | 1 63 | 6 87 |
| 29J | 9 5 | 4 85 | 70 | 11 79 | 22 | 1 75 | 8 73 |
| 29M | 7 36 | 3 93 | 41 | 12 77 | 40 | 2 61 | 7 67 |
| 29N | 7 36 | 5 26 | 172 | 8 29 | 45 | 1 91 | 7 53 |
| 29N3 | 7 36 | 5 29 | 117 | 7 71 | 30 | 1 60 | 7 40 |
| 29N5 | 8 36 | 5 74 | 740 | 5 42 | 55 | 2 40 | 8 47 |
| 29O2 | 5 36 | 5 3 | 568 | 7 15 | 40 | 2 57 | 6 33 |
| 29Q | 5 36 | 4 64 | 48 | 22 41 | 20 | 1 65 | 6 47 |
| 29S | 6 43 | 4 34 | 102 | 6 29 | 50 | 2 57 | 7 67 |
| 29S2 | 20 | 3 43 | 88 | 13 | 60 | 2 25 | 7 67 |
| 29W5 | 5 36 | 5 09 | 27 | 3 56 | 45 | 3 58 | 6 60 |
| 29W6 | 5 36 | 4 32 | 266 | 3 07 | 50 | 4 31 | 10 27 |
| 29X2 | 7 36 | 4 07 | 14 | 5 54 | 95 | 2 08 | 8 73 |
| 29X5 | 7 36 | 5 91 | 299 | 2 17 | 40 | 3 52 | 8 47 |
| 36B2 | 8 36 | 4 3 | 19 | 15 43 | 100 | 1 35 | 0 |
| 36C2 | 8 36 | 4 58 | 18 | 9 78 | 28 | 1 24 | 0 |
| 36G | 7 43 | 4 18 | 22 | 13 02 | 15 | 1 17 | 0 |
| 36H | 7 43 | 4 99 | 20 | 2 04 | 60 | 1 26 | 0 |
| 36I | 8 36 | 4 05 | 24 | 11 46 | 60 | 1 22 | 0 |
| 36J | 8 36 | 5 54 | 21 | 5 9 | 100 | 1 16 | 0 |
| 36K | 8 36 | 4 34 | 18 | 14 14 | 50 | 1 87 | 0 |
| 36L | 7 36 | 4 18 | 24 | 22 26 | 25 | 1 49 | 0 |
| 37 | 8 36 | 4 83 | 18 | 12 32 | 50 | 2 25 | 0 |
| 38 | 9 5 | 4 55 | 23 | 4 52 | 35 | 1 94 | 0 |
| 38E | 8 36 | 4 16 | 24 | 11 86 | 60 | 1 50 | 0 |
| 39 | 20 | 5 66 | 52 | 2 25 | 43 | 0 86 | 0 |
| 39E | 20 | 4 78 | 20 | 3 56 | 28 | 0 86 | 0 |
| 39F | 8 36 | 5 14 | 37 | 7 22 | 75 | 1 01 | 0 |
| 39N | 6 5 | 6 05 | 43 | 3 5 | 55 | 1 23 | 0 |
| 39O | 8 36 | 4 76 | 24 | 10 02 | 26 | 1 00 | 0 |
| 39Q | 5 43 | 7 21 | 49 | 3 72 | 100 | 1 04 | 0 |
| 39R | 7 36 | 5 14 | 29 | 6 04 | 18 | 0 96 | 0 |
| 39S | 8 36 | 5 23 | 35 | 2 32 | 27 | 1 62 | 0 |
| 39T | 7 36 | 4 46 | 19 | 6 84 | 48 | 1 70 | 0 |
| Average | 8 75 | 4 83 | 130 | 8 65 | 51 | 1 74 | 5 45 |
| Minimum | 5 36 | 3 43 | 10 | 2 04 | 14 | 0 86 | 0 |
| Maximum | 20 00 | 7 21 | 1231 | 26 02 | 175 | 4 31 | 4 31 |