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SPATIOTEMPORAL ANALYSIS OF GENE FLOW PATTERNS AMONG WOODLAND SALAMANDER POPULATIONS INHABITATING CONTRASTING LANDSCAPES

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Spatiotemporal analysis of gene flow patterns among woodland salamander populations inhabiting contrasting landscapes

ABSTRACT

Dispersal is a fundamental evolutionary process that serves as a mechanism by which local populations remain connected through space. Habitat loss and fragmentation remain widespread threats to biodiversity globally, and therefore it is imperative to understand how dispersal patterns are affected by anthropogenic modifications of the environment. Using a panel of 10 novel microsatellite loci, I estimated gene flow patterns over historical and contemporary timescales among populations of Eastern Red-backed Salamanders (*Plethodon cinereus*) in a previously unstudied portion of the species range. Four focal populations reside within a highly fragmented urban center whereas the remaining four focal populations persist in a relatively continuous landscape. Among fragmented populations, I observed weak genetic structuring, primarily driven by a highly divergent population. In contrast, populations in the continuous landscape were genetically homogeneous. Temporal analysis of gene flow patterns within the fragmented landscape revealed little difference between historical and contemporary estimates, as well as gene flow estimates comparable to those observed in continuous habitat. These results suggest that the observed genetic differentiation is not a result of reduced gene flow following fragmentation. In the continuous landscape, temporal changes in gene flow indicate a re-routing in the directionality of the major source of historical migrants, likely corresponding to historical land use practices. In both landscape types, I found the contribution of historical processes to be important in shaping contemporary gene flow patterns, as well as gene flow occurring on a large scale within a fragmented landscape.

1. Introduction

A central theme in evolutionary biology is the understanding of how attributes of life-history affect evolutionary processes and patterns within and among populations (Newman 1992; Stearns 1992). Dispersal is a key life history trait influencing patterns of genetic variation over time and space, which directly contributes to the course of evolution a population experiences (Hillman et al. 2014). In the most general sense, dispersal can be described as the permanent movement of organisms away from an origin (Lowe and McPeek 2014). Often, dispersal events are followed by reproduction, which results in gene flow or the transfer of genetic material from one population to another. The exchange of migrants among populations prevents local isolation, representing a critical process by which populations separated through space are able to remain connected. In addition, gene flow mitigates the effects of inbreeding depression and genetic drift while simultaneously contributing to the maintenance of a population's adaptive potential through the conservation and introduction of genetic diversity (Frankham et al. 2012).

Landscape connectivity can be broadly defined as the degree to which structural features of the landscape either facilitate or impede the movement of organisms (i.e. structural connectivity), and often plays an important role in dispersal (Spear et al. 2010). The destruction and fragmentation of habitat can result in a reduction of landscape connectivity, thus altering the dispersal patterns of the organisms that inhabit fragmented environments (Clobert et al. 2001). Habitat fragmentation divides populations into spatially patchy units, often causing populations to become isolated locally, which can

limit dispersal to the extent that mating between populations can become non-random (Hanski 1998). Moreover, the genetic consequences of reduced gene flow associated with habitat fragmentation (i.e. decreased genetic diversity and increased inbreeding) weaken the viability of metapopulations, threatening the persistence of local populations and increasing the probability of regional extinction occurring at the metapopulation level (Templeton et al. 2001; Cushman et al. 2016). Numerous studies have now shown that decreased landscape connectivity created by habitat fragmentation leads to a reduction in genetic connectivity, threatening the persistence and adaptive potential of populations, in many different taxa (Epps et al. 2005; Coulon et al. 2006; Cushman 2006; Vandergast et al. 2007).

Habitat loss and fragmentation are known to be major contributors in the global decline of amphibians (Almeida and Rocha 2014). Amphibians are characterized as having low vagility due to a number of ecological and physiological traits, in addition to many species exhibiting site fidelity (Duellman and Trueb 1986). Therefore, it is generally assumed that amphibians have poor dispersal capabilities and often exist as metapopulations (Alford and Richards 1999; but see Marsh and Trenham 2001; Smith and Green 2005) that rely on periodic long-distance dispersal events in order to maintain gene flow (Semlitsch 2008). Poor dispersal capacity, coupled with metapopulation structuring, are factors that often magnify the effects of reductions in landscape and genetic connectivity that are often experienced by amphibians as a result of habitat fragmentation (Gibbs 1998a; Bowne and Bowers 2004).

Currently, there is limited knowledge of dispersal patterns in woodland salamanders (genus *Plethodon*), although, like most amphibians, this group is generally

thought to be dispersal limited (Liebgold et al. 2011). Dispersal limitation is likely linked to the physiological constraints imposed by being lungless, especially for fully terrestrial species. Woodland salamanders in the genus *Plethodon* occupy moist terrestrial habitats to maintain cutaneous respiration, which constrains the extent to which terrestrial species can move as a consequence of the risk of desiccation. Moreover, the small body size of salamanders in the genus *Plethodon* likely contributes to limited dispersal ability as maximum dispersal distance and vagility exhibit a positive relationship with body mass among amphibians (Hillman et al. 2014). Evidence suggests that throughout eastern North America, populations of salamanders in this genus appear to be in decline (Highton 2005), and several species range from near-threatened to endangered (e.g., *P. hubrichti, P. shenandoah, P. nettingi*, *P. sherando;* Highton and Larson 1979; Bayer et al. 2012; Kroschel et al. 2014; Sutton et al. 2014). Studying the dispersal of these imperiled taxa can be difficult as consequence of low abundance and logistical constraints associated with their narrow geographic distributions. Furthermore, population genetic studies involving declining species often are limited to drawing broad management conclusions based on few individuals or populations, which can reduce the applicability of the results (Lowe and Allendorf 2010). Understanding the dispersal capabilities of an abundant, geographically - widespread species that is ecologically similar to rare imperiled species, can aid conservation biologists in understanding how population dynamics are altered in fragmented landscapes, resulting in more efficient land-use strategies. The Eastern Red-backed Salamander (*Plethodon cinereus*) is an excellent candidate species for a study of this scope.

Plethodon cinereus is a small, terrestrial woodland species distributed throughout eastern North America. This salamander has been used as a model organism in the study of behavioral and community ecology (Jaeger and Forester 1993; Mathis et al. 1995; Anthony and Pfingsten 2013). However, even in this well studied species, dispersal patterns remain unclear. Without robust estimates of migration rates across homogeneous landscapes, it becomes difficult to determine what effect habitat fragmentation has on dispersal and gene flow. Research involving the use of molecular techniques suggests the dispersal rates and patterns of gene flow vary geographically in *P. cinereus*. Populations in continuous habitat exhibit weak, but detectable differentiation at distances as close as 200 m (Virginia: Cabe et al. 2007), but in more northerly parts of the range populations can be genetically homogeneous at distances up to 4.1 km (Quebec: Noël et al. 2007). The effects of habitat fragmentation also have been shown to vary geographically such that low genetic diversity and minimal differentiation have been observed in fragmented patches (Indiana: Jordan et al. 2008); however, strong genetic structure also has been reported in urban isolated pockets (Quebec: Noël et al. 2007).

The objective of this study was to investigate patterns of genetic diversity and gene flow in previously unstudied portions of the range of *P. cinereus*, in continuous and fragmented landscapes, using a panel of 10 novel microsatellite loci. Unlike previous research, the current study investigates gene flow over historical and contemporary timescales, to explicitly address the idea that contemporary gene flow patterns in fragmented landscapes may be coincidental (e.g. resulting from historical differentiation associated with colonization history), rather than due to fragmentation itself (Jordan et al. 2008; Chiucchi and Gibbs 2010).

2. Materials and Methods

2.1 Locality Description

The study area was divided into two landscape types, fragmented and continuous, with four focal populations located in each. The four fragmented populations are essentially analogous to islands, located in a heavily urbanized portion of Cuyahoga County, Ohio (Fig. 1A). These sites include: a cemetery (Lakeview Cemetery (LV); 114 ha; 41.510389° N -81.585018° W); a reclaimed golf course (Acacia Reservation (AR); 47 ha; 41.504196° N -81.494007° W); a reclaimed bluestone quarry (Euclid Creek (EC); 140 ha; 41.543354° N -81.527190° W); and an urban nature preserve (Doan Brook Valley (DB); 113 ha; 41.493723° N -81.593795° W). These sites were established during the late 19th century and are separated by distances ranging from 1.9–8.4 km (Fig. 1A). The continuous populations are located within the Alleghany National Forest, Forest and Warren Counties, Pennsylvania (325 ha; Fig. 1B), which was also established in the early 20th century. The Minister Creek Recreation area was used as a reference point for mirroring the arrangement of sampled populations in Ohio, as best as possible, in attempt to eliminate geographic distance as a confounding variable for comparison of gene flow estimates (Minister Creek Site 1(MC1): 41.6244324° N -79.1599585° W; Minister Creek Site 2(MC2): 41.6395770° N -79.1579901° W; Minister Creek Site 3(MC3): 41.6715618° N -79.2225819° W; Minister Creek Site 4(MC4): 41.6122014° N -79.2239427° W). Distances between the sampled populations within the Allegheny National Forest range from 1.7–6.7 km (Fig. 1B).

2.2 DNA Isolation and PCR–based Genotyping

I obtained 120 tail tips from the focal Ohio populations (Acacia Reservation: n = 30; Doan Brook: n = 30; Euclid Creek: n = 30; Lakeview Cemetery: n = 30) and focal Pennsylvania populations (Minister Creek 1: n = 30; Minister Creek 2: n = 30; Minister Creek 3: n = 30; Minister Creek 4: n = 30) between 23 March and 5 May of 2015. *Plethodon cinereus* is polymorphic for dorsal color and recent studies have suggested that color morphs may differ in their movement behavior (Venesky and Anthony 2007) and in their fidelity to territories (Reiter et al. 2014). I only sampled monomorphic (striped) populations in both Ohio and in Pennsylvania to eliminate color morph as a factor in dispersal. All tissues were collected in compliance with scientific collecting permits issued by the Ohio Department of Natural Resources (Permit No. 16-80) and the Pennsylvania Fish and Boat Commission (Permit No. 2015-01-0040). Genomic DNA was extracted from tail tips using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. I assayed ten newly described microsatellite loci for *P. cinereus* isolated from a population in Virginia (Cameron et al., in prep), which consisted of one pentanucleotide repeat motif (*Pc3*), four tetranucleotide repeat motifs (*Pc13*, *Pc14*, *Pc25*, *Pc28*), and five trinucleotide repeat motifs (*Pc7*, *Pc16*, *Pc17*, *Pc34*, *Pc37*).

All genotyping reactions followed a nested PCR protocol described by Schuelke (2000), which had final volumes of 25µl and contained 2µl of DNA template (concentrations ranged from 5-50 ng/µl), 1x buffer, 1.5mM $MgCl_2$, 0.2mM of each dNTP, 0.1 µM non-M13(-21)-twinned primer, 0.1 µM FAM labeled M13(-21) primer, 0.025 µM M13(-21)-twinned primer, and 0.125 units of GoTaq polymerase (Promega).

PCRs were performed under the following conditions: 2 min at 94° C; followed by 25 cycles of 94°C for 30 sec; 30 sec at 62°C decreasing -0.5°C per cycle; and 72°C for 40 sec; followed by eight cycles pf 94°C for 30 sec; 53°C for 30 sec; and 72°C for 40 sec; followed by a final extension cycle of 30 min at 72°C. Successful amplification was confirmed via electrophoresis using 2% agarose gels, and fragment analysis was performed using an Applied Biosystems 3730 (Arizona State University) with GENESCAN 600 as the internal sizing standard. I then used Geneious version 9.1.2 to manually score, bin, and assign genotypes to all individuals.

2.3 Quality Control, Summary Statistics and Population Bottlenecks

I tested for the presence of null alleles, large allele dropout, and scoring errors using MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004). I estimated observed (H_O) and expected (H_E) heterozygosity to determine significant departures from Hardy-Weinberg proportions using GENEPOP version 4.3 (Rousset 2008). Additionally, GENEPOP was used to test for genotypic disequilibrium and the Weir and Cockerham (1984) estimator of F_{IS}. Finally, I calculated a variety of statistics for characterizing genetic diversity in GENEALEX version 6.5 (Peakall and Smouse 2012), which included the number of alleles per locus, number of effective alleles, number of private alleles, and POPGENKIT for the calculation of allelic richness (Paquette 2012).

I tested for evidence of recent population bottlenecks in both continuous and fragmented populations by examining deviations from expected heterozygosity at drift-mutation equilibrium using the program BOTTLENECK (Piry et al. 1999). Deviations were assessed under the stepwise mutation model (SMM), infinite alleles model (IAM) and the two-phase mutation model (TPM). Under the TPM, I assumed 95% of all

mutations were single-step mutations with 12% of the variance within multistep mutations, based on the recommendations of Piry et al. (1999). I ran 1,000 iterations and used the Wilcoxon signed-rank test and a sign test implemented in BOTTLENECK to determine whether there were significant deviations between Hardy-Weinberg and drift-mutation equilibrium heterozygosity. Additionally, BOTTLENECK was used to implement a Mode–shift test in order to examine observed allele frequency distributions. In a population under mutation–drift equilibrium, alleles of low frequency classes are more abundant than alleles of intermediate frequency classes, producing an L–shaped frequency distribution (Luikart et al. 1998). Following a population bottleneck, alleles of low frequency classes become less abundant in comparison to alleles of intermediate frequencies, causing a mode–shift distortion in the observed allele frequency distribution (Luikart et al. 1998). I also investigated population bottlenecks by calculating *M*-ratios (Garza and Williams 2001). The *M*-ratio test is based on the number of microsatellite alleles (k) to the range in allele size in repeat units (r), and in a population that has experienced a recent bottleneck, k is expected to decrease faster than r. *M*-ratios were calculated using the output from GENEALEX [Number of alleles / (Max – Min / Repeat Unit) +1] and were assessed against the recommended critical value of 0.68 (Garza and Williams 2001).

2.4 Population Structuring and Genetic Differentiation

In order to estimate the number of genetic clusters present within each landscape type, I used STRUCTURE version 2.3.4 (Pritchard et al. 2000). STRUCTURE implements a Bayesian framework to infer the number of genetic clusters (*K*) by probabilistically assigning individuals to groups that maximize conformity to Hardy-

Weinberg equilibrium (HWE) while minimizing linkage disequilibrium based on multilocus genotypes (Pritchard et al. 2000). I ran ten independent runs of STRUCTURE, each with a randomly generated seed, for values of K =1 to K =5. Each Markov Chain Monte Carlo (MCMC) run consisted of 350 000 iterations that were discarded as burn-in with an additional 350,000 iterations for sampling. I used the admixture model with the correlated frequencies prior, the LOCPRIOR and the LOCISPOP prior, with a fixed λ and inferred α. When appropriate, I repeated this procedure within the genetic clusters identified by STRUCTURE to detect secondary genetic structuring. I then used STRUCTURE HARVESTER web version 0.6.94 (Earl and vonHoldt 2012) to calculate ΔK (Evanno et al. 2005) and CLUMPP version 1.1.2 (Jakobosson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004) to align and visualize the replicate runs of the ΔK with highest likelihood. In addition, I also performed an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in GENEALEX to investigate the hierarchical partitioning of genetic variation (1) among STRUCTURE clusters (2) among individuals within STRUCTURE clusters, and (3) within individuals. Given that the AMOVA was performed on the clustering results from STRUCTURE, I do not report the associated *P*-values due to issues of non-independence (Meirmans 2015).

I measured the degree of genetic differentiation between *P. cinereus* populations in each landscape type using two G–statistics. I calculated global and pairwise comparisons of G_{ST} values based on Nei and Chasser's (1983) unbiased estimators of H_S (i.e. the Hardy-Weinberg expected heterozygosity averaged across all populations) and H_T (i.e. the Hardy-Weinberg expected heterozygosity in the total population ignoring subdivision), where $G_{ST} = (H_T - H_S)/H_T$. Additionally, I calculated global and pairwise

comparisons of G''_{ST}, which is a modified version of Hendrick's G'ST correcting for the tendency of G'_{ST} to underestimate the degree of subdivision and is formulated to equal one when populations have non-overlapping allele sets irrespective of the level of genetic diversity (Meirmans and Hendrick 2011). Both G-statistics were calculated in GENEALEX based on 9, 999 permutations.

2.5 Contemporary Gene Flow

Estimates of contemporary gene flow (*m*: proportion of migrants per generation) were generated using BAYESASS v 3.0 (Wilson and Rannala 2003). The software BAYESASS generates a complete matrix of migration rates between populations that is generally assumed to reflect the last 5 generations (Chiucchi and Gibbs 2010; Converse et al. 2015). I used the clusters identified by STRUCTURE as an *a priori* population assignment (Converse et al. 2015). For each landscape type, I ran 10 independent runs with random starting seeds for 50,000,000 iterations, sampling every 2,000 iterations with the first 5,000,000 discarded as burn-in. Chain mixing parameters were adjusted during a series of pilot runs to maintain a state-change acceptance rate between 20-40% (Rannala 2011). Convergence on the stationary distribution was assessed visually for each run in TRACER v 1.5 (Rambaut and Drummond 2007). I also used a Bayesian Deviance measure (Spiegelhalter et al. 2002) to determine which of the ten independent runs best fit the data in R (Meirmans 2014). Estimates of contemporary migration that were selected for interpretation for each landscape type showed a visual sign of convergence, had the lowest Bayesian Deviance score, and an effective sample size (ESS) for all parameters > 200.

2.6 Historical Gene Flow

I used MIGRATE v 3.6.11 (Beerli 2008; 2009) to estimate a historical gene flow (*M*: proportion of migrants per generation scaled by mutation rate). MIGRATE utilizes a coalescent model, and in the current study, a Bayesian framework to estimate gene flow over long time periods (~$4N_e$ generations). I used a Brownian motion model to approximate a step-wise mutation model, with relative mutation rates for each locus estimated from the data. I then used slice sampling for three replicate long chains for 5,000,000 iterations sampling every 200 iterations with the first 1,000,000 iterations discarded as burn-in. Estimates of *M* and θ were modeled with a uniform prior with and lower and upper boundaries of 0 and 3000 for *M*, and 0 and 100 for θ. F_{ST} values were used for initial estimates of both *M* and θ. I generated a complete migration model in order to allow for comparison of migration rates generated by BAYESASS. Values of the estimated parameters were considered accurate if the ESS was ≥ 1000 (Converse et al. 2015).

2.7 Comparison of Contemporary and Historical Gene Flow Estimates

To compare the historical gene flow estimates generated by MIGRATE ($M = m_h/\mu$) to contemporary estimates generated by BAYESASS, I multiplied the *M* values generated in MIGRATE by a standard mutation rate of microsatellite loci 5×10^{-4} (Garza and Williamson 2001). I then subtracted the historical values from the contemporary estimates generated by BAYESASS ($\Delta m = m - m_h$). The value of Δm denotes temporal changes in gene flow, where negative values denote a reduction in present gene flow, positive values denote increased gene flow, and values near zero indicate no change.

3. Results

3.1 Quality Control, Summary Statistics and Population Bottlenecks

Two loci, *Pc13* and *Pc14* were found to be monomorphic in 6 of the 8 sampled populations (Table 1). I removed the two loci from the dataset for downstream analyses as these loci contained no information for the majority of the populations. Holm's (1979) correction for multiple tests was performed by treating the tests associated with each population as a family of tests. After correction, there were two statistically significant deviations from HWE, AR at *Pc25* and MC1 at *Pc7*. MICRO-CHECKER detected evidence for null alleles at *Pc7* in MC1, *Pc16* in AR, *Pc17* in AR and MC2, *Pc25* in AR, EC and LV, *Pc28* in LV, and *Pc37* in MC3. Even though *Pc25* and *Pc7* departed from HWE proportions as well as presented evidence for null alleles, the exclusion of *Pc25* and *Pc7* did not affect my results and I therefore opted to include the two loci in the final data set. There was no statistical evidence of genotypic disequilibrium among any pairs of loci in both landscape types after correcting for multiple tests.

Across all ten loci used in the current study, I recorded 68 alleles among the Ohio populations and 48 alleles among the Pennsylvania populations. Populations within the fragmented landscape tended to have slightly higher heterozygosity (Obs: 0.366–0.509; Exp: 0.378–0.596; Table 1) compared to continuous populations (Obs: 0.303–0.341; Exp: 0.319–0.359; Table 1). In addition, Ohio populations also tended to have higher allelic richness and a greater number of effective alleles compared to Pennsylvania populations (Table 1). Levels of inbreeding were consistent among both sets of populations (Table 1).

There was relatively little statistical evidence for population bottlenecks from the results of the heterozygosity excess tests (BOTTLENECK) and the *M*-ratios (Table 2). Results from the Wilcoxon signed-rank test and the Sign test detected significant heterozygosity excess in two Ohio populations (Acacia Reservation and Lakeview Cemetery) and in two Pennsylvania populations (Minister Creek 1 and Minister Creek 3); however, the results were not consistent across all three mutation models (Table 2). Additionally, the Mode–shift test revealed that none of the sampled populations exhibited a distorted allele frequency distribution as would be expected following a bottleneck. Finally, none of the calculated *M*-ratios were below the critical value of 0.68, although two Ohio populations (Euclid Creek and Lakeview Cemetery) and all four Pennsylvania populations were within one SEM of the critical value (Table 2).

3.2 Population Structuring and Genetic Differentiation

Results from STRUCTURE revealed contrasting patterns of population differentiation among landscape types. For the fragmented landscape in Ohio, the optimal solution was a $\Delta K = 2$, with almost no admixture between the two discrete genetic groupings (Fig. 2A). Further analysis of the cluster that consisted of Euclid Creek, Lakeview Cemetery, and Doan Brook also revealed the optimal solution to be $\Delta K = 2$, with the presence of weak secondary structuring (Fig. 2B). The secondary structuring indicates that Lakeview Cemetery and Doan Brook likely represent one sub-population that is weakly differentiated from Euclid Creek. Therefore, for further analyses, I opted to combine individuals from Lakeview Cemetery and Doan Brook into a single deme on the basis of the population sub-structuring detected by STRUCTURE. Contrastingly, the optimal solution in the undisturbed populations in Pennsylvania was a $\Delta K = 2$; however,

the majority of individuals were maximally assigned to one cluster, which is indicative of a lack of population structure (Fig. 2C). No alternative solutions for ΔK were explored for the Pennsylvania populations, as $\Delta K = 2$ was over an order of magnitude greater than all other explored values of K.

Results of the AMOVA for the fragmented populations in Ohio are consistent with the presence of weak genetic structuring with 15% of the genetic variation partitioned to differences among clusters. Presumably the variation attributed to differences among clusters is largely driven by the genetic dissimilarity of Acacia Reservation compared to the remaining populations, as indicated from the STRUCTURE results (Fig. 2A). Eight percent of the genetic variation was partitioned to differences among individuals within clusters, while 77% of the variation was explained within individuals (i.e. differences between two alleles within a diploid genotype; Table 3). The AMOVA results for the continuous landscape illustrated the absence of even slight population structuring with only 1% of the genetic variation being partitioned to differences among clusters, 10% among individuals within clusters, and 89% of variation partitioned to differences within individuals (Table 3).

Locus-specific estimates of G_{ST} in the fragmented landscape ranged from 0.025-0.263 and were all highly statistically significant after multiple testing correction (maximum $P = 0.009$). The degree of differentiation observed for locus-specific G_{ST} estimates in the continuous landscape ranged from -0.008-0.029 with no loci exhibiting statically significant differentiation after correcting for multiple testing (minimum $P = 0.015$). The global G_{ST} value across eight loci was 0.116 (SE = 0.02; $P = 0.001$) in the fragmented landscape and 0.005 (SE = 0.004; $P = 0.056$) in continuous habitat. Locus-

specific estimates of G''_{ST} for the fragmented landscape ranged from 0.057 to 0.712 and were again all highly statistically significant (maximum $P = 0.011$). Similar to the results observed for estimates of G_{ST}, the locus-specific estimates of G''_{ST} for continuous habitat ranged from -0.015 to 0.059, and were not significantly different from zero after correcting for multiple testing. The global estimate of G''_{ST} across eight loci in the fragmented landscape was 0.382 (SE = 0.058; $P = 0.001$) while the global G''_{ST} in continuous habitat was 0.012 (SE = 0.010; $P = 0.056$).

Pairwise comparisons of G_{ST} and G''_{ST} estimates among the clusters located within the fragmented landscape revealed marked differentiation between Acacia Reservation and the remaining clusters (Tables 4-5). In comparing levels of differentiation between landscape types, pairwise comparisons reveal a fivefold and tenfold increase maximum estimates of G_{ST} and G''_{ST} estimates, respectively, when comparing differentiation among clusters within the continuous habitat to clusters located in the fragmented landscape (Tables 4-5).

3.3 Contemporary Gene Flow

Contemporary estimates of gene flow among the three genetic populations identified within the fragmented landscape indicate low rates of migration, with the majority of *m* values ranging between 0.0054 – 0.0301(Fig. 3A). The lowest levels of contemporary gene flow observed was emigration from Acacia Reservation into Doan Brook/Lakeview ($m = 0.0054$) and Euclid Creek ($m = 0.0102$) but immigration into Acacia Reservation from Doan Brook/Lakeview and Euclid Creek are slightly higher ($m = 0.0301$ and $m = 0.0218$, respectively). Gene flow into Doan Brook/Lakeview from Euclid Creek was markedly higher than all other estimates ($m = 0.2174$) but appears to be

asymmetric in that gene flow into Euclid Creek from Doan Brook/Lakeview is much lower (m = 0.0204). Not surprisingly, the two populations receiving the least amount of gene flow had a higher proportion of self-recruitment (Acacia Reservation 95% and Euclid Creek 97%; Fig. 3A). Gene flow estimates among the four populations located within a continuous landscape tended to be slightly higher than the values observed in the fragmented landscape, where the majority of values for m ranged from 0.0175 – 0.0789 (Fig. 4A). Interestingly, Minister Creek 3 (MC3) contributes a large proportion of migrants to the remaining 3 populations (m = 0.2133 into MC1; m = 0.2496 into MC2; m = 0.2712 into MC4; Fig. 4A). All three populations that receive migrants from Minister Creek 3 exhibit a lower degree of self-recruitment (70%) compared to self-recruitment in the population supplying the immigrants (MC3 = 88%; Fig. 4A).

3.4 Historical Gene Flow

Estimates of historical gene flow revealed similarly low levels of migration among populations in both landscape types (Fig. 3B; Fig. 4B). In the fragmented landscape, values of M ranged from 0.0045 to 0.0145 (Fig. 3B), which is similar to the range for the continuous landscape, where M ranged from 0.003 to 0.0150 (Fig. 4B). Across both landscapes, the proportion of the population that migrates per generation did not exceed 1.5% with the exception of gene flow in the continuous landscape from Minister Creek 2 into Minister Creek 4 (M = 0.139; Fig. 4B).

3.5 Comparison of Contemporary and Historical Gene Flow Estimates

Among the populations within a fragmented landscape, four rates of gene flow were found to increase through time (Fig. 3C). The greatest increase in gene flow occurred from Euclid Creek into Doan Brook/Lakeview (m = + 0.2029) with a marginal

increase in gene flow from Doan Brook/Lakeview (DV) into Euclid Creek (m = + 0.0069). Gene flow into Acacia Reservation from Doan Brook/Lakeview and Euclid Creek also increased over time (m = + 0.0256 and m = + 0.0173, respectively). Gene flow out Acacia Reservation into Doan Brook/Lakeview and Euclid Creek was observed to have decreased over time, albeit the values are small enough to suggest that no temporal change in gene flow has occurred (m = - 0.0021 and m = - 0.0013, respectively). Temporal changes in gene flow among the populations distributed in the continuous landscape revealed only one decrease in gene flow, which took place in the route where the greatest historical exchange of migrants occurred, from Minister Creek 2 into Minister Creek 4 (m = - 0.1196; Fig. 4C). There were four increases that likely represent no change (m = + 0.0025 + 0.009) and four moderate increases in gene flow (m + 0.0226 to + 0.0739). The most notable increase in exchange of migrants is the substantial increase in gene flow out of Minister Creek 3, where contemporary values of m out of Minister Creek 3 are anywhere from 20 to 70 times greater than historical migration estimates (Fig. 4C).

4. Discussion

I analyzed 8 polymorphic microsatellite loci from *P. cinereus* populations inhabiting landscapes contrasting in the degree of fragmentation in order to investigate the influence of landscape connectivity on genetic attributes of salamander populations. My analyses show the presence of genetic differentiation, as well as weak genetic structuring and sub-structuring within a fragmented landscape while *P. cinereus* populations within a continuous landscape appeared genetically homogeneous. My results expand on previous genetic research of *P. cinereus* in that I estimated differences

in historical and contemporary migration rates to examine how the alteration of landscape connectivity has affected gene flow and subsequently influenced population genetic structure. Interestingly, my results suggest that among the fragmented populations examined, a temporal decrease in gene flow does not provide support for the genetic divergence that was observed. Moreover, the highest estimate of contemporary gene flow recovered in the fragmented landscape is similar to the upper estimates observed in the continuous habitat, suggesting that *P. cinereus* is able to disperse through a modified landscape effectively. These results contribute to a growing body of literature documenting the absence of a uniform response at the population genetic level to the effects of urbanization within a single amphibian species (Rowe et al. 2000; Newman and Squire 2001; Crosby et al. 2008; Purrenhage et al. 2009; Safner et al. 2011; Furman et al. 2016).

Previous genetic studies on the responses of populations of *P. cinereus* to habitat fragmentation have yielded inconsistent results. Although the populations in my study are separated by similar geographic distances to those in Noël et al. (0.6-4.1 km; 2007), I did not observe a similar pattern of all urban populations being genetically differentiated from one another. Rather, my results appear to be more consistent with the results of Jordan et al. (2008) and Noël and Lapointe (2010), which document the presence of weak genetic differentiation among some, but not all, populations, and clustering algorithms indicating weak or no population structure in fragmented landscapes. Both of the aforementioned studies found populations separated by substantially greater geographic distances, relative to the current study, to be undifferentiated (35 km, Noël and Lapointe 2010; 70km, Jordan et al. 2008). Despite having detected genetic divergence among *P.*

cinereus populations located within a fragmented landscape, the majority of genetic dissimilarity observed in the current study was exhibited by a single population (Acacia Reservation). The remaining sampled populations were either genetically indistinguishable (e.g. Doan Brook and Lakeview Cemetery; DV; Fig. 2B; Fig. 3A) or weakly differentiated (Euclid Creek). *Plethodon cinereus* populations can reach high densities, which is an attribute that decreases the magnitude of genetic drift, and may offer a potential explanation as to why I observed inconsistent patterns of differentiation within the fragmented landscape. However, the portion of the fragmented landscape where I observed the most genetic similarity has been urbanized for ~ 80 years, therefore fragmentation is not so recent, that drift has not had time to operate. Thus my data may offer an alternative explanation for the lack of genetic differentiation observed in this studies and others. Instead of large population sizes shielding fragmented *P. cinereus* populations from genetic drift, the presence of low resistance corridors among *P. cinereus* populations may facilitate dispersal and, subsequently may prevent genetic differentiation.

Plethodon cinereus have been documented dispersing through open fields (Marsh et al. 2004), which is far more inhospitable habitat than the quality of matrix habitat between the populations where I observed the most genetic similarity (Doan Brook to Lakeview Cemetery; Doan Brook to Euclid Creek; Fig.2B; Fig.3A). *Plethodon cinereus* has also been documented at high densities in disturbed habitat (Anthony and Pfingsten 2015 and citations within), and exhibit much less sensitivity to fragmentation compared to other forest dwelling salamander species (e.g. *Ambystoma maculatum*, *Notophthalmus viridescens*; Gibbs 1998b). Although the long term persistence of *P. cinereus* populations

within degraded habitat remains unclear, small patches of suboptimal habitat within modified landscapes may serve as stepping stones between large source populations. This is especially true for amphibian species that are not reliant on water for reproduction, as is the case for terrestrial members of family Plethodontidae. It is not implausible that the suburban landscapes surrounding source populations foster patches of useable microhabitat. For example, the Oregon Slender Salamander (*Batrachoseps wrighti*) is a federally listed species that is typically associated with late–successional Douglas-fir forests; however, reproducing populations of relatively high densities have been found in several degraded riparian zones within suburban residential developments (Guderyahn et al. 2010).

In the case of *P. cinereus*, the occupation of small habitat fragments may be driven by density-dependent dispersal. The high densities at which *P. cinereus* can occur potentially offsets the high mortality rate of dispersal associated with reduced permeability of the landscape (Gibbs 1998b). Furthermore, despite suburban areas having a high road density, there is evidence to suggest that smaller roads, typical of the sort associated with residential areas, would not pose strong genetic barriers to *P. cinereus* (Marsh et al. 2008). With increasing competition within source populations, individuals may be forced to disperse into adjacent habitat fragments. Subsequently, the colonized habitat fragments act as "pseudo–sinks", or populations that are not entirely dependent upon immigrants. Recurrent immigration from multiple source populations into habitat fragments during high recruitment years acts as the mechanism by which fragments act to promote admixture, and indirectly connect sources populations. The spatial variation in the presence of stepping stone habitats within, and among urbanized landscapes may help

to explain the observed variation in the genetic response of *P. cinereus* to habitat fragmentation.

Although one sampled population (Acacia Reservation) within the fragmented landscape was strongly differentiated (Tables 4 and 5; Fig. 2A), I observed a negligible temporal change in gene flow emigrating from Acacia Reservation. Moreover, gene flow into Acacia Reservation increased over time, suggesting the high degree of differentiation observed is not attributed to a reduction of historical gene flow due to fragmentation. Several hypotheses could provide an explanation for the differentiation of Acacia Reservation. Although outside of the scope of this study, the genetic divergence of Acacia Reservation may be a combination of isolation by distance and isolation by environment. The probability of dispersal to any given habitat patch can be viewed as a function of the distance between the origin and destination (Wright 1943). Isolation by distance (IBD) occurs when genetic differentiation between populations increases with geographic distance (because of declines in gene flow across larger distances). The greatest geographic distance separating populations occurs between Doan Brook/Lakeview and Acacia Reservation which may account for the strong differentiation observed between these two populations (Fig. 1A; Tables 4 and 5). However, I observed stronger differentiation between Euclid Creek and Acacia Reservation, which are closer in proximity (Fig. 1A; Tables 4 and 5). Therefore, the structural features of the landscape between Acacia Reservation and the remaining populations may cause isolation by environment (IBE; Wang and Bradburd 2014). In other words, the geographic distance between populations alone is not the limiting factor, but rather, the physical cost of dispersal exerted by the landscape (i.e. absence of

permeable habitat) isolates populations, which has been documented to occur in much larger-bodied species of Plethodontidae (*Phaeognathus hubrichti*, Apodaca et al. 2012; *Plethodon albagula*, Peterman et al. 2014).

An alternative explanation as to why Acacia Reservation is strongly differentiated from the remaining populations may be due to the size of habitat patch in which this population is located. Previous research has documented that within urban landscapes, *P. cinereus* populations inhabiting smaller habitat patches (< 1.5 ha) were strongly differentiated whereas populations persisting in larger patches exhibited minimal-to-no differentiation (Noël and Lapointe 2010). Acacia Reservation was the smallest patch of habitat examined in the current study, measuring 47 hectares; however, adequate salamander habitat represents only a small fraction of the total reservation. Smaller amounts of habitat support smaller population sizes (Farhig 2003) and recent evidence suggests that habitat quantity is a better predictor of population genetic structure than the spatial configuration of habitat (Jackson and Farhig 2016). It is plausible that the effective population of Acacia Reservation is small and has been for some time, therefore making Acacia Reservation subject to strong drift-induced differentiation. Infrequent genetic exchange with surrounding differentiated populations, as indicated by the contemporary gene flow analysis (Fig. 3A), would increase the total number of observed alleles making the detection of a population bottleneck with utilized methods difficult, potentially eliminating the signature of a reduction in population size (Cornuet and Luikart 1996). However, this scenario would result in an elevated level of inbreeding, greater than the values observed for Acacia Reservation.

Finally, the presence of multiple metapopulations may explain the pronounced

differentiation of Acacia Reservation. Extinction and recurrent recolonization of the habitat present within Acacia Reservation could intensify drift-induced differentiation, a process often associated with pond breeding amphibians (Zellmer and Knowles 2009). In other words, Acacia Reservation may have been colonized by individuals belonging to a metapopulation that is differentiated from the local deme to which Doan Brook/Lakeview and Euclid Creek belong. Genetic drift acts only to differentiate Acacia Reservation from the founding metapopulation and further the genetic divergence from the surrounding demes. Alternatively, adequate gene flow into the Acacia Reservation from the metapopulation that colonized the habitat initially may be present, and the absence of admixture between the two metapopulations could produce genetic divergence similar in magnitude to the levels observed. High indices of genetic diversity within Acacia Reservation, and a lack of statistical evidence of a population bottleneck, suggests that the genetic divergence may be attributed to the presence of multiple metapopulations, reflecting historical differences in the colonization of landscape.

The importance of historical land use on contemporary patterns of gene flow is evident in the results from the continuous landscape within the Allegheny National Forest. Temporal analyses of gene flow illustrate a change in the directionality and magnitude of migration patterns in the continuous habitat. The major rerouting of gene flow likely corresponds to the clear cutting events and subsequent recolonization of the Allegheny National Forest that ended in the early 20th century. Minister Creek Site 3 supplies a large number of migrants to the remaining populations, and is in close proximity to 49 hectares of old growth forest (Hearts Content Recreational Area, Warren County, PA; 41.692583 N -79.254222 W). This old growth forest likely served as a local

refugium, and as secondary succession occurred, once suboptimal habitat was recolonized. This pattern of recolonization would explain the observed directionality of contemporary gene flow and may account for the low genetic diversity and lack of differentiation among the populations. Following clear cutting events in the Southern Appalachians, salamander densities have been reported to equal, or exceed, pre-disturbance densities in as few as 20 to 24 years (Ash 1997); this may explain why I found little evidence of a population bottleneck as populations have had ~ 96 years to recover in size.

My results demonstrate that a dispersal-limited species is able to maintain sufficient levels of gene flow within a fragmented landscape. Insight from the attributes of the fragmented landscape in Ohio that contribute to the genetic connectivity among *P. cinereus* populations potentially stands to inform land management decisions and the conservation of threatened Plethodontid species. First, the presence of moderately-sized habitat patches were found to support viable populations that were absent of signs of eroded genetic health. The ability of plethodontid salamander populations to persist in small amounts of habitat may be linked to kin recognition (Cabe et al. 2007; but see Gibbons et al. 2003), which potentially acts as a mechanism to reduce levels of inbreeding and maintain genetic diversity in small populations.

Therefore, even small patches of habitat may represent a conservation priority with respect to terrestrial woodland salamanders, especially for species occupying restricted ranges (i.e. *P. punctatus*, *P. sherando*, and *P. nettingi*). Additionally, despite the habitat surrounding the sampled populations being comprised of plant communities atypical of traditional salamander habitat (e.g. non-native plant species), the temperature

and moisture conditions may be similar to those experienced in mature forests, and appropriate of the species. The residential areas surrounding the populations may act to buffer migrating individuals from increased temperatures associated with altered light regimes, reducing the risk of desiccation during migration. Salamanders that occupy habitat that is buffered retain higher allelic diversity as well as improved body condition, relative to salamanders that occupy unbuffered habitat (*Phaeognathus hubrichti*, Apodaca and Godwin 2015).

Effective management strategies of mountain-top salamanders are already in practice. For example, the primary habitat of *Plethodon hubrichti* is protected from logging entirely, whereas logging within areas of secondary habitat must occur outside of seasons of salamander surface activity. Additionally, seedlings must be established before mature trees can be removed, and coarse woody debris must be retained (Bayer et al. 2012). The combination of established management practices with the conservation of small and large habitat patches, and buffering areas of conservation priority, may aid in restoring landscape connectivity to some extent, helping to restore genetic homogeneity among isolated populations. Future work should focus on identifying specific attributes of the landscape that may act as dispersal corridors, as well as determining if fitness costs are incurred in small populations associated with patches of suboptimal habitat. Developing our understanding of the long term persistence and viability of salamander populations occurring in small habitat fragments represents critical information for improving future conservation efforts.

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Table. 1 Genetic diversity indices and summary statistics of the 10 microsatellite loci use for genotyping. The four fragmented populations (Acacia, Doan Brooke, Euclid Creek, and Lakeview) tended to exhibit higher genetic diversity across all metrics relative to the populations sampled within a continuous landscape (Minister Creek 1, Minister Creek 2, Minister Creek 3, Minister Creek 4).

Pop/Locus	N	No. Alleles	Obs. Het	Exp. Het	F_{IS}	Allelic Richness	Private Alleles	Effective Alleles
Acacia (AR)								
Pc3	30	2	0.267	0.231	-0.1373	2	0	1.301
Pc7	30	7	0.633	0.737	0.1568	7	5	3.797
Pc13	30	2	0.367	0.299	-0.2083	2	1	1.427
Pc14	30	2	0.2	0.231	0.1512	2	0	1.301
Pc16	30	10	0.733	0.859	0.1633	10	4	7.115
Pc17	30	7	0.567	0.811	0.3167	7	3	5.294
Pc25	30	11	0.4	0.832	0.5316	11	4	5.96
Pc28	30	5	0.733	0.702	-0.0274	5	0	3.358
Pc34	30	8	0.567	0.633	0.1212	8	4	2.723
Pc37	29	6	0.621	0.626	0.0251	6	2	2.67
Pop Mean	29.9	6	0.509	0.596	0.10929	6	2.3	3.495
SEM		1.03	0.06	0.08	0.07	1.03	0.62	0.65
Doan Brook (DB)								
Pc3	30	2	0.633	0.455	-0.3775	2	0	1.835
Pc7	30	2	0.2	0.18	-0.0943	2	0	1.22
Pc13	30	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	30	4	0.5	0.517	0.0502	4	0	2.071
Pc17	30	4	0.667	0.652	-0.0061	3.97	0	2.871
Pc25	30	7	0.433	0.636	0.3333	7	0	2.744
Pc28	30	5	0.5	0.553	0.1131	5	0	2.239
Pc34	30	6	0.667	0.596	-0.1016	6	1	2.476
Pc37	30	4	0.7	0.709	0.0287	4	0	3.429
Pop Mean	30	4	0.43	0.43	-0.0067	3.597	0.1	2.088
SEM		0.59	0.08	0.08	0.06	0.6	0.09	0.23

Pop/Locus	N	No. Alleles	Obs. Het	Exp. Het	F_{IS}	Allelic Richness	Private Alleles	Effective Alleles
Euclid Creek (EC)								
Pc3	30	2	0.6	0.42	-0.4146	2	0	1.724
Pc7	30	2	0.2	0.32	0.3895	2	0	1.471
Pc13	29	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	30	5	0.367	0.414	0.1308	5	0	1.706
Pc17	29	4	0.655	0.648	0.0372	4	1	2.726
Pc25	30	8	0.5	0.636	0.2301	8	0	2.748
Pc28	30	2	0.467	0.357	-0.2889	2	0	1.557
Pc34	30	7	0.667	0.747	0.1239	7	3	3.947
Pc37	29	2	0.2	0.237	-0.098	2.97	0	1.228
Pop Mean	29.7	3	0.366	0.378	0.01375	3.497	0.4	1.911
SEM		0.79	0.08	0.08	0.09	0.77	0.3	0.3
Lakeview (LV)								
Pc3	30	2	0.433	0.34	-0.2609	2	0	1.514
Pc7	30	2	0.233	0.207	-0.1154	2	0	1.26
Pc13	30	1	0	0	N/A	1	0	1
Pc14	30	2	0.167	0.207	0.2077	2	0	1.26
Pc16	30	5	0.433	0.438	0.0258	5	0	1.777
Pc17	30	5	0.6	0.631	0.0654	4.97	1	2.707
Pc25	30	8	0.6	0.731	0.1957	8	0	3.719
Pc28	30	3	0.233	0.376	0.394	3	0	1.603
Pc34	30	5	0.733	0.737	0.0222	5	0	3.805
Pc37	30	4	0.633	0.682	0.0885	4	0	3.147
Pop Mean	30	4	0.407	0.435	0.0692	3.697	0.1	2.179
SEM		0.67	0.075	0.08	0.06	0.67	0.1	0.34

Pop/Locus	N	No. Alleles	Obs. Het	Exp. Het	F_{IS}	Allelic Richness	Private Alleles	Effective Alleles
M. Creek 1 (MC1)								
Pc3	30	3	0.233	0.21	-0.0973	3	1	1.265
Pc7	30	4	0.133	0.381	0.6603	3.97	1	1.617
Pc13	29	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	30	7	0.7	0.665	-0.0357	6.94	1	2.985
Pc17	29	4	0.207	0.25	0.1884	4	0	1.333
Pc25	30	7	0.567	0.649	0.1441	6.97	0	2.853
Pc28	30	4	0.367	0.371	0.0304	3.95	1	1.592
Pc34	30	4	0.533	0.591	0.1137	4	1	2.442
Pc37	30	3	0.367	0.473	0.2405	2.98	0	1.897
Pop Mean	29.8	3.8	0.311	0.359	0.156	3.78	0.5	1.798
SEM		0.64	0.07	0.07	0.08	0.64	0.17	0.23
M. Creek 2 (MC2)								
Pc3	30	2	0.167	0.207	0.2077	2	0	1.26
Pc7	29	4	0.345	0.44	0.2329	4	1	1.786
Pc13	30	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	30	6	0.733	0.637	-0.1352	5.88	1	2.752
Pc17	30	4	0.2	0.362	0.4605	3.96	0	1.567
Pc25	30	6	0.667	0.622	-0.0536	5.96	0	2.651
Pc28	30	3	0.3	0.304	0.0333	2.97	0	1.439
Pc34	30	4	0.7	0.623	-0.1073	4	0	2.651
Pc37	30	2	0.3	0.299	0.0151	2	0	1.427
Pop Mean	29.9	3.3	0.341	0.349	0.081	3.28	0.2	1.753
SEM		0.58	0.08	0.07	0.07	0.57	0.13	0.22

Pop/Locus	N	No. Alleles	Obs. Het	Exp. Het	F_{IS}	Allelic Richness	Private Alleles	Effective Alleles
M. Creek 3 (MC3)								
Pc3	30	2	0.2	0.18	-0.0943	2	0	1.22
Pc7	30	2	0.233	0.207	-0.1154	2	0	1.26
Pc13	30	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	30	5	0.567	0.577	0.0352	4.96	0	2.365
Pc17	30	3	0.233	0.304	0.2509	2.96	0	1.439
Pc25	29	8	0.655	0.686	0.0626	8	2	3.186
Pc28	30	3	0.333	0.283	-0.1623	2.96	1	1.394
Pc34	30	4	0.633	0.638	0.0248	4	0	2.765
Pc37	29	3	0.172	0.314	0.4636	2.94	1	1.456
Pop Mean	29.8	3.2	0.303	0.319	0.058	3.18	0.4	1.708
SEM		0.67	0.07	0.07	0.07	0.66	0.22	0.24
M. Creek 4 (MC4)								
Pc3	30	2	0.1	0.095	-0.0357	2	0	1.105
Pc7	30	3	0.267	0.234	-0.1181	2.97	1	1.307
Pc13	30	1	0	0	N/A	1	0	1
Pc14	30	1	0	0	N/A	1	0	1
Pc16	29	3	0.379	0.541	0.314	3	0	2.176
Pc17	30	4	0.6	0.497	-0.1904	3.97	1	1.989
Pc25	30	6	0.7	0.633	-0.0885	5.94	1	2.727
Pc28	29	3	0.345	0.38	0.1111	3	0	1.614
Pc34	30	3	0.633	0.594	-0.0475	3	0	2.469
Pc37	27	3	0.333	0.367	0.112	3	0	1.581
Pop Mean	29.5	2.9	0.336	0.334	0.007	2.89	0.3	1.697
SEM		0.46	0.08	0.07	0.05	0.45	0.15	0.19

Table 2. Probability values for tests of population bottleneck effects in Ohio and Pennsylvania populations of *Plethodon cinereus* under the Infinite Alleles model (IAM), stepwise mutation model (SMM), and two-phase mutation model (TPM). The probabilities reported for the Wilcoxon Signed-Rank Test are for a one-tailed test of heterozygote access. The mean *M*-ratio and standard error for each population were compared to the critical value of 0.68.

Population	Mutation Model	Sign Test	Wilcoxon Test	Mode-shift	*M*-ratio (SEM)
Fragmented					
Acacia	IAM	**0.021**	**0.004**	L-shaped	0.756
	TPM	0.247	0.687	distribution	(0.064)
	SMM	0.245	0.812		
Doan Brook	IAM	0.459	0.097	L-shaped	0.756
	SMM	0.240	0.769	distribution	(0.054)
	TPM	0.234	0.843		
Euclid Creek	IAM	0.385	0.097	L-shaped	0.711
	SMM	0.569	0.527	distribution	(0.055)*
	TPM	0.559	0.578		
Lakeview	IAM	**0.027**	**0.013**	L-shaped	0.724
	SMM	0.161	0.714	distribution	(0.052)*
	TPM	0.157	0.751		
Continuous					
Minister Creek 1	IAM	0.236	0.727	L-shaped	0.718
	SMM	**0.055**	0.994	distribution	(0.081)*
	TPM	**<0.01**	0.996		
Minister Creek 2	IAM	0.441	0.320	L-shaped	0.748
	SMM	0.077	0.986	distribution	(0.081)*
	TPM	0.075	0.986		
Minister Creek 3	IAM	0.311	0.727	L-shaped	0.706
	SMM	**0.014**	0.994	distribution	(0.086)*
	TPM	**0.014**	0.994		
Minister Creek 4	IAM	0.173	0.191	L-shaped	0.716
	SMM	0.063	0.963	distribution	(0.082)*
	TPM	0.061	0.973		

* Mean standard error (SEM) intersects critical *M*-value.

Table 3. AMOVA results for fragmented and continuous landscapes. The majority of the genetic variation was partitioned to variation within individuals indicating, a lack of genetic structuring within either landscape; however, a greater proportion of genetic variance was explained by differences among clusters in the fragmented compared to continuous landscape.

Landscape Type	Source of Variation	Percent of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Fixation Index
Fragmented						
	Among Clusters	15%	2	64.183	0.395	$F_{ST} = 0.149$
	Within Clusters	8%	117	290.675	0.223	$F_{IS} = 0.099$
	Within Individuals	77%	120	244.500	2.038	$F_{IT} = 0.233$
	Total	100%	239	599.358	2.656	N/A
Continuous						
	Among Clusters	1%	3	8.1	0.012	$F_{ST} = 0.007$
	Within Clusters	10%	116	226.3	0.175	$F_{IS} = 0.099$
	Within Individuals	89%	120	192	1.600	$F_{IT} = 0.105$
	Total	100%	239	464.4	1.788	N/A

Table 4. Pairwise comparisons of G_{ST} estimates across eight polymorphic loci for the fragmented and continuous landscapes. G_{ST} values are above the diagonal and corresponding p-values below. In the fragmented landscape, there was pronounced genetic differentiation observed for the Acacia population, whereas in continuous habitat, there was almost complete overlap in allele frequencies among the sampled populations.

Fragmented	Acacia	Doan Brook/Lakeview	Euclid Creek	
Acacia		0.102	0.127	
Doan Brook/Lakeview	**0.001**		0.026	
Euclid Creek	**0.001**	**0.001**		
Continuous	Minister Creek 1	Minister Creek 2	Minister Creek 3	Minister Creek 4
Minister Creek 1		-0.0002	0.006	0.015
Minister Creek 2	0.481		-0.002	0.003
Minister Creek 3	0.068	0.753		-0.001
Minister Creek 4	0.003	0.185	0.529	

Table 5. Pairwise comparisons of G''_{ST} estimates across eight polymorphic loci for the fragmented and continuous landscapes. G''_{ST} values are above the diagonal and corresponding p-values below. In the fragmented landscape, the Acacia population approaches nearly half of the total amount of differentiation possible based on the heterozygosity that is present, whereas in continuous habitat, the greatest differentiation observed is only five percent of the total possible differentiation.

Fragmented	Acacia	Doan Brook/Lakeview	Euclid Creek	
Acacia		0.484	0.538	
Doan Brook/Lakeview	**0.001**		0.102	
Euclid Creek	**0.001**	**0.001**		
Continuous	Minister Creek 1	Minister Creek 2	Minister Creek 3	Minister Creek 4
Minister Creek 1	*	-0.001	0.020	0.051
Minister Creek 2	0.481	*	-0.009	0.010
Minister Creek 3	0.068	0.753	*	-0.002
Minister Creek 4	0.003	0.184	0.529	*

Figure 1. A) Land cover map and spatial arrangement of urban *P. cinereus* populations located in Cuyahoga Co, OH., Doan Brook (DB); Lakeview Cemetery (LV); Acacia Reservation (AR); and Euclid Creek (EC). Distances between populations range from 1.9–8.4km. **B)** Land cover map and spatial arrangement of continuous populations of *P. cinereus* located in the Allegheny National Forest. Distances between sampled populations range from 1.7–6.7km. Minister Creek campground was used a reference point to mirror the sampling arrangement of fragmented populations.

Figure 2. A) Population structure of *P. cinereus* within the fragmented landscape across 10 replicate runs of STRUCTURE. $\Delta K = 2$ with little admixture present between genetic clusters identified. **B)** Analysis of OH population sub-structure revealed the presence of two distinct genetic clusters, with Lakeview Cemetery and Doan Brook grouping together. **C)** Population structure of *P. cinereus* from continuous forest across 10 replicate runs of STRUCTURE. $\Delta K = 2$ with the majority of individuals maximally assigning to a single genetic cluster, indicative of a lack of genetic structuring.

Figure 3 A) Contemporary gene flow estimates for the genetic populations in OH. The value within each rectangle denotes the proportion of the population that are non-migrants while the proportion of migrants per generation into each population is adjacent to the corresponding arrows. The proportion of the migrants per generation ranged from 0.5 to 3.0% with the exception of migrants from EC into the DV population. **B)** Historical gene flow and estimates of effective population sizes (value inside rectangle) for the genetic clusters identified within the fragmented landscape. The proportion of migrants per generation observed was lower than contemporary estimates, ranging from 0.4 to 1.4%. **C)** Temporal changes in gene flow that were calculated by M_H - M_C, where solid lines indicate an increase in gene flow and dashed lines denote decreases. Decreases or increases <1% likely represent no substantial temporal change in gene flow.

Figure 4 A) Contemporary gene flow estimates for sample localities in PA. The value within each rectangle denotes the proportion of the non-migrant population while the proportion of migrants per generation into each population is shown above and below the corresponding arrows. The proportion of the migrants per generation ranged from 1.3-7.8%. MC3 contributes a large proportion of migrants to the remaining populations, ranging from 21 – 27%. **B)** Historical gene flow and estimates of effective population sizes for the genetic populations in PA. The proportion of migrants per generation observed were lower than contemporary estimates, with over half of the values <1% and the remaining estimates ranging from 1.1-1.5% with the exception of gene flow from MC2 into MC4. **C)** Temporal changes in gene flow that were calculated by M_H - M_C, where solid lines indicate an increase in gene flow and dashed lines denote decreases. Temporal changes in gene flow reveal a clear change in the direction and magnitude of gene flow patterns among the sampled populations.

Figure 1.

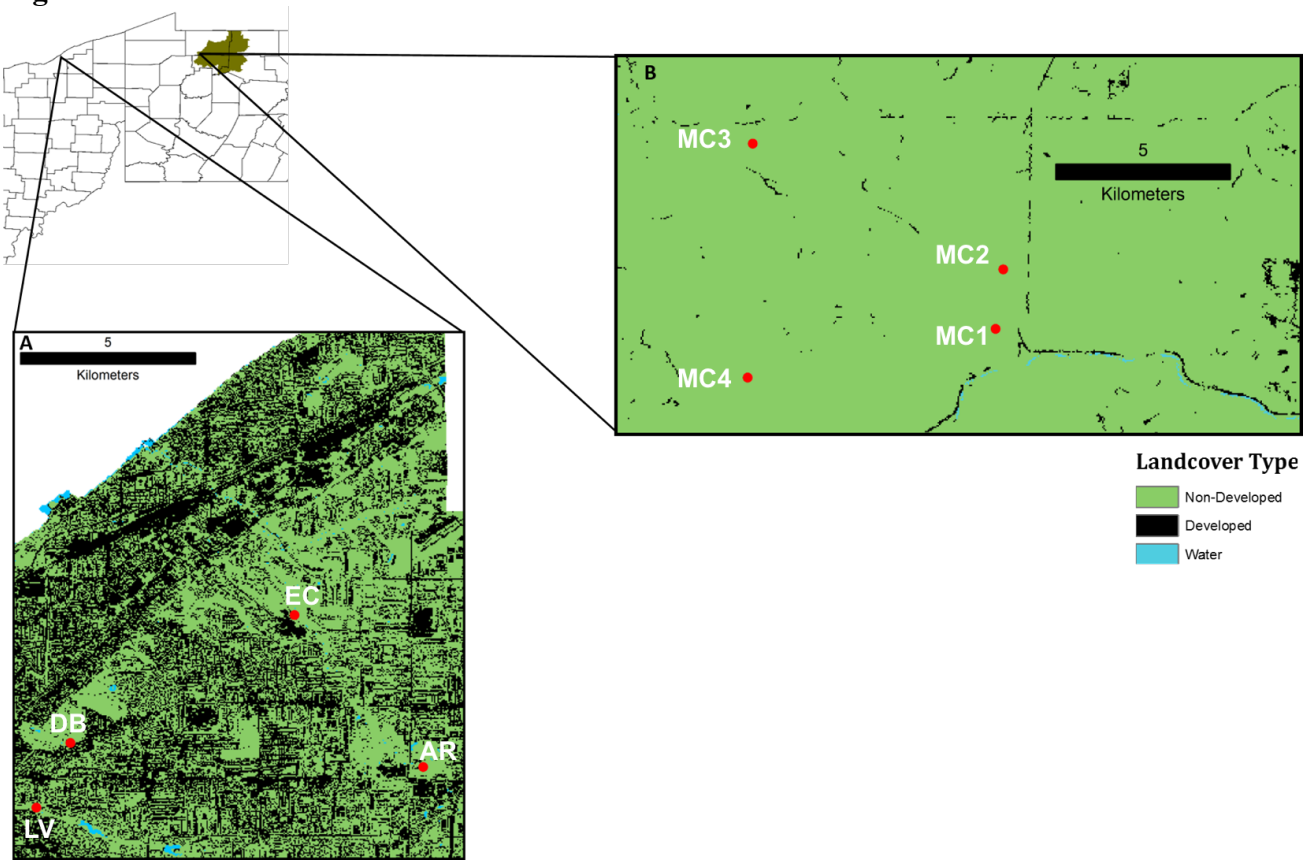

B
MC3
MC2
MC1
MC4
5
Kilometers
Landcover Type
Non-Developed
Developed
Water
A
5
Kilometers
EC
DB
AR
LV

Figure 2.

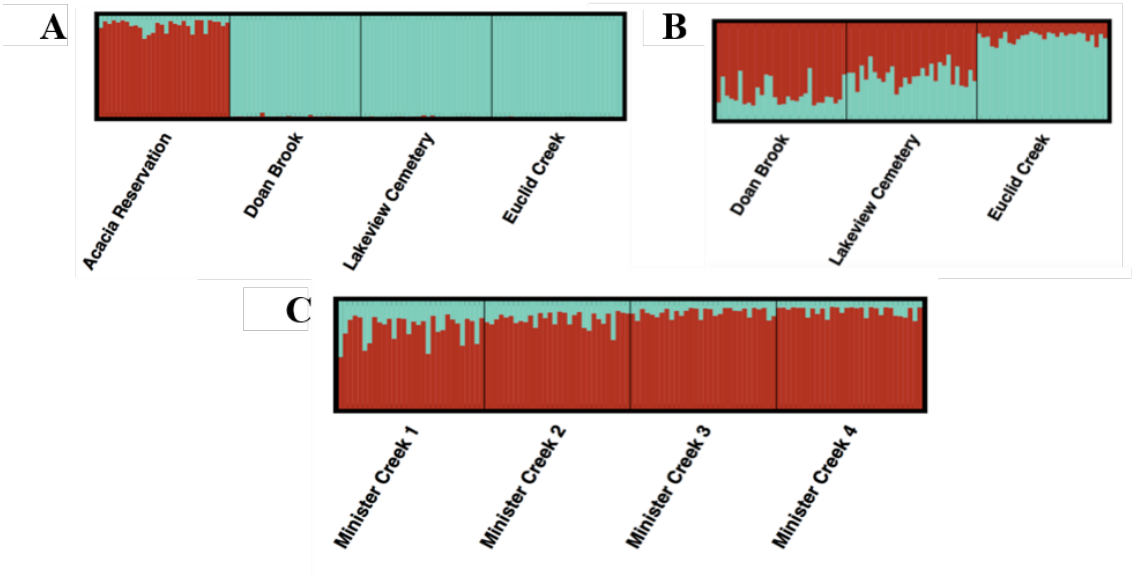

A
Acacia Reservation
Doan Brook
Lakeview Cemetery
Euclid Creek
B
Doan Brook
Lakeview Cemetery
Euclid Creek
C
Minister Creek 1
Minister Creek 2
Minister Creek 3
Minister Creek 4

Figure 3.

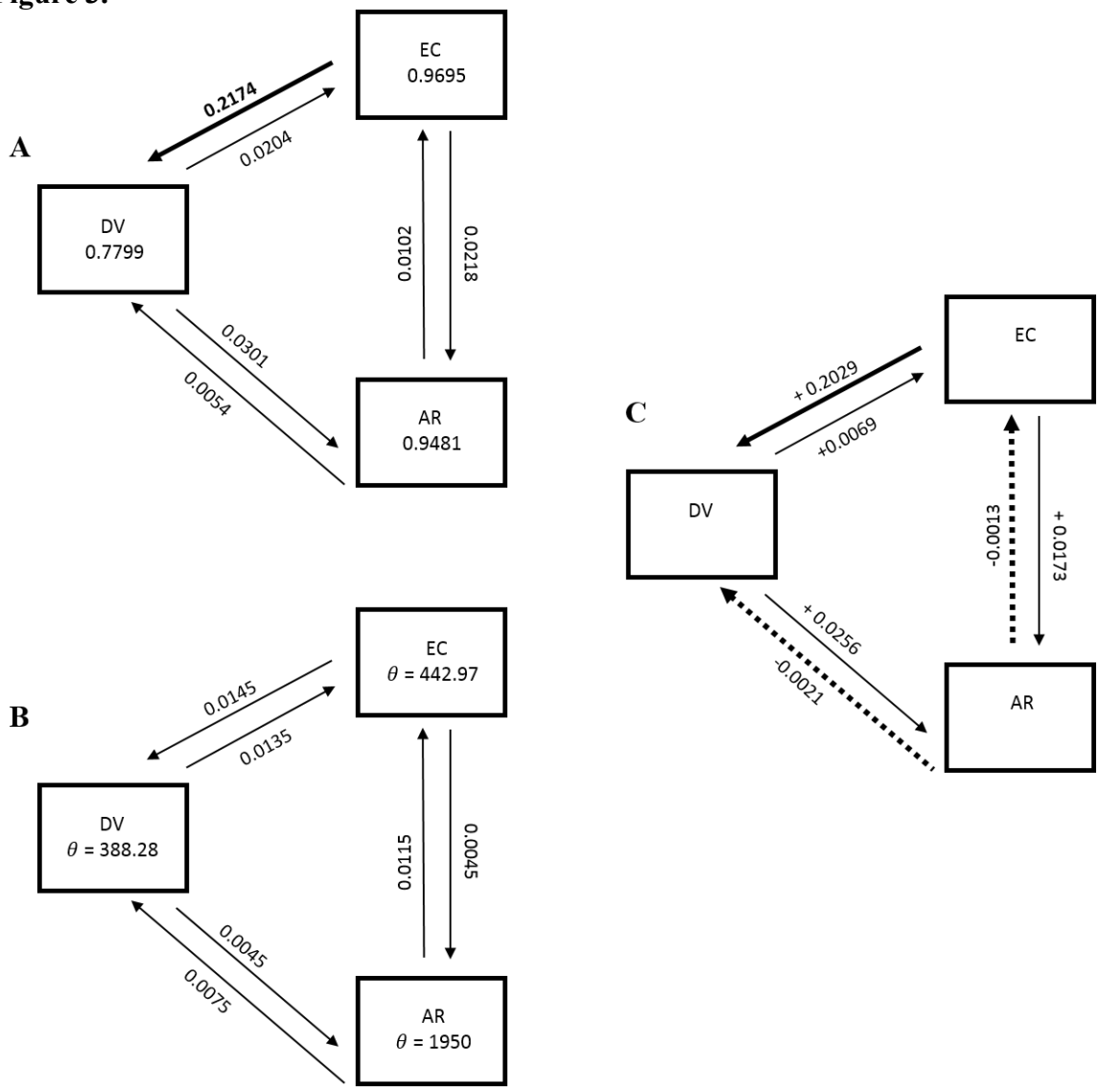
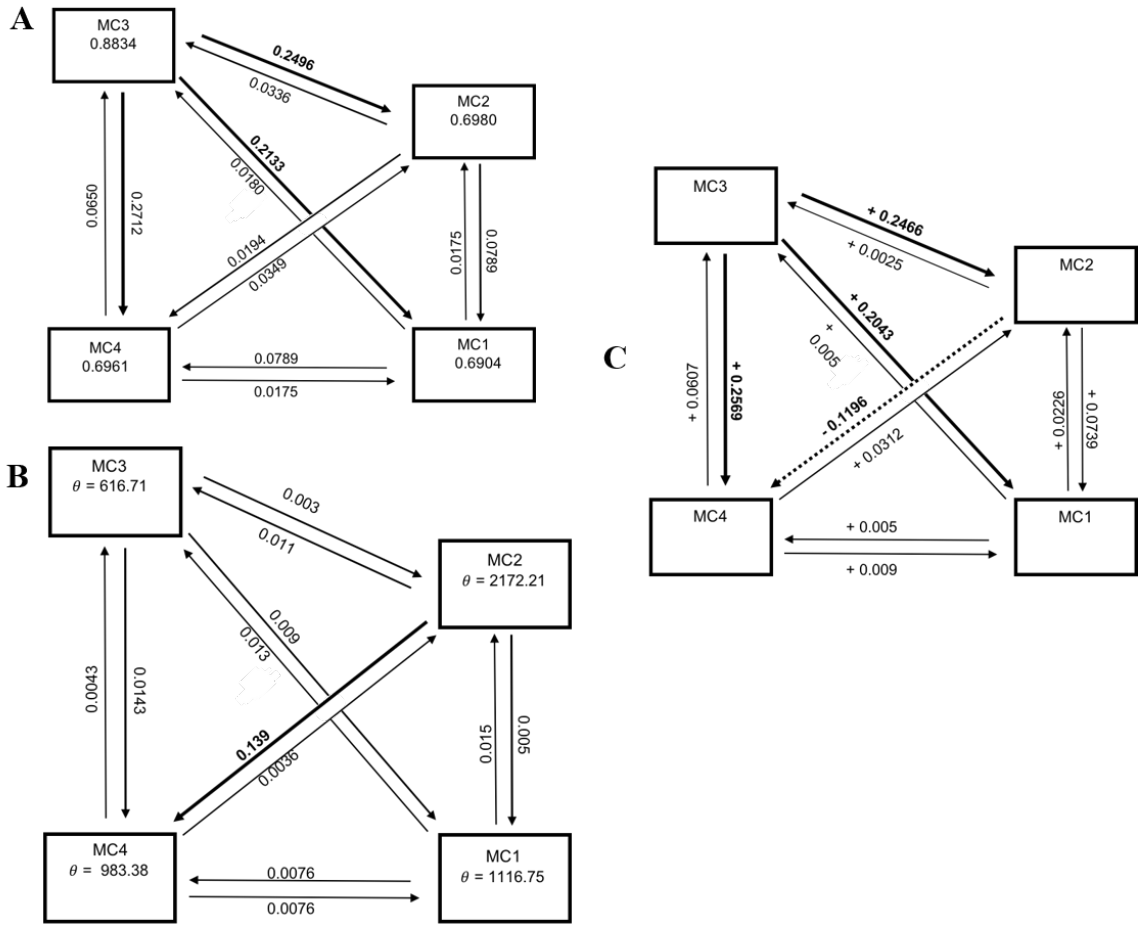

A
EC
0.9695
0.2174
0.0204
DV
0.7799
0.0102
0.0218
0.0301
0.0054
AR
0.9481
B
EC
θ = 442.97
0.0145
0.0135
DV
θ = 388.28
0.0115
0.0045
0.0045
0.0075
AR
θ = 1950
C
EC
+ 0.2029
+0.0069
DV
-0.0013
+ 0.0173
+ 0.0256
-0.0021
AR

Figure 4.


A
MC3
0.8834
MC2
0.6980
MC4
0.6961
MC1
0.6904
0.2496
0.0336
0.2133
0.0180
0.0650
0.2712
0.0194
0.0349
0.0175
0.0789
0.0789
0.0175
B
MC3
θ = 616.71
MC2
θ = 2172.21
MC4
θ = 983.38
MC1
θ = 1116.75
0.003
0.011
0.009
0.013
0.0043
0.0143
0.139
0.0036
0.015
0.005
0.0076
0.0076
C
MC3
MC2
MC4
MC1
+ 0.2466
+ 0.0025
+ 0.2043
+ 0.005
+ 0.0607
+ 0.2569
- 0.1196
+ 0.0312
+ 0.0226
+ 0.0739
+ 0.005
+ 0.009