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## HIGH GENETIC CONNECTIVITY IN WOOD FROGS (*LITHOBATES SYLVATICUS*) AND SPOTTED SALAMANDERS (*AMBYSTOMA MACULATUM*) IN A COMMERCIAL FOREST

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**Abstract.**—We characterized the genetic structure of two pond-breeding amphibian species in a commercial forest to evaluate population connectivity and investigate whether landscape features and timber harvest influenced dispersal and gene flow. We sampled 20 Wood Frog (*Lithobates sylvaticus*) populations and 23 Spotted Salamander (*Ambystoma maculatum*) populations across an area of 40 × 52 km. We estimated genetic diversity and differentiation, and used both a Bayesian clustering approach and a spatial autocorrelation analysis to evaluate genetic structure. We used a least-cost path analysis to examine dispersal and gene flow within each species. In both species, we found high genetic diversity and low differentiation across the study area, and the Bayesian clustering analysis identified a single genetic cluster for each species. The spatial autocorrelation analysis indicated there was greater spatial genetic structure in Spotted Salamanders than Wood Frogs. None of the landscape features measured were significantly related to genetic distance in Wood Frogs, and lakes impeded dispersal in Spotted Salamanders. We attribute the findings of high genetic connectivity in both species to a combination of abundant forest and wetlands with minimal anthropogenic disturbance. These findings suggest that current silviculture practices in the study area do not significantly impede dispersal and gene flow of pond-breeding amphibians.

**Key Words.**—dispersal; forestry; gene flow; landscape genetics.

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### INTRODUCTION

Dispersal is one of the most important processes in population biology because it can impact individual fitness, population dynamics, population genetics, and species distribution (Clobert et al. 2001). Anthropogenic influences such as habitat alteration or fragmentation can limit dispersal by increasing the demographic and genetic isolation of local populations, thereby increasing extinction risks (Keyghobadi 2007). In order to implement effective conservation management, it is therefore important to understand how landscape features affect the movement and dispersal patterns of organisms.

Many pond-breeding amphibians depend on forested habitat for much of their habitat require-

ments and are sensitive to forest fragmentation and loss (deMaynadier and Hunter 1995; Gibbs 1998; Cushman 2006). They may be especially vulnerable to habitat modification due to their limited mobility, high philopatry, patchy distributions, and complex life cycles that include both aquatic and terrestrial stages (Sinsch 1990; Alford and Richards 1999; Cushman 2006). In addition, many amphibians exist as metapopulations and thus depend on periodic dispersal to maintain gene flow and connectivity among populations (Marsh and Trenham 2001; Semlitsch 2008). Therefore, understanding the factors that impact amphibian dispersal and gene flow are important for maintaining population persistence.

Population genetic analyses can characterize dispersal by measuring the cumulative impact

of disturbance on gene flow over generations. When combined with spatial information, these analyses can reveal how environmental factors influence population connectivity. Recent studies have found that despite high levels of gene flow in many amphibian species, geographic distance alone does not explain genetic structure (Crosby et al. 2009; Purrenhage et al. 2009; Gabrielsen et al. 2013). Instead, both natural and anthropogenic factors influence amphibian connectivity. There is evidence that lakes and rivers limit dispersal in some amphibians (Lee-Yaw et al. 2009; Richardson 2012), while elevation and topography influence genetic structure in others (Funk et al. 2005; Giordano et al. 2007). In addition, anthropogenic disturbance such as development, agriculture, or forest management can negatively impact amphibian gene flow (Joly et al. 2003; Crosby et al. 2009; Spear and Storfer 2008; Greenwald et al. 2009b; Gabrielsen et al. 2013).

Land managed for timber production may have negative impacts on amphibians dependent on forest habitat. Research shows that timber harvesting, especially clearcutting, can negatively impact the survival, abundance, and dispersal of local amphibian populations (deMaynadier and Hunter 1998; Patrick et al. 2006; Petranka et al. 1994; Raymond and Hardy 1991). Clearcuts remove canopy, reduce leaf litter and soil moisture, increase soil temperature, alter hydrology, and create a harsh microclimate unfavorable for amphibians (e.g., deMaynadier and Hunter 1995; Semlitsch et al. 2009). This type of forest disturbance may limit amphibian dispersal as individuals must move through less optimal habitat, which increases the risk of desiccation and mortality (Rothermel and Luhring 2005; Rittenhouse et al. 2008). Whether these impacts are manifest in reduced connectivity at the population level may depend on several local factors, including the nature of the forest management practices and the abundance and distribution of the species and their breeding ponds.

In northern New England, there is a long his-

tory of forest management for timber harvest. Forest clearing peaked in the mid-1800s at the height of agricultural production, and timber harvest remains a staple of the regional economy (Foster et al. 1998; North East Foresters Association 2013a). In Maine, the forest product industry is an important livelihood for the state, with 500,000 acres and 7.86 million cords harvested annually (Maine Forest Service, Department of Conservation 2010; North East Foresters Association 2013b). To promote sustainable harvest, best management practices are encouraged (e.g., protect the integrity of water bodies, minimize exposed soil, etc.; see Moesswilde 2004) and regulations limit the size and distribution of clearcuts. Today, Maine is 89% forested and dominated by northern hardwood and spruce-fir forest types. Abundant vernal pools also characterize the forest. In the context of the largely forested New England landscape, the impact of timber harvesting on vernal pool-breeding amphibians is unknown. Given the history of forest management, it is important to understand how this type of disturbance affects amphibian movement and dispersal.

In this study, our objectives were to characterize the population genetic structure of two amphibian species in a commercial forest managed for timber harvest and identify landscape features that impact dispersal and gene flow within each species. The study area offered an excellent opportunity to examine factors that influence amphibian connectivity because it consisted of a mosaic of natural and human-created landscape features with breeding ponds dispersed throughout. We studied the Wood Frog (*Lithobates sylvaticus*) and the Spotted Salamander (*Ambystoma maculatum*) – two abundant, forest-dependent species. The two species have similar life cycles, but differ in demographic patterns, microhabitat selection, and dispersal capabilities (Redmer and Trauth 2005; Savage and Zamudio 2005), and they may respond differently to forest fragmentation. The Wood Frog is more mobile, with the maximum distance recorded from a breeding

pond of 2.5 km, as compared to 467 m in Spotted Salamanders (Berven and Grudzien 1990; Montieth and Paton 2006). We predicted that anthropogenic landscape features such as forest management, roads, and agriculture impede dispersal for both species. In addition, we predicted that some natural landscape features such as lakes would impede dispersal, while others such as wetlands would facilitate dispersal via stepping-stones. Due to the greater mobility of Wood Frogs as compared to Spotted Salamanders, we expected to find less genetic differentiation and fewer landscape influences on gene flow in Wood Frogs.

#### MATERIALS AND METHODS

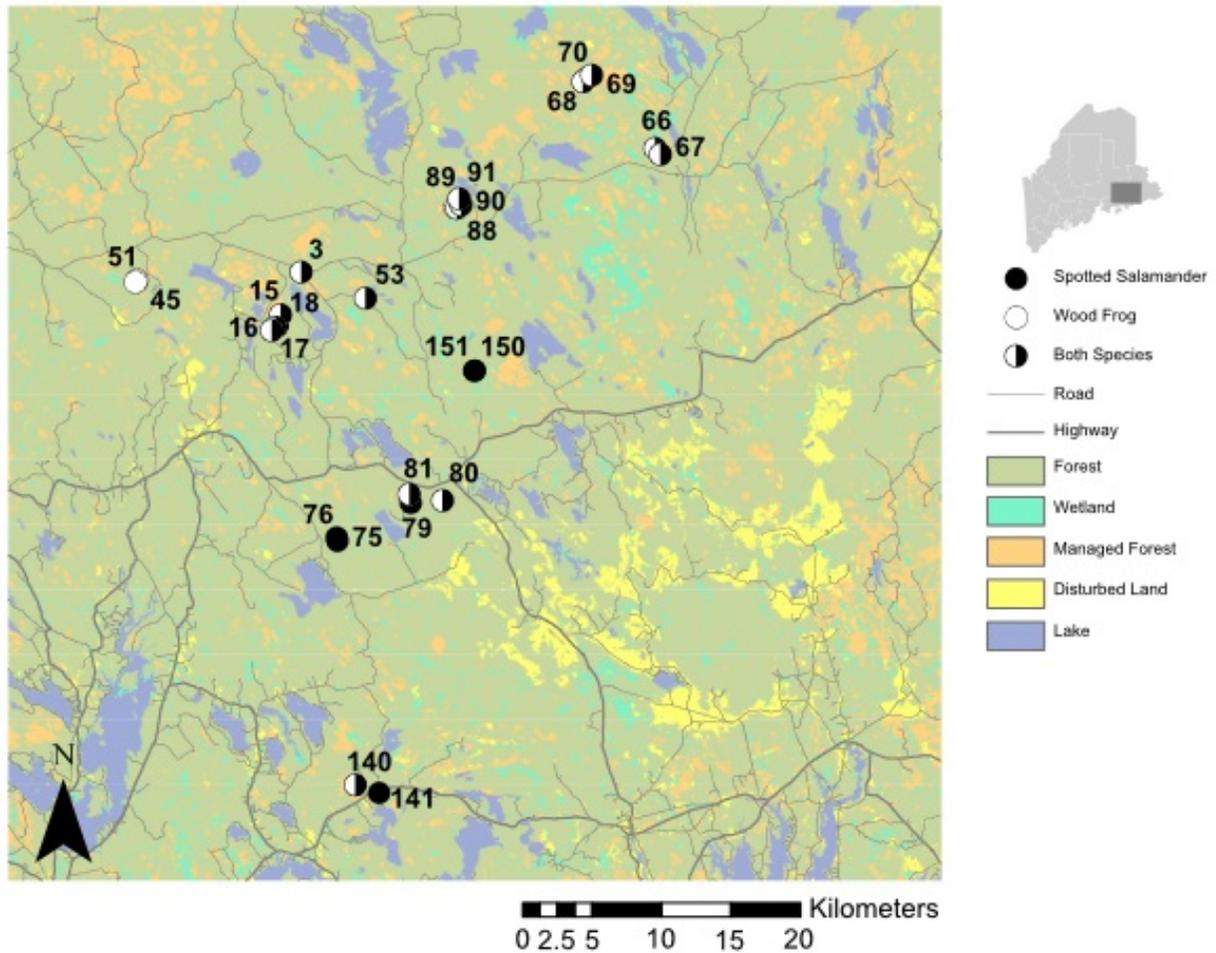
**Study area and sampling.**—We conducted this research in central Maine within the counties of Penobscot, Hancock, and Washington (Fig. 1). The study area (40 × 50 km) was predominantly forested (72%) with numerous wetlands and lakes (16%), and limited agriculture (4%) and development (0.7%). The forest was largely privately owned commercial timberland and consisted of actively logged second growth forest. The species composition included northern hardwoods such as Beech (*Fagus grandifolia*), Yellow Birch (*Betula alleghaniensis*) and Sugar Maple (*Acer saccharum*) and softwoods including White Pine (*Pinus strobus*), Red Spruce (*Picea rubens*), and Balsalm Fir (*Abies balsamea*; Hasbrouck and Knight 1994).

Seven percent of the study area was made up of patches that have experienced various forest management treatments between 1995–2004, including partial, clearcut, and regenerating patches. Clearcuts made up < 1% of the study area and were composed of forest patches with > 90% overstory removal; patch size ranged from 2–20 ha with a mean of 9 ha. Heavy partial cuts made up 3% of the study area and were composed of forest where > 50% of the trees were harvested; patch size ranged from 0–192 ha with a mean of 7 ha. Light partial cuts made up 2%

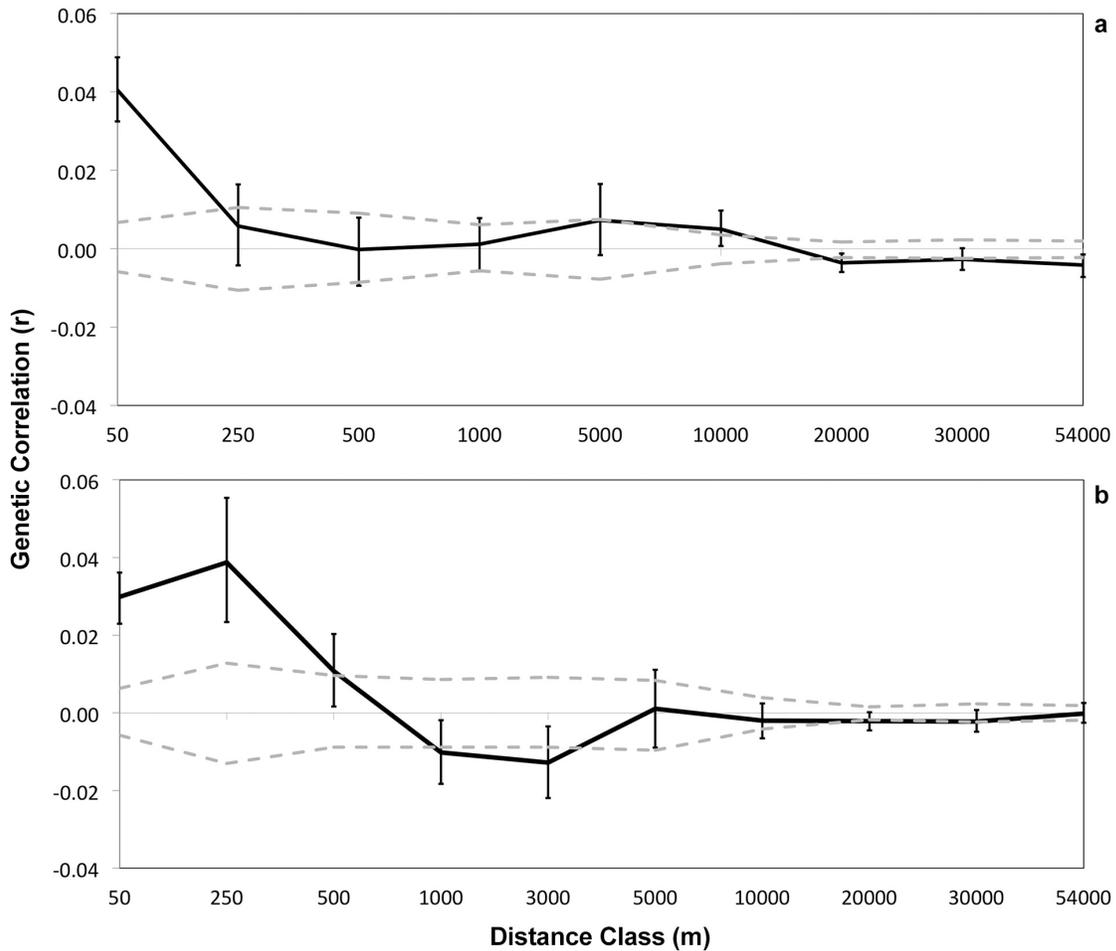
of the study area and were composed of forest where < 50% of the overstory had been removed; patch size ranged from 0–88 ha with a mean of 4 ha (derived from Maine Land Cover Data Set, MeLCD. 2004; Maine Office of Geographic Information Systems; available from <http://megis.maine.gov/catalog/>).

To identify potential breeding ponds for sampling, we consulted US Fish and Wildlife Service National Wetlands Inventory (NWI) maps. We selected breeding ponds distributed across the study area, so as to include potential landscape barriers such as lakes and highways (Fig. 1). In the spring of 2004 and 2005, we sampled a total of 26 ponds and found Spotted Salamanders at 23 and Wood Frogs at 20 of the ponds. The nearest neighbor distance between sampled ponds ranged from 60 m to 4 km and the maximum pairwise distance across all ponds was 54 km. At each pond we collected one embryo from every egg mass with a target of 15 individuals per pond, and stored them in vials with 95% ethanol until DNA extraction. For six ponds, where larva had already hatched, we used dipnets to collect samples from different areas of the pond to minimize possible collection of related individuals (two collections of *A. maculatum*, four collections of *L. sylvaticus*).

**DNA extraction and microsatellite analysis.**—We extracted DNA from the embryos and larvae using QIAGEN QIAamp DNeasy Blood and Tissue® kit (QIAGEN, Hilden, Germany). For both Spotted Salamanders and Wood Frogs, we amplified DNA by polymerase chain reaction (PCR) at six polymorphic, species-specific, tetranucleotide microsatellite loci, using the published protocols of Julian and King (2003) and Julian et al. (2003). We used Spotted Salamander loci D287, D315, D184, D321, D99, C40 (Julian et al. 2003) and Wood Frog loci C41, D32, C52, D20, D77, C11 (Julian and King 2003). We used fluorescent-dye-labeled primers (HEX, FAM, NED) in two multiplexes for each species. Amplified products were



**FIGURE 1.** The location and identification number of ponds sampled for Wood Frogs (*Lithobates sylvaticus*) and Spotted Salamanders (*Ambystoma maculatum*) in a commercial forest in central Maine.



**FIGURE 2.** Correlogram plots of the genetic correlation coefficient ( $r$ ) as a function of distance for a) Wood Frogs (*Lithobates sylvaticus*) and b) Spotted Salamanders (*Ambystoma maculatum*). The extent of positive spatial genetic structure as measured by the  $\times$  intercept is 490 m for Wood Frogs and 756 m for Spotted Salamanders. The null hypothesis of a random distribution of genotypes is bounded by 95% CI (dashed line) with error bars determined by bootstrapping. Significant positive spatial genetic structure is inferred for distance classes at which the correlation coefficient ( $r$ ) is above the 95% CI and the error bars do not overlap zero.

electrophoresed in an automated DNA sequencer (ABI 377 genetic analyzer, Applied Biosystems, Foster City, California, USA) with positive and negative controls. We manually scored and binned the genotypes using Genotyper (Applied Biosystems).

**Genetic diversity.**—We checked the data set for scoring errors, null alleles, and large-allele dropout using the program MICROCHECKER (Van Oosterhout et al. 2004). We estimated null allele frequencies in MICROCHECKER using the Brookfield 1 estimator (Brookfield 1996). To measure genetic diversity by locus and population, we used the programs FSTAT (Goudet 1995) and GDA (Lewis and Zaykin 2002; Available from <http://www.eeb.uconn.edu/people/plewis/software.php>). Specifically, we measured the number of alleles, allelic richness, observed and expected heterozygosity ( $H_O$  and  $H_E$ ), the inbreeding coefficient ( $F_{IS}$ ), and locus-specific population differentiation ( $F_{ST}$ ). We tested for Hardy-Weinberg equilibrium and linkage disequilibrium using FSTAT (1,000 permutations) with Bonferroni correction ( $\alpha = 0.05$ ) for multiple tests.

**Population genetic structure.**—To characterize genetic differentiation between ponds, we calculated pairwise fixation indices ( $F_{ST}$  values) in FSTAT and tested for significance (using 1,000 permutations with Bonferroni correction). We also calculated  $G'_{ST}$ , a metric related to  $F_{ST}$  that is standardized for within-population variability, in order to compare genetic differentiation between the two species (Hedrick 2005). Between each pair of ponds, we also calculated chord distance ( $D_C$ ; Cavalli-Sforza and Edwards 1967), which is a distance metric based on allele frequency distributions that assumes differentiation is caused by genetic drift rather than mutation. We used the program MSA (Dieringer and Schlötterer 2003) to calculate  $G'_{ST}$  and  $D_C$ .

To characterize genetic structure across the study area, we used the program BAPS (Coran-

der et al. 2003; Corander and Marttinen 2006). This program uses Bayesian models of population structure to estimate the number of subpopulations ( $K$ ) based on the genetic similarity of individuals. By incorporating spatial information, it is possible to assign a biologically relevant, non-uniform prior distribution to increase power and detect underlying population structure (Corander et al. 2008). We therefore used the model that accounted for spatial clustering of groups, and ran five replicates with a maximum  $K$  value equal to the number of ponds occupied by each species. To visualize genetic structure, we used a principal coordinates analysis (PCA) conducted in GenAlEx 6.4 (Peakall and Smouse 2006, 2012; see Appendix 1 and 2).

To evaluate whether genetic differentiation was a function of geographic distance (i.e., an isolation by distance model), we tested the correlation of geographic distance with linearized  $F_{ST}$  [ $F_{ST}/(1/F_{ST})$ ]. We calculated this using Mantel tests implemented in R statistical software (R Core Team 2011) using the ecodist package (Goslee and Urban 2007) with a Pearson's correlation and 10,000 permutations. We estimated the 95% CI for the Mantel tests using 1,000 bootstraps. We also conducted a spatial autocorrelation analysis to evaluate the fine-scale spatial genetic structure of each species. Spatial autocorrelation estimates the correlation ( $r$ ) between genetic and geographic distance matrixes for pairs of individuals across a number of predetermined distance classes, thereby describing the geographic extent to which genetic relatedness is detected (Smouse and Peakall 1999). In the resulting correlogram, the distance at which  $r$  intercepts the x-axis indicates the extent of genetic relatedness, and beyond this distance, gene flow does not connect populations (Peakall et al. 2003). For each species, we performed spatial autocorrelation analyses in GenAlEx 6.4 with 999 permutations and 1,000 bootstraps, and up to 10 distance classes that spanned the extent of the study area in incrementally larger classes and had a

minimum of 600 pair-wise comparisons in each class (50, 250, 500, 1,000, 3,000, 5,000, 10,000, 20,000, 30,000, 54,000 m). For Wood Frogs, we omitted the 3,000 m distance class due to small sample size within that distance class.

***Landscape features as barriers or facilitators of dispersal.***—We used a least-cost path analysis (Adriaensen et al. 2003) to assess the potential of certain landscape features to act as barriers or facilitators of dispersal for Spotted Salamanders and Wood Frogs. To do this, we evaluated two methodologies for assigning resistance costs to landscape features, including univariate and multivariate models. The goal of the univariate approach was to identify specific landscape features that influenced genetic structure of amphibians, whereas the goal of the multivariate approach was to test a model that simultaneously parameterized the relative effects of different landscape variables on dispersal based on amphibian ecology.

To create a land cover map for the analysis, we obtained land cover data from the 2004 Maine Landcover Dataset (MeLCD 2004 op. cit.; 5 m resolution). As this land cover had limited wetland information, we used the Spatial Analyst extension in ARCGIS 10 (Environmental Science Research Institute, Redlands, USA) and merged the land cover with the NWI map and a map of stream orders derived from the National Hydrography Data Set Plus (NHDPlus, US Environmental Protection Agency. 2006. Available from <http://www.epa.gov/waters> 2006). To isolate different road classes, we merged a road layer provided by the landowner, which had more detail than the state databases.

In the univariate approach, we mapped each landscape variable separately in a binary friction grid and assigned either high or low resistance to movement to each variable (Pérez-Espona et al. 2008). We identified eight landscape classes of interest: geographic distance, lakes, wetlands, streams, forestry, roads, slope, and disturbed land (see Appendix Table A1 for details). To investi-

gate dispersal barriers we isolated each land cover class and assigned high resistance (50) to the variable of interest and low resistance (1) to the background. In contrast, we assumed wetlands facilitated gene flow and thus assigned them a low resistance (1) and assigned the background a high resistance (50). For the multivariate model in the least-cost path analysis, we used the expert opinion model of Compton et al. (2007) with the addition of classes describing forestry treatments, bare ground, and wetland type (e.g., vernal pool, scrub-shrub wetlands, etc.). We reclassified the data into 19 categories and for all comparable classes used the resistance values from Compton et al. (2007) multiplied by a factor of 10 (Table 1). For the few classes that were not represented in Compton et al. (2007), we estimated resistance values by evaluating relevant ecological knowledge about dispersal relative to the other classes.

We used program PATHMATRIX (Ray 2005) in ArcView 3.3 (Environmental Science Research Institute, Redlands, California, USA) to identify the least-cost paths, by minimizing the sum of resistances of all cells along the path between pairs of locations. Effective distances were measured as lengths of the least-cost paths between each of the breeding ponds, as opposed to the total sum of costs along the path, because the least-cost length is more biologically interpretable (Ray 2005; Broquet et al. 2006). For each landscape variable, we tested for correlations between genetic distance metrics ( $F_{ST}$  and  $D_c$ ) and least-cost path length using a partial Mantel test controlling for geographic distance. We performed these analyses in R using Pearson's correlation with 10,000 permutations in the *ecodist* package ( $\alpha = 0.05$ ) and 1,000 iterations for the bootstrapped 95% confidence limit. In addition to models based on the landscape and topographic variables, we also tested a geographic distance model, which is expected to be the best fitting model if straight line Euclidean distance alone explains the greatest variation in genetic distance. We did not use a Bonferroni correction procedure to account for multiple tests in the landscape genetics analysis

**TABLE 1.** Resistance values for landcover used in the multivariate model, with values comparable to Compton et al. (2007) multiplied by a factor of 10.

Landcover type	Resistance value
Vernal pool	10
Forest	10
Wetland: Freshwater forest/Scrub-shrub/riverine	10
Wetland: Freshwater emergent	30
Scrubland	31
Bare Ground/Gravel pit	48
Development: Low density residential	68
Cropland/pasture/grassland	102
Open water	220
Estuarine	400
Forestry: Regenerating/light partial cut	40
Forestry: Clearcut/heavy partial cut	103
Road: minor	72
Road: major	164
Road: highway	326
Stream: 1st order	13
Stream: 2nd order	28
Stream: 3rd order	126
Stream: 4th order	330

because this metric is too conservative and increases type II error (Perneger 1998; Cabin and Mitchell 2000; Moran 2003), an important consideration given that Mantel tests often produce small but significant correlations (Dutilleul et al. 2000; Legendre and Fortin 2010).

## RESULTS

**Genetic diversity and population genetic structure.**—We genotyped 9–16 Wood Frog samples per pond for a total of 287 individuals, and 8–27 Spotted Salamander samples per pond for a total of 411 individuals. The loci were highly polymorphic, with a mean of 9.5 and 19.5 alleles in Spotted Salamanders and Wood Frogs, respectively (Appendix Table A2). MICROCHECKER detected no loci with null alleles in Wood Frogs. Two loci were found to have null alleles in Spotted Salamanders (Ama D99 at 5% and Ama D321 at 8%). As the overall percent of null alleles

was < 10%, and null alleles have been found to have minor impacts on genetic distance estimates (Chapuis and Estoup 2007), we retained these loci in further analyses.

Observed heterozygosity was also high in both species with a mean  $H_O = 0.71$  in Spotted Salamanders and  $H_O = 0.87$  in Wood Frogs (Tables 2 and 3). Pond specific observed heterozygosity ranged from 0.65–0.80 in Spotted Salamanders (Table 2) and 0.76–0.94 in Wood Frogs (Table 3). Pond specific FIS values ranged from 0–0.12 in Spotted Salamanders and 0–0.11 in Wood Frogs (Tables 2 and 3) and were not significantly different from zero, indicating no sampling bias due to within-pond relatedness. For Wood Frogs, there were no deviations from Hardy-Weinberg equilibrium, and linkage disequilibrium was detected in 1% of tests after Bonferroni correction. For Spotted Salamanders, no pairs of loci were in linkage disequilibrium; and D321 and D99 were out of Hardy-Weinberg equilibrium, consistent

**TABLE 2.** Metrics describing the genetic diversity of Spotted Salamanders (*Ambystoma maculatum*) from 23 populations in central Maine. N is the number of individuals sampled per population. The number of alleles, allelic richness (AR), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and  $F_{IS}$  are averaged across 6 microsatellite loci per pond. Pond locations are shown in Fig. 1.

Pond	N	Alleles	AR	$H_O$	$H_E$	$F_{IS}$
69	19	6.7	4.6	0.73	0.73	-0.01
70	16	5.7	4.3	0.75	0.71	-0.05
68	19	6.5	4.6	0.69	0.70	0.01
66	20	6.7	4.4	0.70	0.73	0.03
67	15	6.0	4.5	0.78	0.73	-0.06
90	26	6.8	4.5	0.67	0.73	0.08
91	13	6.7	4.8	0.73	0.75	0.04
89	11	5.3	4.2	0.65	0.72	0.10
15	14	6.2	4.5	0.72	0.76	0.06
17	12	4.8	4.0	0.69	0.66	-0.04
16	13	7.0	4.9	0.70	0.75	0.07
53	16	6.2	4.4	0.67	0.75	0.12
3	22	6.8	4.4	0.70	0.72	0.03
18	27	7.2	4.5	0.69	0.72	0.04
151	21	6.7	4.5	0.74	0.73	-0.01
150	22	6.5	4.4	0.67	0.75	0.11
76	10	5.5	4.6	0.78	0.76	-0.03
75	8	4.7	4.1	0.78	0.73	-0.07
79	22	6.2	4.4	0.80	0.73	-0.10
81	20	5.5	4.1	0.66	0.68	0.04
80	22	6.8	4.6	0.66	0.71	0.07
140	23	7.2	4.6	0.66	0.73	0.10
141	20	6.3	4.6	0.74	0.74	0.01

with the low frequency of null alleles detected in these loci. The lack of significant non-zero pond-specific  $F_{IS}$  values, however, further supported our rationale for retaining these loci in the analyses.

We found a general pattern of high genetic connectivity in both species with an overall  $F_{ST}$  value of 0.016 ( $\pm$  0.002) in Spotted Salamanders and 0.023 ( $\pm$  0.003) in Wood Frogs. In Spotted Salamanders, pairwise  $F_{ST}$  ranged from 0–0.08 and  $D_c$  ranged from 0.18–0.41, and in Wood Frogs, pairwise  $F_{ST}$  ranged from 0–0.09 and  $D_c$  ranged from 0.31–0.58 (Appendix Tables A3 and A4). Significance tests of pairwise  $F_{ST}$  values indicated only 5% of the pond pairs were significantly differentiated in Spotted Salamanders and 24%

were differentiated in Wood Frogs. Standardized  $G'_{ST}$  indicated Wood Frogs had slightly higher overall genetic differentiation (0.32) than Spotted Salamanders (0.16). The analyses using BAPS assigned all breeding ponds to one population cluster for both Spotted Salamanders ( $Pr = 1.0$ ) and Wood Frogs ( $Pr = 0.71$ ).

The tests of isolation by distance found no correlation between genetic and geographic distance in Spotted Salamanders ( $r = -0.051$ ,  $P = 0.60$ ) or Wood Frogs ( $r = -0.20$ ,  $P = 0.98$ ). The spatial autocorrelation analysis revealed that Spotted Salamanders had greater fine-scale spatial genetic structure than did Wood Frogs (Fig. 2). For Spotted Salamanders, the permutation and bootstrap methods in the spatial

**TABLE 3.** Metrics describing the genetic diversity of Wood Frogs (*Lithobates sylvaticus*) from 20 populations in central Maine. N is the number of individuals sampled per population. The number of alleles, allelic richness (AR), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and  $F_{IS}$  are averaged across 6 microsatellite loci per pond. Pond locations are shown in

Pond	N	Alleles	AR	$H_O$	$H_E$	$F_{IS}$
69	14	10.3	8.8	0.89	0.87	-0.03
68	16	10.8	8.5	0.78	0.83	0.06
70	15	10.2	8.4	0.92	0.87	-0.06
66	16	11.2	8.7	0.76	0.85	0.11
67	16	10.5	8.3	0.84	0.84	-0.01
88	16	8.8	7.3	0.84	0.81	-0.04
89	12	5.8	5.4	0.82	0.76	-0.09
90	10	7.8	7.6	0.85	0.86	0.03
91	16	10.7	8.3	0.80	0.83	0.03
3	12	8.8	7.8	0.86	0.83	-0.04
16	15	10.2	8.4	0.91	0.87	-0.05
15	15	11.0	8.7	0.89	0.85	-0.04
17	15	10.0	8.4	0.89	0.87	-0.03
53	16	11.0	8.7	0.89	0.84	-0.05
18	15	11.5	9.2	0.86	0.88	0.03
51	15	11.5	9.2	0.87	0.87	0.00
45	15	10.8	8.9	0.94	0.88	-0.07
80	14	8.8	7.7	0.90	0.86	-0.06
81	9	6.5	6.5	0.89	0.81	-0.10
140	15	11.0	8.9	0.89	0.89	0.00
140	15	11.0	8.9	0.89	0.89	0.00

autocorrelation analysis confirmed significant positive spatial structure within the 50, 250, and 500 m distance classes ( $x$  intercept = 756 m). For Wood Frogs, significant genetic structure was found only within the 50 m distance class ( $x$  intercept = 490 m), suggesting little spatial association of related individuals beyond this distance.

**Landscape features as barriers or facilitators of dispersal.**—The least-cost path analyses found a limited number of significant correlations between landscape features and genetic distance metrics. In Wood Frogs, none of the univariate models were significant using either distance metric, indicating no detectable relationship of the landscape features we tested and gene flow

at this scale. In Spotted Salamanders, none of the univariate models were significant using  $F_{ST}$ , though roads, lakes, streams, and disturbed land had a marginally significant positive correlation with least-cost path length. For the  $D_C$  metric, there was a significant positive correlation between lakes and least-cost path length in salamanders, indicating a negative relationship with gene flow (Table 4). The multivariate resistance model based on expert opinion was not significantly correlated with genetic distance for either species.

## DISCUSSION

**Population structure.**—We found high genetic connectivity with minimal genetic structure for

**Table 4.** Results from the partial Mantel tests of effective and genetic distance ( $F_{ST}$  and  $D_C$ , respectively) for Wood Frogs (*Lithobates sylvaticus*) and Spotted Salamanders (*Ambystoma maculatum*). Includes the partial correlation coefficient (R), the p value (P), and the 95% confidence interval around the correlation coefficient (LL, lower limit; UL, upper limit).

Model	$D_C$ R	$D_C$ P	95% CI-LL	95% CI-UL	$F_{ST}$ R	$F_{ST}$ P	95% CI-LL	95% CI-UL
<b>Wood Frog</b>								
IBD	-0.10	0.74	-0.18	0.03	-0.05	0.58	-0.12	0.06
Disturbed	-0.02	0.58	-0.10	0.05	-0.04	0.67	-0.12	0.02
Forestry	-0.20	0.90	-0.27	-0.14	-0.17	0.85	-0.21	-0.13
Roads	-0.02	0.58	-0.10	0.06	-0.05	0.69	-0.12	0.01
Lakes	-0.08	0.75	-0.13	0.02	-0.11	0.82	-0.17	-0.03
Slope	0.04	0.38	-0.06	0.11	-0.04	0.54	-0.11	0.05
Wetlands	-0.21	0.96	-0.32	-0.07	-0.21	0.96	-0.32	-0.06
Streams	-0.01	0.55	-0.09	0.07	-0.05	0.68	-0.13	0.02
Multivariate	-0.13	0.82	-0.19	0.00	-0.13	0.82	-0.22	0.01
<b>Spotted Salamander</b>								
IBD	-0.13	0.86	-0.21	-0.03	-0.20	0.98	-0.26	-0.13
Disturbed	0.05	0.18	-0.01	0.10	0.08	0.08+	0.02	0.14
Forestry	-0.10	0.66	-0.14	-0.04	0.05	0.32	0.01	0.12
Roads	0.04	0.24	-0.01	0.10	0.08	0.10+	0.02	0.14
Lakes	0.16	0.03*	0.06	0.26	0.12	0.06+	0.04	0.22
Slope	-0.02	0.52	-0.09	0.05	0.00	0.47	-0.06	0.06
Wetlands	0.04	0.36	-0.02	0.11	-0.05	0.69	-0.13	0.02
Streams	0.05	0.22	-0.01	0.11	0.08	0.09+	0.02	0.14
Multivariate	0.07	0.27	-0.03	0.20	0.06	0.30	-0.03	0.19

\*P ≤ 0.05  
+P ≤ 0.10

both Spotted Salamanders and Wood Frogs across the study area. A lack of genetic differentiation at this scale was indicated by low pairwise  $F_{ST}$  values, a lack of detectable structure using Bayesian clustering analysis, and limited evidence that landscape features impact dispersal patterns. For both species, individuals from ponds showed genetic similarity across distances of 40–50 km. Previous studies have found these species have high levels of gene flow at similar spatial scales (e.g., Newman and Squire 2001; Zamudio and Wieczorek 2007; Crosby et al. 2009; Greenwald et al. 2009b; Purrenhage et al. 2009).

High genetic connectivity may be a result of a number of factors, including higher dispersal rates or longer dispersal distances than commonly reported, extinction-recolonization dynamics that minimize genetic structuring, few landscape barriers to dispersal, or anthropogenic fragmentation that is too recent to affect genetic variation (Smith and Green 2005; Purrenhage et al. 2009). In this study, we attribute the high amount of gene flow to a predominately forested landscape with abundant wetlands and few landscape features that act as absolute dispersal barriers.

***Landscape influences on gene flow.***—Despite high levels of gene flow, the pairwise  $F_{ST}$  analyses revealed some genetic differentiation among some ponds for both species. The isolation by distance analyses indicated that these genetic discontinuities were not explained by geographic distance, which suggests that landscape influences may be shaping genetic patterns across the study area. Although the least-cost path analyses revealed a lack of detectable associations between the landscape variables and gene flow in Wood Frogs, the models indicated lakes, streams, roads, and disturbed land limit gene flow in Spotted Salamanders. The negative relationship between these landscape variables and gene flow is consistent with biological constraints on salamander dispersal. Lakes and streams impede salamander movement because salamanders have diffi-

culty crossing large and fast-moving water bodies (Marsh et al. 2007; Richardson 2012). The presence of fish further makes these habitats inhospitable as juveniles and adults risk predation if using these wetlands as stepping-stones for dispersal (Pilliod and Peterson 2001). Roads are often associated with mortality (Gibbs and Shriver 2005) and can act as physical or psychological barriers to movement (deMaynadier and Hunter 2000). Open-canopied habitat such as agriculture, development, and bare ground (labeled as ‘disturbed land’ in this analysis) increases the risk of desiccation and reduces dispersal ability and distance (Rothermel and Semlitsch 2002; Greenwald et al. 2009a).

Complex interactions between environmental factors at a local and landscape scale may also influence gene flow. For example, conditions at the breeding pond such as hydroperiod, temperature, density, and predators, vary from year to year and are known to influence productivity, growth rates, and larval survival (Berven 1990; Skelly 1996; Werner and Glennemeier 1999; Marsh and Trenham 2001; Babbitt 2005). These local factors create variation in population dynamics that influence the number of metamorphs successfully dispersing between ponds and thus influence metapopulation processes at a larger scale. Although population fluctuations can increase genetic differentiation by reducing dispersal among ponds, in a landscape with abundant ponds and healthy amphibian populations, there are plenty of metamorphs dispersing to nearby ponds to maintain high gene flow across the broader landscape.

Recognizing that genetic distance metrics can vary in their sensitivity to detect genetic changes over short time scales, we used both  $F_{ST}$  and  $D_C$  as response variables in the landscape analysis. We expected  $D_C$  to be sensitive to more recent landscape change such as forestry, roads, and disturbed land, while  $F_{ST}$  might only detect the influence of landscape features with a longer-term presence such as lakes, streams, or slope. For Wood Frogs, we found that both contemporary

and historical distance metrics had little relationship to landscape pattern. For Spotted Salamanders, lakes were correlated with both  $D_C$  and  $F_{ST}$ , and disturbed land, roads, and streams were correlated with  $F_{ST}$  but not  $D_C$ . Therefore, our findings suggest that in this analysis,  $D_C$  was not more sensitive to recent landscape change than  $F_{ST}$ .

Though this study took place in a commercial forest, we found no negative impacts of forest management on gene flow at the scale we measured. Similarly, Spear and Storfer (2008, 2010) found a relatively high amount of gene flow in two species of tailed frog (*Ascaphus*) in managed forests in the northwestern US. Current regulations governing forest management practices in Maine may contribute to patterns of high gene flow. Public concern over timber liquidation in conjunction with salvage logging in the 1970s–1980s prompted the state to pass the Forest Practices Act in 1989 (Sader et al. 2003). This act effectively restricted clearcuts over 50 ha and required clearcuts to be separated by patches of forest that cannot be cut for a decade, while also promoting partial and shelterwood harvest techniques (Forest Practices Act, Maine Forest Service. 1989. Title 12, Part 11, Chapter 805, Augusta, Maine, USA). These forest management practices may contribute to the connectivity of amphibians in this landscape because current clearcut patches are noncontiguous, constrained in size, distributed throughout the area, and make up a small percentage of the landscape. Our findings suggest that the current level of forest disturbance in the study area is minimal enough to maintain population level gene flow.

Alternatively, the lack of an effect of timber harvest on gene flow may result from a temporal lag in the genetic response (Keyghobadi et al. 2005; Holzhauer et al. 2006). Simulation studies suggest that the time to detect the genetic effects of landscape change depends on the dispersal distance of the species and the generation time. In species with low dispersal ability ( $\geq 10$  km), such as amphibians, it generally takes longer to

detect landscape change. In least-cost path analyses using Mantel tests, simulations showed it can take up to six generations to detect a dispersal barrier and  $> 500$  generations to detect its removal (Landguth et al. 2010). These findings indicate that for these less mobile species, genetic patterns may not reflect recent landscape change, but instead may reflect past landscape conditions. In their study of harvest impacts on gene flow in the Coastal Tailed Frog (*Ascaphus truei*), Spear and Storfer (2008) found that the effects of forest management on amphibian gene flow took several generations (20+ years) to influence genetic structure. The land cover map used in our analysis depicted timber harvest cut up to a decade before genetic sampling, which is time for one Spotted Salamander generation and two to three Wood Frog generations to pass (Spotted Salamander longevity up to 20 years, mode 7 years [Flageole and Leclair Jr. 1992]; Wood Frog longevity 3–5 years [Redmer and Trauth 2005]). Given this time frame, there may not have been enough time to detect an effect of recent forest management on gene flow.

Timber harvest may be a temporary disturbance that operates on a time scale too short to influence genetic patterns. In the study area, vegetation in clearcut patches regenerates quickly, with saplings and shrubs growing up to 1 m tall within a year, providing shaded microhabitats that facilitate dispersal (Patrick et al. 2006; Popescu et al. 2012). Recent evidence suggests that for juvenile Wood Frogs in Maine, abundance and survival within clearcuts increases just a few years after harvest (Patrick et al. 2008; Popescu et al. 2012). In addition, both species are capable of moving through clearcuts to reach forested patches (Veysey et al. 2009; Freidenfelds et al. 2011). Consequently, the impacts of forest management on dispersal may be minimal, with more permanent landscape features more likely to influence genetic patterns for these two species. Our findings confirm this, in that the only significant dispersal barrier we identified was lakes. However, in developed

areas, where anthropogenic landscape features are long lasting, these factors may have a stronger negative influence on gene flow (Crosby et al. 2009; Gabrielsen et al. 2013).

**Species comparison.**—By comparing multiple species within the same landscape, it is possible to identify species-specific responses and assess if patterns are consistent across species (Steele et al. 2009; Goldberg and Waits 2010; Richardson 2012). In this study, we found similarly high levels of gene flow in both focal species, but different landscape influences. The varied patterns may be related to the differences in mobility of the two species. Wood Frogs are capable of traveling farther distances than Spotted Salamanders (Berven and Grudzien 1990; Montieith and Paton 2006). Therefore, Wood Frogs are more likely to move through an unfavorable patch or find ways around a potential dispersal barrier and we would expect to find less genetic differentiation and fewer landscape influences on gene flow. Richardson (2012) found evidence that supported this hypothesis, with greater mobility in Wood Frogs contributing to higher levels of gene flow as compared to Spotted Salamanders. Our results corroborate this hypothesis as no landscape features were significantly correlated with genetic distance patterns in Wood Frogs, while several landscape features were correlated in Spotted Salamanders. In addition, greater Wood Frog mobility was supported by the spatial autocorrelation analysis, in which Wood Frogs showed less fine-scale spatial genetic structure, indicating higher dispersal rates than Spotted Salamanders. Wood Frogs, however, exhibited higher overall  $G'_{ST}$ , which implies higher average genetic differentiation between ponds.

**Methodological considerations.**—Aspects of our experimental design may limit the strength of the conclusions that can be drawn. First, in landscape genetics the scale of analysis and sampling scheme are important considerations be-

cause both factors can impact the ability to detect a genetic signal of landscape change (Cushman and Landguth 2010; Jaquiere et al. 2011; Oyler-McCance et al. 2013). Studies conducted at scales many times larger than the dispersal distance of an animal may be detecting patterns that emerge at a regional scale, rather than identifying landscape features that impact individual movement patterns. Assuming a maximum dispersal distance of 2.5 km for Wood Frogs and 1 km for salamanders (Berven and Grudzien 1990; Gamble et al. 2007), our study included ponds within and up to 50 times the dispersal distance for these species. Investigating the influences of landscape features on gene flow at a smaller spatial scale may improve the power of the analysis (Cushman and Landguth 2010). Further, our sampling scheme incorporated several clusters of ponds with a range of intervening distances distributed across the extent of the study area, rather than ponds sampled systematically and consistently throughout the landscape, as is considered optimal for fine-scale landscape genetics studies (Storfer et al. 2007). A more systematic and intensive sampling scheme may be better at detecting patterns of isolation by distance and landscape influences on gene flow (Oyler-McCance et al. 2013).

The number of microsatellite loci (six per species) used in this study may also have limited the power of our analyses. While conducting the study with additional loci may have increased the sensitivity to detect patterns, it has been shown theoretically that the power to detect genetic differentiation is a function of not just the number of loci used, but more specifically the total number of alleles, as well as the sample sizes (Kalinowski 2002; Ryman et al. 2006). The loci used in this study were highly polymorphic, with high levels of heterozygosity and a large number of alleles per locus (total of 117 and 57 alleles were found for the 6 loci in Wood Frogs and Spotted Salamanders, respectively). The high polymorphism of the loci, combined with relatively large sample sizes, despite relatively low  $F_{ST}$ s, suggest that

the low levels of genetic differentiation we found were not due to power limitations, but rather were characteristic of this highly connected system. Other studies using more loci have also shown similar findings of high rates of gene flow and weak landscape influences in these species in northeastern North America (Crosby et al. 2009; Richardson 2012; Gabrielsen et al. 2013; Coster et al. 2015). Lastly, although null alleles were detected in two loci in the Spotted Salamander dataset, they were present at low frequencies (< 10%), which are not expected to bias genetic distance metrics (Chapuis and Estoup 2007), and therefore should not have influenced our results.

The choice of resistance values may also affect the ability to detect a relationship between gene flow and landscape features (Jaquiere et al. 2011; Cushman et al. 2013). In this study, the multivariate expert opinion model performed poorly. The lack of relationship between effective and genetic distance in the multivariate model may indicate that the expert assigned resistances we used were not appropriate in this landscape. These resistance values were initially generated for ambystomatid salamanders in southern New England, in a landscape with more development and less forestry (Compton et al. 2007). Using univariate models can reduce some of the uncertainty associated with expert opinion models by eliminating the question of relative effects and instead focusing on which landscape variables are most important to dispersal (Broquet et al. 2006; Pérez-Espona et al. 2008; Schwartz et al. 2009). However, the univariate models in this study similarly detected weak relationships between gene flow and landscape pattern. Therefore, the lack of power may not be a methodological problem, but instead reflect the high connectivity due to abundant habitat. Simulation studies confirm there is less power to detect landscape influences on gene flow in homogenous landscapes where habitat is extensive (Cushman and Landguth 2010; Cushman et al. 2012, 2013).

**Conclusion.**—We found high genetic connectivity and limited influences of landscape features on the dispersal of Wood Frogs and Spotted Salamanders in a commercial forest. Results from our study suggest that the legislation in Maine restricting the size of clearcuts and promoting partial harvest techniques may be compatible with amphibian dispersal and gene flow. These forest management practices ultimately result in a largely forested landscape that favors successful dispersal for Spotted Salamanders and Wood Frogs. However, we caution against extrapolating these findings to commercial forests in other areas where moisture is limiting, wetlands are isolated, or management regimes are different.

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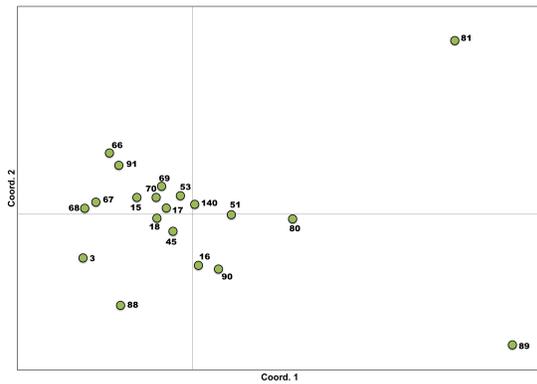
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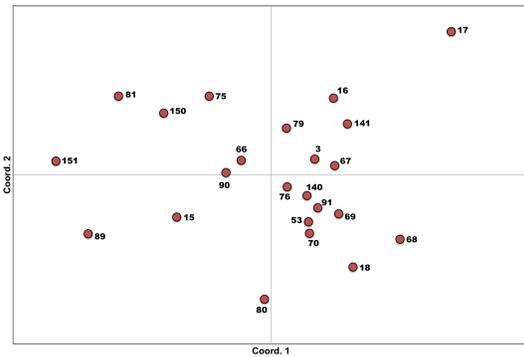
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APPENDICES



**APPENDIX 1.** Principal coordinates analysis (PCA) of Wood Frog (*Lithobates sylvaticus*) populations based on six microsatellite loci. Numbers indicate pond identification numbers.



**APPENDIX 2.** Principal coordinates analysis (PCA) of Spotted Salamander (*Ambystoma maculatum*) populations based on six microsatellite loci. Numbers indicate pond identification numbers.

**TABLE A1.** Description of landscape classes investigated in binary least-cost path analyses.

Layer	Data Source	Description
Geographic Distance (IBD)	GPS coordinates	Distance derived from least-cost paths between GPS coordinates.
Lakes	National Wetlands Inventory (NWI)	Lakes were isolated from NWI
Wetlands	NWI	Wetlands were classified as all water bodies excluding lakes
Roads	Maine Department of Transportation	Roads included highways and industrial access roads.
Slope	USGS Digital Elevation Model	Percent slope was calculated using Spatial Analyst in ArcGIS 10, and natural breaks with two classifications were used to represent high (20%) and low (80%) resistance to movement.
Streams	NHDPlus	Streams orders 3 and 4 were included.
Landcover	MeLCD 2004	See below for details.
Forest Management		Forestry included classes that were identified as clear-cut, heavy partial cut, light partial cut, or forest regeneration.
Disturbed land		Disturbed land included agriculture (cultivated land, blueberry field, pasture/hay), development, and bare ground).

**TABLE A2.** Metrics describing the genetic diversity of Wood Frog (*Lithobates sylvaticus*) and Spotted Salamander (*Ambystoma maculatum*) populations in central Maine. Each locus is presented and the number of alleles, allelic richness (AR), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ),  $F_{IS}$ , and pairwise  $F_{ST}$  along with their standard error in parentheses are averaged across populations.

Locus	Alleles	AR	$H_O$	$H_E$	$F_{IS}$	$F_{ST}$
Wood Frog						
C41	11	4.8	0.65	0.65	-0.019 (0.041)	0.014 (0.008)
D32	18	8.9	0.87	0.90	0.013 (0.028)	0.016 (0.008)
C52	27	10.8	0.90	0.93	-0.005 (0.019)	0.036 (0.009)
D20	16	8.9	0.91	0.90	-0.039 (0.023)	0.021 (0.007)
D77	24	10.8	0.93	0.93	-0.017 (0.021)	0.022 (0.007)
C11	21	10.1	0.93	0.91	-0.038 (0.020)	0.025 (0.009)
Spotted Salamander						
D287	11	5.2	0.87	0.83	-0.055 (0.020)	0.013 (0.006)
D315	9	4.2	0.77	0.74	-0.051 (0.029)	0.015 (0.008)
D184	12	5.9	0.85	0.87	0.003 (0.018)	0.009 (0.006)
D321	12	5.2	0.68	0.82	0.165 (0.029)	0.015 (0.007)
D99	10	4.9	0.72	0.81	0.102 (0.027)	0.021 (0.012)
C40	3	2.1	0.39	0.38	-0.047 (0.035)	0.034 (0.016)

**TABLE A3.** Pairwise  $F_{ST}$  values (above diagonal) for the Wood Frog (*Lithobates sylvaticus*) populations with bold indicating significance after Bonferroni correction. Geographic distance between populations (km) is below the diagonal. Numbers on top and left indicate pond identification numbers that correspond to those shown in Fig. 1 and Table 3.

	69	68	70	66	67	88	89	90	91	3	16	15	17	53	18	51	45	80	81	140	
69																					
68	0.001																				
70	0.022	-0.001																			
66	0.016	0.019	0.033																		
67	7.1	7.1	7.0	7.6	0.6																
88	13.2	12.9	13.7	15.0	15.3	0.018															
89	13.2	12.9	13.7	14.9	15.2	0.027	0.056														
90	12.8	12.5	13.2	14.5	14.8	0.033	0.064	0.021													
91	12.5	12.3	13.0	14.6	14.9	0.019	0.074	0.019	0.029												
3	24.8	24.5	25.3	27.0	27.3	0.025	0.075	0.020	0.006	0.023											
16	28.4	28.2	28.9	30.1	30.2	0.023	0.066	0.016	0.011	0.013	0.022										
15	27.8	27.5	28.3	29.5	29.7	0.023	0.066	0.021	0.043	0.018	0.037	0.040	0.026								
17	28.7	28.4	29.1	30.3	30.4	0.1	0.054	0.021	0.068	0.071	0.055	0.072	0.066	0.050							
53	22.4	22.1	22.9	23.5	23.7	0.5	0.039	0.028	0.039	0.028	0.012	0.020	0.007	0.015	0.001	-0.002	0.027	0.020	0.063	0.008	
18	29.0	28.7	29.5	30.6	30.8	0.7	0.5	0.024	0.034	0.024	0.034	0.020	0.030	0.007	0.024	0.018	0.038	0.046	0.060	0.032	
51	35.4	35.2	35.9	38.5	38.8	12.0	12.5	12.6	12.6	12.6	0.020	0.019	0.018	0.017	0.011	0.018	0.028	0.040	0.075	0.025	
45	35.6	35.3	36.1	38.7	38.9	15.3	15.4	16.0	16.0	4.2	0.020	0.008	0.020	0.019	0.018	0.019	0.019	0.019	0.067	0.010	
80	32.1	31.9	32.5	29.7	29.5	14.8	14.8	15.3	15.4	3.4	0.8	0.008	0.013	0.009	0.005	0.005	0.012	0.023	0.057	0.018	
81	32.6	32.3	33.0	30.6	30.5	15.6	15.6	16.0	16.2	4.4	0.2	1.0	0.014	0.009	0.004	0.009	0.011	0.025	0.048	0.009	
140	53.6	53.3	54.0	50.8	50.5	42.3	42.3	42.6	43.0	37.3	33.6	34.4	33.6	35.1	33.4	39.7	39.6	21.5	21.3	21.3	

**Table A4.** Pairwise  $F_{ST}$  values (above diagonal) for the Spotted Salamander (*Ambystoma maculatum*) populations with bold indicating significance after Bonferroni correction. Geographic distance between populations (km) is below the diagonal. Numbers on top and left indicate pond identification numbers that correspond to those shown in Fig. 1 and Table 2.

69	0.007	-0.001	0.016	0.000	0.016	0.016	-0.007	0.033	0.006	0.040	0.023	-0.004	0.015	0.007	0.041	0.022	0.014	0.024	0.000	0.041	0.027	0.003	0.007
70	0.5	0.000	0.008	-0.004	0.010	0.010	-0.003	0.023	0.007	0.035	0.024	-0.008	0.011	-0.003	0.035	0.025	-0.006	0.024	0.017	0.027	0.009	0.004	0.012
68	0.3	0.8	0.006	-0.005	0.010	0.002	0.040	0.016	0.037	0.022	0.014	-0.002	0.006	0.056	0.038	-0.001	0.019	0.011	0.046	0.011	0.000	0.008	
66	7.1	7.0	7.1	0.004	0.000	0.008	0.025	0.007	0.043	0.024	0.019	0.001	0.022	0.034	0.020	0.004	-0.008	0.006	0.012	0.025	0.009	0.017	
67	7.7	7.6	7.7	0.6	0.003	0.003	-0.010	0.021	0.003	0.013	-0.002	-0.001	-0.001	0.027	0.011	-0.009	0.008	0.006	0.028	0.016	-0.015	-0.009	
90	12.8	13.2	12.5	14.5	14.8	14.8	-0.004	0.013	-0.002	0.043	0.025	0.019	0.000	0.022	0.031	0.011	-0.001	-0.019	0.006	0.022	0.017	0.003	
91	12.5	13.0	12.3	14.6	14.9	14.9	0.5	0.019	-0.007	0.034	0.003	-0.001	-0.002	0.009	0.020	0.020	-0.008	-0.005	-0.005	0.039	0.006	-0.015	
89	13.2	13.7	12.9	14.9	15.2	15.2	0.4	0.7	-0.016	0.080	0.046	0.020	0.022	0.027	0.009	0.022	0.020	0.013	0.030	0.032	0.005	0.020	
15	27.8	28.3	27.5	29.5	29.7	29.7	15.3	15.4	14.8	0.060	0.020	-0.002	0.005	0.018	0.021	0.009	-0.012	0.001	0.005	0.032	0.012	0.007	
17	28.7	29.1	28.4	30.3	30.4	30.4	16.0	16.2	15.6	0.027	0.034	0.029	0.032	<b>0.079</b>	0.044	0.054	0.041	0.030	0.065	<b>0.057</b>	0.025	0.007	
16	28.4	28.9	28.2	30.1	30.2	30.2	15.8	16.0	15.4	0.2	0.2	0.023	0.003	0.032	0.035	0.013	0.009	0.011	0.028	0.027	0.019	-0.006	
53	22.4	22.9	22.1	23.5	23.7	23.7	9.6	9.9	9.2	6.6	6.6	5.0	0.016	0.004	0.033	0.014	-0.003	0.035	0.014	<b>0.033</b>	0.032	0.010	
3	24.8	25.3	24.5	27.0	27.3	27.3	12.5	12.6	12.1	3.4	4.4	4.2	5.0	0.013	0.036	0.018	-0.004	-0.003	-0.001	<b>0.030</b>	0.017	-0.002	
18	29.0	29.5	28.7	30.6	30.8	30.8	16.4	16.5	16.0	1.3	0.4	7.2	4.7	0.045	0.032	0.010	0.033	0.023	<b>0.052</b>	0.006	0.001	0.011	
151	22.6	23.0	22.3	20.7	20.6	20.6	12.0	12.5	11.8	14.5	14.7	9.4	14.4	14.9	0.012	0.028	0.017	0.028	<b>0.027</b>	0.031	0.024	0.035	
150	22.5	22.9	22.2	20.6	20.6	20.6	12.0	12.5	11.8	14.6	14.6	9.5	14.4	14.9	0.1	0.011	0.002	0.011	0.030	<b>0.046</b>	0.019	0.023	
76	37.6	38.1	37.4	36.3	36.2	36.2	25.6	26.0	25.3	16.6	15.8	17.4	19.4	15.7	15.6	15.6	0.2	0.003	0.008	0.042	0.014	0.003	
75	37.8	38.3	37.6	36.4	36.3	36.3	25.8	26.2	25.5	16.8	16.1	17.6	19.6	15.9	15.7	15.8	0.2	0.003	0.001	0.026	0.021	-0.002	
79	33.1	33.5	32.8	31.1	31.0	31.0	21.8	22.3	21.5	16.5	16.0	15.2	18.5	16.0	10.6	10.6	5.9	6.0	<b>0.032</b>	0.027	0.005	0.002	
81	32.6	33.0	32.3	30.6	30.5	30.5	21.2	21.7	20.9	15.9	15.5	14.5	17.9	15.4	10.1	10.1	6.1	6.2	0.7	<b>0.049</b>	0.034	<b>0.043</b>	
80	32.2	32.6	31.9	29.7	29.6	29.6	21.4	21.9	21.2	17.8	17.4	15.6	19.4	17.4	9.7	9.7	8.0	8.1	2.2	2.4	2.4	0.004	
140	53.6	54.0	53.3	50.8	50.5	50.5	42.6	43.0	42.3	34.4	33.6	35.1	37.2	33.4	31.1	31.1	17.9	17.7	20.7	21.3	21.4	0.015	
141	53.6	54.0	53.4	50.6	50.4	50.4	42.9	43.3	42.6	35.2	34.5	35.7	38.0	34.3	31.2	31.3	18.7	18.5	21.0	21.7	21.5	1.8	



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