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Landcover predicts isolation in *Ambystoma* salamanders across region and species

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ABSTRACT

Anthropogenic modification of the landscape can have important consequences for dispersal of terrestrial organisms. If certain landcover types consistently act as barriers to movement, their occurrence could serve as a proxy for population isolation. We tested the fit of a general *a priori* landcover model on genetically-inferred isolation in ambystomatid salamander species. The landcover model included terms for pond size, geographic isolation, and amount of surrounding agriculture. In all cases, agriculture was associated with increased population isolation, while deciduous forest was almost always associated with decreased isolation. The full model described over 70% of the variation in a genetically-inferred measure of isolation in the system for which it was developed, and 30–45% of variation in novel systems. We suggest that landscape analyses can serve as a proxy for population isolation in ambystomatid salamanders. Our findings suggest that preserving well-forested regions without substantial agriculture will protect salamanders through increased population connectivity. These results have important implications for conservation, as genetic analyses are costly and time-consuming, and decisions to purchase or preserve land must often occur quickly.

1. Introduction

Range reductions and population declines of amphibian species have been well documented over the past two decades, with the consensus that there is no single global cause (Beebee and Griffiths, 2005; Blaustein et al., 1994; Collins and Storfer, 2003; Houlahan et al., 2000; Storfer, 2003; Stuart et al., 2004; Wake, 1991); however, as is the case with many taxa of conservation concern, habitat loss and fragmentation are major contributing factors (Stuart et al., 2004). While other factors such as disease have played a role in some regional declines (Berger et al., 1998; Lips, 1999), habitat loss associated with region-specific land use by humans is a consistent threat to population ecology, genetic diversity, and persistence.

Habitat destruction can be especially detrimental for the many amphibian species that require both aquatic and terrestrial habitats (Beebee, 1997; Hitchings and Beebee, 1997) because the philopatric nature of many pond-breeding amphibians (Gamble et al., 2007; Shoop, 1965) reduces the probability of rescue for populations threatened by local extinction. Low vagility may also play an important role in areas of high disturbance, with altered habitat such as clearings and roads either dissuading movement or contributing to high mortality during dispersal (de Maynadier and Hunter, 2000; Fahrig et al., 1995; Marsh et al., 2005; Rittenhouse and Semlitsch, 2006; Rothermel and Semlitsch, 2002; Semlitsch et al., 2006). The resulting isolation of local populations can have important (and potentially detrimental) effects on demography (Burke and Nol, 2000; Kuussaari et al., 1998; Lloyd et al., 2005) and population genetic structure (Keller and Largiadèr, 2003; Stow et al., 2001; Wauters et al., 1994).

To mediate risks such as adverse genetic effects and stochastic local extinctions caused by landscape change, conservation biologists require tools for predicting the impacts of habitat alteration among populations (Cushman, 2006). Predicting which landscape variables will influence effective dispersal (i.e., gene flow) is essential for a host of management goals, including reserve design, habitat restoration and corridor construction (Epps et al., 2007; Joly et al., 2003; Ray et al., 2002). For pond-breeding amphibians, pond size, interpond distances, and upland land use are key landscape variables; however, the relative importance of these variables may differ among species and regions. The development of spatially explicit Geographic Information Systems (GIS) models is becoming increasingly important for conservation planning (e.g., Gustafson et al., 2001). Nevertheless, spatially explicit models are rarely tested beyond the study species for which they are developed, which makes it difficult to assess whether a given model can be generalized to other species or landscapes. The development of general models would enhance multiple-species conservation planning,

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which is currently a primary approach to regional conservation (Barrows et al., 2005). Specifically, the general model described here could facilitate economical prioritization of land acquisition and restoration decisions targeting wetland fauna.

A recent study demonstrated the utility of landcover models to reliably predict population isolation in the marbled salamander (*Ambystoma opacum*; Greenwald et al., 2009). On two spatial scales (300 m and 1 km radii), agriculture and greater average interpond distance were associated with higher levels of population isolation, whereas forest cover and larger pond size were associated with less isolation of populations. Here we explore the generality of these results in related salamander taxa in other landscapes. We tested amongst alternative landscape variables to infer whether patterns of population structure are associated with particular landscape features. We examined model performance on landscape datasets for three species of the North American *Ambystoma* salamanders, a clade that contains several species under rapid decline and risk of extinction (Stuart et al., 2004). Most ambystomatids occupy vernal ponds for breeding and larval development, and upland areas for the terrestrial life stage, leading to a naturally patchy distribution at both local and regional scales.

In this study we tested amongst several factors that affect the spatial distribution of *Ambystoma* salamander populations. Specifically, we examined: (1) whether a landcover model developed for one species (*A. opacum*) is suitable for describing population isolation in two related species, and (2) whether the same landcover metrics are associated with the observed distribution of population genetic structure in each dataset. In testing across the different datasets, we correlated genetic assignment tests with the variables that best described landscape genetic structure in *A. opacum* and compared these results to other candidate landscape models. Our ultimate goal was to determine whether there are landscape variables that consistently explain population genetic structure across different regions and species, because identifying this kind of general relationship would be invaluable in guiding species management and the allocation of limited conservation resources.

2. Materials and methods

2.1. Datasets and genetic analyses

We used four population genetic datasets from ambystomatid salamanders in the Midwestern and eastern United States (Table 1, Fig. 1; names reflect species and location). Each dataset consisted of one focal species (either *A. opacum*, *Ambystoma maculatum*, or *Ambystoma laterale*) that ranged from 251 to 665 individuals from 14 to 29 sites, averaging 18–24 individuals/site. Individuals were genotyped at a minimum of eight (Amac-OH) to a maximum of 17 (Alat-MN) microsatellite DNA loci per dataset. Methodological details on sampling and microsatellite analysis can be found in the associated publication for each dataset (Table 1).

Estimates of recent migration events are one approach to characterizing population structure because the resulting inferences are informative about the extent of likely dispersal (Berry et al., 2004; Manel et al., 2005; Waser and Strobeck, 1998). For these inferences we used the assignment test approach implemented in GeneClass2 (Piry et al., 2004) to determine the number of individuals of recent immigrant ancestry at each local population (represented by individual genotypes for each of the four datasets independently). High numbers of correct assignments (*i.e.*, individuals sampled in deme A and genetically assigned to that deme) are one indication that recent migration is rare, and this provides an index of population isolation (Greenwald et al., 2009). We used the standard assignment of individuals for datasets consisting of adults (Amac-OH, 12 sites from Amac-NY); for the Amac-NY dataset, larvae and adult resident assignments results were combined. For datasets composed of larval samples (Aop-OH, Alat-MN, 19 sites from Amac-NY) we used the "detection of first generation migrants" function with a Bayesian computation method (Rannala and Mountain, 1997) and Monte-Carlo resampling (Paetkau et al., 2004) assuming a conservative alpha value of 0.01. For each site in each dataset, the proportion of individuals assigned as residents with $\geqslant$95% probability was used as the response variable in testing the landscape GIS models.

We also performed a least-cost path analysis wherein we compared pairwise distance measures to pairwise genetic measures (see below). We used GENEPOP 3.4 (Raymond and Rousset, 1995) to generate a matrix of pairwise F_{ST} values for all sites in each dataset for this analysis.

2.2. GIS and statistical analyses

We used Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson, 2002) to select landcover models that best explain isolation across the sites. For each dataset at each scale we assembled a suite of models potentially explaining isolation (see below), and then used the AIC approach to discriminate among them. We consider models with $\Delta AIC_c < 2$ to be perform as well as the best model (Burnham and Anderson, 2002); such models are listed along with the best models ($\Delta AIC_c = 0$) and the a priori *opacum* model for comparison (Table 2). We also report a pseudo-r^2 (predicted versus observed genetic isolation) to describe the fit of the model (explained variance). In all cases the observational unit was the pond; therefore the sample size for each study system corresponds to the number of sampled ponds.

We included three metrics to describe pond size and physical isolation: pond perimeter, average pond distance, and nearest pond distance. Pond perimeter (PP) was either directly measured (Aop-OH), or estimated from field maps and detailed satellite images (Amac-NY, Amac-OH). For estimated pond sizes, we used the following categorical classification: very small (area < 200 m^2), small (200–500 m^2), medium (500–1000 m^2), large (1000–3000 m^2), and very large (>3000 m^2); these areas were then converted to perimeters assuming wetlands were roughly circular. Pond size was not included as a predictor in the models for the Alat-MN dataset because ponds were uniformly very small (<200 m^2). Average pond distance (APD) is the mean distance from the focal pond to all other ponds in the study area; nearest pond distance (NPD) is the distance to the sampled pond closest to the focal pond (nearest neighbor distance).

To examine effects of the surrounding landscape, we determined landcover composition within circular areas centered on each of the sampled ponds at two scales (300 m and 1 km radii) in ArcView 9.3 (ESRI, Inc.). The 300 m scale was chosen to encompass the recommended wetland buffer zone for salamanders (Semlitsch and Bodie, 2003), while the larger scale was chosen to allow inclusion of more landcover types and to account for the possibility of long-distance dispersal (Gamble et al., 2007). For both scales we determined the proportion of each circular area comprised of each landcover category using the 30 m resolution National Landcover 2001 Database (NLCD; http://seamless.usgs.gov/), and grouped landcover categories based on previously published landscape resistance values (Compton et al., 2007; Greenwald et al., 2009). The resistance values fell into five categories named to reflect the dominant landcover type as follows: FOR (Deciduous Forest, Mixed Forest, Evergreen Forest, Woody Wetlands, all with a resistance of 1.0); SHRUB (Emergent Herbaceous Wetlands, Scrub/Shrub and Barren Land, all with a resistance of 3.0–3.4); LODEV (Developed, low intensity and Developed, medium intensity, both with a resistance of 6.8); AG (Grassland/Herbaceous, Pasture/Hay and

Table 1
Descriptions of four *Ambystoma* salamander datasets included in this study. The samples row describes the number of individual larvae (L) and/or adults (A) sampled for each dataset. resident asst range describes the range (across all sites) of the proportion of individuals assigned as residents. Highly isolated sites have high numbers assigned as residents. Spatial Extent is the range of pairwise distance between sampled sites for each dataset. The landcover overview rows list the average (range in parentheses) proportion of land within a 1 km radius comprised of low, medium–low, medium, medium–high and high resistance landcover types (FOR, SHRUB, LODEV, AG and HIDEV, respectively), from the National Landcover Database.

Dataset	Aop-OH	Amac-NY	Amac-OH	Alat-MN
Species	*A. opacum*	*A. maculatum*	*A. maculatum*	*A. laterale*
Location	Ohio	New York	Ohio	Minnesota
Samples	478 L	404 L/261 A	407 A	251 L
Sites	21	19 L/12 A	17	14
Loci	9	11	8	17
Overall fst	0.056	0.069	0.050	0.011
Resident asst range	0.16–1.00	0.35–1.00	0.05–0.75	0.18–0.53
Spatial extent (km)	1.0–68.2	1.6–46.9	0.1–55.2	0.1–28.9
Landcover – 300 m				
FOR	0.81 (0.38–1.00)	0.67 (0.03–1.00)	0.85 (0.55–1.00)	0.91 (0.80–0.98)
SHRUB	0.00 (0.00–0.02)	0.08 (0.00–0.76)	0.00 (0.00–0.00)	0.05 (0.00–0.20)
LODEV	0.12 (0.00–0.29)	0.05 (0.00–0.62)	0.07 (0.00–0.17)	0.04 (0.00–0.10)
AG	0.05 (0.00–0.49)	0.19 (0.00–0.86)	0.06 (0.00–0.27)	0.00 (0.00–0.00)
HIDEV	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.02)	0.00 (0.00–0.00)
Landcover – 1 km				
FOR	0.81 (0.34–0.98)	0.62 (0.12–1.00)	0.68 (0.49–0.94)	0.94 (0.88–0.98)
SHRUB	0.01 (0.00–0.04)	0.07 (0.00–0.32)	0.00 (0.00–0.01)	0.03 (0.00–0.06)
LODEV	0.08 (0.02–0.28)	0.05 (0.00–0.37)	0.18 (0.04–0.42)	0.02 (0.01–0.03)
AG	0.09 (0.00–0.32)	0.25 (0.00–0.69)	0.10 (0.01–0.37)	0.00 (0.00–0.00)
HIDEV	0.00 (0.00–0.02)	0.00 (0.00–0.06)	0.02 (0.00–0.13)	0.00 (0.00–0.00)
Reference	Greenwald et al. (2009)	Zamudio and Wieczorek (2007)	Purrenhage et al. (2009)	Savage (2009), Julian et al. (2003a,b)

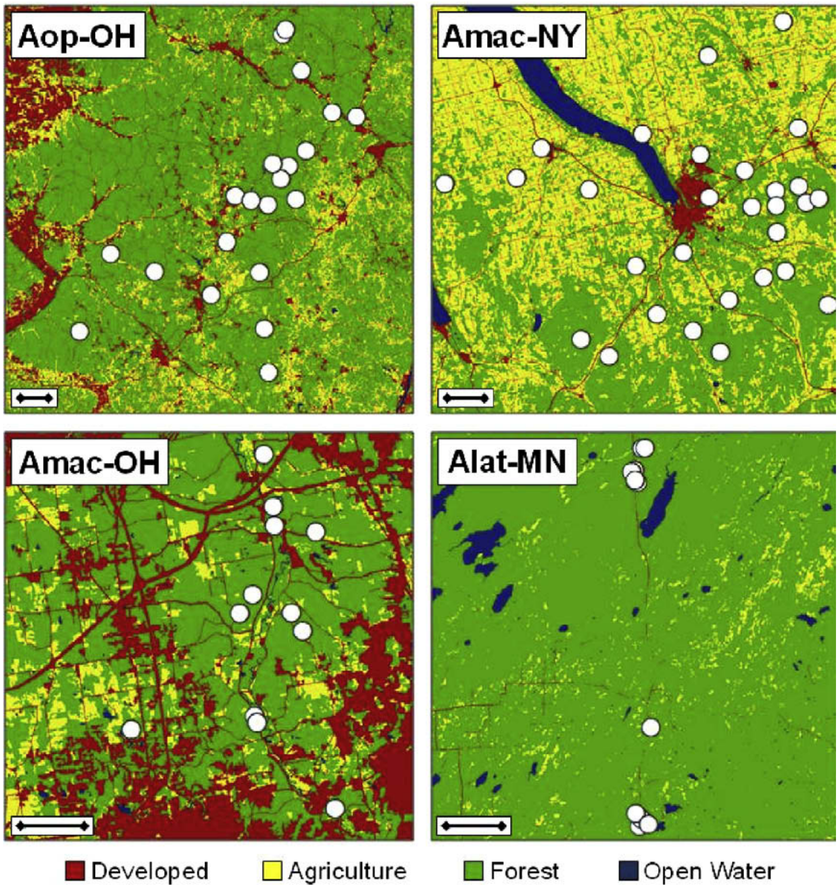

Fig. 1. Landcover maps for the four *Ambystoma* salamander datasets used in this study. White circles represent sampled ponds. Scale bars represent 5 km in all cases.

Cultivated Crops, all with a resistance of 9.2–10.2); and HIDEV (Developed, medium intensity and Developed, high intensity, both with a resistance of 12.6). Open water (WATER) was included as a variable on its own, due to its very high resistance value (22.0).

For the AIC_C analysis we included all possible model subsets for each dataset at each spatial scale, which resulted in a maximum of 512 models when all nine variables were permuted (PP; APD; NPD; FOR; SHRUB; LODEV; AG; HIDEV; WATER); however, in most cases

Table 2
Best performing models ($\Delta AIC_c < 2$) for all datasets. The direction of the regression coefficient and significance for each individual term in the model is given in parentheses. The fit between the model-predicted data and observed (genetic) data is given by r^2. Variables are as follows: pond perimeter (PP); average pond distance (APD); nearest pond distance (NPD); expert-assigned resistance groups of increasing resistance: forest (FOR), shrub/scrub (SHRUB), low-intensity development (LODEV), agriculture (AG), and high-intensity development (HIDEV). The a priori *opacum* model (PP, APD, AG) is included for all datasets regardless of ΔAIC_c value for comparison purposes.

Data	Scale	Model (β direction, *p*-value)	ΔAIC_c	r^2
Aop-OH	300 m	PP (Neg, <0.001) + APD (Pos, 0.037) + AG (Pos, 0.001)	0.00	0.783
		PP (Neg, <0.001) + FOR (Neg, 0.002)	1.74	0.722
Aop-OH	1 km	PP (Neg, <0.001) + FOR (Neg, 0.007)	0.00	0.683
		PP (Neg, <0.001) + APD (Pos, 0.102) + FOR (Neg, 0.010)	0.09	0.730
		PP (Neg, <0.001) + APD (Pos, 0.059) + AG (Pos, 0.023)	1.98	0.705
Amac-NY	300 m	PP (Pos, <0.001) + APD (Pos, 0.001) + LODEV (Neg, 0.044)	0.00	0.533
		PP (Pos, 0.001) + APD (Pos, .002)	1.86	0.449
		PP (Pos, 0.003) + APD (Pos, 0.005) + AG (Pos, 0.659)	4.57	0.453
Amac-NY	1 km	PP (Pos, 0.001) + APD (Pos, 0.002)	0.00	0.449
		PP (Pos, <0.001) + APD (Pos, 0.002) + LODEV (Neg, 0.222)	1.18	0.481
		PP (Pos, 0.005) + APD (Pos, 0.007) + AG (Pos, 0.805)	2.87	0.450
Amac-OH	300 m	PP (Pos, 0.142)	0.00	0.138
		AG (Pos, 0.216)	0.73	0.100
		APD (Neg, 0.149) + AG (Pos, 0.061)	1.59	0.229
		FOR (Neg, 0.421)	1.77	0.044
		LODEV (Neg, 0.486)	1.96	0.033
		PP (Pos, 0.097) + LODEV (Neg, 0.272)	1.97	0.212
		PP (Pos, 0.133) + APD (Neg, 0.083) + AG (Pos, 0.069)	2.65	0.356
Amac-OH	1 km	PP (Pos, 0.142)	0.00	0.138
		APD (Neg, 0.074) + AG (Pos, 0.042)	0.83	0.263
		HIDEV (Neg, 0.272)	1.11	0.080
		AG (Pos, 0.321)	1.37	0.066
		LODEV (Neg, 0.346)	1.49	0.059
		PP (Pos, 0.404) + APD (Neg, 0.109) + AG (Pos, 0.129)	4.00	0.303
Alat-MN	300 m	NPD (Pos, 0.044) + FOR (Neg, 0.043)	0.00	0.414
		SHRUB (Pos, 0.100)	0.13	0.210
		NPD (Pos, 0.116) + SHRUB (Pos, 0.064)	0.88	0.376
		FOR (Neg, 0.190)	1.35	0.139
		NPD (Pos, 0.195)	1.39	0.136
Alat-MN	1 km	NPD (Pos, 0.195)	0.00	0.136
		LODEV (Neg, 0.655)	1.80	0.017
		SHRUB (Pos, 0.729)	1.90	0.010

one or more of the landcover variables was not present within the buffer area, so the total model set was reduced. We report all good models ($\Delta AIC_c < 2$) along with the a priori *opacum* model, which consists of PP + APD + AG.

We also performed a least-cost path analysis (Adriaensen et al., 2003) to determine whether landscape resistance influenced gene flow on a larger spatial scale. We generated three pairwise distance metrics for each dataset: (1) Euclidean distance, (2) path length, and (3) path cost. For the latter two, we used landscape and slope (independently and together) to calculate least-cost paths between pairs of sites using the extension PATHMATRIX (Ray, 2005) in ArcView 3.2. We determined the actual length of the least-cost path between each pair of sites (path length) as well as the total accumulated cost to move along the path (path cost). Landscape resistance values were based on Compton et al. (2007); we used their *A. opacum* values for datasets Aop-OH and Alat-MN, and their *A. maculatum* values for datasets Amac-NY and Amac-OH. Percent slope was calculated for each cell from the National Elevation Dataset (NED) (Spear et al., 2005). In order to combine landcover resistance and slope resistance into one layer, we log-transformed percent slope and then multiplied by a constant such that the range of its values was equivalent to the range of values observed in the landcover resistance matrix. In this way we insured that landcover and slope contributed evenly to the combined resistance matrix, while also accounting for the possibility of a nonlinear relationship between landcover resistance and slope. We performed Mantel tests with 10,000 permutations to test for correlations between pairwise genetic distances (F_{ST}) and each of the three pairwise landscape distance metrics (Euclidean distance, Path length, and Path cost).

3. Results

3.1. Assignment test results

Assignment tests revealed a large amount of variation among datasets (Table 1). The Alat-MN dataset had the narrowest overall range of resident assignments, with all sites falling between 18 and 53% of individuals assigned as residents, which is a likely indication that gene flow is relatively high across the small sample area. The other three datasets had sites with much higher resident assignment percentages (75%, 100% and 100% for Amac-OH, Amac-NY and Aop-OH, respectively). The lowest overall F_{ST} (0.011) was estimated from the Alat-MN dataset, followed by

Amac-OH, Aop-OH and Amac-NY (0.050, 0.056 and 0.069, respectively; Table 1).

3.2. Model performance across datasets

Overall, the *opacum* model (PP + APD + AG) performed reasonably well at describing population isolation across datasets (Table 2). At the 300 m scale, the *opacum* model described 36% and 45% of the variation in isolation in datasets Amac-OH and Amac-NY, respectively, and at the 1 km scale it described 30% and 45% of the variation. This model could not be tested on dataset Alat-MN because there was no agriculture in that study region. A reduced model with APD and FOR described 13.9% of the variation in population isolation for dataset Alat-MN at the 300 m scale, but only 0.4% of the variation at the 1 km scale.

Certain landcover variables consistently performed well across datasets. Pond size was often a good predictor; however it was positively related to genetic isolation for the *A. opacum* dataset (Aop-OH), but negatively related in the *A. maculatum* datasets (Amac-NY, Amac-OH). Agriculture (AG) was consistently positively associated with genetic isolation, while forest (FOR) was negatively related to genetic isolation. Pond distance metrics (APD and NPD) were fairly consistent but generally weak predictors of isolation. Two variables (SHRUB and WATER) were weak predictors of population structure.

3.3. Individual datasets

For dataset Aop-OH, the best model at the 300 m scale was the *opacum* model (PP + APD + AG; $r^2 = 0.783$), as shown in previous work (Greenwald et al., 2009), with only one alternative good model (PP + FOR; $\Delta AIC_c = 1.74$; Table 2). At the 1 km scale the best model was PP + FOR ($r^2 = 0.683$); however, there were two additional good models, the *opacum* model and PP + APD + FOR (Table 2). Larger ponds were significantly associated with lower levels of genetic isolation ($p < 0.001$), likely due to supporting larger population sizes. APD and isolation were positively correlated and the relationship was consistent at both scales ($p = 0.037$ at the 300 m scale; $p = 0.059$ at the 1 km scale). Agriculture (AG) was consistently and significantly associated with increased isolation ($p = 0.001$ at the 300 m scale; $p = 0.023$ at the 1 km scale). Forest (FOR) was consistently associated with decreased isolation, and was individually significant in the second best model at the 300 m scale ($p = 0.002$) and the best model at the 1 km scale ($p = 0.007$; Table 2). There was no significant isolation-by-distance (IBD) relationship for this dataset (Table 3).

For dataset Amac-NY, the best models were PP + APD + LODEV (300 m; $r^2 = 0.533$) and PP + APD (1 km; $r^2 = 0.449$). Larger ponds were significantly associated with *higher* population genetic isolation ($p < 0.001$ at the 300 m scale; $p = 0.001$ at the 1 km scale; Table 2). APD again had a consistently significant relationship, with higher distances associated with higher levels of isolation ($p = 0.001$ at the 300 m scale; $p = 0.002$ at the 1 km scale; Table 2). LODEV was significantly negatively related to isolation at the 300 m scale ($p = 0.044$) but not the 1 km scale ($p = 0.222$), although this negative relationship was driven by one large outlier. In the *opacum* model, AG was not individually significant but was again associated with increased isolation at both scales. This dataset had a significant IBD relationship ($p < 0.001$; Table 3).

Dataset Amac-OH had six good models at the 300 m scale, all with relatively low r^2 values (Table 2). As with dataset Amac-NY, pond size had a consistently positive regression coefficient, meaning that larger ponds were significantly associated with more isolation. Agriculture was again consistently positively related to isolation in this dataset, and was a significant individual predictor in a model with APD at the 1 km scale ($p = 0.042$) and approached significance in the *opacum* model at 300 m ($p = 0.069$). Both development variables (LODEV and HIDEV) had negative coefficients, meaning that these variables were associated with *less* isolation in this dataset. Although good models, the relationships were weak ($r^2 = 0.059$ and 0.080 for LODEV and HIDEV at 1 km, respectively). At both scales in the *opacum* model APD had a negative relationship with isolation; however, this is a very weak relationship ($r^2 \sim 0.000$ at both scales). There was no significant IBD relationship for this dataset (Table 3).

Dataset Alat-MN was from a region with no agriculture and very little development, and had a significant IBD relationship ($p = 0.001$). The relatively natural and homogenous landscape allowed for very little predictability using our GIS framework. There were five good models at the 300 m scale, and three at the 1 km scale (Table 2). The only individually significant predictors were for the best model at 300 m: NPD (positive; $p = 0.044$) + FOR (negative; $p = 0.043$). An *a posteriori* AIC analysis including all unreduced landcover categories confirmed that no particular landcover category had superior predictive ability (data not shown). At the 300 m scale, shrub, deciduous forest and low-intensity development were all good models; however, every remaining landcover category had $\Delta AIC_c < 2.9$. At the 1 km scale, every landcover category had $\Delta AIC_c < 0.5$, meaning that they were all equally likely predictors of genetic isolation.

3.4. Least cost path analysis

Incorporating landscape resistance did not increase correlations between distance and F_{ST} (Table 3). Dataset Alat-MN had the highest r^2 values, with ~20% of the variation in F_{ST} explained by the path length and least-cost distance metrics. All *p*-values were significant at $\alpha = 0.002$ (following Bonferroni correction), and the F_{ST} values were not significantly improved by incorporating elevation or landcover data. Rather, they were driven by simple geographic distance, and hence, explained by the null expectation of isolation-by-distance (i.e., no cost beyond physical distance). Datasets Aop-OH and Amac-NY had intermediate r^2 values. For dataset Aop-OH, explanatory power was not significantly increased by

Table 3

Results from least-cost path analysis. Pairwise F_{ST} values were compared with seven pairwise distance metrics. For each dataset, we show the correlation (r^2) and *p*-value (in parentheses) based on a Mantel test with 10,000 permutations comparing pairwise F_{ST} to each of the distance metrics. These metrics are (a) Euclidean (geographic) distance; (b) path length based on slope; (c) path cost based on slope; (d) path length based on landcover; (e) path cost based on landcover; (f) path length based on both slope and landcover; (g) path cost based on both slope and landcover. Bolded *p*-values ($p < 0.002$) remain significant following Bonferroni correction.

Data	Aop-OH	Amac-NY	Amac-OH	Alat-MN
Euclidean	0.025 (0.138)	0.121 (**<0.001**)	0.001 (0.575)	0.189 (**<0.001**)
Slope PL	0.042 (0.081)	0.165 (**<0.001**)	0.003 (0.496)	0.190 (**<0.001**)
Slope PC	0.016 (0.161)	0.088 (0.006)	0.008 (0.211)	0.173 (**0.001**)
Landcover PL	0.027 (0.126)	0.065 (0.003)	0.000 (0.579)	0.192 (**<0.001**)
Landcover PC	0.030 (0.117)	0.051 (0.011)	0.000 (0.551)	0.192 (**<0.001**)
Slope + cover PL	0.015 (0.219)	0.013 (0.146)	0.000 (0.579)	0.191 (**<0.001**)
Slope + cover PC	0.030 (0.131)	0.022 (0.090)	0.000 (0.540)	0.190 (**<0.001**)

including slope or landcover resistance, and there were no statistically significant relationships between distance metrics and F_{ST}. For Amac-NY, both Euclidean distance and slope path length significantly explained F_{ST} ($p < 0.001$), with a maximum r^2 of 0.165 for slope path length (Table 3). Including landcover resistance but not slope resulted in lower explanatory power ($r^2 = 0.065$). Dataset Amac-OH had models with the lowest explanatory power, with a maximum $r^2 = 0.008$ for the path-cost values based on slope, and no significant comparisons based on Mantel tests.

4. Discussion

Our landscape genetic approach yielded five key findings. (1) The *opacum* model performed well at describing the factors influencing population genetic structure in two other *Ambystoma* datasets. (2) Individual landscape variables had qualitative influences on population structure: agriculture is consistently associated with higher population genetic isolation, while forest was consistently associated with lower isolation. Development was less important than agriculture and forest cover, and when it was a good model, the direction was opposite than predicted by previous research. (3) The direction of the relationship between population isolation and pond size varies by species: bigger ponds are less isolated in *A. opacum*, but more isolated in *A. maculatum*. (4) Landscape data predicted assignment test results more reliably than it did pairwise F_{ST} values. (5) In less-modified landscapes, gene flow is mediated by geographic distance. Overall, we conclude that straightforward landcover analysis provides some ability to predict population isolation in these salamanders, with certain landcover categories (e.g., forest, agriculture) having consistent effects across region and species.

4.1. Performance of the opacum model

The *opacum* landscape model (pond size, geographic isolation, and agriculture) performed well at both the 300 m and 1 km scales, and its application as a generalized model is supported by its overall performance in explaining 30–45% of the variation in population genetic structure. Although alternative models were selected by AIC, the fit between predicted and observed data was not substantially improved over the *opacum* model. The *opacum* model was developed for the Aop-OH dataset, so not surprisingly, it appears as a good model at both scales for this dataset. For the Amac-NY dataset, pond size and average pond distance (two of the three variables from the *opacum* model) appear in all good models. For the Amac-OH dataset, the top two models at both scales include all three of the variables from the *opacum* model (pond perimeter, agriculture, and average pond distance), either independently or in pairs. Overall we conclude that some combination of these variables can act as a good proxy for genetic isolation in ambystomatid salamander populations.

4.2. Influence of individual landscape variables

Forest cover was consistently associated with reduced population isolation, while agriculture was consistently associated with increased population isolation. The inverse relationship between forest and isolation is not unexpected, given that these ambystomatid species are woodland specialists (Pfingsten and Downs, 1989). The importance of forest is corroborated by our inclusion of a dataset which lacked barriers to natural dispersal (Alat-MN), resulting in effective movement across the landscape and a significant isolation-by-distance relationship (Slatkin, 1987, 1993). Agriculture was positively associated with higher population isolation, and in some cases (Aop-OH at both scales, Amac-OH at 300 m) it was at or near significance as an individual predictor in the *opacum* model. These results complement work demonstrating that ambystomatids suffer higher mortality and reduced mobility when moving through open field versus forest habitat, and that movement is biased away from field and toward forest (Rittenhouse and Semlitsch, 2006; Rothermel, 2004; Rothermel and Semlitsch, 2002; Walston and Mullin, 2008). Our study suggests that maintenance of forest habitat between breeding populations is the single management action most likely to support natural population dynamics by retention of population connectivity.

Development (mostly roads and residences) was included as a good model for two datasets (Amac-NY and Amac-OH); however, for the former, the relationship was driven by one large outlier. In both cases higher levels of development were associated with *less* isolation. This is surprising due to numerous studies showing that paved roads and other types of development lead to decreased species diversity, abundance, and movement (de Maynadier and Hunter, 2000; Fahrig et al., 1995; Gibbs, 1998; Porej et al., 2004; Rubbo and Kiesecker, 2005; Semlitsch et al., 2006). These relationships were weak for the Amac-OH dataset, but previous analyses also showed unusual patterns of gene flow in this system (Purrenhage et al., 2009). The core ponds in the Amac-OH dataset are located within the Cuyahoga Valley National Park (CVNP), which is centered around the Cuyahoga River and its tributaries and is fragmented by roads and residences. The reduced isolation attributed to development in these models could instead reflect the influence of some other landscape features spatially autocorrelated with development and roadways, such as riparian corridors in the Cuyahoga watershed. The positive relationship between development and connectivity could alternatively be explained by salamanders using road-associated ditches during dispersal. A wide variety of amphibians have been shown to use ditches, even in more heavily agricultural landscapes (Maes et al., 2008). This result may also reflect high historical gene flow and/or large recent population sizes that counteract the effects of drift and mutation.

4.3. Influence of pond size

The relationship between pond size and genetic isolation varied by species. In marbled salamanders (dataset Aop-OH), the relationship between pond size and population structure was negative: larger ponds were less isolated. However, in spotted salamander datasets (Amac-NY, Amac-OH), larger ponds were *more* isolated. (It should be noted, however, that small and large ponds are relative terms – very few sites were over 300 m in circumference.) An interesting question is whether this result could be due to differences in breeding biology. Marbled salamanders, which lay eggs on land before ponds fill in the fall, could prefer larger ponds because females may compete for low- or intermediate-elevation nest sites along the future pond edge (Croshaw and Scott, 2006; Petranka and Petranka, 1981), or for preferred cover objects (Croshaw and Scott, 2006). Spotted salamanders breed aquatically, in large explosive aggregations in the ponds during early spring (Myers and Zamudio, 2004; Pfingsten and Downs, 1989); smaller ponds may allow individuals to locate mates more successfully. These differing ecologies – competition for nest sites versus mate location or localization – may drive the observed differences in the role of pond size on population genetic structure.

4.4. Relationship of landcover with assignment tests versus F_{ST}

Landcover is a good predictor of the short-term population isolation metric (number of individuals assigned as residents), but not of longer-term gene flow (which we measured using F_{ST}). This may be due to the timescale that these measures describe; F_{ST} is a coarse measure of subdivision, and a poor discriminator of recent versus historical gene flow inference (Whitlock and McCauley, 1999). Allele loss, fixation, or mutation (new alleles) is dependent on population size, among other assumptions generally violated in natural populations, which can obscure the genetic impacts of anthropogenic changes in the habitat. Spear et al. (2005) found that F_{ST} in tiger salamander populations was well-described by distance and elevation; however, their focal landscape was not impacted by habitat modification so this result is possibly the result of long-term dynamics of restricted gene flow. Land uses such as agriculture have clearly not been in place for comparable lengths of time as geologic features (i.e., elevation). It is therefore reasonable to suggest that the signature of anthropogenic change would be more detectable by recent migration rate estimates (i.e., assignment tests) than by coarse measures of substructure (F_{ST}).

4.5. Anthropogenic modification, gene flow, and geographic distance

The signal of a significant isolation-by-distance relationship may be an indication that landscapes are less impacted by anthropogenic disturbance, or depending on the spatial scale it could also be due to nonequilibrium population structure (Slatkin, 1993). In a relatively unaltered and natural landscape, population genetic isolation is mediated by physical distance among populations (Wright, 1943). This relationship (IBD) was strongest for the Alat-MN (*A. laterale*) dataset, which was sampled from a contiguous landscape with no large-scale barriers to dispersal. The significant IBD relationship in the Amac-NY dataset is not as easily explained given the opposite finding in the Amac-OH dataset. However, these landscapes are quite different in the type and extent of anthropogenic disturbance, with much more agriculture and less development in the region of the Amac-NY dataset (Table 1). Our results suggest that agriculture may be less isolating for *A. maculatum* than it is for *A. opacum*; spotted salamanders in an agriculture-heavy landscape (Amac-NY) are able to disperse frequently enough to maintain an IBD relationship, whereas the signal of population structure for marbled salamanders (dataset Aop-OH) indicates more restricted movement patterns. The lack of IBD in the Amac-OH dataset suggests that roads and associated development may disrupt natural dispersal processes, and therefore gene flow, a result that has been observed previously in other amphibians with very important consequences for the maintenance of natural population dynamics (Hitchings and Beebee, 1997; Reh and Seitz, 1990).

5. Conclusions

Genetic diversity among populations, governed by the stochastic nature of gene flow and genetic drift, is an important factor to consider when prioritizing conservation action for protecting wetland associated fauna. However, population genetic data requires a great deal of resources, which may not always be economically or temporally feasible. Although genetic data and landcover models are most powerful when used in combination, our results show that spatially explicit models that incorporate pond characteristics and landcover variables are good predictors of genetic isolation of populations. Our study demonstrates that landscape GIS models can be used to guide management decisions, measure the anticipated costs to species in environmental impact statements, and inform land managers at the onset of proposed landscape modification.

We demonstrate the utility of a predictive model incorporating landcover variables and pond size to explain a large proportion of the variation in population genetic structure. Several general relationships were evident across both the regions and species examined. In a region with minimal anthropogenic impact, populations were relatively well-connected and genetic structure was best described by geographic proximity, whereas in regions impacted by human activity, agriculture was consistently associated with increased population isolation. Based on our results, protecting habitat characterized by minimal agriculture and maximal forest cover and wetlands will likely be an important measure to sustain *Ambystoma* populations. Because we found that the relationship between pond size and population isolation is specific to the focal species, a management approach that incorporates varying sizes of wetlands could benefit more than a single species. Our finding that salamander species in disparate areas are affected similarly by habitat modification supports the conclusion that it is possible to effectively conserve multiple species using comparable management strategies.

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