

Altered functional connectivity and genetic diversity of a threatened salamander in an agroecosystem

John A. Crawford · William E. Peterman ·
Andrew R. Kuhns · Lori S. Eggert

Received: 10 November 2015 / Accepted: 5 May 2016
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Abstract

Context Amphibian metapopulations have become increasingly fragmented in the Midwestern United States, with wetland-breeding salamanders being especially dependent on intact, high-quality forested landscapes. However, the degree to which amphibian populations are isolated, the factors that influence dispersal and, ultimately, functional connectivity remain areas in need of investigation.

Objectives and methods We combined population demographic and genetic approaches to assess how a

landscape fragmented by agriculture influences functional connectivity and metapopulation dynamics of a locally threatened salamander (*Ambystoma jeffersonianum*).

Results We found that the allelic richness and heterozygosity of this species was significantly related to the level of connectivity with other occupied breeding wetlands and that decreased connectivity resulted in increased genetic differentiation. We also found that effective population size appears to be declining and, while correlative, our focal landscape has experienced significant losses of forested upland habitats and potential wetland breeding habitats over the last 200 years.

Conclusions By combining population and landscape genetic analyses with an assessment of regional wetland occupancy, our study has uniquely synthesized genetic and metapopulation processes, while also incorporating the effects of the landscape matrix on dispersal, connectivity, and population differentiation. The significant relationship between connectivity with heterozygosity, allelic richness, and genetic divergence observed in this study reinforces empirical observations of long distance dispersal and movements in ambystomatid salamanders. However, our results show that protection of core habitat around isolated wetlands may not sufficiently minimize genetic differentiation among populations and preserve critical genetic diversity that may be essential for the long-term persistence of local populations.

Electronic supplementary material The online version of this article (doi:[10.1007/s10980-016-0394-6](https://doi.org/10.1007/s10980-016-0394-6)) contains supplementary material, which is available to authorized users.

J. A. Crawford (✉)
National Great Rivers Research and Education Center,
One Confluence Way, East Alton, IL 62024, USA
e-mail: joacrawford@lc.edu

W. E. Peterman · L. S. Eggert
Division of Biological Sciences, University of Missouri,
105 Tucker Hall, Columbia, MO 65211, USA

A. R. Kuhns
Illinois Natural History Survey, Prairie Research Institute,
University of Illinois, 1816 South Oak Street, Champaign,
IL 61820, USA

Present Address:
W. E. Peterman
School of Environment and Natural Resources, Ohio State
University, 2021 Coffey Road, Columbus, OH 43210, USA

Keywords Agriculture · *Ambystoma jeffersonianum* · Dispersal · Fragmentation · Metapopulation · Wetland

Introduction

Historically, many wetland-breeding amphibians have been thought to occur in metapopulations, although there has been some disagreement on whether metapopulation structure accurately describes amphibian populations (Semlitsch 2000; Marsh and Trenham 2001; Smith and Green 2005). In the strictest sense, metapopulations exist when geographically separate subpopulations undergo periodic local extinctions followed by recolonizations via dispersal, which maintains regional persistence over time (Hanski 1999; Hanski and Gaggiotti 2004). More broadly applied, the metapopulation designation is used to describe spatially structured populations (Hanski 1998; Smith and Green 2005), source-sink populations (Brown and Kodric-Brown 1977), or rescue-effect populations (Stacey et al. 1997). Considering the wide variation in amphibian population dynamics, Marsh and Trenham (2001) proposed a wetlands-as-patches metapopulation model to use as a starting point for investigations into the population dynamics of wetland-breeding amphibian systems. This model offers a straightforward wetland-based approach to monitoring and management while accounting for landscape conditions and processes, and is especially relevant for systems impacted by habitat alteration.

Habitat degradation, fragmentation, and loss are generally accepted as the primary causative factors for the widespread decline of amphibian species around the world (Cushman 2006). Many amphibian species require both aquatic and terrestrial habitats to complete their life cycle and are therefore sensitive to loss or alteration of either habitat (Semlitsch 2008). Because amphibians are physiologically linked to specific temperature and moisture regimes, landscape features can have particularly strong effects on their distributions and dispersal patterns (Stevens et al. 2006a, b; Peterman and Semlitsch 2013; Peterman et al. 2014). Wetland-breeding amphibians are generally thought to have poor dispersal abilities as evidenced by field studies showing high philopatry (Gamble et al. 2007; Whiteley et al. 2014). Numerous

genetic studies of ambystomatid salamanders have found relatively low levels of gene flow among populations, with significant population differentiation or structure occurring at scales of 1–5 km (Zamudio and Wieczorek 2007; Purrenhage et al. 2009; Whiteley et al. 2014; Peterman et al. 2015). Additionally, patterns of genetic differentiation can be significantly affected by landscape features and habitat (Greenwald et al. 2009a, b; Goldberg and Waits 2010), underscoring the potential impacts of habitat alteration on population structure. The subdivision of a large metapopulation into several smaller, isolated populations through landscape alteration can increase extinction rates and decrease recolonization rates of patches. Therefore, successful conservation of species in highly fragmented landscapes not only requires knowledge of their distribution, but requires information on patterns of gene flow (i.e. connectivity) among populations.

Landscape connectivity is commonly defined as the extent to which a certain landscape impedes or facilitates movement of individuals (Taylor et al. 1993). O'Brien et al. (2006) identified two components of landscape connectivity: structural connectivity and functional connectivity. Structural connectivity is the spatial arrangement of habitat patches, while functional connectivity is the response of organisms to the spatial arrangement of patches (i.e., structurally connected populations only become functionally connected populations when organisms move from one patch to another). As landscapes become increasingly fragmented, the importance of dispersal increases since it is the primary way organisms maintain population viability and genetic diversity within and among metapopulations (Clobert et al. 2012). Thus, quantifying functional connectivity between habitat patches (and local populations) is important for understanding the mechanisms that promote long-term population persistence in fragmented landscapes (Revilla et al. 2004; Van Buskirk 2012). The classical expectation that genetic diversity should decrease due to drift and reduced gene flow as isolation increases has been a constant in landscape genetic studies (e.g., Manel et al. 2003); however there is a lack of information that combines a rigorous assessment of the landscape matrix with known site (i.e., population) occupancy and how that influences functional connectivity among populations.

Amphibian metapopulations have become increasingly fragmented in the Midwestern United States (e.g., Kolozsvary and Swihart 1999; Weyrauch and Grubb 2004), with wetland-breeding salamanders being especially dependent on intact, high-quality forested landscapes (Homan et al. 2004; Porej et al. 2004; Rothermel and Semlitsch 2006). However, the degree to which amphibian populations are isolated, the factors that influence dispersal and, ultimately, functional connectivity remain areas in need of investigation. To that end, we conducted a case study of habitat fragmentation and population isolation effects on genetic diversity of a locally threatened salamander in an agriculturally dominated system. Specifically, the objectives of our study were to: (1) Assess population structure of *Ambystoma jeffersonianum* in a landscape fragmented by agriculture; (2) Determine the effects of the landscape on functional connectivity (i.e. genetic differentiation); (3) Determine the role of metapopulation dynamics (i.e. connectivity among occupied wetlands) in affecting measures of genetic diversity and differentiation; and (4) Estimate historical and contemporary effective population sizes.

Methods

Study species and sampling

Ambystoma jeffersonianum (Jefferson Salamander) are vernal pool-breeding salamanders that inhabit deciduous forests in eastern North America (Petranka 1998). These salamanders can be locally rare, occur in small populations, and are considered a species of conservation concern over portions of their range (Brodman 2005). They require fishless wetlands in deciduous forests for reproduction (Petranka 1998) and have been found to disperse up to 1600 m away from natal wetlands (Bishop 1941; Williams 1973). *Ambystoma jeffersonianum* were first documented in Illinois in 1990, and subsequently listed as a state threatened species because of their restricted range and dependence on rare and vulnerable habitat in a highly fragmented landscape (Illinois DNR 2006). From their discovery in 1990 through 2007, *A. jeffersonianum* were documented from fewer than ten localities along the Illinois-Indiana border in Clark and Edgar Counties, with one disjunct population

13 km from the state border. The demographic structure of this disjunct population in Lincoln Trail State Park, Clark County, Illinois was examined by Mullin and Klueh (2009). More recently, Peterman et al. (2013a) documented *A. jeffersonianum* from 29 new localities in Clark and Edgar Counties and noted the continued presence of *A. jeffersonianum* at four of eight sampled historical locations.

To assess the occupancy and genetic structure of *A. jeffersonianum*, we sampled 57 wetlands in Clark and southern Edgar counties in east-central Illinois, USA (Fig. 1); for site identifications and descriptions see Peterman et al. (2013a). Wetlands were identified through a combination of National Wetland Inventory wetland layers, topographic maps, aerial images, site visits, and landowner contacts. All identified wetlands located in forested patches or within 50 m of forest edges in the study area were sampled. Thirty-one wetlands were sampled in 2008 and 26 wetlands were sampled in 2009. Each wetland was sampled for breeding adults with 6–8 double-throated minnow traps for four consecutive nights during the early spring (February–March) breeding season to calculate the detection probability and occupancy of *A. jeffersonianum* in the region (Peterman et al. 2013a). Tissue samples (0.5 cm tail clip) were collected and stored in 95 % ethanol at -20°C until DNA extraction.

Laboratory procedures

DNA was extracted from tissue samples using chelex-based resin (InstaGene, BioRad) following protocol described by Peterman et al. (2012). We used 11 polymorphic microsatellite loci in two multiplex PCR reactions (Appendix 1 in supplementary material); because *A. jeffersonianum* are contributors to a unisexual hybrid complex, identification of pure *A. jeffersonianum* can be problematic (Petranka 1998). Four loci used in this study (AjeD23, AjeD75, AjeD94, and AjeD283) cross amplify in *Ambystoma laterale* and produce triploid banding patterns (Julian et al. 2003), which allowed us to ensure that only pure *A. jeffersonianum* were present in our study populations. Multiplex reactions were carried out using Qiagen Multiplex PCR Kits with the following modifications to the protocols. Reactions contained 4 μL of Qiagen multiplex mastermix, 0.45 μL primer mix, 0.65 μL BSA, 30 ng template DNA, and water to

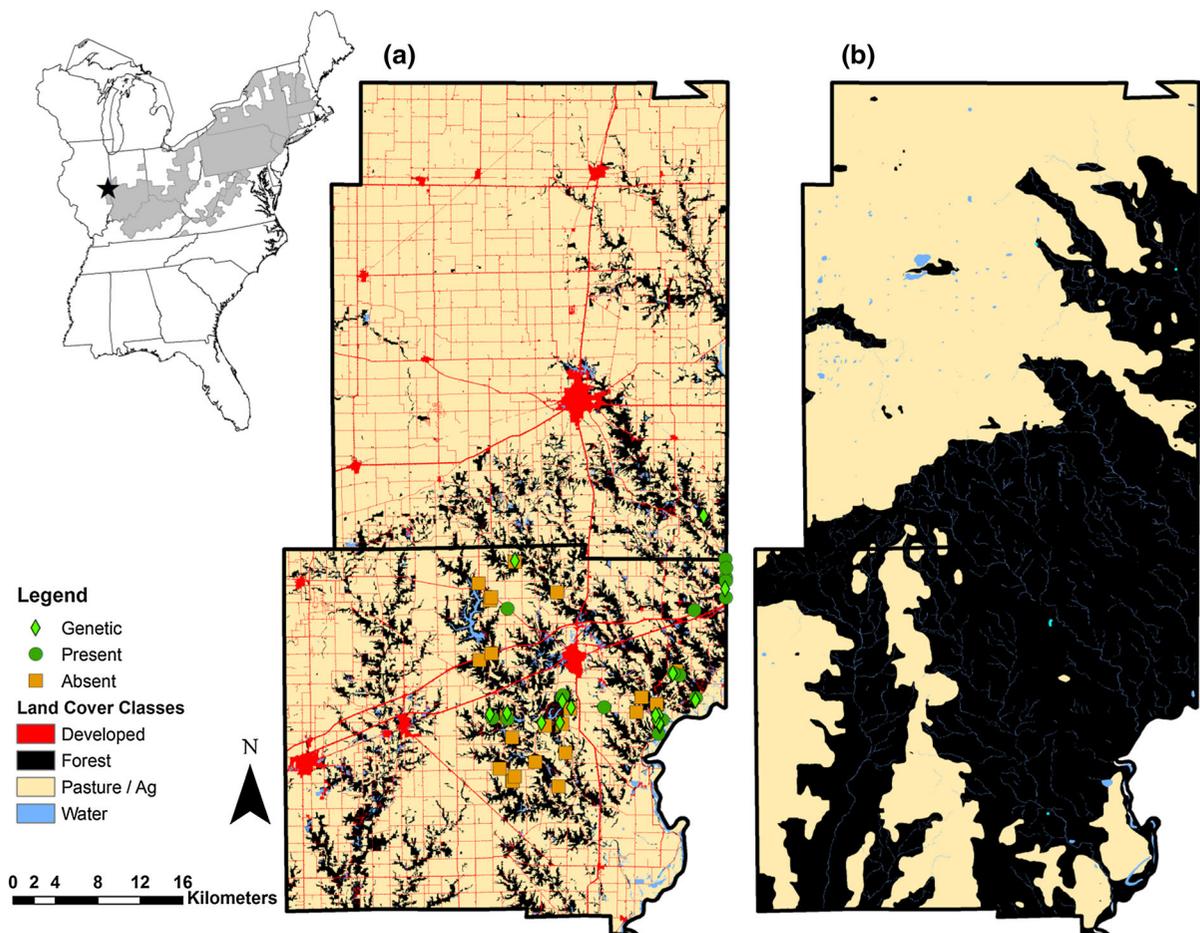


Fig. 1 Map of sampling locations and contemporary land cover within a two county focal area in east-central Illinois, USA. **a** Row crop agriculture dominates the landscape (83.3 %) and

forest comprises only 12.9 % of the current land area. **b** Historically, the two-county focal area was evenly covered by forest (51 %) and prairie (47.5 %)

bring the total volume to 8 μ L. Forward primers were fluorescently labeled with FAM, NED, VIC, or PET (Appendix 1 in supplementary material). Multiplex PCR cycling conditions were: initial denaturing at 95 $^{\circ}$ C for 15 min, followed by 35 cycles of 94 $^{\circ}$ C denaturing for 30 s, 58 $^{\circ}$ C annealing for 90 s, and 72 $^{\circ}$ C extension for 60 s; and a final 30-min extension at 60 $^{\circ}$ C. Samples were run on 96-well plates with negative and positive controls to ensure consistency in allele scoring. Additionally, 12 % of the samples were run twice to check for errors; no inconsistencies were found. PCR products were sized on an ABI 3730 automated sequencer using Liz 600 size standard, and were scored using the software GeneMarker version 1.6 (SoftGenetics, LLC).

Genetic diversity and population differentiation

Genotypic data were analyzed for only *A. jeffersonianum* localities where we were able to collect at least 15 unique tissue samples ($n = 16$). The program CONVERT version 1.31 (Glaubitz 2004) was used to format the data set for subsequent analyses. We used the program GENEPOP version 4.0 (Raymond and Rousset 1995) to assess genetic variability within and among sampled localities, calculate number of alleles per locus, determine observed and expected heterozygosities and test for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between loci. Pairwise F_{ST} values for each locality were calculated using program ARLEQUIN version 3.5 (Excoffier et al. 2005).

Landscape resistance and isolation by distance

We assessed the combined effects of landscape resistance and distance on genetic differentiation using optimization methods described by Peterman et al. (2014), implemented with *ResistanceGA* (Peterman 2014) in R (R Core Team 2015). *ResistanceGA* uses a genetic algorithm (GA; Scrucca 2013) to adaptively optimize resistance surfaces through a series of transformations (continuous resistance surfaces) or by assignment of resistance values (categorical resistance surfaces). We used *ResistanceGA* to optimize a land use, land cover surface (National Land Cover Database, 2006: http://www.mrlc.gov/nlcd06_data.php), which was reclassified into 4 classes prior to optimization: Forest; Row crop/Pasture; Open water; and Urban/Developed. At each iteration of the genetic algorithm, unitless resistance values were assigned to each land cover type (ranging in value from 1 to 2500), and landscape resistance between sample locations was assessed using cost distance assessed along least cost paths (LCP) in *gdistance* (van Etten 2014) and resistance distance with CIRCUITSCAPE (McRae and Shah 2009), which accounts for all possible pathways between locations. To evaluate the relative support for land cover in affecting genetic differentiation, linear mixed effects models were fit with *lme4* (Bates et al. 2014) 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). Linearized pairwise F_{ST} was used as the dependent variable and scaled and centered effective resistance between populations (either least cost distance or resistance distance) was the independent variable. Model fits were assessed using AICc calculated from the linear mixed effects models. Optimization proceeded until no further improvement of AICc could be achieved (for detailed description of *ResistanceGA* see Peterman 2014). In addition to the land cover resistance surface, we assessed Euclidean distance alone (isolation by distance) as well as an intercept only null model. Because distance is implicitly incorporated into the resistance distance and least cost resistance measures calculated by CIRCUITSCAPE and *gdistance*, Euclidean distance was not included as an additional factor in our land cover model.

Effective population size and land cover change

To estimate historic effective population size for each population we used program MIGRATE version 3.1.6 (Beerli and Felsenstein 1999, 2001). MIGRATE uses coalescent theory to estimate long-term gene flow estimates and effective population sizes. Using the Bayesian implementation of MIGRATE, we performed an initial run on our data using F_{ST} to find start parameters. The results of this initial run were used as the starting parameter values for all subsequent runs. Results from different runs were similar indicating that Markov chains had likely converged on the stationary distribution. For our runs, we used the continuous Brownian motion microsatellite model in MIGRATE and ran 5,000,000 generations per long chain with a burn-in of 200,000. We used MIGRATE to calculate effective population size as a function of mutation rate ($\Theta = 4N_e\mu$) and effective number of migrants ($4N_em$), where N_e is effective population size, μ is mutation rate, and m is the migration rate into the population. While the exact mutation rate for each locus is unknown, a mutation rate of 5.4×10^{-4} is generally accepted for vertebrates (Goldstein et al. 1995; Howes et al. 2009). The coalescent approach in MIGRATE estimates historic effective population size, so to generate a contemporary population estimate we used program ONeSAMP version 1.2 (Tallmon et al. 2008). ONeSAMP employs approximate Bayesian computation using sampling and biological parameters to estimate effective population size from microsatellite genotypes. For our runs we used 2 as the lower limit of N_e for simulations and the upper limit was 100 (based on MIGRATE results). Changes in effective population size were assessed using a paired t test. To fully assess the differences between effective population size estimates from MIGRATE and ONeSAMP, we conducted 10,000 Monte Carlo simulations. For each population in each simulation we randomly selected an effective population size estimate for MIGRATE and OneSAMP from within the predicted 95 % confidence interval. We then determined the mean and 95 % confidence interval for the difference in effective population size, t -statistic, and p -value from the 10,000 simulations. We calculated changes in land cover from the early 1800s to present day, using data obtained from the Illinois Natural History Survey web site (<http://www.>

inhs.illinois.edu/resources/gis/). Changes in wetland area were obtained from Suloway and Hubbell (1994).

Regression analyses

Wetland area measurements were taken with a 50 m tape reel at each of the 16 breeding wetlands (Table 1). We assessed wetland connectivity using an incidence function that accounts for distance to all occupied wetlands using a negative exponential dispersal kernel (Hanski 1994; Moilanen and Nieminen 2002). The general form of the incidence function calculates connectivity (S_i) of wetland i as:

$$S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) \quad (1)$$

where α scales the effect of dispersal to cumulative resistance, and d_{ij} is the cumulative resistance along the least cost path between the focal wetland i and source wetland j . We modified Eq. 1 to assess alternative competing hypotheses concerning how the level of connectivity affects average genetic differentiation of salamanders at focal pond i .

$$S_i = \sum_{j \neq i} p_j \exp(-\alpha d_{ij}) \quad (2)$$

$$S_i = \sum_{j \neq i} p_j \exp(-\alpha d_{ij}) A_j \quad (3)$$

$$S_i = A_i \sum_{j \neq i} p_j \exp(-\alpha d_{ij}) A_j \quad (4)$$

$$S_i = \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j \quad (5)$$

$$S_i = A_i \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j \quad (6)$$

In Eqs. 2–4, p is 1 when wetland j is occupied and 0 zero otherwise, which results in connectivity only being assessed to occupied wetlands. In Eqs. 3–6, A is the area (in hectares) of the forest patch that wetland i or j is closest to or found within. Multiplying by forest patch area A_j weights the contribution of wetland j to test the hypothesis that wetlands located in or near larger forest patches have larger population sizes and contribute more to connectivity across the landscape. Multiplying by focal patch area A_i weights the summed connectivity measure of wetland i to assess the hypothesis that a larger focal forest patch is more likely to be reached by individuals dispersing from j wetlands on the landscape. Connectivity of the 16 wetlands used for genetic analyses (Table 1) was

Table 1 Mean sampling and genetic data for 16 populations of *Ambystoma jeffersonianum* in east-central Illinois

Locality no.	Area (m ²)	Connectivity	Allelic richness	H_O^*	H_E^*	F_{ST}
1	441	0.752	4.7	0.571	0.571	0.103
2	306	0.328	5.9	0.682	0.680	0.057
3	810	<0.001	5.6	0.610	0.625	0.134
4	704	0.001	4.6	0.608	0.608	0.138
5	850	1.379	5.9	0.676	0.640	0.064
6	361	0.502	5.6	0.674	0.635	0.063
7	477	0.841	5.8	0.665	0.648	0.068
8	2590	1.871	5.7	0.678	0.697	0.054
9	396	2.098	6.1	0.633	0.665	0.050
10	286	1.087	5.1	0.620	0.619	0.099
11	1598	0.856	6.1	0.711	0.694	0.075
12	600	3.068	5.9	0.704	0.692	0.052
13	250	3.173	6.2	0.715	0.709	0.049
14	1652	3.080	6.0	0.661	0.717	0.055
15	672	3.215	6.7	0.687	0.723	0.046
16	702	2.810	6.1	0.673	0.683	0.048
Average	793.4	1.566	5.8	0.661	0.663	0.072
SD	634.8	1.192	0.5	0.041	0.044	0.030

* H_O and H_E are the observed and expected heterozygosity values, respectively

assessed in relation to the 57 wetlands surveyed (33 occupied) by Peterman et al. (2013a). The detection rate for *A. jeffersonianum* was 0.774 (SE = 0.0395; 95 % CI 0.687–0.842), which, given our sampling regime, gives us 99.7 % confidence in the occupancy status of all wetlands sampled (see Peterman et al. 2013a for detailed description of occupancy sampling and results).

Because we have no biological understanding or expectation of how cumulative resistance affects movement or dispersal in *A. jeffersonianum*, we could not reliably assign a value to α in Eqs. 1–6 above. Therefore, we used optimization to find the value of α that best scaled the effect of cumulative resistance. The objective function in this optimization was the negative log-likelihood of the linear model

$$F_{ST_i} = \beta_0 + \beta_1 S_i, \quad (7)$$

where F_{ST} of i is the average of all pairwise genetic distances between focal wetland i and all other j wetlands. S_i is the connectivity of wetland i , as calculated in Eqs. 1–6. As such, α was optimized uniquely for Eqs. 1–6, and we then used model selection with AICc to assess the level of support for linear models fit to each connectivity scenario. AICc values were calculated using the R package *AICcmodavg* (Mazerolle 2012), and the Brent optimization algorithm in R was used to optimize α (R Core Team 2015).

Finally, we evaluated the relationship between both wetland area and the best-supported connectivity measure with four response variables: effective population size, observed heterozygosity, allelic richness, and mean pairwise F_{ST} . Linear regression models were used to assess each relationship. Because of moderate sample size ($n = 16$), as well as heteroscedasticity in the variance of errors for some models, we also analyzed each regression model using nonparametric bootstrap regression and calculated the accelerated bootstrap confidence interval from 10,000 replicates. All statistical analyses were conducted using R (R Core Team 2015).

Results

Genetic diversity and population differentiation

We captured *A. jeffersonianum* at 33 of the 57 wetlands sampled; however we only used wetlands

with ≥ 15 individuals in the genetic analyses. Thus, a total of 401 individuals were used from 16 breeding wetlands (mean = 25, range 17–29; Appendix 2 in supplementary material). The 11 microsatellite loci used in this study were polymorphic across sampled individuals, ranging from 2 to 17 alleles with a mean of 9.1 ± 5.1 SD. Despite our sampled populations existing at the range margin there was no apparent reduction in genetic diversity at eight microsatellite loci previously used by Julian et al. (2003) in the eastern part of *A. jeffersonianum* distribution (Appendix 1 in supplementary material). Seven of the populations sampled contained at least one private allele (Appendix 2 in supplementary material). None of the 11 loci were significantly out of HWE for the 16 populations sampled and there was no evidence of linkage disequilibrium.

Allelic richness and heterozygosity were similar across populations (Table 1). Pairwise F_{ST} estimates ranged from low (0.000) to relatively high (0.210), and were significantly different from zero ($P < 0.05$) in 109 out of 120 cases after sequential Bonferroni correction for multiple comparisons (Table 2). In general, wetlands within 1100 m Euclidean distance of each other showed no significant genetic differentiation (Table 2), but populations greater than 1100 m apart were significantly differentiated (with one exception).

Landscape resistance and isolation by distance

There was unequivocal support for the effects of land cover on genetic differentiation among sampled populations (Table 3). Optimization using both LCP distance and circuit resistance distance with *ResistanceGA* identified forest land cover as having the lowest resistance (1/1; LCP/Circuitcape), followed by row crop/pasture (91/40), open water (1068/984), and urban/developed (1512/1198). Cumulative resistance calculated along the least cost path of the optimized land cover surface predicted pairwise genetic distance between wetlands well (Fig. 2) with a marginal R^2 of 0.516 for the fitted mixed effects model. Because of qualitative and quantitative congruence between LCP and circuit resistance results, our analysis proceeded with a focus on pairwise least cost distances. Using the landscape resistance values estimated from LCP optimization, we calculated effective distances between wetlands as the length of the least cost path

Table 2 Pairwise F_{ST} values for *Ambystoma jeffersonianum* below the diagonal, Euclidean distance (meters) between populations above the diagonal (first row and first column refer to locality numbers) for 16 populations in east-central Illinois

	1	2	3	4	5	6	7	8	9
1	–	5033	14,523	26,284	6007	3357	17653	15,977	5289
2	0.066*	–	16,012	23,874	1789	1703	12,851	11,592	2075
3	0.210*	0.121*	–	18,154	14,947	15,574	21,474	18,244	14,101
4	0.183*	0.113*	0.139*	–	22,086	24,809	17,585	15,253	22,063
5	0.057*	0.012*	0.154*	0.157*	–	3069	11,647	10,086	1018
6	0.040*	0.021*	0.156*	0.153*	0.011*	–	14,519	13,134	2766
7	0.117*	0.070*	0.126*	0.145*	0.067*	0.069*	–	3327	12,442
8	0.088*	0.052*	0.099*	0.081*	0.062*	0.059*	0.034*	–	10,692
9	0.044*	0.011*	0.142*	0.125*	0.009	0.000	0.049*	0.043*	–
10	0.097*	0.093*	0.164*	0.217*	0.076*	0.053*	0.099*	0.081*	0.059*
11	0.116*	0.064*	0.133*	0.121*	0.084*	0.065*	0.067*	0.052*	0.053*
12	0.097*	0.047*	0.129*	0.136*	0.052*	0.059*	0.043*	0.035*	0.038*
13	0.114*	0.052*	0.094*	0.118*	0.060*	0.066*	0.029*	0.020*	0.049*
14	0.120*	0.051*	0.118*	0.127*	0.059*	0.069*	0.038*	0.041*	0.046*
15	0.089*	0.043*	0.124*	0.141*	0.045*	0.053*	0.033*	0.031*	0.032*
16	0.089*	0.043*	0.124*	0.141*	0.045*	0.053*	0.033*	0.031*	0.032*
	10	11	12	13	14	15	16		
1	1604	23197							
2	6588	19,549	9288	9349	9335	8998	8974		
3	14,841	20,011	19,952	20,747	20,578	20,086	19,727		
4	27,528	8079	19,462	20,409	20,200	19,927	19,515		
5	7611	17,843	8250	8479	8427	8033	7933		
6	4892	20,878	10,980	11,052	11,036	10,696	10,665		
7	19,256	10,156	3699	4114	3999	4117	3986		
8	17,565	8695	4235	5161	4955	4716	4327		
9	6884	18,173	9132	9411	9350	8941	8816		
10	–	24,683	15,800	15,921	15,897	15,541	15,483		
11	0.114*	–	12813	13,645	13,458	13,308	12,955		
12	0.088*	0.049*	–	991	775	495	317		
13	0.074*	0.054*	0.004	–	216	670	1082		
14	0.100*	0.044*	0.000	0.000	–	492	881		
15	0.074*	0.052*	0.000	0.000	0.000	–	438		
16	0.074*	0.052*	0.000	0.002	0.001	0.000	–		

Bolded values represent non-significant F_{ST} values and their corresponding Euclidean distances

Values significantly different from zero are indicated with asterisks

between each pair. On average, least cost path lengths were twice as long as straight-line Euclidean distances between wetlands (range 1–11 times as long), however cumulative cost distances were on average 258 times longer (range 1–950 times as long).

Effective population size and land cover change

Based on effective population size estimates from MIGRATE and ONeSAMP, we observed a significant

decline in effective population size ($t_{15} = -13.740$; $P < 0.001$). This relationship remained significant following 10,000 Monte Carlo simulations (mean [95 % confidence interval]; decline = -13.90 [-16.36 to -11.63], $t_{15} = -12.07$ [-17.44 to -8.63], $P < 0.0001$). On average, there was a 21.8 % decline in effective population size at each wetland (Fig. 3). Estimates of historical effective population size from MIGRATE ranged from 35.35 to 48.48 (mean = 45.01 ± 3.76 SD), while contemporary

Table 3 Model selection results of optimized least cost path resistance surfaces for *Ambystoma jeffersonianum* in east-central Illinois

Model	K ^a	AICc	ΔAICc	ω _i ^b
Land cover	5	-550.22	0.00	1.00
Distance	2	-528.62	21.60	0.00
Null	1	-466.13	84.09	0.00

Genetic differentiation (F_{ST}) was the response variable in all models. Model selection results were the same when resistance surfaces were optimized using resistance distance calculated using CIRCUITSCAPE, with 100 % of the support going to the land cover model

^a Number of estimable parameters in approximating model

^b Probability that the current model (i) is the best approximating model among those tested

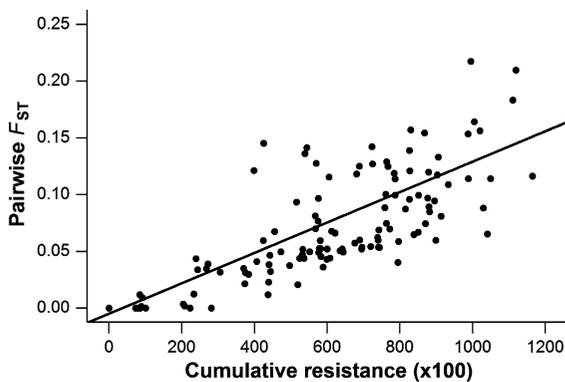


Fig. 2 Relationship between pairwise F_{ST} and cumulative resistance distance ($\times 100$). Cumulative resistance distance was calculated along least cost paths from the best supported resistance surface, which was determined to be land use, land cover with resistance values of forest = 1, row crop/pasture = 91, open water = 1068, and urban/developed = 1512

estimates of effective population size from ONeSAMP ranged from 19.97 to 38.98 (mean = 29.95 ± 4.26 SD). Historically, >149,000 ha of forest and >138,000 ha of prairie covered our two county study area (Illinois Department of Natural Resources and Illinois Natural History Survey 2003; Fig. 1a). Currently, forest occurs on only 37,716 ha of land (12.9 % of area; Fig. 1b), resulting in a 71 % decline in forest land cover. No natural prairie habitat remains in these counties, and the dominant land cover is now row crop/pasture (243,315 ha, 83.3 % of area). Developed land (4209 ha, 1.5 % of area) and open water (6842 ha, 2.3 % of area) make up the remaining portion of the

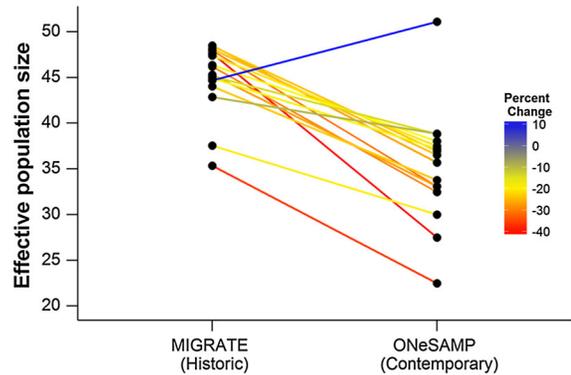


Fig. 3 Paired line plot demonstrating the decline in effective population size (N_e). Line color indicates the percent change in N_e , with the mean change across all populations being -21.8 %. Note that the percent change depicted does not imply a linear rate of change through time

landscape (Illinois Natural History Survey et al. 1996). Additionally, Suloway and Hubbell (1994) found that 90–99 % of natural wetlands within these two counties have been lost.

Regression analyses

Connectivity between wetlands was best modeled using Eq. 2, which calculated connectivity to wetlands occupied by *A. jeffersonianum* (Table 4). There was much less support for Eq. 1, which assessed connectivity to all wetlands on the landscape. There was no support for weighting connectivity by patch area. The optimization procedure resulted in an optimized α parameter of 1.333×10^{-4} , which suggests that the average resistance distance traversed by *A. jeffersonianum* is 7500 (i.e. $1/\alpha$). Overall, there was very little bias in the mean nonparametric bootstrap parameter estimates compared to the parametric linear regression model parameter estimates (Appendix 3 in supplementary material). Therefore, we report results from the significant linear regression models below. Bootstrap results and full regression tables for all analyses can be found in Appendices 3 and 4, respectively. We did not find a significant relationship between breeding wetland area and effective population size, heterozygosity, allelic richness, or mean pairwise F_{ST} . We did find positive relationships between wetland connectivity and heterozygosity ($F_{1,14} = 8.05$; $P = 0.013$; $R^2 = 0.320$; Fig. 4a) and allelic richness ($F_{1,14} = 12.81$; $P = 0.003$; $R^2 = 0.440$; Fig. 4b), and a negative relationship with

Table 4 Model selection results for linear models fit with optimized connectivity estimates

Equation	K ^a	AICc	ΔAICc	ω _i ^b
Equation 2	3	-88.80	0.00	0.85
Equation 1	3	-85.33	3.47	0.15
Equation 5	3	-76.52	12.28	0.00
Equation 3	3	-75.55	13.25	0.00
Equation 4	3	-66.29	22.51	0.00
Equation 6	3	-65.99	22.81	0.00

Average genetic differentiation (F_{ST}) was the response variable in each linear model, and connectivity was calculated using Eqs. 1–6

^a Number of estimable parameters in approximating model

^b Probability that the current model (i) is the best approximating model among those tested

mean pairwise F_{ST} ($F_{1,14} = 72.27$; $P < 0.001$; $R^2 = 0.826$; Fig. 4c); there was no significant relationship between wetland connectivity and effective population size.

Discussion

Our case study of *Ambystoma jeffersonianum* has highlighted the importance of maintaining a network of suitable wetlands within connected forested habitat. Specifically, the future evolutionary potential (i.e. allelic richness) of this species was significantly related to the level of functional connectivity with other occupied breeding wetlands. We also found that decreased functional connectivity resulted in increased genetic differentiation. These results demonstrate the role of dispersal and metapopulation dynamics in limiting divergence and maintaining genetic diversity among populations. Importantly, our measure of functional connectivity was scaled to effective distance, and the effective inter-wetland distance accounted for the effects of the predominantly agricultural matrix. We also found that effective population size appears to be declining, and while correlative, our focal landscape has experienced significant losses of forested upland habitats and potential wetland breeding habitats over the last 200 years. Surprisingly, there was no relationship between effective population size and wetland area, forest patch size or functional connectivity. This finding likely indicates that there are unmeasured

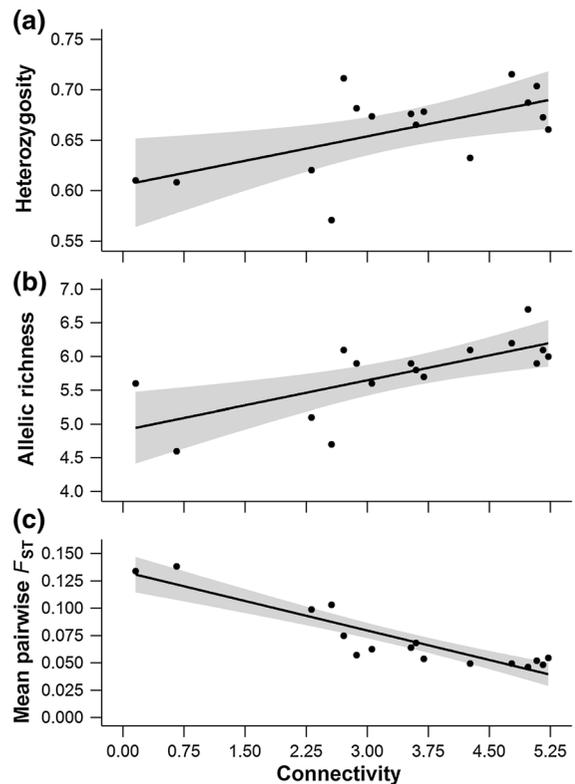


Fig. 4 Response plots showing significant effect of connectivity on heterozygosity (a), allelic richness (b), and mean pairwise F_{ST} (c) of *Ambystoma jeffersonianum*. Gray shading represents the 95 % confidence interval around the predicted mean

local-, landscape- and wetland-level features critical to maintaining effective population size. Effective future management strategies for *A. jeffersonianum* (as well as other wetland-breeding salamanders) will require strategic placement of wetlands on the landscape that considers the forested distance to other breeding wetlands, as well as incorporate (yet unknown) wetland and landscape features that promote large breeding populations.

Numerous studies have demonstrated the effects of patch occupancy, connectivity and habitat area on population genetic and demographic processes. For example, a simulation study conducted by Gibbs (2001) found that the primary demographic process affecting heterozygosity was the proportion of patches that were occupied through time. In a study of butterflies, Keyghobadi et al. (2005) found that heterozygosity increased as habitat patches became more connected, and Zuckerberg et al. (2014) found that haplotype and nucleotide diversity of birds

increased as forests became more connected. In salamanders, Cosentino et al. (2011) found that genetic differentiation increased and allelic richness decreased as connectivity among populations decreased. By combining population and landscape genetic analyses with a rigorous assessment of regional wetland occupancy, our study has uniquely synthesized genetic and metapopulation processes that have previously been described independently, while also explicitly incorporating the effects of the landscape matrix (as opposed to simple Euclidean distances) on dispersal, functional connectivity, and population genetic differentiation. The significant relationship between functional connectivity with heterozygosity, allelic richness, and genetic divergence observed in this study reinforces empirical observations of long distance dispersal and movements in ambystomatid salamanders (Fig. 4). However, our results also show that protection of core habitat around an isolated wetland (Semlitsch 1998) may not sufficiently minimize genetic differentiation among populations and preserve critical genetic diversity that may be essential for the long-term persistence of local populations.

Research on other ambystomatid salamander species by Cosentino et al. (2011) and Wang et al. (2011) found significant effects of wetland area on genetic diversity, differentiation, and effective population size. In addition to wetland area, Cosentino et al. (2011) also found that genetic divergence and diversity depended on connectivity; small, isolated wetlands exhibited lower genetic diversity and greater genetic divergence. In contrast, we did not find a relationship between wetland area and effective population size, heterozygosity, allelic richness, or genetic divergence. But like Cosentino et al. (2011), we did find that allelic richness and genetic divergence were affected by connectivity. Wetland area may not have been influential in this study due to the limited variation in area among our sampled wetlands. Wetland area ranged from 0.025 to 0.259 ha in this study (Table 1), while area ranged from 0.07 to 3.80 ha in Cosentino et al. (2011) and 0.032–7.84 ha Wang et al. (2011). Additionally, Wang et al. (2011) found the relationship between wetland area and effective population size was much greater for seasonal vernal pools than permanent wetlands, but most sampled wetlands in this study had semi-permanent to permanent hydroperiods.

Successful dispersal of individuals (and subsequent transfer of their genes) is critical to the maintenance of genetic diversity across the landscape, and necessitates functional connectivity among populations. When habitats connecting clusters of breeding wetlands are fragmented, local populations become isolated from each other, and the rescue effect becomes difficult or impossible to achieve (Laan and Verboom 1990; Reh and Seitz 1990; Goldberg and Waits 2010). In addition, isolated or peripheral populations are more vulnerable to loss of genetic diversity (Eckert et al. 2008; Whiteley et al. 2010; Peterman et al. 2013b), and the likelihood of extinction may increase as a result (Saccheri et al. 1998). We assessed connectivity as a function of distance, modulated by the landscape matrix. Unsurprisingly, we found that pairwise genetic differentiation in the forest-dependent *A. jeffersonianum* was best predicted by distance measures that accounted for landscape resistance. Our focal landscape in this study is ~13 % forested, and this forested habitat is predominantly confined to ravines that are unsuitable for agriculture. Historically, >50 % of our focal landscape was covered by large tracts of continuous forest (Fig. 1a). Concurrent with the loss and fragmentation of forests, >90 % of natural wetlands also have been lost (Suloway and Hubbell 1994). The current landscape configuration results in the effective distance between breeding wetlands being up to 950 times greater than if the entire landscape were forested. The ultimate effect of these large interpopulation distances has resulted in most *A. jeffersonianum* breeding populations being significantly differentiated, and thus, largely isolated from one another due to the vast expanses of agricultural matrix. The positive effects of forest and negative impacts of agriculture are recurring themes for many ambystomatid salamanders. In Ohio, Porej et al. (2004) found that *A. jeffersonianum* were positively associated with amount of forest cover within their core habitats (defined as the terrestrial habitat within 200 m of the breeding wetland), and in Illinois, breeding wetland occupancy for *A. jeffersonianum* is significantly correlated with canopy cover (Peterman et al. 2013a). More generally, studies of four other forest-dependent ambystomatid salamanders have shown that an agricultural matrix can have a prominent negative effect on population isolation (Greenwald et al. 2009a, b; Richardson 2012).

Despite the comparatively equivalent levels of genetic diversity in our *A. jeffersonianum* populations as compared to contiguous populations in the east, we found evidence for significant reductions in effective population size. Our contemporary estimates of effective population size are comparable to estimates made for other ambystomatid species in North America (Funk et al. 1999; Whiteley et al. 2010; Wang et al. 2011). This congruence may reflect the carrying capacity and natural population sizes of ambystomatid salamanders, and/or may indicate similar levels of population decline due to habitat loss and fragmentation. Regardless, the estimated decline in effective population size is coincident with landscape fragmentation and the loss of >70 % of available forested habitat and >90 % of wetland breeding habitat.

In our study, we found that heterozygosity, allelic richness, and genetic differentiation were all significantly affected by the level of functional connectivity to other *A. jeffersonianum* populations, and that effective population sizes have been significantly reduced. Likely underlying each of these findings is the extreme fragmentation and loss of habitat that has occurred in this landscape. However, our findings highlight clear and tractable applied management strategies that can be implemented to prevent further erosion of effective population sizes and genetic diversity, and minimize population isolation and genetic drift. Namely, increase functional connectivity and create/restore wetland breeding habitat. Restoration of forested habitats is critical to bolstering population connectivity. While core habitat requirements have been delineated for *A. jeffersonianum*, and other ambystomatid salamanders (Semlitsch 1998; Faccio 2003), our results demonstrate that these salamanders are dependent on more than protection of forested habitat immediately surrounding individual breeding populations; thus management should occur at the landscape scale. Long-term persistence of *A. jeffersonianum* depends on habitats that connect local populations as species persistence appears to rely on metapopulation dynamics to withstand fluctuating environmental conditions. To foster metapopulation dynamics, it will be necessary to create and restore suitable breeding wetlands within forested habitats. In doing so, local populations experiencing decline or extirpation can be rescued or recolonized by salamanders from neighboring populations.

More generally, our study provides a clear demonstration of the importance of functional connectivity and the need to carefully consider the added resistance to movement created by matrix habitat. Genetic diversity and differentiation are expected to increase with population isolation, but isolation is often subjective or based solely on Euclidean distance. By incorporating landscape resistance, patterns of population occurrence on the landscape, and population genetic data into estimates of connectivity, a more complete understanding of how landscape configuration and composition affect movement can be determined.

Acknowledgments We thank R. Jansen (IDNR Heritage Biologist, District 14) & D. Hiatt (NRCS-Martinsville, IL) for their work in compiling contacts and suggesting potential sampling sites, and the numerous landowners who graciously granted us access to their wetlands. S. Bales, N. Marioni, A. Price and J. Tiemann assisted in the field and J. Tunnage provided assistance in the lab. Funding for this project was provided by both the Illinois Endangered Species Protection Board and the Illinois Wildlife Preservation Fund as administered by the Illinois Department of Natural Resources. All research was conducted in accordance with animal care protocols of the University of Illinois animal care and use committee (permit # 08228).

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