



# Regional replication of landscape genetics analyses of the Mississippi slimy salamander, *Plethodon mississippi*

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## Abstract

**Context** Landscape genetics can identify habitat features that facilitate or resist gene flow, providing a framework for anticipating the impacts of land use changes on dispersal of individuals. To inform management, a better understanding of how inferences derived from one study region are applicable to other regions is needed.

**Objectives** We investigated the manner in which five landscape variables correlated with gene flow among *Plethodon mississippi* populations in two study regions. We compared order of importance, direction (facilitation vs. resistance of gene flow) and scale of effect, and functional relationships of variables within each study area.

**Methods** In forests in Mississippi and Alabama, USA, we tested individual-based genetic distances derived from microsatellite genotypes against effective distances caused by agriculture, hardwoods, pine,

manmade structures, and wetlands that were optimized for both scale and transformation using maximum likelihood population effects modeling.

**Results** Of the landscape variables, agriculture and wetlands ranked at the top of both study areas' models. In both forest regions, agriculture was consistently associated with resistance, whereas pine was inferred to facilitate gene flow. However, we found region-specific differences in effects of wetlands, hardwoods, and manmade structures. Configuration of the latter landscape variables differed between forest regions, which may explain the contrasting outcomes.

**Conclusions** Our results underscore the value of metareplication in revealing which components of landscape genetics models may be consistent across different portions of a species' range, and those that have context-dependent impacts on gene flow. We also highlight the need to consider habitat configuration when interpreting the results of landscape genetics analyses.

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## Introduction

All species have areas of preferred habitat interspersed with areas of sub-optimal or unsuitable habitat (i.e., a

matrix; Fahrig and Merriam 1985). In order to maintain genetic connectivity among local populations that reside within different habitat patches, individuals must be able to traverse the intervening matrix. However, such areas are increasingly altered by anthropogenic influences (Jules and Shahani 2003). Modifications of natural areas are occurring at an accelerating rate due to the direct effects of a growing human population and expansion of urban areas. This is compounded by indirect effects such as alteration of natural disturbance regimes, introduction of exotic species, and climate change (Vitousek et al. 1997; Schmitz et al. 2015; Parisien et al. 2016). As a result, areas that were previously comprised mostly of suitable habitat have become increasingly “hostile” to free movement. This change in the permeability of the habitat matrix can lead to long-term isolation among small populations and random loss of genetic diversity due to the predominance of drift over selection (Keyghobadi 2007). As inbreeding becomes unavoidable in small isolated populations, this can give rise to inbreeding depression. In turn, these negative effects on individual fitness further diminish population size and growth rate (Allendorf et al. 2013). Indeed, these population-level changes can interact with other threatening processes (e.g., rapid changes in the abiotic environment, or emergence of wildlife disease) leading to local extinction (Gilpin and Soulé 1986), and by extension, an overall reduction in a species’ long-term viability (Sork and Waits 2010).

Knowledge about the relationship between organisms and their environments is a cornerstone of natural resource management. Wildlife conservation must consider the consequences of population isolation in the design of protected area networks and corridors, and this requires an understanding of the effect of specific landscape features on dispersal of individuals, and gene flow among populations. For decades, techniques such as capture-mark-recapture and radio telemetry have been used to gain such insights (e.g., Ovaska 1988; Riecken and Rath 1996). These methods are valuable, but do have some notable limitations. For example, capture-mark-recapture studies are time and labor intensive, and data points are acquired only from individuals that are re-encountered (Berry et al. 2004). Furthermore, the probability of recapturing marked individuals that have dispersed large distances is very low, creating an observation

bias toward detection of short-distance dispersal events (Koenig et al. 1996). Similarly, radio telemetry and passive integrative transponder tagging are also time and labor intensive, and often involve expensive equipment such that data are typically obtained from relatively few individuals (Hebblewhite and Haydon 2010; Connette and Semlitsch 2015). While these methods can provide high-resolution information on fine-scale movement, given that all data are usually acquired from a single cohort of individuals, capture-mark-recapture and radio telemetry usually provide only a short temporal snapshot. Accordingly, inferences may be influenced by abnormal environmental conditions, and thus could be unrepresentative (Bailey et al. 2004).

In principle, a robust understanding of how individuals perceive and move through a habitat matrix would be drawn from a large number of individuals sampled over a range of spatial and temporal scales, with at least two tiers of temporal insights: those reflecting very recent dispersal events (i.e., within the past generation or two), and those based on the accumulated effects of many generations of repeated movement. Molecular approaches have been used for these purposes, using individual-based comparisons of multilocus genotypes to determine recent dispersal, and population-based allele frequencies to detect the effects of repeated movement over time (e.g., Sunnucks 2000; Epps et al. 2013, 2018). When employing a landscape genetics approach, molecular data can be used to estimate genetic distances between individuals or populations, which may then be compared to corresponding distances based on the permeability of intervening heterogeneous habitats (Manel et al. 2003). For example, in an early landscape genetics study of gene flow among European roe deer in a fragmented landscape, Coulon et al. (2004) found an ecologically informed “resistance distance” that maximized use of wooded corridors provided a significantly better fit to inter-individual genetic distances based on microsatellite data than straight line distances, showing that roe deer dispersal is strongly tied to wooded areas.

Today, landscape genetics studies have become more analytically advanced, but the same basic principles apply. That is, the hypothesized resistance to dispersal caused by landscape variables such as land cover, topography, or bioclimatic measures (i.e., potential predictor variables) is tested against

empirically derived genetic distances (i.e., the response variable) to determine which landscape features most strongly resist (or facilitate) gene flow. Findings from these investigations can be used to plan for the impacts of recent and future land use changes upon an individual's ability to disperse, thereby providing spatially explicit guidance for conservation management (e.g., Cleary et al. 2017).

There are many spatial data types available for landscape genetics analyses, but of these, land cover classifications, presence or absence of roads, and topographic data are among the most commonly used (Zeller et al. 2012). The decision to include a given landscape variable in analyses and the associated choices regarding its hypothesized resistance to gene flow are typically informed by expert opinion and literature reviews (Beier et al. 2008). While these approaches have value, they may overlook relationships that are counterintuitive given the current understanding of a species' natural history (e.g., Peterman et al. 2014). An additional source of potential bias relates to idiosyncrasies associated with the chosen study region. Indeed, understanding the transferability (i.e., applicability to other areas) of landscape genetics models is critical to their use in conservation (Keller et al. 2014), and as such, metareplication is a powerful approach for distinguishing between site-specific vs. species-wide processes.

In order to minimize biases due to oversimplification of models or erroneous a priori assumptions, optimization can be performed on model parameters. For instance, the geographic scale at which individuals perceive habitat quality can be variable and difficult to ascertain (Mayor et al. 2009). A large scale may apply to some landscape features, where a small scale is relevant to others (e.g., if a road 500 m away impedes movement owing to its far reaching effects, but a pine ridge has little impact until it is within 100 m). In the past, the same scale has been applied to all landscape variables under consideration (McGarigal et al. 2016). However, a more suitable approach would be to consider several alternative geographic scales for each landscape variable in order to determine the appropriate fit (Galpern et al. 2012; McGarigal et al. 2016; Wan et al. 2017; Zeller et al. 2017). Another approach to model optimization focuses on the functional relationship between a landscape variable and its level of resistance. To date, the most typical functional

relationship between gene flow and landscape variable has been negative and linear (e.g., greater amounts of agriculture between two individuals correlate with less gene flow). However, several studies, including those with genetic response variables (Cushman 2006; Zeller et al. 2017) and with physical animal tracking (Trainor et al. 2013; Keeley et al. 2017), have found support for non-linear functional relationships between landscape variables and resistance to movement. For instance, in the above example, a precipitous reduction in gene flow may be observed only when the amount of agriculture between two individuals reaches moderate levels. Accordingly, transformations of functional relationships can be beneficial.

Replicated empirical analyses, or metareplications, have the ability to determine how transferable landscape genetics models are across a species' range. Metareplication study design requires replicate study areas that at least partly differ in land use characteristics (e.g., contrasting habitat configurations), so as to enable conclusions about how the focal species responds to landscape variables generally (Johnson 2002). Importantly, when researchers compare the best-fit optimizations of model parameters (e.g., geographic scale and function) that are independently derived from multiple study areas, cases exist in which even relatively minor changes in local environmental conditions can alter the influence that a given landscape variable has on gene flow owing to "threshold or connectivity effects" (see Cushman et al. 2011; Shirk et al. 2014). Through replicated analyses, limiting factors such as presence of human disturbance (Reddy et al. 2019), patch size of disturbed areas (Shirk et al. 2014), habitat connectivity (Castillo et al. 2016), and land cover heterogeneity (Vergara et al. 2017) have been found to affect how landscape variables contribute to resistance or facilitation of gene flow. For this reason, metareplication in landscape genetics is particularly powerful for extending the scope of insights that can be gained.

In this study, we conducted separate landscape genetics analyses of a terrestrial salamander in two southeastern deciduous forests within Mississippi and Alabama, USA, to understand the extent to which inferences drawn from one location are transferable to the other, and to examine the effect of optimization on transferability. Given that even simulated stochastic data would likely result in models with some differences, we focused on broad-scale similarity, along

several different axes for which comparisons are possible. Specifically, we asked if the landscape variables that were assessed (1) show a similar rank or order of importance (particularly with respect to the most influential variables), (2) have the same direction of effect (i.e. facilitate vs. impede gene flow), (3) have the same or similar scale of effect, and (4) exhibit the same or similar functional relationship.

## Methods

### Focal species and study regions

The present study focused on a species distributed throughout eastern Mississippi and western Alabama, the Mississippi slimy salamander (*Plethodon mississippi* Highton 1989). Plethodontid salamanders are low-mobility, ecologically specialized taxa that have several life history traits that make them well-suited for landscape genetics studies. These salamanders inhabit cool, moist environments, and exhibit direct development, meaning their offspring do not need an aquatic environment to metamorphose into the adult form (Petranka 1998). Without the need to disperse to aquatic environments for reproduction, it is hypothesized that they disperse very little over their lifetimes, which may cause genetic differentiation among populations over a relatively small geographic area. Furthermore, due to *P. mississippi*'s short generation time (females and males reach sexual maturity in two years and three years respectively; Highton 1962), the effect on dispersal by changes in the landscape may be detected over relatively short time scales.

The geographic range of *P. mississippi* spans the 63,131 ha Holly Springs National Forest (HSNF) in northern Mississippi, and the 73,653 ha Bankhead National Forest (BNF) approximately 190 km to the east in northern Alabama. These two forest regions encompass similar land use types, with both containing bottomland hardwood forests, forested wetlands, upland pine and silviculture, agricultural fields and pastures, and manmade structures such as roads, buildings, and parking lots. While composition of these forest regions is similar and both are managed by the U.S. Department of Agriculture Forest Service (USFS), they differ in that only BNF includes a large protected Wilderness area (over 10,000 hectares) and roughly 1400 ha of old growth (USFS 2004, 2012).

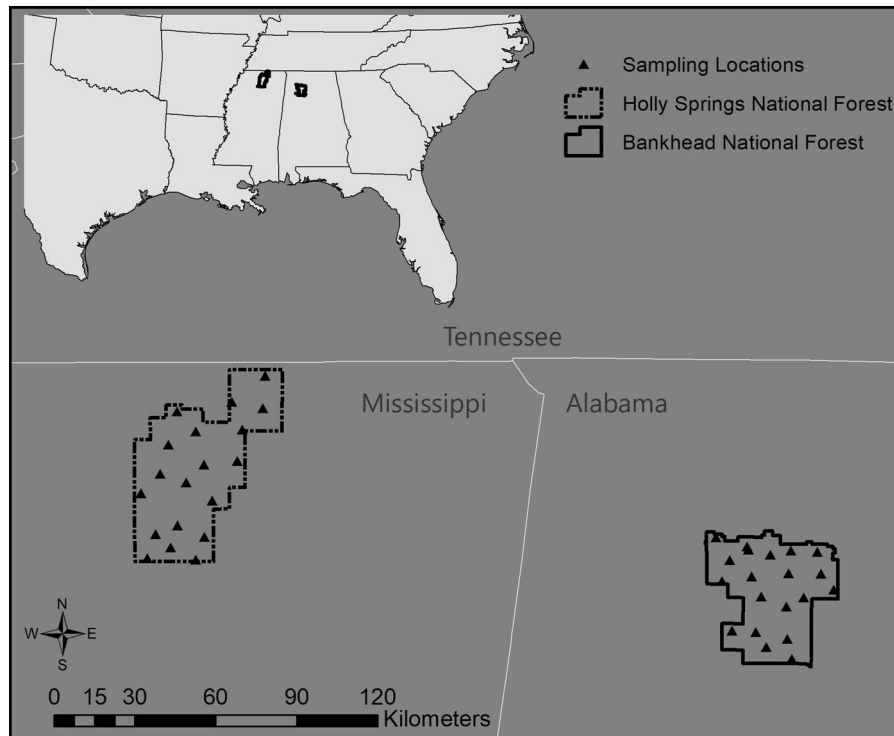
### Population sampling

Tail tip tissues were collected from 113 *P. mississippi* individuals at 19 locations in HSNF in northern Mississippi, and 110 individuals at 20 locations in BNF in northern Alabama (Fig. 1). Sampling locations were chosen to span the entirety of each of the two forest regions, and spaced approximately 8 km apart. At least five individuals were sampled at each location. Because *P. mississippi* is a completely terrestrial species that is likely continuously distributed, population units cannot be readily delimited a priori. Accordingly, we conducted individual-based analyses (Shirk et al. 2018).

### Genetic analysis

Genomic DNA was extracted from tail tips using a DNeasy Blood and Tissue kit (Qiagen, Valencia CA, USA) following manufacturer's recommendations. Individuals were genotyped using eight microsatellite loci described by Spatola et al. (2013; see Supplementary Material for PCR amplification conditions, allele-calling approaches, and quality control measures). At three locations in HSNF and one location in BNF we collected 9–11 individuals. These four sample sets were tentatively assumed to each represent panmictic groups for the purpose of testing for null alleles, Hardy–Weinberg Equilibrium, and linkage disequilibrium, using Genepop v 4.2 (Raymond and Rousset 1995). Based on the full genetic dataset, the R (R Core Team 2019) package PopGenReport (Admack and Gruber 2014) was used to quantify percent missing data, number of alleles per locus, and mean allelic richness in each forest region. An examination of overall population structure within each forest region was performed via genotypic clustering, using STRUCTURE v. 2.3.4 (Prichard et al. 2000; see Supplementary Material for details of STRUCTURE analysis). Also within each forest region, we used GenAIEx v. 6.503 (Peakall and Smouse 2012) to test for spatial autocorrelation using 999 permutations, 999 bootstrap replicates, and tests for heterogeneity. For these analyses, a distance class (i.e., bin size) size of 3 km was chosen to encompass the smallest distances between sampling locations, which were greater than 3 km.

To determine pair-wise individual-based genetic distances within each forest region, we conducted a



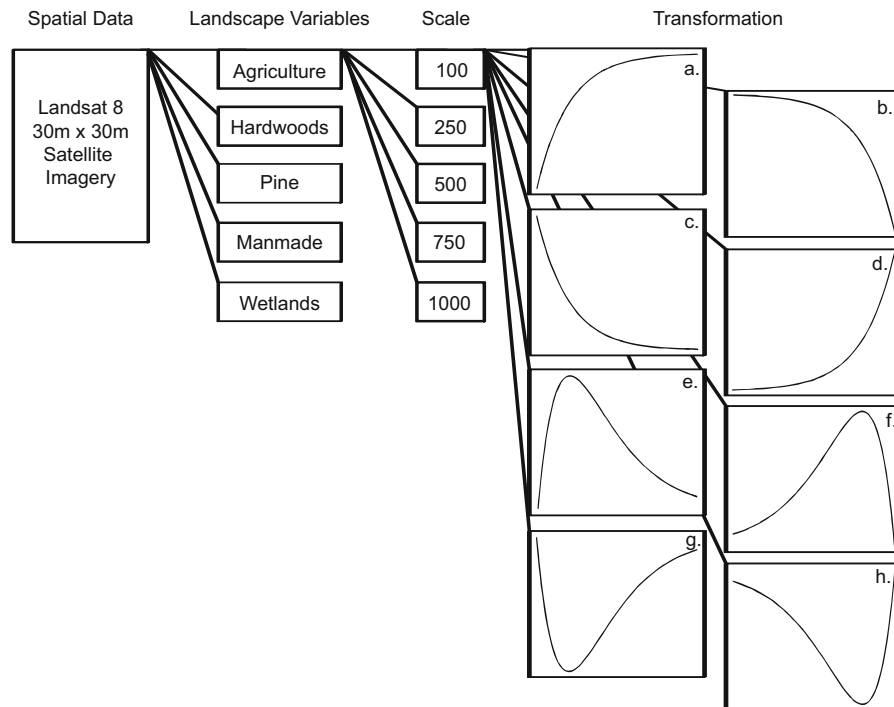
**Fig. 1** Sampling locations of *P. mississippi* within Holly Springs National Forest (HSNF) and Bankhead National Forest (BNF). Five or more individuals were sampled at each of 19 locations in HSNF, and 20 locations in BNF

principal components analysis (PCA) and calculated Euclidean distance between the first 64 axes of the ordination using the *ade4* package (Dray and Dufour 2007) in R. The use of Euclidean distances derived from a representative number of PCA axes (i.e., axes that, in total, represent a large portion of the variation found within a dataset) has been shown to perform better than others when genetic structure is weak and low sample sizes are low (Shirk et al. 2017). Pairwise genetic distances among individuals from the same sampling location were removed from further analyses, to avoid skewing landscape genetics models.

#### Landscape analysis

To examine the extent to which land use type influences gene flow, we initially classified spatial data into six distinct land use classes: agriculture, hardwoods, pine, manmade structures, wetlands, and water, using multi-spectral raster files from the NASA Landsat 8 satellite (see Fig. 2, left panel; see Supplementary Material for details regarding classification). Upon identification of correlation between the wetland

and water land use classes, wetlands (but not water) were retained for further analysis. We used the final maps to calculate for each landscape variable amount of habitat, patch density, correlation length, clumpiness, patch cohesion, and an aggregation index using the software FRAGSTATS v 4.2 (McGarigal et al. 2012). We then conducted a series of univariate moving window analyses on the classified images using five separate kernel sizes for each land use class with the PLAND calculation in FRAGSTATS (see Fig. 2, middle panels). Each pixel in the resulting maps (a total of five maps for each land use class) reflected the percent of a given land use class within 100, 250, 500, 750, and 1000 m (i.e., if a 100 m square surrounding a given pixel is completely made up of agriculture, that pixel would be given a value of 100). These values were then transformed using the eight transformations found in the R package *ResistanceGA* (named and illustrated in Fig. 2, right panels; also see Peterman 2018). We calculated transformed values using  $\text{max} = 100$  and  $\text{shape} = 2$  as *ResistanceGA*'s genetic algorithm optimization method would be computationally restrictive in our large study areas.



**Fig. 2** Depiction of landscape classification and optimization for scale and transformation. Landsat 8 imagery was classified into five landscape variables. Each variable was tested for five scales (100, 250, 500, 750, and 1000 m) and eight transformations ((a) monomolecular, (b) reverse monomolecular, (c) inverse monomolecular, (d) inverse-reverse monomolecular, (e) ricker, (f) reverse ricker, (g) inverse ricker, and

(h) inverse-reverse ricker) and a linear relationship, for a total of 45 univariate tests per landscape variable. The transformation graphs show the relationship between the original resistance value (i.e., a value 0–100, indicating the percent of the given landscape variable within 100, 250, 500, 750, or 1000 m) on the x-axis, and the new resistance value as a result of transformation on the y-axis

Using the *gDistance* package (van Etten 2017) in R, we computed pairwise random-walk distance between individuals for each map, resulting in 45 distance calculations for each land use class. We also created a raster file that had a uniform pixel value of one to calculate a random-walk distance that would represent the geographic distance between points and could be used to test for isolation by distance (IBD) (Emel and Storfer 2012). To remove the effect of geographic distance from our land use class random-walk calculations, we performed a series of simple linear regressions of the uniform pixel distance and each random-walk calculation using the *lme4* package in R (Bates et al. 2015). The residuals from these linear regressions were then used in model testing.

#### Model testing

To optimize each land use type for both transformation and scale, we ran a series of univariate maximum

likelihood population effects (MLPE) models (Clarke et al. 2002). These linear random effects models account for the lack of independence between pairwise comparisons. This method was the most robust among seven regression-based model selection methods tested using inter-individual landscape genetics simulations (Shirk et al. 2018). Univariate models were ranked using corrected Akaike Information Criterion (AICc; Hurvich and Tsai 1989). The most strongly supported scale and transformation of each land use class (i.e., that with the lowest AICc score) was used for final model testing.

We tested several hypotheses of resistance with MLPE models (Table 1). Each model included the geographic distance variable derived from a uniform raster, as well as a combination of land use variables. Models were then ranked using AICc. Summaries of the best-fit models were examined to determine the sign of effect for each model component (i.e., each landscape variable). A positive sign of effect indicated

**Table 1** Maximum likelihood population effects models and AICc scores and delta ( $\Delta$ ) AICc values

Model name	Variables included	Holly Springs National Forest		Bankhead National Forest	
		AICc	$\Delta$ AICc	AICc	$\Delta$ AICc
Full model	Geographic Distance, Agriculture, Manmade, Pine, Hardwood, Wetlands	<b>29467</b>	<b>0</b>	<b>25809</b>	<b>0</b>
Isolation by distance	Geographic Distance	30089	620	26324	515
Modified habitat	Geographic Distance, Agriculture, Manmade	29870	401	25990	181
Moderate habitat	Geographic Distance, Pine, Agriculture	29879	410	25937	128
Forest cover	Geographic Distance, Pine, Hardwood, Wetlands	29914	445	26192	383
Agriculture	Geographic Distance, Agriculture	29952	483	26018	209
Manmade	Geographic Distance, Manmade	29991	522	26249	440
Pine	Geographic Distance, Pine	30025	556	26269	460
Hardwood	Geographic Distance, Hardwood	29954	485	26209	400
Wetlands	Geographic Distance, Wetlands	29893	424	26104	295

The lowest AICc scores for each forest are in bold

that the variable resisted gene flow, whereas a negative sign of effect indicated the variable facilitated gene flow (Row et al. 2017).

## Results

### Genetic analysis

Multilocus genotypes were produced from 113 of 114 individuals in HSNF with 1.7% missing data, and 107 of 109 individuals in BNF with 4.1% missing data. One individual in HSNF and two individuals in BNF were excluded from the dataset because they repeatedly failed to amplify at greater than two loci. The locus *402* failed to amplify reliably in BNF, and was therefore removed from analysis in BNF but not HSNF. The locus *B8DRY* was found to be monomorphic in HSNF but not BNF, so it was removed from analysis in the former forest region only. Loci within the HSNF dataset had 4–32 alleles with a mean allelic richness of 14.5, and loci within BNF had 10–29 alleles with a mean allelic richness of 18.9. Tests for departures from HWE showed all loci were in HWE except one (*QWZ*) in HSNF and one (*43 M*) in BNF. Tests for linkage disequilibrium and null alleles found no linkage, and only one possibility of null alleles (*43 M*) in BNF. *QWZ* was in HWE in BNF, and *43 M* was in HWE and showed no indication of null alleles in HSNF, therefore both loci were kept in the

dataset. STRUCTURE analysis supported  $K = 1$  cluster in HSNF, and  $K = 1$  cluster in BNF (see Table S4 for mean log likelihood and delta  $K$  scores); Evanno et al.'s (2005) method used in STRUCTURE HARVESTER (Earl and vonHoldt 2012) calculates a delta  $K$  value which by definition cannot be calculated for  $K = 1$ , therefore we relied on the highest mean estimated log likelihood, following Pritchard et al. (2000). There was significant spatial autocorrelation within both forests (test for heterogeneity  $p < 0.001$ ), with the  $x$  intercept at 7.38 km in HSNF and 16.28 km in BNF (Figures S2 and S3). The 64 axes of ordination generated through PCA explained 93.75% and 90.70% of the variation in HSNF and BNF respectively.

### Landscape analysis

Kappa statistic calculations of the supervised classifications for HSNF and BNF were 0.85 and 0.86 respectively (see Table S5 for rates of commission and omission by class). Supervised classification of the Landsat 8 imagery and subsequent analysis with FRAGSTATS revealed HSNF and BNF have several similarities in the amount and distribution of landscape variables as well as a number of differences (Table 2). There are similar amounts of hardwood, manmade, and wetland areas in both forests. However, in HSNF, pine patches are ten times more abundant and the patches are denser than those in BNF. HSNF pine patches also had a higher correlation length,

**Table 2** Comparison of habitat amount (km<sup>2</sup>), patch density (number of patches per 100 ha), correlation length, Clumpiness Index, patch cohesion, Aggregation Index for Holly Springs National Forest (italics) and Bankhead National Forest (bold)

Landscape type	Amount of habitat	Patch density	Correlation length	Clumpiness Index	Patch cohesion	Aggregation Index
Hardwood	<i>1339.41</i>	<i>14.04</i>	<i>353.68</i>	<i>0.59</i>	<i>93.24</i>	<i>68.73</i>
	<b>1382.44</b>	<b>9.96</b>	<b>912.92</b>	<b>0.59</b>	<b>97.52</b>	<b>79.60</b>
Pine	<i>2230.41</i>	<i>17.22</i>	<i>562.51</i>	<i>0.61</i>	<i>95.53</i>	<i>76.65</i>
	<b>236.40</b>	<b>6.41</b>	<b>211.13</b>	<b>0.69</b>	<b>89.43</b>	<b>71.51</b>
Agriculture	<i>958.83</i>	<i>8.03</i>	<i>295.24</i>	<i>0.69</i>	<i>92.13</i>	<i>74.53</i>
	<b>271.55</b>	<b>4.10</b>	<b>258.56</b>	<b>0.73</b>	<b>92.09</b>	<b>75.42</b>
Manmade	<i>291.75</i>	<i>1.42</i>	<i>5511.04</i>	<i>0.53</i>	<i>98.80</i>	<i>55.50</i>
	<b>311.57</b>	<b>10.66</b>	<b>3838.83</b>	<b>0.51</b>	<b>98.61</b>	<b>56.48</b>
Wetlands	<i>245.30</i>	<i>11.59</i>	<i>91.31</i>	<i>0.41</i>	<i>73.57</i>	<i>43.67</i>
	<b>441.08</b>	<b>17.71</b>	<b>279.73</b>	<b>0.55</b>	<b>89.24</b>	<b>62.20</b>

which is a measure of the distance an individual could travel and remain in a single patch when dropped in a random location and traveling in a random direction (McGarigal et al. 2012). The amount of agriculture in HSNF was higher than in BNF, but the patches were at similar densities and correlation lengths. Areas containing manmade structures were considerably denser in BNF, however in HSNF they had a higher average correlation length, suggesting manmade structures in HSNF are mainly roads, and in BNF they are more commonly buildings and paved lots. Hardwood patches were denser in HSNF, but had a higher correlation length in BNF, and wetlands in BNF had a much higher correlation length than in HSNF.

### Model testing

The full model had the lowest AICc and was thus the best-fit model for both forests, indicating all of the tested landscape variables contribute to the genetic distances found in *P. mississippi* (Table 1). The rank of model components differed between forests (Table 3), with agriculture and wetlands the top two components for both forests and the remaining variables contributing less to *P. mississippi* genetic distance variability. Agriculture, pine, and hardwoods had the same sign of effect in both forests, with agriculture and hardwoods resisting gene flow of *P. mississippi* and pine facilitating gene flow. In HSNF, manmade structures correlated with facilitation of gene flow, whereas in BNF they correlated with resistance to gene flow. The opposite was true for

**Table 3** Rank and model coefficients for each landscape variable in the most supported maximum likelihood population effects model for each forest region

Holly Springs NF		Bankhead NF	
Wetlands	0.91	Agriculture	1.14
Agriculture	0.69	Wetlands	− 0.48
Hardwoods	0.65	Manmade	0.47
Manmade	− 0.43	Pine	− 0.40
Pine	− 0.25	Hardwoods	0.26

As the models have been optimized to both scale and transformation separately for each forest region, model coefficients in HSNF cannot be directly compared to those found in BNF. However, the overall rank-ordering of variables provides a means for comparison

wetlands, which correlated with resistance to gene flow in HSNF and facilitation of gene flow in BNF (Table 4).

Univariate tests for scale and transformation resulted in few similarities between the forests (Fig. 3). Only one landscape variable, pine, was optimized to the same transformation (inverse-reverse ricker). The presence of pine on the landscape consistently correlated with increased gene flow in both forest regions. In HSNF, gene flow was at its highest when 20% of the area within 1000 m was comprised of pine. As the amount of pine increased within the 1000 m area, correlation with facilitation of gene flow decreased until the amount of pine reached 80%, at which point facilitation of gene flow



**Table 4** Comparison of scale, transformation, and sign of effect for Holly Springs National Forest (italic) and Bankhead National Forest (bold)

Landscape type	Scale	Transformation	Sign of effect
Hardwood	500 m	<i>Inverse ricker</i>	+
	<b>100 m</b>	<b>Inverse–reverse ricker</b>	+
Pine	1000 m	<i>Inverse–reverse ricker</i>	–
	<b>750 m</b>	<b>Inverse–reverse ricker</b>	–
Agriculture	500 m	<i>Inverse–reverse ricker</i>	+
	<b>500 m</b>	<b>Inverse ricker</b>	+
Manmade	250 m	<i>Inverse ricker</i>	–
	<b>1000 m</b>	<b>Ricker</b>	+
Wetlands	1000 m	<i>Inverse ricker</i>	+
	<b>100 m</b>	<b>Monomolecular</b>	–

A negative sign of effect indicates the variable facilitates gene flow, and a positive sign of effect indicates the variable resists gene flow

exponentially increased. The scale at which this occurred in BNF was 750 m.

Only one landscape variable, agriculture, was optimized to the same scale (500 m) in both study areas. Agriculture correlated with resistance to *P. mississippi* gene flow in both study regions. The transformation, or function of the relationship between gene flow and the amount of agricultural land, differed between forest regions. In HSNF, the area within 500 m comprised of 20% agriculture correlated with the highest resistance, and the lowest amount of resistance was seen when 80% of the surrounding area was agriculture. Conversely, in BNF, correlation with the lowest resistance to gene flow occurred when 20% of the surrounding landscape was agricultural.

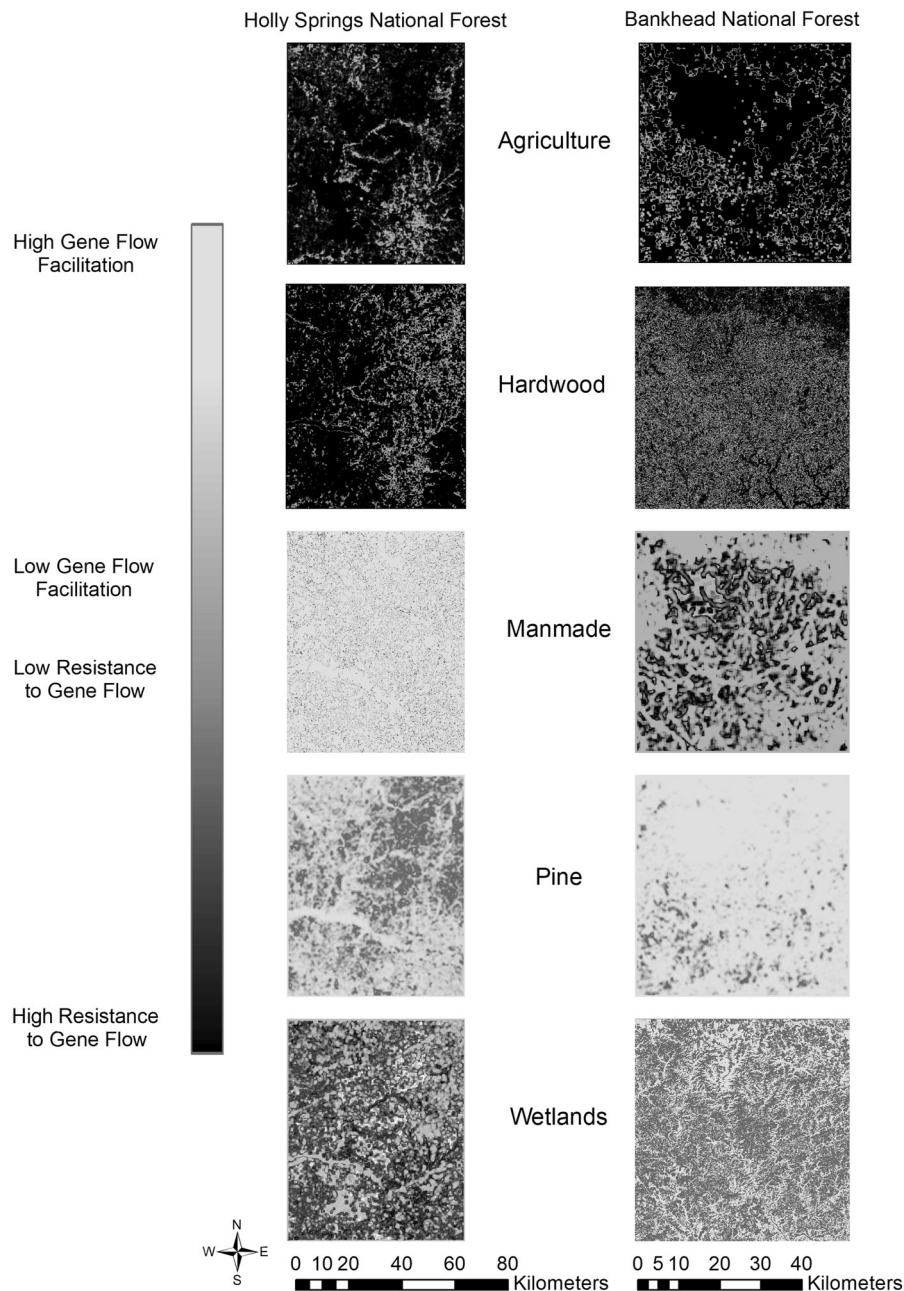
The largest difference in scale was found in wetlands, with HSNF optimized to 1000 m and BNF optimized to 100 m. Wetlands in HSNF correlated with resistance to gene flow, with increasing resistance from 20 to 100% wetlands within 1000 m. In contrast, wetlands in BNF correlated with facilitated gene flow at a scale of 100 m with increasing facilitation with an increasing presence of wetlands.

The manmade structures landscape variable showed the largest difference in transformation, with HSNF optimized to an inverse ricker transformation and BNF optimized to a ricker transformation. In HSNF, manmade structures correlated with facilitation of gene flow, whereas in BNF, manmade structures correlated with resistance to gene flow. However, facilitation is at its lowest when manmade structures comprise 20% of the landscape in HSNF, and resistance is at its highest when manmade structures comprise 20% of the landscape in BNF.

Hardwoods correlated with resistance to *P. mississippi* gene flow in both forest regions, however both the scale and function (i.e., transformation) of their effect was different. The scale of effect in HSNF was at 500 m, with increasing resistance from 20 to 100% of the immediate area consisting of hardwoods. In BNF, the scale of effect was at 100 m, showing decreasing resistance from 0 to 80% hardwoods.

## Discussion

In this study, landscape genetics models for the Mississippi slimy salamander were evaluated in each of two forest regions that are qualitatively similar in many respects (e.g., forest types, land uses, management). This metareplication allowed us to understand the extent to which landscape genetics inferences are transferable to neighboring regions. Indeed, due to the general similarity between the two forest regions, the present study represents a case where transferability is potentially quite high. Nonetheless, we recognize that at least some differences between the best-fit landscape genetics models are likely given that there are many alternative ways in which discordance can arise (cf. only a single path to complete congruence), and even the two datasets generated with the same simulated yet stochastic conditions would likely result in non-identical results. Accordingly, we focused on broad elements of model similarity, along several axes for which comparisons were possible. We found that in both forest regions, the most strongly supported MLPE model included all five of the landscape variables under consideration, indicating they all influence *P. mississippi* gene flow in non-negligible



**Fig. 3** Comparison of the facilitation or resistance to gene flow created by land use types in Holly Springs National Forest versus Bankhead National Forest. Maps illustrate the optimized

scale, transformation, and sign for each land use type to give a visual representation of the effect of each land use type on gene flow of *P. mississippi*

ways, even if the magnitude of influence is weak for some. We found that the rank ordering of variable effects was different between forests, and so the notion of a single landscape genetics model that is broadly applicable across the species' range was not supported. Notably, wetlands and agriculture were

consistently at the top of the rankings for both regions. Furthermore, some variables affected gene flow in the same way across the two forest regions (e.g., agriculture correlated with resistance to gene flow), suggesting some generality in the responses of *P. mississippi* individuals to characteristics of the intervening matrix.

That said, there were several variables, including some of large effect (e.g., wetlands), which differentially affected gene flow, indicating context-dependent responses that may make extrapolation of landscape genetics models difficult. Below, we discuss the variables in descending order of rank of effect, and compare our findings with those of other relevant studies. We close by considering some limitations of our work, and point to future directions for understanding the effects of landscape genetics model optimization upon generating broadly applicable inferences about how individuals perceive and move through a habitat matrix.

Based on our data, agriculture was ranked as one of the top two most influential variables affecting gene flow in the best-fit landscape genetics models for *P. mississippi* in both HSNF and BNF. We found that agricultural areas correlated with resistance to gene flow at a relatively intermediate spatial scale of 500 m. Notably, the most prominent agricultural practices within both study regions include a rotation of corn, wheat, and soybeans. In all of these cases, the crops are grown as monocultures, and harvesting/planting times are such that there is high probability of bare earth during spring and fall, which are the most active seasons for *P. mississippi* in terms of dispersal of individuals (Petranka 1998; Salmerón et al. 2016; S. Burgess personal observation). The lack of vegetative cover and increased ground disturbance associated with planting and harvest activities may explain the resistance to gene flow correlated with agricultural areas. Abundance surveys of plethodontid salamanders have shown a direct relationship between the number of individual salamanders and the amount of herbaceous cover (Riedel et al. 2008) and recent landscape genetics analyses of marbled newts have shown an increase in population structure due to agricultural areas (Costanzi et al. 2018).

Like agriculture, wetlands also ranked in the top two most influential variables. However, the effects of wetlands on gene flow differed between the two forest regions; wetlands within 1000 m correlated with resistance to gene flow in HSNF, whereas wetlands correlated with facilitation of gene flow at a much smaller scale (100 m) in BNF. These contrasting outcomes may be due to differences in the shape and connectedness of wetland patches across each forest region. For instance, in BNF, the correlation lengths of wetland patches (i.e., a metric of the amount of time an

individual can move forward from a random starting point in a random direction and stay within the same patch) are considerably higher than in HSNF. In this context, simulations by Cushman et al. (2011, 2013) are particularly relevant, as these authors explored how the distribution of landscape variables within a study area impacts their effect on gene flow, finding the most prominent effects from differences in correlation length and patch cohesion, (i.e., a metric indicating the physical connectedness of patches). Thus, our data suggest that in BNF, individual *P. mississippi* can move relatively large distances without exiting wetland areas, but not in HSNF. Overall, our data underscore the idea that if wetlands are well connected, they facilitate gene flow in terrestrial salamanders such as *P. mississippi*.

Our analyses showed that the magnitude of influence on gene flow of hardwood, manmade, and pine landscape variables were consistently ranked relatively low in the best-fit MLPE models for both forest regions. While hardwoods consistently correlated with resistance to gene flow in both forest regions, the function of resistance (i.e., the transformation) differed. Specifically, in HSNF, there was a positive relationship between amount of hardwoods and amount of resistance, whereas in BNF, the reverse was true. As with wetlands, the correlation lengths of hardwood patches in BNF were higher than in HSNF. One possible explanation for these relationships is that *P. mississippi* typically reside in bottomland hardwood forests (Petranka 1998) and thus may be unprompted to leave. Smith and Rissler (2010) reported that hardwood dominated understories were characteristic of “pristine” habitat for terrestrial heterpetofauna in Talladega National Forest, Alabama, and the population genetic effects of philopatry (natal or otherwise) have been reported for plethodontid salamanders in continuously forested habitat (e.g., *P. cinereus*; Cabe et al. 2007). Thus, in the case of *P. mississippi*, the reduction in gene flow, and associated apparent “resistance” correlated with hardwoods in the best fit landscape genetics models, should not be equated to that caused by agriculture. Indeed, Richardson et al. (2016) cautioned that even when a set of landscape variables each show evidence of resistance, the underlying reasons may be ecologically different.

In HSNF, we found that manmade structures correlated with facilitation of gene flow among *P.*

*mississippi*, whereas in BNF they correlated with resistance to gene flow. These effects were found to be at different scales and different transformations in each study area. Notably, patches of manmade structures in HSNF are considerably less dense, but have a greater correlation length, suggesting their distribution across the landscape is more linear (i.e., more roads than buildings and paved lots). Studies of salamander gene flow have found variable responses to roads, showing both resistance to gene flow (Marsh et al. 2008) and no effect (Purrenhage et al. 2009). Our analysis was unique due to the inclusion of manmade structures, however our results support separating roads and other manmade structures in the future to differentially determine the effect of each land use type.

Although pine ranked as one of the least influential landscape variables in each best-fit MLPE model, it did have the most consistent effect across the two forest regions (i.e., same sign, scale, and transformation). This consistent correlation with facilitation of gene flow by a landscape type that is less than ideal habitat for *P. mississippi* (Petranka 1998) may seem counter to predictions based on the species' natural history. However, increased dispersal—and by extension, gene flow—through moderately hostile habitat has been reported for salamanders (Wang 2009; Peterman et al. 2014; Prunier et al. 2014). In each of the two forest regions studied here, pine is typically found immediately adjacent to hardwoods and, as a consequence of being evergreen, provides year-round canopy cover. Furthermore, a considerable amount of the pine habitat found in both forest regions is routinely burnt via prescribed low-intensity fire (USFS 2004, 2012), resulting in a reduction of pine litter, which has been experimentally shown to increase the movement of an ambystomatid salamander (*Ambystoma talpoideum*, Moseley et al. 2004). Thus the combination of these factors—close proximity to ideal habitat, protection by year-round canopy cover, and potential to increase movement—may explain the consistent increase in gene flow found in response to pine habitat.

Due to the nature of metareplications, there are a number of potential limitations that arise both during analysis and in the interpretation of results. Because the goal of metareplication is to compare the effect of each landscape variable in multiple areas, researchers must include in their analysis environmental and

landscape variables that have both the potential to affect the genetic structure of their study organism (Keller et al. 2014) and are also present in all study locations (Short Bull et al. 2011; Castillo et al. 2016; Vergara et al. 2017). When results are intended to apply to conservation throughout a species range (Row et al. 2015) researchers may focus on the inclusion of a smaller number of landscape variables in an attempt to strike a balance between the number of parameters evaluated and the transferability of model inferences. By focusing on a smaller number of landscape variables, researchers may fail to identify a landscape feature that affects gene flow. There is also a potential source of error in the interpretation of metareplication results. Because metareplications must, by definition, occur in separate geographic areas, they inherently include the potential for erroneous conclusions due to unidentified phylogeographic breaks between study areas. Divergent lineages can at times be cryptic, abrupt, and even counter to morphologic differences (e.g., Jones and Weisrock 2018). If a phylogeographic break exists, any inferred location-specific differences between study regions (e.g., response to a particular landscape variable) may instead be due to deeply divergent lineages and separate evolutionary histories. In the present study, we have prioritized the optimization of a limited number of landscape variables to maintain the ability to compare their effects across forest regions while attaining detailed information about the scale and function (i.e., transformation) of those effects. While the possibility of an unidentified phylogeographic break exists between *P. mississippi* in our two forest regions, both HSNF and BNF lie well within the range delineated for *P. mississippi* by Highton (1989).

#### Management implications

Through metareplication, we have obtained information about the relationships between *P. mississippi* and their environment that would not have been apparent with analysis of a single study area which can be used to generate management recommendations for the species. The consistent resistance to gene flow by agriculture, coupled with the consistent facilitation of gene flow by pine, indicates that land managers overseeing multi-use areas could increase *P. mississippi* gene flow by prioritizing silviculture over agricultural leasing. Our results also indicate that the

connectivity of wetland patches (as seen in our analyses as a high correlation length) is an important factor in their ability to facilitate *P. mississippi* gene flow. Thus, managers should focus efforts on improving the connectivity of wetlands, potentially through targeted restoration.

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**Data availability** Genotypic data are available from DRYAD Repository entry <https://doi.org/10.5061/dryad.h18931zgh>.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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