

Article

## Upland Habitat Quality and Historic Landscape Composition Influence Genetic Variation of a Pond-Breeding Salamander

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**Abstract:** Understanding the temporal and spatial scale at which habitat alteration impacts populations is important for conservation and management. Amphibians have declined more than other vertebrates, and pond-breeding species are particularly susceptible to habitat loss and fragmentation because they have terrestrial and aquatic life stages. One approach to management of pond-breeding species is protection of core upland habitat surrounding the breeding pond. We used genetic variation as an indicator of population status in a common amphibian species, spotted salamanders (*Ambystoma maculatum*), to determine how amount of suitable upland habitat relates to population status in the greater Charlotte, North Carolina, USA metropolitan area. We developed candidate models to evaluate the relative influence of historical and contemporary forested habitat availability on population genetic variation at two spatial scales of upland area (164 m and 2000 m) at four time intervals over the past seven decades (1938, 1978, 1993, 2005). We found that historical land cover best predicted contemporary allelic richness. Inbreeding coefficient and observed heterozygosity were not effectively predicted by forest cover at either spatial or temporal scales. Allelic richness was best predicted at the smaller spatial scale in the 1993 time interval. Predicting and understanding how future landscape configuration affects genetic

variation of common and rare species is imperative for the conservation of amphibian and other wildlife populations.

**Keywords:** population genetics; *Ambystoma maculatum*; upland core habitat; historic landscapes; microsatellite DNA

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## 1. Introduction

Populations of most species are distributed patchily across the landscape, and abundance is highest where the local environment meets niche requirements [1]. Regional persistence of species requires that sufficient amounts of suitable habitat remain on the landscape, and long-term persistence is enhanced when this habitat maintains some level of connectivity [2]. Anthropogenic land-use changes over the past several decades have resulted in habitat loss and fragmentation, which are the primary causes of biodiversity loss and population declines [3,4]. For example, urbanization decreases the amount of natural habitat and increases the number of impervious surfaces (e.g., roads) that divide natural landscapes, creating multiple isolated habitat fragments [5]. Reduced habitat availability will decrease population viability because fewer individuals can be supported, and reduction or loss of connectivity to other populations restricts gene flow and might prevent re-colonization following local extinctions [6,7].

Species that occupy multiple habitat types throughout their life cycle are more susceptible to negative effects of habitat alteration, loss, and fragmentation. Amphibian populations have declined at faster rates than most other organisms for this and other reasons [8]. This is especially true for amphibian species that breed in ponds because these species typically reside in terrestrial habitats during juvenile and adult stages and undergo embryonic and larval stages in freshwater aquatic habitats [9–11]. Habitat availability and connectivity is positively related to survival, population recruitment, and dispersal success; therefore, changes over time likely influence viability and distribution of amphibian populations [9–12]. However, more studies are needed to evaluate the relative influence of historical and contemporary landscape heterogeneity on amphibian populations [13,14].

Understanding the minimum habitat requirements needed to maintain populations is important for conservation and management [9,15]. Legislation passed in most U.S. states requires that a small buffer (15–30 m) of terrestrial habitat must remain intact around bodies of water [9]. However, these buffers may not include the types of terrestrial habitat required by organisms that use the aquatic habitats [9,16]. Some investigators have suggested that a minimum amount of terrestrial habitat (termed “core habitat”) is needed around wetlands to maintain local populations [15–18]. For example, Semlitsch [18] suggested that a core terrestrial habitat encompassing an area within a 164-m radius of a wetland is necessary to maintain at least 95% of most *Ambystoma* populations and other species of amphibians with low vagility. However, a single population-focused approach might not be enough to maintain regional persistence [19].

Population status and likelihood of persistence can be assessed using population genetic data, and both are positively related to the amount of genetic variation within and among populations [20–23]. Information about the extent of genetic variation of populations in combination with maintenance of minimum habitat requirements may be useful for better understanding and improving conservation

status of amphibians in fragmented habitats [23,24]. Although many researchers have estimated the size of minimum core habitats [17,18], additional studies are needed to evaluate the relationship between the genetic variability of amphibian populations and the likelihood that those populations can persist in fragmented habitats. Our objectives were to use geographical information system (GIS) data and genetic analyses to (1) examine the relationship between genetic variability of *Ambystoma maculatum* (spotted salamander) and the amount of forest cover surrounding a wetland within recommended core terrestrial habitat and at a larger scale; and (2) determine if historic or contemporary landscapes better explain current genetic variability.

## 2. Experimental Section

We studied six populations of *A. maculatum* across the greater Charlotte area (Mecklenburg and Cabarrus counties) of North Carolina, USA (Figure 1). Sites were selected based on the presence of *Ambystoma maculatum* egg masses and variability in upland habitat. Most mole salamanders (genus *Ambystoma*) provide excellent models for studying the effects of habitat fragmentation on populations. They breed in distinct, clearly defined areas (*i.e.*, ponds) that can be easily sampled and are widespread and able to survive in a human-altered environment. Adult salamanders typically use fish-free ponds for breeding and forested upland habitat for feeding and over-wintering, and aquatic larvae metamorphose and disperse to surrounding upland habitat until maturation occurs [25].

**Figure 1.** Map of study sites across the greater Charlotte area (Mecklenburg and Cabarrus counties) of North Carolina, USA. Inset map of the United States depicts location of counties in North Carolina.



At each wetland, we collected 5 eggs from each of 30 egg clutches (or from every clutch when fewer than 30 existed) from 6 February to 25 March 2005. Eggs were preserved and stored in 95% ethanol until DNA extraction. We extracted DNA from one egg per clutch using the Qiagen DNeasy blood and tissue protocol (Qiagen, Valencia, CA, USA). Each individual was genotyped for 5 microsatellite DNA loci from Julian *et al.* [26] (D367, D321, D49, D287, D99) using PCR and an ABI 310 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Allele lengths were scored using GeneScan Analysis Software

version 3.1.2 (Life Technologies, Carlsbad, CA, USA). We tested for deviations from Hardy Weinberg Equilibrium (HWE) for each locus and population using global tests with Bonferroni corrections and for linkage disequilibrium using Fisher's exact test with Bonferroni corrections in FSTAT 2.9.3.2 [27]. We calculated allelic richness, inbreeding coefficient ( $F_{IS}$ ), observed heterozygosity ( $H_O$ ), and expected heterozygosity ( $H_E$ ) for each population using FSTAT 2.9.3.2 [27].

Use of aerial imagery from different time periods can be used to investigate how historic landscapes impacted population structure and to provide support for interpretation of genetic variation. Aerial imagery (*i.e.*, photographs) was obtained from Mecklenburg County Geospatial Information Services and Cabarrus County Soil and Water Conservation District offices. Little cloud cover appeared on these images and seasonal variation in vegetation did not affect our ability to discern among cover types.

To quantify habitat availability at each site, we used ArcGIS v.9.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) for analyses of habitat surrounding each wetland. Aerial imagery was available for the years 1938, 1978, 1993, and 2005 and was georeferenced to determine habitat change over time at each site. Using digitized imagery we determined the dominant land cover types surrounding each wetland. Land cover types included urban (*i.e.*, human development), agriculture (*i.e.*, pasture and crops), and forest (*i.e.*, natural habitat). Land cover type was calculated as a percentage of total land cover (e.g., percent forest) at two spatial scales represented as 164-m and 2000-m radii surrounding breeding ponds. Semlitsch [18] recommended a 164-m-radius core, forested habitat around wetlands for protection of 95% of adult *A. maculatum* individuals within a population. A 2000-m distance was used for comparison because it better represents the larger landscape. For analyses, we focused on proportion of forest cover at each site and time period because *A. maculatum* prefer forest to open habitat and have significantly lower survival in open habitat [28,29].

We used an information-theoretic approach to generalized linear modeling [30] to examine the relationship between population genetic diversity and forest cover at multiple spatial and temporal scales. We focused on forest cover because it is the most important land cover type for spotted salamanders and because forest cover was correlated to agriculture and urban land cover. We evaluated eight single-variable candidate models for allelic richness,  $F_{IS}$ , and  $H_O$ . Because  $H_E$  and allelic richness were correlated ( $r = 0.919$ ;  $p = 0.009$ ), we excluded  $H_E$  from analyses. Other measures of genetic variation were not correlated. Each model corresponded to percent forest cover at both 164-m and 2000-m scales for each time period (*i.e.*, 1938, 1978, 1993, and 2005). The best candidate models were defined as models with the lowest AIC values and highest weights [30]. Parameter estimates ( $\pm$ standard error) and  $p$ -values for each variable were also presented.

### 3. Results

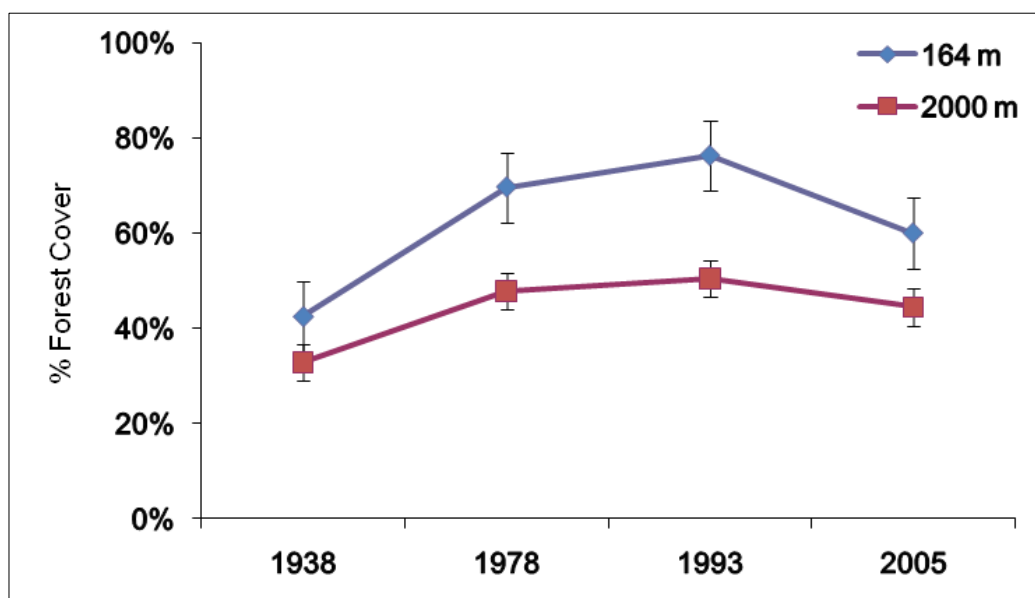
The six populations exhibited a wide range of genetic variation (Table 1). No significant deviations from linkage equilibrium were detected, but 10 of 30 tests were significant for deviation from HWE. Percent forest cover surrounding each wetland varied over time and space whereby forest cover at the 164-m and 2000-m spatial scales increased from 1938 to 1993, but decreased again in 2005 (Figure 2). At the 2000-m scale, there was less recovery of forest cover over time and less variability in forest cover among sites and years (Figure 2). Land cover within the 164-m area primarily remained as forest and agriculture over our study period. At the 2000-m scale, urbanization appeared after the 1938 time period and increased over time at all sites at varying intensities. The best candidate model for predicting allelic

richness included the variable percent forest cover within 164 m in the year 1993 (AICc weight = 0.767; Table 2). Allelic richness was positively associated with forest cover in this model (parameter estimate =  $7.60 \pm 1.97$ ). Contemporary forest cover at both the 164-m and 2000-m spatial scale were poor predictors of allelic richness (Table 2). In terms of  $F_{IS}$ , forest cover during 1993 at the 2000-m and 164-m scales were the highest supported models (Table 2). However, parameter estimates for each were not statistically different from zero. In general, forest cover was a poor predictor of  $H_o$ ; all models had nearly identical AICc weights and parameter estimates were not statistically significant.

**Table 1.** Observed genetic variation of six *Ambystoma maculatum* populations in the greater Charlotte area of North Carolina, USA.

Population	Allelic Richness	Inbreeding Coefficient	Observed Heterozygosity	Expected Heterozygosity
A	8	0.26	0.589	0.803
B	5.8	0.30	0.648	0.731
C	7.8	0.20	0.639	0.794
D	7.2	0.11	0.650	0.618
E	8.8	0.38	0.471	0.787
F	6.2	0.08	0.588	0.69
Mean + 1 SE	7.3 + 0.46	0.22 + 0.05	0.565 + 0.036	0.737 + 0.030

**Figure 2.** Average ( $\pm 1$  SE) percent forest cover within circular areas of 164-m and 2000-m radii for six wetlands in the greater Charlotte area of North Carolina, USA from 1938–2005.



**Table 2.** Akaike’s Information Criterion of candidate models that examine habitat availability and time on genetic variables ( $F_{IS}$  = inbreeding coefficient,  $H_O$  = observed heterozygosity,  $H_E$  = expected heterozygosity).

Genetic variable	Model	AICc	$\Delta AICc$	$w_i$	Parameter Estimate ( $\pm 1$ St. Err)	$p$ -value
Allelic richness	1993: 164 m	14.32	0.00	0.767	7.601 ( $\pm 1.973$ )	0.018
	1938: 164 m	17.67	3.36	0.143	3.177 ( $\pm 1.221$ )	0.060
	1978: 164 m	19.89	5.57	0.047	6.392 ( $\pm 3.444$ )	0.137
	2005: 164 m	22.78	8.46	0.011	2.381 ( $\pm 3.083$ )	0.483
	1993: 2000 m	23.08	8.77	0.010	2.323 ( $\pm 3.821$ )	0.576
	1978: 2000 m	23.60	9.29	0.007	0.298 ( $\pm 3.816$ )	0.942
	2005: 2000 m	23.60	9.29	0.007	-0.261 ( $\pm 3.293$ )	0.941
	1938: 2000 m	23.61	9.30	0.007	0.087 ( $\pm 7.911$ )	0.992
$F_{IS}$	1993: 2000 m	-7.43	0.00	0.341	0.538 ( $\pm 0.301$ )	0.148
	1993: 164 m	-5.47	1.96	0.128	0.415 ( $\pm 0.379$ )	0.336
	1978: 164 m	-5.43	2.01	0.125	0.449 ( $\pm 0.418$ )	0.343
	1938: 164 m	-5.15	2.28	0.109	0.175 ( $\pm 0.182$ )	0.390
	1978: 2000 m	-4.75	2.68	0.089	0.279 ( $\pm 0.359$ )	0.480
	1938: 2000 m	-4.39	3.04	0.075	0.447 ( $\pm 0.767$ )	0.592
	2005: 2000m	-4.20	3.23	0.068	0.1465 ( $\pm 0.325$ )	0.675
	2005: 164 m	-4.13	3.31	0.065	-0.127 ( $\pm 0.328$ )	0.718
$H_O$	1993: 2000 m	-8.23	0.00	0.175	-0.248 ( $\pm 0.281$ )	0.428
	2005: 164 m	-7.75	0.47	0.138	0.156 ( $\pm 0.242$ )	0.555
	1978: 2000 m	-7.68	0.55	0.133	-0.169 ( $\pm 0.282$ )	0.582
	1978: 2000 m	-7.68	0.55	0.133	-0.169 ( $\pm 0.282$ )	0.582
	1938: 164 m	-7.42	0.81	0.117	0.063 ( $\pm 0.151$ )	0.696
	1993: 164 m	-7.31	0.91	0.111	0.105 ( $\pm 0.325$ )	0.764
	2005: 2000 m	-7.25	0.49	0.108	-0.060 ( $\pm 0.252$ )	0.823
	1978: 164 m	-7.26	0.96	0.108	0.093 ( $\pm 0.359$ )	0.807
1938: 2000 m	-7.26	0.96	0.108	-0.158 ( $\pm 0.604$ )	0.807	

#### 4. Discussion

The trends in percent forest cover we documented for 1938–2005 corresponded to previous research in the Piedmont region of the southeastern United States. This region was historically forested but was mostly converted to pastures and agricultural land during the 1800s [31,32]. Beginning around 1940, many of these croplands and pastures were abandoned and allowed to reforest [31,32]. Beginning in the 1970s, urbanization increased dramatically, which led to a significant reduction of forested lands [33,34]. We found a positive relationship between allelic richness and amount of suitable, forested habitat at the finer (164-m) scale. Because inherent variability in forest cover at the 2000-m scale was less than the finer scale (Figure 2), we had lower ability to detect its influence. Regardless, forest cover is generally important for *A. maculatum* because they prefer forest substrates to other substrates [29] and can suffer high mortality rates when traveling through non-forested habitats [10]. Although we did not detect any

significant patterns for other measures of genetic variability, our study might be limited in power to detect these differences because of number of loci used and number of study sites.

Historic habitat availability was a better predictor of genetic variability than current landscape configuration. Our results suggest there is a time lag between when habitats are altered and when population genetic variability responds, and it contributes to a growing literature supporting that caution must be taken in using contemporary genetic variability as an indicator of current population status, especially in landscapes continuing to lose habitat [13,14,24,35]. One turnover in generations (and potentially multiple) might be required before the time lag response in genetics reflects land-use change. Spotted salamanders have an average generation time of 4–5 years [22,25]. Therefore, the lag time we detected represents 2–3 generations. Similarly, Purrenhage *et al.* [22] found that the amount of habitat fragmentation had no significant effect on genetic structure of spotted salamander populations and suggested that fragmentation might have been too recent to observe changes in genetic structure.

Most amphibian species that breed in this wetland type (isolated, depressional wetlands) have a similar requirement for upland forested habitat, so our results are applicable to these amphibians and other organisms with similar habitat requirements. Thus other species should exhibit a positive relationship between amount of upland habitat surrounding wetlands where they breed and population genetic variability. As habitat is degraded, there will be a time lag in genetic signature, so contemporary variability within each species might overestimate population status. Additionally, because *A. maculatum* is a common species that tends to survive in human-altered environments more so than many other amphibian species, we feel that our study might overestimate response to land-use change by less tolerant species.

Although regional biodiversity is maintained by landscape-level, among-population dynamics, many of our management techniques continue to focus on individual populations. Sound management requires integration of perspectives that emphasize factors operating at local and landscape scales [19,36]. Populations of wetland species will go extinct if either the wetland or surrounding upland habitat is severely degraded or lost. Therefore, management must focus on preserving both habitat types [17,18]. However, although this management philosophy will maintain local populations in the short term, it ignores the long-term, evolutionary perspective. To conserve wetland species in the long term and regionally, we not only need to conserve upland buffers surrounding wetlands, but also we must preserve ecological connectivity among these breeding populations [17,18]. This not only allows for recolonization when local population extinctions occur, but also allows for maintenance of genetic diversity across the landscape via within-population processes and low-level dispersal rates among populations [20].

As land-use changes continue to occur and rural land is transformed by urban sprawl, *A. maculatum* and other species' populations will continue to be negatively affected. The most severe land-cover change is urbanization, which is associated with more endangered species in the United States than is any other human activity [3]. Unlike other habitat alterations (e.g., agricultural cultivation, pastureland creation, forest clearing) urbanization is more permanent. From a practical perspective, many landscape contexts, especially urban areas, present difficulty in maintaining large corridors through matrix between patches and may not be logistically or economically feasible. Even in areas with severely disturbed habitat between populations, genetic variation and connectivity can be maintained using innovative techniques, including corridors and green spaces in urban areas, wildlife fences and tunnels under roads, and strategically placed artificial wetlands [37,38]. Additional studies are necessary to

determine the effect of current and future rates of habitat loss on genetic structure and species diversity and to determine best management practices across these landscapes [39].

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### Conflicts of Interest

The authors declare no conflict of interest.

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