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RESEARCH ARTICLE

Differential dispersal shapes population structure and patterns of genetic differentiation in two sympatric pond breeding salamanders

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Abstract Understanding patterns of dispersal, gene flow, and population differentiation are critical to making informed management and conservation decisions. By assessing these processes in multiple sympatric species, we can increase the generality and applicability of management plans. In this study, we assess patterns of genetic differentiation and population structure in two ecologically similar ambystomatid salamanders in Missouri, USA. *Ambystoma annulatum* (ringed salamander) and *A. maculatum* (spotted salamander) are both dependent upon forested habitats and fishless ponds for reproduction, but differ in their breeding phenology. In comparing these species, we assessed the support for five different processes that we hypothesized to affect genetic differentiation: (1) resistance of landscape features to movement, (2) distribution of breeding habitat, (3) dispersal propensity, (4) dispersal ability, and (5) breeding habitat quality. Of these hypotheses, we found support for differences in dispersal ability and propensity. In both species, there was a strong pattern of isolation-by-distance. However, *A. annulatum* exhibited greater overall differentiation ($F'_{ST} = 0.31$), had a greater rate of differentiation increase with distance, and were grouped into three spatially congruent genetic clusters. In contrast, *A. maculatum* consisted of a single population cluster and overall F'_{ST} was 0.047. We estimated the mean genetic dispersal distance of *A. annulatum* and *A. maculatum* to be 1,693 m and 2,050 m, respectively. Our results underscore the importance of considering multiple species when developing management criteria to better account for differences in dispersal ability.

Keywords *Ambystoma annulatum* · *Ambystoma maculatum* · Amphibian · Landscape genetics · Life history · Ozark · Military installation

Introduction

Dispersal of individuals and their genes are processes critical to population demography, evolutionary potential and long-term viability (Hanski and Gilpin 1997). Understanding how species differ in their dispersal ability and in their response to the landscape matrix is a critical step toward making informed management and conservation decisions (Pittman et al. 2014). Direct observation of dispersal is a challenging, if not impossible task, and even if individuals are observed moving between populations, there is no guarantee that they will successfully reproduce to transfer their genes. To gain a more complete understanding of dispersal and population connectedness across the landscape, analyses must incorporate spatial genetic, local habitat, and environmental data.

A clear understanding of gene flow is important for management and conservation, but the vast majority of studies focus on a single species. To make genetic-based inferences more applicable to management it is important to understand their generality (Schwenk and Donovan

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2011). There is a growing literature demonstrating that sympatric species often have starkly different responses to the landscape matrix and exhibit different patterns of genetic differentiation (Brede and Beebee 2004; Manier and Arnold 2006; Olsen et al. 2011). Even ecologically similar taxa can exhibit substantially different patterns of genetic differentiation and divergent relationships to landscape features (Goldberg and Waits 2010; Richardson 2012; Van Buskirk 2012; Whiteley et al. 2014).

In this study, we compare patterns of genetic differentiation and population structure in two sympatric pond-breeding ambystomatid salamanders in Missouri, USA. *Ambystoma annulatum* (ringed salamander) and *A. maculatum* (spotted salamander) are both generally dependent upon forested habitats, and breed in fishless ponds (Petranka 1998). *Ambystoma annulatum* is a species endemic to the interior highlands of the Ozark and Ouachita mountains of Missouri, Arkansas and Oklahoma, while *A. maculatum* is widely distributed throughout the forested midwest and eastern US (Petranka 1998). Although these species are sympatric and utilize similar breeding habitat, *A. maculatum* breed in the late winter—early spring whereas *A. annulatum* breed in autumn (Hocking et al. 2008).

We assess the support for five different processes, which we hypothesize have the potential to affect genetic differentiation of *A. annulatum* and *A. maculatum*: (1) resistance of landscape features to movement, (2) distribution of breeding habitat, (3) dispersal propensity, (4) dispersal ability, and (5) breeding habitat quality. There is minimal empirical evidence to make informed predictions concerning how most of these processes will affect gene flow, or how they will differ between species. Given the ecological similarity of the two focal species, we hypothesized that differences between species would be minimal, with one exception: resistance of landscape features to movement. Peterman et al. (2014a) found that *A. annulatum* had a greater tendency to breed in ponds that were not located in closed canopy forest, and Brussock and Brown (1982) observed *A. annulatum* in Arkansas, USA breeding in open pastures. As such, there is potential for *A. annulatum* to more readily disperse through open grassland habitat, compared to *A. maculatum*, which has strong tendencies to orient toward, and utilize, closed canopy forested habitat (Rothermel and Semlitsch 2002, 2006; Pittman and Semlitsch 2013). We predict that *A. maculatum* will show a greater relationship with forested habitat, and that non-forest habitat will have a greater resistance to movement. In contrast, we predict that forest and non-forest habitats will not differentially affect dispersal of *A. annulatum*. Finally, we predict that there will be no differences between the species with regard to dispersal ability, dispersal propensity, distribution of breeding habitat, or breeding habitat quality.

Methods

Study area and sample collection

Sampling occurred at Fort Leonard Wood (FLW), an active military training facility in the Ozark Highlands, Pulaski County, MO, USA (Fig. 1; 37.92°N, 92.17°W). FLW encompasses 24,686 ha that is 80 % forested, and has an extensive road system (both paved and gravel) throughout much of the military base. Active year-round military training from all five branches of the armed forces occurs onsite. More than 500 constructed and unintentional bodies of water (i.e. tire ruts), exist at FLW, primarily in the form of small (<0.04 ha) fishless, manmade wildlife ponds (Peterman et al. 2014a). We collected 12–53 *A. annulatum* from 20 ponds and 10–54 *A. maculatum* from 23 ponds within a 7,140 ha focal area (Fig. 1). Tail clips were sampled from late stage larvae of *A. annulatum*, and to minimize sampling of siblings, larvae were systematically sampled from the entire perimeter of the pond. For *A. maculatum*, one late stage embryo was sampled from an egg mass. All collections were conducted March–April 2012.

Genetic analyses

We extracted DNA using a chelex-based resin (Instagene, BioRad) following the protocol detailed by Peterman et al. (2012), and genotyped both species at 19 species-specific microsatellite loci (Peterman et al. 2013a, b). Locus Am_60 was not included in Peterman et al. (2013a). Details of this primer are in Table S1. Primers were fluorescently-labelled and arranged into two multiplex reactions for each species as described in Peterman et al. (2013a, b). Amplification products were sized on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using Liz 600 size standard at the University of Missouri DNA Core Facility, and results were scored using GENEMARKER (v.1.97; Softgenetics, State College, PA, USA). Before proceeding with analyses we tested for, and removed, full siblings from our data set using COLONY (Jones and Wang 2010). In COLONY, we set both male and female mating to polygamous without inbreeding, used a long run with full likelihood and high precision, and did not include a sibship prior.

Genepop 4.2 (Raymond and Rousset 1995; Rousset 2008) was used to test for significant deviations from expected heterozygosity values under Hardy–Weinberg equilibrium (HWE) and to test for linkage disequilibrium among pairs of loci. Both tests were conducted using 250 batches with 2,500 iterations following a burn-in of 2,500. Significance of all tests was assessed following Bonferroni correction for the number of comparisons (Rice 1989). We

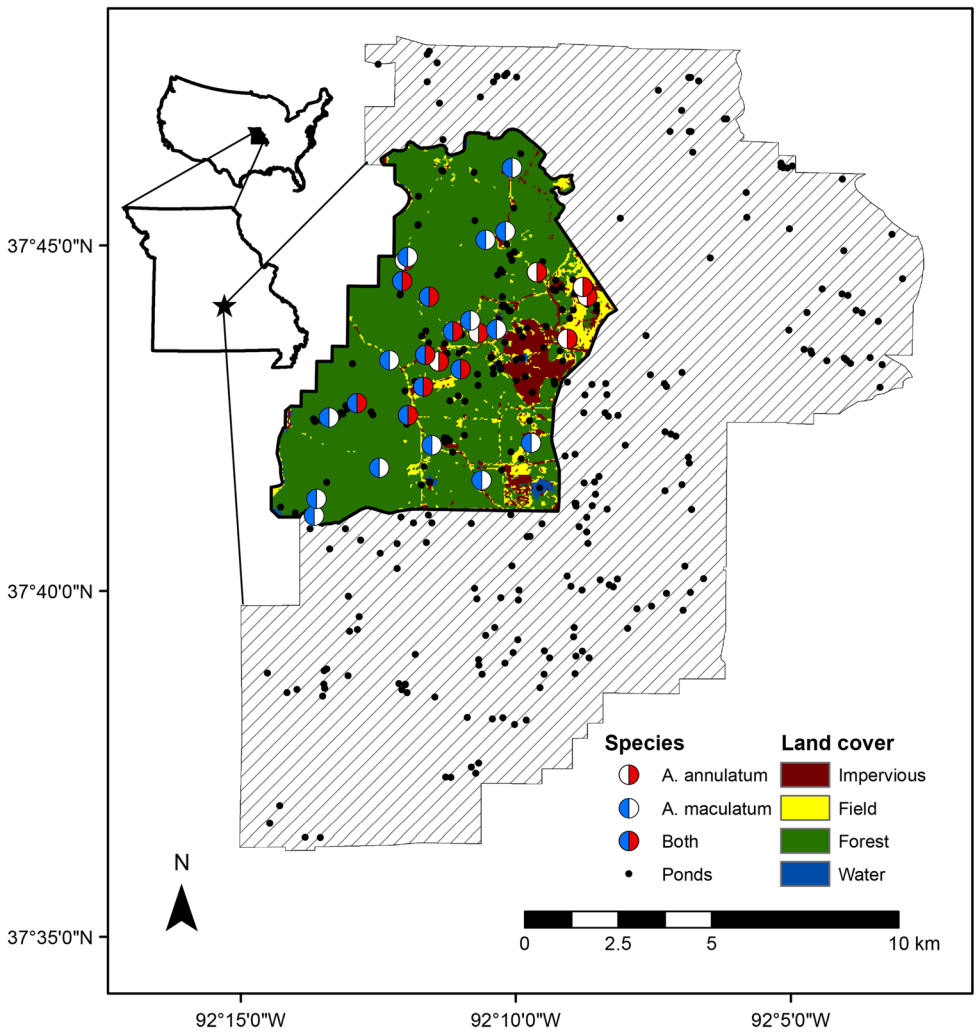

Fig. 1 Map of Fort Leonard Wood, MO with land cover detail for the 7,140 ha focal area landscape assessed in this study

tested for presence of null alleles using PopGenReport (Adamack and Gruber 2014). We calculated rarefied allelic richness, observed and expected heterozygosity, and the standardized fixation index F'_{ST} using GenoDive v2.02b24 (Meirmans and Van Tienderen 2004). We use F'_{ST} throughout this paper so that equitable comparisons can be made between the two species (Meirmans and Hedrick 2011).

We tested for spatial population structure using BAPS v6 (Corander et al. 2003, 2008). We assessed spatial structure using BAPS, rather than STRUCTURE, because of known limitations of STRUCTURE when isolation-by-distance is present (Pritchard et al. 2000; Schwartz and McKelvey 2009). In BAPS, the most likely number of genetic partitions for each species was assessed using a two-step analysis. We first used a spatial clustering of individuals to allocate each sample into its most likely genetic group. We then refined the results by assessing admixture (Corander and Marttinen 2006). This approach uses the spatial coordinates of each sample location, Voronoi tessellation, and Markov Random fields to determine the maximum number of population clusters (K). For *A. annulatum* and *A. maculatum*, we tested for 2–15 and 2–22 clusters using ten replicates at each potential cluster number.

Landscape resistance

To test the hypothesis that species differ in their response to the landscape, we conducted a landscape genetics analysis. We assessed the joint effects of landscape resistance and distance on genetic differentiation using optimization methods described by Peterman et al. (2014b), implemented in *ResistanceGA* (Peterman 2014). Briefly, we iteratively optimized resistance surfaces using CIRCUITSCAPE 4.0-Beta (McRae 2006). To evaluate the relative support for each resistance surface, we fit linear mixed effects models using a maximum likelihood population effects (MLPE) parameterization to account for the non-independence of values within pairwise distance matrices (Clarke et al. 2002; Van Strien et al. 2012). Mixed effects models were fit by maximum likelihood using *lme4* (Bates

et al. 2014). Pairwise F'_{ST} was used as the dependent variable while scaled and centered effective resistance between populations was the independent variable. Because distance is implicitly incorporated into the effective resistance measure calculated by CIRCUITSCAPE, Euclidean distance was not included as an additional factor in our models. Model fits were assessed using AIC_c calculated from the linear mixed effects models. Resistance surfaces assessed included land cover (forest, open water, field, and developed), potential relative radiation (Pierce et al. 2005), distance from ravine, topographic position index, and topographic wetness index. Land cover data was obtained from the United States Geologic Survey (USGS, http://viewer.nationalmap.gov). All other surfaces were derived within a GIS (ArcGIS v.9.3, ESRI, Redlands, CA) using 30-m resolution elevation data obtained from USGS, following methods described by Peterman and Semlitsch (2013).

Distribution of breeding habitat

Independent of landscape features, there is the potential for the locations of ponds on the landscape to affect genetic differentiation. To test this hypothesis we assessed the distance between breeding ponds used by each species in both 2012 and 2013. Breeding ponds used in this analysis were identified during standardized surveys of 169 ponds located within the focal area (Peterman et al. 2014a). Differences between species and years were assessed using a linear model with nearest neighbor distance as the response, with species, year, and species by year interaction as predictors.

Dispersal propensity

The propensity of a species to disperse, in combination with their dispersal ability and the landscape matrix, can significantly affect patterns of spatial genetic differentiation and relatedness. We assessed patterns of isolation by distance between the two species using mixed effects models with MLPE parameterization. Global F'_{ST}, and the inbreeding coefficient, F'_{IS}, were also assessed as potential indicators of philopatry and dispersal propensity.

Habitat quality

To test hypotheses concerning the effects of within-pond characteristics, as well population isolation (metapopulation hypotheses), we utilized population-specific, metapopulation-based analyses (Pflüger and Balkenhol 2014). Using the geometric mean of all pairwise F'_{ST}, we obtained a unique, population-specific F'_{ST} value for each population that represents the differentiation of a pond subpopulation to all other pond subpopulations (Gaggiotti and Foll 2010; Pflüger and Balkenhol 2014). Population-specific F'_{ST}, values were modelled as a function of three connectivity indices (S) for each pond (i):

(1) $S_i = C_i$
(2) $S_i = \Sigma[exp(-kd_{ij})]$
(3) $S_i = C_i * \Sigma[exp(-kd_{ij})]$

where C_i is an attribute of focal pond i (see below), d_{ij} is the Euclidean distance between focal pond i and pond j, summation is across all subpopulations ($j \neq i$), and k is a scaling parameter equal to 1/average dispersal distance (Moilanen and Nieminen 2002). The first index represents the hypothesis that dispersal and genetic differentiation are determined by local pond attributes only, while the second index corresponds to the hypothesis that genetic connectivity is solely dependent on the distance among populations. The third index assumes that pond attributes and distance are both influencing the genetic structure of the metapopulation. For local pond attributes, we assessed factors previously found to influence larval abundance in each species (Peterman et al. 2014a). For *A. annulatum* we tested invertebrate predator richness at each pond (number of invertebrate predator species observed during amphibian sampling), the number of ponds within 300 m of each focal pond, and the percentage of the landscape that is forested within 300 m of each pond. For *A. maculatum* we tested the percentage of forest cover within 300 m of each pond and the percent canopy cover over each focal pond.

Dispersal ability

Because the average dispersal distance (necessary for k) is unknown for both species, we estimated it from our data. Using the Brent optimization algorithm in R (R Core Team 2014), we used an optimization function to minimize the squared sum of errors between our pond-averaged F'_{ST}, and connectivity index estimates. We estimated the mean dispersal distance for each species by resampling our data with replacement 10,000 times. The scaling parameter k was then calculated as 1/mean dispersal distance, and was subsequently used to calculate connectivity indices 2 and 3.

Results

Genetic diversity

Prior to conducting analyses, we identified and removed full siblings from our data so that a family group was represented by a single individual. We had a very high frequency of full siblings among our *A. annulatum* data, which resulted in

Table 1 Population genetic summary statistics for *Ambystoma maculatum* from 22 sample ponds at Fort Leonard Wood, MO, USA

Population	N	A_R	H_O	H_E	F'_{IS}
2	21	3.54	0.610	0.643	0.051
11	25	3.39	0.619	0.636	0.026
122	23	3.42	0.628	0.629	0.002
152	35	3.03	0.542	0.576	0.059
200	10	2.74	0.559	0.575	0.027
228	35	3.04	0.605	0.604	−0.001
229	33	3.12	0.586	0.603	0.028
238	49	3.20	0.585	0.609	0.040
246	41	3.27	0.581	0.600	0.032
251	17	2.92	0.608	0.605	−0.004
264	43	3.24	0.589	0.605	0.026
274	47	2.90	0.557	0.578	0.035
294	10	2.70	0.616	0.548	−0.124
356	12	2.86	0.583	0.586	0.005
387	51	3.04	0.573	0.580	0.011
393	30	3.11	0.577	0.600	0.038
407	32	3.13	0.592	0.604	0.020
408	15	3.01	0.578	0.592	0.024
415	10	2.86	0.533	0.555	0.039
66	14	3.06	0.599	0.588	−0.020
71	35	2.96	0.592	0.586	−0.011
8	54	3.21	0.611	0.603	−0.014
Avg	29.18	3.08	0.587	0.596	0.013

N is the number of samples after removal of full siblings, A_R is the mean rarefied allelic richness, H_O is observed heterozygosity, H_E is expected heterozygosity and F'_{IS} is the inbreeding coefficient. Population is a unique identification number for each pond

Table 2 Population genetic summary statistics for *Ambystoma annulatum* from 15 sample ponds at Fort Leonard Wood, MO, USA

Population	N	A_R	H_O	H_E	F'_{IS}
120	35	3.71	0.724	0.705	−0.027
127	40	3.66	0.624	0.688	0.093
152	32	3.67	0.694	0.704	0.014
228	10	3.34	0.653	0.694	0.059
229	11	3.51	0.655	0.713	0.082
238	15	3.10	0.609	0.646	0.058
246	13	3.14	0.641	0.666	0.038
264	12	3.27	0.637	0.660	0.034
315	10	3.11	0.674	0.662	−0.018
331	30	3.58	0.629	0.681	0.076
380	15	3.87	0.711	0.715	0.006
400	32	3.47	0.603	0.685	0.120
407	13	3.14	0.631	0.640	0.015
66	23	3.51	0.643	0.655	0.018
71	15	3.52	0.709	0.694	0.009
Avg	20.40	3.44	0.656	0.681	0.038

N is the number of samples after removal of full siblings, A_R is the mean rarefied allelic richness, H_O is observed heterozygosity, H_E is expected heterozygosity and F'_{IS} is the inbreeding coefficient. Population is a unique identification number for each pond

44 % of the 547 field collected samples being removed from our data set. As a result of sibling removal, 15 of the 22 sampled ponds had ≥10 samples remaining (306 samples total), and only these 15 ponds were retained for downstream analysis. Only 1.5 % of the 667 *A. maculatum* samples were removed due to sibship, and one *A. maculatum* pond had only 6 samples. This pond was removed, and 661 samples from 22 ponds were used in downstream analyses.

No population or locus deviated significantly from expected genotype frequencies under HWE after Bonferroni corrections for *A. maculatum*, and there was no evidence of null alleles in any locus. One locus (Am_60) was monomorphic and was omitted from the data set. The remaining 18 loci had 3–17 alleles (mean, ±standard deviation; 7.67 ± 3.96; Table S2). Observed heterozygosity at each pond ranged from 0.53 to 0.63 (0.59 ± 0.025; Table 1), and pairwise estimates of F'_{ST}, ranged from 0 to 0.216 (0.045 ± 0.038; Table S3). In *A. annulatum*, four loci (Aa_37, Aa_45, Aa_31, and Aa_4) had significantly fewer heterozygotes than expected under HWE (Table S4), and each of these loci showed evidence of null allele frequencies exceeding 0.13. Following removal of these loci and Bonferroni correction, no populations deviated from HWE expectation (Table 2). The remaining 15 loci were polymorphic, and had 3–14 alleles (7.46 ± 3.62; Table 2). Observed heterozygosity in *A. annulatum* ranged from 0.60 to 0.72 (0.66 ± 0.038; Table 1), and pairwise estimates of F'_{ST} ranged from 0.018 to 0.280 (0.125 ± 0.055; Table S5). There was no evidence of linkage among any loci in either species.

Population structure and differentiation

Spatial clustering of individuals with BAPS suggested a 100 % probability that *A. maculatum* ponds sampled comprised a single genetic cluster. In contrast, there was a 96 % probability that the *A. annulatum* individuals sampled at 15 ponds consisted of three genetic clusters (Fig. 2).

Support for hypotheses

Landscape resistance

We found that none of the tested resistance surfaces substantially differed from distance alone in explaining pairwise genetic differences in either species. All models had a $\Delta AICc < 0.80$ from the top model; therefore we conclude that none of the factors we tested contribute meaningfully to landscape resistance at the scale we sampled (Table 3).

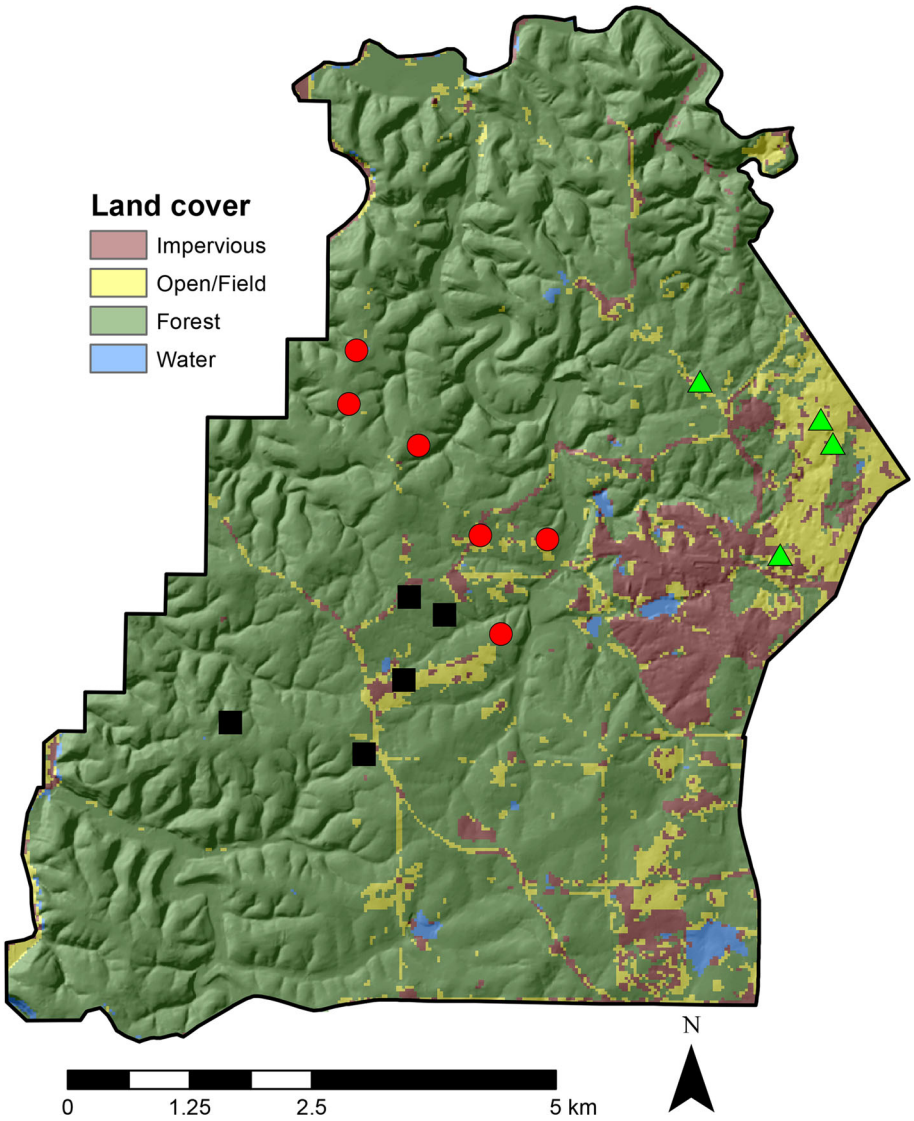

Fig. 2 Genetic clusters (K = 3) of *Ambystoma annulatum* as identified by BAPS

Interpond distance

A total of 151 different ponds were used for breeding in 2012 and 2013, and *A. annulatum* and *A. maculatum* overlapped at 31.1 % and 42.4 % of the ponds, respectively. We found no significant differences between years in terms of nearest-neighbor distance between utilized breeding ponds (Fig. 3; Table S6). There was a significant interaction between *A. maculatum* and year, with interpond distance being significantly greater during the drought spring of 2012. The mean distance between all ponds included in genetic analyses of this study (minimum–maximum) was 2,922 m (277–6,799 m) for *A. annulatum* and 3 773 m (448–10,710 m) for *A. maculatum*.

Dispersal propensity

In both species, there was a strong and significant signal of isolation-by-distance (Fig. 4; Table S7). However, *A. annulatum* had greater differentiation across all pairwise distance comparisons and had a greater rate of differentiation as distance between ponds increased (Fig. 4). Overall F'_{ST} was 0.131 (95 % CI 0.101–0.165) and F'_{IS} was 0.037 (95 % CI 0.002–0.087) in *A. annulatum*, while overall F'_{ST} was 0.047 (95 % CI 0.034–0.062) and F'_{IS} was 0.014 (95 % CI −0.010–0.043) in *A. maculatum*. As such, F'_{ST} was 2.8 times greater and F'_{IS} was 2.6 times greater in *A. annulatum* than in *A. maculatum*. However, we note that the estimated F'_{IS} are low for both species.

Dispersal capabilities

Following 10,000 bootstrap iterations, the mean dispersal distance for *A. annulatum* was estimated to be 1,693 m (95 % confidence interval = 1,645–1,740 m), and the mean dispersal distance for *A. maculatum* was estimated to be 2,050 m (95 % confidence interval = 2,009–2,091 m). The mean

Table 3 Model selection results for landscape resistance optimization

Surface	Type	K	AICc	ΔAICc	ω
A. annulatum					
TPI	Continuous	3	−421.69	0.00	0.21
Distance	Uniform	1	−421.62	0.08	0.20
LULC	Categorical	4	−420.94	0.76	0.15
TWI	Continuous	3	−421.00	0.70	0.15
Ravine distance	Continuous	3	−420.97	0.73	0.15
PRR	Continuous	3	−420.91	0.78	0.14
A. maculatum					
Distance	Uniform	1	−1,155.02	0.00	0.19
TPI	Continuous	3	−1,154.99	0.04	0.18
TWI	Continuous	3	−1,154.95	0.08	0.18
PRR	Continuous	3	−1,154.87	0.15	0.17
Ravine distance	Continuous	3	−1,154.68	0.35	0.16
LULC	Categorical	4	−1,154.28	0.75	0.13

No optimized resistance surfaces explained pairwise genetic variation better than distance alone

TPI topographic position index, *TWI* topographic wetness index, *LULC* land use, land cover, *PRR* potential relative radiation

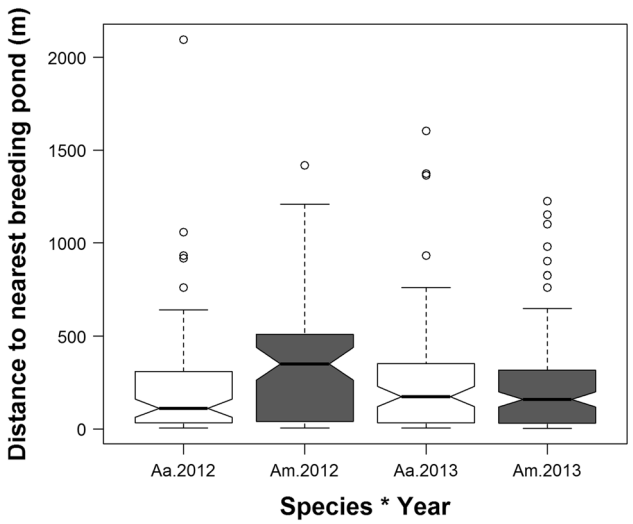

Fig. 3 Notched boxplot demonstrating the median (*dark black line*), 95 % confidence interval around the median (notch), and the interquartile range of the distance to nearest breeding pond for each species in 2012 and 2013. Aa = *Ambystoma annulatum*, Am = *A. maculatum*

dispersal values were used to calculate the rate parameter (k = 1/mean dispersal distance) used in Eqs. 2 and 3.

Habitat quality and breeding pond isolation

Of the three metapopulation hypotheses considered: pond characteristics alone, isolation alone, or both pond characteristics and isolation, isolation alone was best supported for both species (Table 4, Table S8). None of the tested pond-level parameters were significant predictors of genetic differentiation for either species (Table S8).

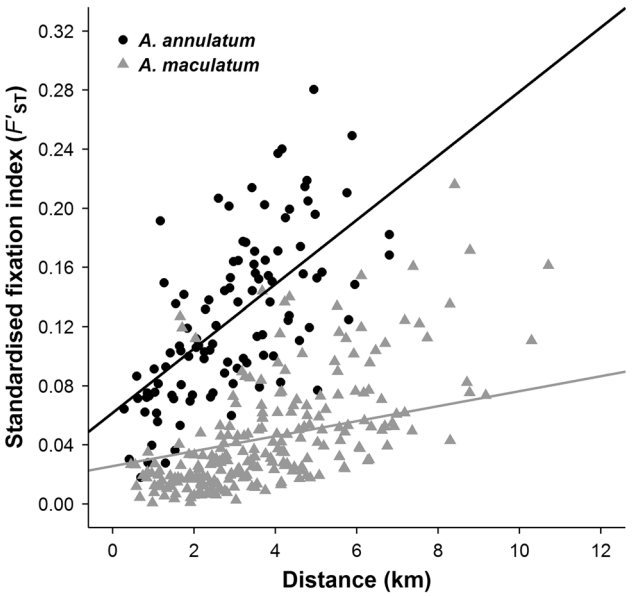

Fig. 4 Scatter plot demonstrating significant isolation-by-distance relationship for both *Ambystoma annulatum* and *A. maculatum*. Fit lines were determined from mixed effects models using a maximum likelihood population effects parameterization (see Table S7 for parameter estimates)

Table 4 AIC_c model selection results from linear models assessing the effects of pond-level characteristics, isolation, and both pond characteristics and isolation on genetic differentiation

Hypothesis	Species	
	A. annulatum	*A. maculatum*
Pond characteristics	−49.32	−104.2
Isolation	**−63.75**	**−123.89**
Both	−57.07	−120.24

Isolation alone (bolded) was the best predictor of genetic differentiation for both species

Discussion

Ambystoma annulatum and *A. maculatum* appear to be very similar ecologically, differing only in breeding phenology and oviposition site selection. Despite the apparent similarities, we identified stark genetic differences between the two species. We used our findings to assess the support for five hypotheses to identify the processes underlying differences between *A. annulatum* and *A. maculatum*. We found no support for differential effects of landscape resistance, distance to nearest neighbor breeding pond, or

pond-level covariates on observed genetic differentiation. Only pond isolation significantly predicted genetic differentiation, and the differences between *A. annulatum* and *A. maculatum* appear to be driven by species-specific specific dispersal distances, with average *A. annulatum* dispersal being 350 m shorter than *A. maculatum*. There are also notable differences between the overall genetic differentiation between the two species, with *A. annulatum* exhibiting greater overall differentiation and a greater rate of differentiation as distance increases. A potential process contributing to this pattern may be that *A. annulatum* are more likely to return to their natal pond, as suggested by the higher level of inbreeding measured in *A. annulatum*. However, we note that the observed levels of inbreeding were low for both species, and that factors such as migration and population size may also influence F'_{IS}.

Dispersal is a difficult life history attribute to measure in any organism, but is made more challenging by the small size of juvenile salamanders. Using population averaged genetic differentiation and pond isolation, we estimated the mean dispersal of *A. annulatum* to be 1,693 m and *A. maculatum* to be 2,050 m. These estimates may at first seem high, but significant differentiation among ambystomatid salamander populations often occurs at distances much greater than the perceived dispersal abilities of salamanders (e.g., Zamudio and Wieczorek 2007; Purrenhage et al. 2009; Whiteley et al. 2014). It is important to be cautious when literally interpreting connectivity and dispersal based solely on genetic measures (Lowe and Allendorf 2010). Gene flow, as inferred through population differentiation, is a multi-generational estimate of the successful dispersal and reproduction and does not incorporate individual movement behavior. While estimated dispersal distances are likely to differ between direct observations and genetic measures, there are direct observations of juvenile *A. opacum* dispersing up to 1,300 m in Massachusetts, USA (Gamble et al. 2007), and dispersing juveniles and migrating adults of several ambystomatid species have been documented making movements in excess of 1 km (Smith and Green 2005). It is worth noting that these long distance movement observations often covered the full extent of the study area, suggesting that longer movements, although likely rare, may go undetected.

We have focused on testing hypotheses predominantly related to dispersal, and to a lesser extent, on characteristics of breeding ponds. While we did not find evidence for landscape resistance affecting gene flow in either species, our study occurred over a relatively small spatial scale and on a predominantly forested landscape. At a broader spatial scale and with more extensive landscape variation, it is possible that landscape resistance could become a meaningful driver of genetic differentiation beyond distance alone (e.g., Goldberg and Waits 2010; Richardson 2012). It is also possible that the observed patterns in genetic variation reflect historical landscape features (Spear and Storfer 2008). Historically, much of the Ozark region was an open forest savannah landscape with frequent fires (Jacobson and Primm 1997). However, extensive logging, grazing, agriculture and fire suppression dominated the landscape during the nineteenth and early twentieth centuries (Jacobson and Primm 1997). Perhaps the most prominent addition to the landscape are human-made wildlife ponds, which account for the vast majority of fishless water bodies used by salamanders for reproduction at FLW (Peterman et al. 2014a). Depending upon historical distribution of ponds and the spatial locations of created ponds in time, the addition of this critical resource to the FLW landscape undoubtedly has influenced the spatial genetic structure of salamanders.

In addition to dispersal and breeding habitat, there are other life history traits that have the potential to affect genetic differentiation which should also be examined. For instance, population size, generation time, and duration of larval period are potential mechanisms underlying interspecific differences in genetic differentiation that have been considered in previous studies of sympatric amphibians (Richardson 2012; Whiteley et al. 2014). Neither differences in population size or generation times are consistent with our data or seem plausible in our system. Previous research has shown that larvae of *A. annulatum* are more widely distributed and numerically dominate at FLW (Peterman et al. 2014a), and while empirical evidence is lacking, there is no reason to conclude that generation times differ substantially between these two species in Missouri (Petranka 1998; Lannoo 2005). However, there are distinct differences in larval periods. In Missouri, the larval period of *A. annulatum* averages 250 days (Semlitsch et al. 2014) and *A. maculatum* averages 155 days (unpublished data). While the longer larval period of *A. annulatum* could result in reduced larval survival and recruitment, data do not indicate that this is the case. Anderson et al. (*in review*) estimated the average rates of larval survival to be 0.0016 and 0.0025 for *A. annulatum* and *A. maculatum*, which were not significantly different across monitored ponds or years ($t_{20} = -1.25$, $P = 0.23$).

The differences observed in our study occurred over a small spatial scale (<11 km). However, our finding that spatial genetic structure is more pronounced in *A. annulatum*, and that genetic differentiation increases at a greater rate with distance, may also lend insight into biogeographical differences between the two species. *Ambystoma maculatum* have one of the broadest distributions of any salamander in North America (Petranka 1998), but have achieved this widespread distribution following rapid expansion from glacial refugia (Zamudio and Savage

2003). In contrast, *A. annulatum* have a relatively restricted distribution that is confined to the Interior Highlands of the Ozark and Ouachita Mountains. There is also evidence to suggest that *A. annulatum* expanded their distribution from a southern refugium in the last 8,000–4,000 years (Phillips et al. 2000). Given that the current distribution of both species reflects dispersal post-glaciation, it is logical to conclude that life history characteristics of *A. maculatum* favor dispersal and colonization.

There are two other aspects of our study that warrant further discussion. First, our inferences in this study have been drawn from samples collected at 15 and 22 ponds for *A. annulatum* and *A. maculatum*. Across FLW, these species have respectively been found to breed in 142 and 133 ponds. As such, there are numerous breeding ponds ("populations") that have not been sampled. It has been shown that unsampled or "ghost" population can significantly affect estimates of migration and spatial connectivity, largely because of stepping stone dispersal through unsampled populations (Beerli 2004; Slatkin 2005; Naujokaitis-Lewis et al. 2012; Koen et al. 2013). However, it is unclear what effects these usampled populations have on estimates of F-statistics and dispersal. Our sampling of breeding ponds generally resulted in a uniform distribution of pairwise distance between ponds (Fig. 2), and encompassed the variation in landscape features present at FLW (Fig. 1). Assuming that the populations sampled are representative of all breeding populations at FLW, our inferences should be robust regardless of unsampled populations. Second, we have observed variable use of breeding habitats in space and time. *Ambystoma* spp. are long lived (5–10 years; Petranka 1998), but also highly philopatric. In the most detailed population study to date, Gamble et al. (2007) found that 91 % of *A. opacum* returned to breed in their natal pond, and that 96 % of experienced breeders returned to breed in the same pond every year. However, observation of breeding in the field suggest that *A. maculatum* may be more opportunistic, often breeding in tire ruts and shallow depressions that become inundated following snow melt and spring rains (personal obs, WEP). In contrast, *A. annulatum* reproduction is generally restricted to larger, more permanent wetlands. If such opportunistic reproduction by *A. maculatum* is occasionally successful, this could greatly increase gene flow across the landscape and reduce overall genetic differentiation. As indicated by our analysis of interpond distance of utilized breeding ponds, there can be significant differences among years that correspond to local precipitation patterns (Fig. 4, Table S7).

Conservation implication

Understanding similarities and differences among sympatric species is critical to forming and implementing comprehensive management strategies (Nicholson and Possingham 2006; Schwenk and Donovan 2011; Peterman et al. 2014a). Rarely is there a one size fits all solution (Caro 2003; Ficetola et al. 2007). At FLW, previous research has demonstrated that the distribution and abundance of larval salamanders differs substantially among species and in relation to different local and landscape level covariates (Peterman et al. 2014a). The existence of fine scale genetic structure in *A. annulatum* may make this species more sensitive to habitat fragmentation and population isolation. An effective management strategy for pond breeding amphibians, when done correctly, is wetland construction (Shulse et al. 2010). Increasing the number of wetlands and decreasing the distance between ponds may promote dispersal and gene flow across the landscape, making the metapopulation more resilient to local perturbations (Trenham et al. 2003). Placement of created wetlands on the landscape can also significantly impact the colonization of a wetland by amphibians (Shulse et al. 2010; Pittman et al. 2014), and species' specific dispersal capabilities and habitat preferences should be taken into account when determining where to place wetlands. Although landscape features and pond attributes did not emerge as significant factors affecting patterns of genetic differentiation, future management of both species should carefully consider the location of existing breeding ponds as well as terrestrial habitat, and then construct ponds with appropriate hydroperiods to maximize benefit for all species (Peterman et al. 2014a). Our study further reinforces the importance of understanding species' differences, and adds to the growing literature of multispecies genetic studies that show contrasting differences among sympatric amphibian species (Brede and Beebee 2004; Steele et al. 2009; Goldberg and Waits 2010; Richardson 2012; Sotiropoulos et al. 2013; Whiteley et al. 2014).

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