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The importance of terrestrial dispersal for connectivity among headwater salamander populations

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Abstract. Many organisms are primarily constrained to the channels of the upstream terminus of river networks with limited capacities for both in-stream and overland dispersal. Dispersal is believed to contribute to gene flow and the demographic stability of headwater populations. While assumed, the importance of overland dispersal to gene flow is largely unexplored in headwater salamanders. Six microsatellite markers were used to assess patterns of genetic population structure for a headwater salamander, Desmognathus fuscus. Tissue samples were collected using a nested hierarchical study design, which specifically addressed the contribution of overland movements to the genetic connectivity of headwater populations. Genetic divergence was significant among all populations (Fst = 0.027–0.405) and at all hierarchical spatial scales. However, lower degrees of genetic population structure were observed among proximal streams that shared no downstream hydrologic connections. Mantel tests indicated a significant relationship between genetic divergence and overland distance, suggesting that D. fuscus populations conform to an isolation-by-distance model of gene flow. Estimated dispersal among headwater streams was limited, with an average of less than one effective migrant per generation moving between paired headwater streams. These results indicate that regional patterns of genetic population structure are influenced by overland dispersal and suggest the importance of terrestrial movements in maintaining population connectivity among D. fuscus populations.

Key words: amphibians; Desmognathus fuscus; genetic population structure; movement; river networks; streams.

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INTRODUCTION

Dispersal plays a key role in determining the population dynamics and genetic structure of organisms with patchy distributions. The capacity of an organism to disperse is driven largely by its ability to move between habitat patches across a landscape matrix characterized by suboptimal habitat (Turner et al. 1995). There may be risks to individual fitness associated with moving across the landscape, such as death or injury, energy costs, or the possibility of settling in a habitat patch that was less suitable than the source (Bonte et al. 2012). Although dispersal provides an opportunity to colonize unoccupied habitat patches (Fagan 2002), it may stabilize or destabilize populations depending on the rate of dispersal among populations (Brown and Kodric-Brown 1977, Labonne et al. 2008, Grant et al. 2010). Emigration may provide an opportunity for individuals to avoid competition, particularly with kin groups (Bowler and Benton 2005). Gene
flow associated with dispersal also increases genetic relatedness among subpopulations (Bohonak 1999) and may promote or preserve genetic diversity by facilitating gene flow among subpopulations (Lacy 1987) and augmenting within-patch population densities (Lowe and Allendorf 2010).

For many stream species, the ability to disperse among streams is directly related to physiological tolerance of terrestrial environments. Dispersal for organisms such as fish is constrained to the river channel due to morphological traits, such as gills, which preclude most fishes from terrestrial movement (Hughes et al. 2009). Therefore, patterns of genetic population structure are largely shaped by the hierarchical spatial structure of stream networks. In contrast, many headwater species are not completely constrained to the stream channel and are capable of varying degrees of movement outside the channel depending on environmental conditions and tolerance of desiccation. Finn et al. (2007) demonstrated how limited overland movement would create neighborhoods of relatedness, with dispersal of crawling stream insects occurring mainly among closely associated headwaters. Flying stream insects may have highly mobile adult stages, which can easily move over large areas among streams and often exhibit an isolation-by-distance pattern of genetic population structure (Wilcock et al. 2003). However, the importance of terrestrial dispersal to gene flow is largely unexplored for vertebrate headwater species that may be prone to desiccation while moving through upland areas.

Our purpose was to investigate the importance of overland dispersal to gene flow for a species of headwater salamander, *Desmognathus fuscus*. *Desmognathus fuscus* has a biphasic life cycle, with a distinct aquatic larval phase and an adult phase capable of overland movements (Danstedt 1975). A previous mark-recapture study of *D. fuscus* found approximately 12% of overland movements were among neighboring branches of second-order watersheds separated by 300–500 m (Grant et al. 2010). These results suggested that overland dispersal was occurring among neighboring streams, similar to that observed with crawling invertebrates. However, the contribution of dispersers to gene flow has not been investigated. Additionally, the presence of long-distance in-network dispersal, while unlikely, could not be ruled out due to shared downstream hydrologic connections.

Here, we used microsatellite markers to infer the degree of terrestrial dispersal occurring among headwater populations of *D. fuscus* from patterns of genetic population structure. Due to their small size and small limbs, we expected that movement among streams by *D. fuscus* would be relatively rare and result in significant genetic structuring, even among populations occupying closely associated streams. Additionally, we predicted that headwater populations of *D. fuscus* would show neighborhoods of relatedness similar to crawling aquatic insects, as overland dispersal would make significant contributions to gene flow among headwater populations of *D. fuscus*.

**METHODS**

We sampled salamanders from eight watersheds throughout the Washington, D.C., and Baltimore Metropolitan region (Fig. 1). Each watershed flows independently into the Chesapeake Bay, which we assumed to be an impassible barrier for *D. fuscus*. Watersheds were arranged into four replicate pairings so that headwaters of neighboring watersheds were in proximity to one another. Therefore, genetic similarities among paired populations from different watersheds would result from overland movement (assuming the Chesapeake Bay is a barrier to movement within the channel). Two streams were sampled in each watershed, one upstream and one downstream reach (Fig. 1). Within each watershed, streams were separated by the main river channel, which represented suboptimal habitat for *D. fuscus*. Our study design resulted in populations paired between watersheds being separated by 0.9–4.4 km of upland habitat and populations paired within watershed being separated by 10.7–19.7 km of stream channel.

We collected tissue samples from June through October 2012 by removing a small portion of tail from juvenile and adult salamanders. In general, about 20 tissue samples were collected on a single visit. However, several stream reaches were sampled over the course of several weeks in order to obtain the targeted sample size. In order
Fig. 1. Map of sampled streams in central Maryland region. Paired watersheds are indicated on regional map from north to south: (A) Bynum Run–Winters Run; (B) Little Gunpowder Falls–Gunpowder Falls; (C) Jones Falls–Gwynns Falls; (D) Patuxent River–Seneca Creek. A graphical representation of hierarchical sampling scheme is shown in bottom inset.
to avoid sampling the same individual more than once, we did not sample salamanders that were missing the distal portion of the tail on subsequent visits. Genomic DNA was extracted from all samples using the Promega Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Six tetranucleotide microsatellite primers developed for *Desmognathus auriculatus* (Croshaw and Glenn 2003) and *Desmognathus ocoee* (Adams et al. 2005) were found to consistently amplify and exhibit polymorphism (Appendix A). Fluorescently labeled forward primers were used in the polymerase chain reaction (PCR) amplification of microsatellite loci. Allele identities of labeled amplicons were assigned using a Beckman Coulter Genetic Analyzer (CEQ8000 platform; Beckman Coulter, Brea, California, USA).

To describe the degree of genetic differentiation among populations we used the program FSTAT (v.2.9.3; Goudet 1995) to calculate $F_{st}$ following Weir and Cockerham (1984). In order to validate the degree of genetic divergence estimated using $F_{st}$, and for comparison with other studies, we calculated other measures of genetic differentiation (Nei 1973, Nei and Chesser 1983, Jost 2008) using the DEMETics package (v.0.8-7; Gerlach et al. 2010) in program R (R Core Team 2013). For all measures of genetic differentiation among populations, we assessed significance using resampling (1000 bootstraps) followed by sequential Bonferroni corrections (Holm 1979).

To determine how watershed boundaries influence gene flow, we estimated the hierarchical partitioning of genetic population structure by performing a four-level hierarchical analysis of molecular variance (AMOVA) using the hierfstat (Goudet 2005) program in R (R Core Team 2013). Hierarchical $F$-statistics were estimated at the following four levels: (1) among individuals within reaches, (2) among reaches within watersheds, (3) among watersheds within paired watersheds, and (4) among watershed pairs. We determined significance at each level using a likelihood-ratio statistic based on 1000 permutations.

To assess region-wide patterns of genetic population structure, we used Bayesian assignment tests implemented in STRUCTURE (v.2.3.4; Pritchard et al. 2000). We tested ten levels of $K$ with 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions after a burn-in of 200,000 steps, with 10 replicates for each level of $K$. We evaluated the probability of each level of $K$ using the posterior probability method described by Pritchard et al. (2000) and created bar plots using DISTRUCT (v.1.1; Rosenberg 2004).

We estimated coalescent-based migration rates ($M$) and effective population size parameters ($\Theta$) using a maximum-likelihood approach in LAMARC (v.2.0; Kuhner 2006). All estimates were carried out using 10 initial MCMC chains of 10,000 trees and two final chains of 200,000 trees each. Each chain had a burn-in period of 1000 steps. All loci were assumed unlinked. We used these parameters to estimate effective migration rates ($N_e m$) among streams in watershed pairings in order to investigate the relative importance and probability of out-of-network gene flow. We calculated effective migration rates by multiplying the value of $M$ by the $\Theta$ of the recipient population, and dividing by four. This value gives an estimate of the total number of migrants from a source population, which contributes to the gene pool of a recipient population per generation (Beerli and Felsenstein 2001).

To evaluate the relationships between genetic distances and two different measurements of physical distance among sites (stream distance and geographic distance), we preformed Mantel tests with 1000 permutations implemented using the package ecodist (v.1.2.9; Goslee and Urban 2007) in R (R Core Team 2013). We estimated stream distance, defined as the shortest possible in-network path between paired sites, using the National Hydrography Dataset and the Network Analyst Extension in ARCGIS 10.1 (ESRI 2011). We estimated geographic distance, defined as the shortest straight-line distances among sites, using geographic coordinates and the RASTER package (v. 2.2-12; Hijmans and van Etten 2012) in R (R Core Team 2013).

**Results**

Within populations most loci were in Hardy-Weinberg equilibrium (94.8%) and there was no evidence of significant linkage among loci (Appendix B). Pairwise $F_{st}$ values among *D. fuscus*
populations ranged from 0.027 to 0.405 (median = 0.137) and were all significantly different from zero (Appendix C). All other measures of genetic distance were consistent with the patterns of divergence observed with $F_{st}$ (Appendix C); therefore, the following analyses use only $F_{st}$ values. Hierarchical measures of genetic differentiation indicated greater genetic population structuring among streams within watersheds ($F_{st} = 0.091$), than among paired watersheds ($F_{st} = 0.027$; Table 1). The Bayesian STRUCTURE analysis grouped individuals into five genetic clusters. Individuals among watersheds located at the north and south ends of the sampling region had high probabilities of being assigned to genetic clusters representative of their respective watershed pairings (Fig. 2). In contrast, watersheds occurring at intermediary points along the north-south gradient exhibited lower genetic structure, which suggests greater gene flow among these watersheds.

Overall, migration among streams within each watershed pair was low ($N_{em} = 0.11$ to 0.51), but significant. Average effective migration rates were similar among paired watersheds ($\bar{x} = 0.30$) and within individual stream networks ($\bar{x} = 0.26$). Migration was often asymmetrical between stream pairings, with no real relationship emerging with either directionality or magnitude along dispersal corridors (Appendix D).

Mantel tests indicated significant relationships between genetic distance ($F_{st}$) and both geographic distance ($P = 0.001$) and stream distance ($P = 0.019$; Appendix C). Neither geographic distance nor stream distance explained a large proportion of genetic variance ($R^2 = 0.193$ and 0.157, respectively). The slope of the isolation-by-geographic distance model ($\beta = 0.00134$; 95% CI = 0.00084–0.00184) was significantly greater than the slope of the isolation-by-stream distance model ($\beta = 0.00018$; 95% CI = 0.00010–0.00025).

**DISCUSSION**

Using microsatellite genotypes, we provide evidence of overland dispersal and its contribution to gene flow among *D. fuscus* populations associated with headwater stream channels. If movements were restricted to the stream hierarchy, watershed boundaries would be expected to act as a distinct barrier to gene flow, increasing
genetic divergence among neighboring watersheds despite the shorter overland distance among adjacent watersheds. In this case a strong relationship between genetic distance and the hierarchical stream network is expected (Hughes et al. 2009). Instead, patterns of genetic population structure for *D. fuscus* indicated a neighborhood effect, similar to that observed in crawling aquatic insects (Finn et al. 2007). The results of an AMOVA indicated that genetic divergence was lowest among headwater stream populations from neighboring watersheds ($F_{st} = 0.027$) when compared to those populations paired within watersheds ($F_{st} = 0.091$). Additionally, Bayesian assignment tests revealed associations among neighboring watersheds (Fig. 2) in a number of cases and we found a significant but weak isolation-by-geographic distance relationship among all populations (Appendix C: Fig. C2).

Although patterns of genetic population structure indicated the significance of overland movements, our results also suggest that the extent of dispersal via overland or within channel movement was limited. Pairwise $F_{st}$ comparisons were significant among all populations indicating limited outbreeding, even among the most closely associated headwater populations. Additionally, average migration rates among sites were all less than one effective migrant per generation but significantly different from zero, suggesting that dispersal among headwaters and along stream channels that results in successful reproduction are relatively rare events. Grant et al. (2010) observed overland movement probabilities of approximately 11.5% and within channel movement probabilities of approximately 16.9% over a two-year period; rates that are substantially higher than suggested based on our genetic data. They also found that movement within and among stream reaches occurred almost exclusively in recently metamorphosed juveniles, but survival to maturity among juvenile salamanders was very low (7.5%). Therefore, the low effective migration rates observed in our study may have resulted from higher rates of dispersal of juveniles with low rates of survival to maturity and breeding success.

Differences between studies in landscape structure and disturbance by humans may also be responsible for differences in estimated dispersal rates. Distances among populations were much greater in our study when compared to that of Grant et al. (2010); distance between paired headwaters was between 300 to 500 m in the Grant et al. study and from 0.9 to 4.4 km in our study. Grant et al. (2010) also conducted their study in the Shenandoah National Park where headwater streams are connected by upland forest. In contrast, our study was conducted in a landscape dominated by human land uses that may impede salamander movement both within and outside of the channel. Open spaces, roads, building, fences and walls all have the potential to limit dispersal of amphibians in urban landscapes (Hamer and McDonnell 2008), but no studies have addressed limitations on dispersal imposed by urban infrastructure.

Although our study design does not isolate in-stream dispersal, our results suggest that in-stream dispersal events are important in determining genetic population structure. Within our study design, salamanders could move between populations within the same watershed via the stream channel or via overland dispersal between headwater streams (i.e., using headwater streams as stepping stones). However, the significantly lower slope for the relationship between stream distance and genetic distance when compared to the relationship between geographic distance and genetic distance suggests that movements within watersheds are via the stream channel. Consistent with the results from mark recapture studies (Grant et al. 2010), our results suggest that the probability of dispersal is similar for in-network and out-of-network routes, but in-network resistance may be less than out-of-network resistance.

The limited dispersal abilities and reliance on out-of-network dispersal of *D. fuscus* may be related to their relatively small size and lack of external respiratory structures. Desmognathine salamanders are small salamanders that rely on cutaneous respiration as juveniles and adults (Petranka 2010) and our results indicate limited dispersal along both within and out-of-network pathways. Other stream-associated salamanders with larger body sizes (e.g., *Cryptobranchus alleganiensis*; Unger et al. 2013) or that maintain external gills as juveniles and adults (e.g., *Dicamptodon copei*; Steele et al. 2009), exhibited
reduced genetic population structuring ($F_{st} < 0.001–0.281$) and conformed more closely to a stream hierarchy model when compared to *D. fuscus*, suggesting that larger size or external gills, or both may constrain dispersal to the stream corridor but allow greater movement within these corridors. The limiting role of external gills is also supported by genetic population structure of *D. tenebrosis*, a species of giant salamander that can exhibit a biphasic life cycle with a distinct, terrestrial adult stage and that exhibits no significant isolation between neighboring watersheds, unlike its predominantly paedomorphic conspecific (*D. copei*; Steele et al. 2009). This suggests that terrestrial dispersal is a common characteristic of biphasic salamanders, in spite of differences in the extent of dispersal that are most likely related to body size (*D. fuscus*: $F_{st} = 0.027–0.405$; *D. tenebrosis*: $F_{st} = 0.000–0.104$).

Worldwide, many amphibians are experiencing widespread declines. However, small headwater salamanders, such as *D. fuscus*, seem to have much more stable populations than pond or riverine amphibians (Green 2003). One factor that may contribute to the relative stability of these populations is the use of multiple dispersal pathways and in particular, overland dispersal (Grant et al. 2010). Large river-dwelling salamanders that are apparently not capable of overland dispersal, such as *C. alleganiensis*, seem to be rare (Petranka 2010). While overland dispersal may not be the only factor at play, Grant et al. (2010) was able to demonstrate, using a simulation model, that even limited overland dispersal dramatically increased the probability of population persistence for desmognathine salamanders under scenarios with low to moderate extinction rates of local populations.

Current management plans, which focus predominantly on the conservation of stream habitat and the closely associated riparian buffer, may inadequately address issues of population connectivity for headwater salamanders given the potential importance of overland dispersal. Integrative approaches that incorporate the conservation of upland habitat may be very important for maintaining connectivity among populations of headwater species with capacities for overland dispersal. The results of our Mantel tests indicated that simple isolation-by-distance models alone fail to account for a large proportion of the genetic variance observed ($R^2 < 0.20$). Several streams also exhibited relatively high pairwise $F_{st}$ estimates ($F_{st} > 0.20$), a sign of possible population isolation. These results suggest that factors other than distance alone may be influencing the extent of dispersal among headwaters. Habitat alterations related to fragmentation, deforestation, agriculture, and urbanization can degrade the quality of stream and upland ecosystems (Allan 2004), and thus may negatively impact the dynamics of headwater populations. The relative importance of different dispersal routes may also vary throughout the range of a species (Dudaniec et al. 2012, Trumbo et al. 2013). Addressing how upland disturbance influences the extent of overland dispersal is a critical next step in understanding the dispersal dynamics of headwater salamanders.

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SUPPLEMENTAL MATERIAL
ECOLOGICAL ARCHIVES

Appendices A–D are available online: http://dx.doi.org/10.1890/ES15-00302.1.sm