Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat

P. R. Cabe, R. B. Page, T. J. Hanlon, M. E. Aldrich, L. Connors, and D. M. Marsh

Department of Biology
Washington and Lee University
Lexington VA 24450

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Correspondence: David M. Marsh. Fax: 549-458-8012. E-mail: marshd@wlu.edu

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Abstract

Several recent studies have shown that amphibian populations may exhibit high genetic subdivision in areas with recent fragmentation and urban development. Less is known about the potential for genetic differentiation in continuous habitats. We studied genetic differentiation of red-backed salamanders (*Plethodon cinereus*), a species with direct-development, across a 2 km transect through continuous forest in Virginia, USA. Mark-recapture studies suggest very little dispersal for this species, while homing experiments and post-pleistocene range expansion both suggest greater dispersal abilities. We used six microsatellite loci to examine genetic differentiation between eight subpopulations of red-backed salamanders at distances from 200 m to 2 km. We also used several methods to extrapolate dispersal frequencies and test for sex-biased dispersal. We found small, but detectable differentiation among populations, even at distances as small as 200 m. Differentiation was closely correlated with distance and both Mantel tests and assignment tests were consistent with an isolation-by-distance model for the population. Extrapolations of intergenerational variance in spatial position ($\sigma^2 < 15$ m^2) and pairwise dispersal frequencies ($4Nm < 10$) both suggest very limited gene flow. Additionally, tests for sex-biased dispersal imply that dispersal frequency is similarly low for both sexes. We suggest that these low levels of gene flow and the infrequent dispersal observed in mark-recapture studies may be reconciled with homing ability and range expansion if dispersing animals rarely succeed in breeding in saturated habitats, if dispersal is flexible depending on the availability of habitat, or if dispersal frequency varies across the geographic range of red-backed salamanders.

Introduction

The study of population genetic structure in fragmented environments has become a cornerstone of ecological genetics research. Studies of genetic differentiation in fragmented habitats can determine the extent to which genetic diversity is lost in isolated populations (Avise & Hamrick 1996; Burns et al. 2004; Funk et al. 2005) and identify the landscape features that restrict dispersal and gene flow (Hitchings & Beebee 1997; Lampert et al. 2003; Funk et al. 2005). These studies can also identify appropriate management units and determine the extent to which individual sub-populations should be protected as distinct genetic units (Moritz 1994; Shaffer et al. 2000; Lecis & Norris 2004). Given the accelerating pace of fragmentation across the globe, most large-scale studies of population genetics are *de facto* studies of habitat fragmentation. As a result, for many organisms we often know less about patterns of genetic differentiation in continuous habitats than in recently fragmented habitats. This is unfortunate, because an understanding of patterns of genetic structure in continuous habitats is essential for determining how these patterns are altered by fragmentation. In addition, continuous environments provide the appropriate historical backdrop for the evolution of many species.

Terrestrial salamanders have been models for the study of behavioral evolution and community ecology (see Jaeger & Forester 1993, Petranka 1998 for reviews), and a better understanding of genetic structure in a continuous environment could inform the study of behavior and ecology of this group. For example, most behavioral studies of red-backed salamanders (*Plethodon cinereus*) have used salamanders from a single area in Southwestern Virginia (e.g. Jaeger 1981; Mathis 1990, 1991; Jaeger et al. 1995; Gillette 2003). The potential for local adaptation in behavior and the scale over which observed behaviors are ecologically relevant will depend on the extent of dispersal, gene flow, and genetic differentiation in these animals. Additionally, terrestrial salamander populations are increasingly being monitored as an index of forest health (e.g. Amphibian Research and Monitoring Initiative, North American Amphibian Monitoring Program, Partners in Amphibian and Reptile Conservation). An improved understanding of dispersal and gene flow could help determine the appropriate scale for selection of monitoring units.

Dispersal and gene flow are currently something of a paradox for terrestrial salamanders. Terrestrial salamanders defend cover objects on the forest floor and oviposit at these sites (Mathis 1989, 1991). Because eggs hatch to small terrestrial juveniles, migration to aquatic sites is not a part of the life-history of these animals. Mark-recapture studies consistently show small home ranges (5 -25 m^2) and very little movement among cover objects (Kleeberger & Werner 1982; Mathis 1991; Marvin 1998), even across multiple years (Gillette 2003). However, Kleeberger & Werner (1982) showed that adult red-backed salamanders could home successfully at distances up to 90 m – much larger distances than any observed home range. Additionally, Marsh et al. (2004) found that young adult red-backed salamanders readily colonized experimental plots in open fields with movements on the order of 30 or 40 m. Finally, red-backed salamanders have expanded their distribution considerably since the end of Pleistocene glaciation, and their current distribution goes north to Quebec and Nova Scotia and west to Minnesota and Wisconsin. Assuming a period of range expansion of 18,000 years, their rate of expansion is approximately 80 m/yr. Thus, ecological data alone present contradictory suggestions about dispersal frequencies in red-backed salamanders.

A few previous studies have used genetic markers to investigate population structure in red-backed salamanders. Highton and Webster (1976) used protein electrophoresis to document high levels of divergence among populations throughout the range, though their closest populations were 32 km apart, and most population samples were separated by hundreds of kilometers. Using the same genetic markers, Highton (1977) also showed marked genetic differences on a finer scale (0.5-35 km). Gibbs (1998), using RAPD DNA markers, found that the degree of differentiation among samples was affected by recent forest fragmentation. He also found that in continuous forest, genetic divergence was weakly correlated with distance (Gibbs 1998). Finally, Sites et al. (2004) analyzed mitochondrial DNA and found that red-backed salamanders formed a monophyletic clade with some differentiation across Virginia, West Virginia, and Kentucky, USA.

Collectively, these studies suggest that red-backed salamander dispersal and gene flow are limited at the scale of kilometers. However because these studies were on relatively large scales, they cannot resolve whether terrestrial salamanders regularly disperse moderate distances (as suggested by homing and range expansion) or rarely move at all (as suggested by mark-recapture studies).

We used microsatellite markers to examine genetic population structure in red-backed salamanders along a 2 km transect passing through continuous forest in the Appalachian mountains of Southwestern Virginia. In addition to passing through continuous forest, this transect contained no streams, ridges, or other likely barriers to dispersal. We asked whether subpopulations separated by a range of distances from 200 m – 2 km were locally differentiated. We also used several methods to extrapolate dispersal rates from these data. Finally, we used patterns of relatedness to determine whether dispersal in red-backed salamanders appears to differ between males and females.

Materials and Methods

Study Area

Our study site is located in Giles County, Virginia, USA in the Jefferson National Forest at approximately 37.41 N and –80.50 W. It ranges in elevation from 1150 to 1250 m. The site consists of mixed deciduous hardwood forest and has no obvious heterogeneity in stand age or potential dispersal barriers such as streams and rocky ridges. At the study site, we established a straight transect 2 km long with the aid of a GPS receiver. Along the transect, we marked off eight 50 m^2 plots with plots separated by either 200 m or 300 m (Figure 1). This design was used as a compromise between the need for a range of pairwise distances between plots and the need for replicates at each distance.

Collection and Laboratory Methodology

We collected 48 red-backed salamanders on each plot between 24 April and 6 August 2003. Salamanders were found by searching under naturally occurring cover objects, such as rocks and logs, and by sifting through the leaf litter. For all but one plot, all salamanders were

collected on a single day to avoid recapturing individuals. We determined the sex of as many individuals as possible in the field by checking for the presence of testes as described by Gillette & Peterson (2001). Juvenile salamanders were not sexed. From each captured salamander, we removed approximately 1 cm of tail tissue with forceps and placed it in a sterile 1.5 ml microtube containing collection buffer (10 mM Tris, 10 mM EDTA, pH 8). Following tissue collection, salamanders were released at their points of capture. We placed samples on ice during transport to the laboratory, and performed DNA extraction within 24 h. Genomic DNA was extracted using the reagents and suggested protocols from the Promega Wizard Genomic DNA Purification Kit. We ground tissue samples in 500 µl of Nuclei Lysis Solution, incubated at 65° C for 20-30 minutes, treated with RNase at 37° C for 25 minutes, treated with 170 µl of Protein Precipitation Solution, and centrifuged. The supernatant was decanted and the DNA was precipitated using isopropanol. The DNA pellet was then washed with 70% ethanol, dried, and rehydrated with TE. DNA samples were stored in a freezer and dilutions of this stock (1:4 or 1:9) were used as templates for PCR.

We amplified five microsatellite loci (PcI16, PcLX16, PcLX23, PcJX06, and PcFX08) following the protocols detailed in Connors & Cabe (2003). While the original protocols specified multiplex reactions, some loci were amplified independently to increase yields. One additional locus, PcXD23 (primers HEX/GCAAAACAGCAACAAGACAAC, AACCTTGATGTTTGGCAAGG, Genbank accession number AY151376) was amplified using similar protocols (Cabe, unpublished).

After verifying the success of our PCR via agarose gel electrophoresis, PCR products were shipped to the Advanced Genetics Analysis Center at The University of Minnesota where they were sized using ABI 377 or 3100 DNA sequencers and GENESCAN software. Genotypes were determined in our laboratory using GENOTYPER software. We manually inspected each allele call, and manually binned alleles.

Data Analysis

We used Microsatellite Analyzer (MSA) to obtain observed and expected heterozygosities, allele counts, and size ranges (Dierenger & Schlotterer 2003). We then used GenePop, Version 3.4 (Raymond & Rousset 1995) to test each locus for Hardy-Weinberg proportions. The probabilities from these tests were adjusted for an experiment-wise probability of 0.05 using the sequential Bonferroni correction suggested by Weir (1990). We tested for linkage disequilibrium for each pair of loci in the same manner.

Fixation indices (F_{ST}) were calculated across all loci and for each locus in Arlequin, Version 2.000 (Schneider et al. 2000) using an Analysis of Molecular Variance (AMOVA) framework (Weir & Cockerham, 1984; Excoffier et al. 1992). In addition, we used Arlequin to calculate pair-wise estimates of F_{ST} between all pairs of plots. The significance of these estimators were assessed using a non-parametric data permutation approach (Excoffier et al 1992; Schneider et al. 2000). We chose to calculate F_{ST} values rather than R_{ST} values because of the better performance of F_{ST} estimates when divergence among samples is expected to be low (Balloux & Goudet 2002; Balloux & Lugon-Moulin 2002).

We also used Arlequin to assess our ability to assign the genotypes of individuals to the plots from which they came. This procedure determines the log-likelihood of observing each multilocus genotype in each of a series of plots. Log-likelihoods are calculated by using the allelic frequencies from the observed data for each plot in the series. The "global individual likelihood" of an individual coming from a given plot is the product of the likelihood of that individual coming from this plot for each locus of the individual's genotype (Schneider et al. 2000).

The relationship between genetic and geographic distance was investigated with a Mantel test performed with ZT software (Bonnet & Van de Peer 2002). Using this procedure, we examined the correlation between the pair-wise F_{st} distance matrix and the geographic distance matrix.

Estimates of gene flow and genetic neighborhood size ($4D\pi\sigma^2$ or 4Nm respectively) were obtained via two methods. First, we used Rousset's (1997) methodology for estimating these parameters for two-dimensional habitats. This procedure regresses $F_{ST}/(1-F_{ST})$ on the natural logarithm of distance. Second, we estimated 4Nm using maximum-likelihood estimation in Migrate, Version 1.5 (Beerli & Felsenstein 1999, 2001). To obtain these estimates, we followed the recommendations of Beerli (2002): (1) we did an initial run on our data set using F_{ST} to find the start parameters, (2) we used the output of the initial run as the start parameters of our second run. Because there were only minor differences between the outputs from the first run and the second run, we stopped after the second run, and present output from the second run in the results below.

Both of these methods for estimating migration rates may have important limitations with respect to our data set. The F_{ST}-based approach is limited because of the numerous theoretical assumptions that must be made in order to equate F_{ST} and gene flow (see Beerli & Felsenstein 2001; Neigel 2002). The maximum-likelihood approach is limited by the practical problems associated with estimating migration rates via maximum likelihood when there are several samples, several loci, and a large number of individuals (Beerli 2002; Neigel 2002). Despite these limitations, we used both methods in the hope that broad, qualititative inferences about dispersal would be consistent across methods.

Finally, we examined the data for evidence of sex-biased dispersal. Within each plot, we calculated a relatedness coefficient, R (Queller & Goodnight 1989) for each pair of individuals of the same sex, and then compared mean R-values for each sex using a T-test paired by plot. In general, the sex which disperses less would be expected to show higher average relatedness (Queller & Goodnight 1989). Statistical significance for this test was determined by 1000 random permutations of genotypes among individuals. In addition, we used assignment indices in Fstat, Version 2.9.3.2 (Goudet 2001) as an alternative approach for investigating sex-biased dispersal. This method involves comparing the means of each sex's corrected assignment index (AIc) (Goudet 2001). The assignment index is based on the probability of a genotype being found in a given sample and is "corrected" (AIc) for differing levels of allelic diversity among samples (Goudet 2001). The sex with the higher AIc would be expected to disperse less, and we tested the significance of differences in AIc values between sexes with 10,000 permutations of genotypes.

Results

Summary Statistics

All loci within each sample plot were in Hardy-Weinberg (H-W) proportions with a single exception, locus PcLX16 in plot S, which had an excess of heterozygotes. However, deviations from H-W of this locus in other sample plots did not approach statistical significance. There was also no evidence for genotypic disequilibrium. Descriptive statistics for the entire study site are given in Table 1 and plot-specific statistics are provided in Table 2.

AMOVA indicated that the majority of the genetic variation (98.1%) resided within plot samples. However, the tests for genetic structure yielded a small but highly significant site-wide fixation index ($F_{ST} = 0.019$, $P<0.00001$). More surprisingly, the majority (23 of 28) of the pair-wise plot comparisons also yielded significant F_{ST} values (Table 3). The estimated probabilities imply strong significance with eighteen of the comparisons yielding $P<0.001$. Three of these non-significant comparisons were between adjacent plots and a single plot separated the remaining two.

Our ability to correctly assign individuals to plots ranged from 0.82 for plots separated by 200 m to 0.94 for plots separated by 2 km (Figure 2). The strong positive relationship between correct assignment probability and distance is consistent with the results of the Mantel test. In this latter test, we found a strong positive correlation between F_{ST} and geographic distance ($r = 0.723$, $P = 0.001$, Figure 3).

The regression of $F_{ST}/(1-F_{ST})$ on ln(distance) yielded a slope of 0.008 (Figure 3). This slope was used to estimate $1/(4D\pi\sigma^2)$, where D is the population density and σ^2 is the intergenerational variance in spatial location (i.e. dispersal distance). This yields an estimate of $4D\pi\sigma^2 \approx 125$ individuals. Mathis (1991) estimated the density near our study site as 2.82 individuals per m^2, giving an estimate of 3.53 for σ^2. However, densities on our study site may be somewhat lower than those at Mathis' site and effective population size is likely to be less than absolute population size. Therefore, we also consider that the effective population size may be half or even 25% of the Mathis (1991) estimate. These assumptions yield values for σ^2 of 7.05 and 14.11 respectively.

The mean maximum likelihood estimate of 4Nm for pairs of plots that were 200 m apart was 7.94 with a range from 2.07 to 18.04 ($n = 8$); the mean maximum likelihood estimate of 4Nm for the pair of plots 2 km apart was 4.13 and the range was 3.31 to 4.96 ($n = 2$). This extrapolates to roughly 2 migrants exchanged per generation at 200m and 1 exchanged per generation at 2 km.

Neither our sex-based comparisons of R nor AIc provide evidence of sex-biased dispersal. Mean pair-wise R-values for males (0.23) and females (0.21) were nearly identical ($T = 0.48$, $P = 0.95$). Similarly, the mean AIc of the 131 males (0.247) and the 109 females (-0.297) were not significantly different ($P = 0.137$). We note that the high values of R simply reflect the

levels of differentiation across the transect and do not imply that individuals within a plot are necessarily close relatives (Queller & Goodnight 1989).

Discussion

Analysis of microsatellite data from red-backed salamanders suggests a continuously-distributed population with very limited dispersal. Differentiation was small (F_{ST} 0.01 to 0.06) but statistically significantly even for some populations separated by as little as 200 m. A Mantel test and Bayesian assignment tests were both consistent with an isolation-by-distance model for population structure. Extrapolations of the second moment of dispersal distance from F_{ST} values (Rousset 1997) suggested mean intergenerational dispersal of less than 20 meters. Similarly, maximum-likelihood estimates of dispersal frequencies (Beerli & Felsenstein 1999) suggest the equivalent of a relatively small number of migrants per generation for plots separated by 200 m. Although estimating dispersal rates from patterns of genetic differentiation is notoriously difficult (see Bohanak 1999; Whitlock & McCauley 1999 for reviews), all our analyses were consistent in their suggestion of limited dispersal for red-backed salamanders within our study site.

This interpretation of the genetic data is consistent with mark-recapture data showing very limited dispersal for nearby populations of red-backed salamanders (Mathis 1991; Gillette 2003). However, these data are more difficult to reconcile with red-backed salamanders ability to colonize new habitats (Marsh et al. 2004), their long-distance homing abilities (Kleeberger and Werner 1982; Marsh et al. 2004), and their substantial post-pleistocene range expansion (Petranka 1998). There are several possibilities that could explain this apparent discrepancy. The first is that dispersal is not necessarily the same as gene flow. Field studies have suggested the presence of a large-proportion of non-territorial floaters who may lack the opportunity to breed (Mathis 1991). Thus, young adults may move fairly long distances but fail to breed in areas that are already near carrying capacity (Jaeger 1980). This scenario could account for the fact that the majority of colonists observed by Marsh et al. (2004) were newly mature adults, and could also explain how salamanders expand their range when suitable habitat becomes available. A related possibility is that dispersal behavior is flexible for individual salamanders. That is, salamanders may have the potential to disperse over a very large area, but use this ability only when suitable but unsaturated habitat is available. This could explain the homing ability of these animals, along with their colonization ability and potential for range expansion. Finally, it is possible that dispersal behavior varies throughout the range of red-backed salamanders. Salamanders in Virginia and near the core of the range may disperse minimally, as most available habitats have likely been colonized. Conversely, closer to range margins, salamanders may have a greater propensity for dispersal. Some variation in behavior is known to exist across the range of red-backed salamanders. Territorial behavior in red-backed salamanders is well-documented from Virginia populations (Mathis 1989, 1991), but appears to be absent from populations in Michigan (Quinn & Graves 1999). Models and empirical results for other species suggest that dispersal may be selected for at range margins, leading to greater dispersal ability at margins relative to core areas (Travis & Dytham 2002, Simmons & Thomas 2004). Thus, there are several potential explanations for the apparently contradictory picture of red-backed salamander dispersal, and each of these possibilities merits further investigation.

Amphibians with direct-development (i.e., no aquatic larval phase) have greatly diversified across North America, South America, and Asia, and may account for 20% of all amphibian species (Duellman & Trueb 1994). In addition to *Plethodon*, this group includes the neotropical frog genus *Eleutherodactylus*, which is currently the most speciose genus of vertebrates (Crother 1999). The one previous set of studies of a genetic structure in a species with direct-development showed that Australian frogs of the genus *Geocrinia* had extremely high levels of genetic differentiation on the scale of a few kilometers (Driscoll 1998a, 1998b, 1999). Using allozymes, Driscoll observed F_{ST} values in the range of 0.30 to 0.69 and genetic neighborhoods as small as 30 m. The more numerous studies of genetic structure in pond-breeding amphibians have found genetic differentiation to be less pronounced, but highly variable among species. Several previous studies have reported detectable genetic differentiation on spatial scales only somewhat larger than the scale of our study (Shaffer et al. 2000, Lampert et al. 2003, Palo et al. 2004). Other studies using microsatellites have found much less genetic structure on these small scales and have suggested that some amphibians may disperse farther than previously recognized (Newman and Squire 2001, Burns et al. 2004). Thus, our study puts terrestrial salamanders near the low end of the range for gene flow in amphibians. Further studies of population structure in other genera with direct-development are needed (e.g. *Eleutherodactylus*, *Philautus*), though it is reasonable to expect that genetic structure may generally be more pronounced for these species.

We found little evidence for a sex-biased dispersal, despite the use of multiple statistical methods and relatively large sample sizes (six loci for approximately 400 individuals). The few other genetic studies of sex-biased dispersal in amphibians suggest female-biased dispersal in some species (Austin et al. 2003, Palo et al. 2004), and male-biased dispersal in others (Lampert et al. 2003). Mark-recapture data on sex-biased dispersal also indicate substantial variation among amphibian species (Marsh & Trenham 2001). Our study suggests that red-backed salamanders may have little sex-biased dispersal, which is perhaps not surprising considering that both sexes are often territorial in this species (Mathis 1991). However, given that dispersal appears limited for both sexes but no evidence for inbreeding (e.g. lower than expected heterozygosities) was found, it appears that red-backed salamanders may have some mechanism for inbreeding avoidance. One possibility is that these salamanders possess a kin recognition mechanism used in mate selection. Although kin recognition has not been demonstrated in *Plethodon*, it is known from several other amphibian taxa (Walls & Roudebush 1991, Pfennig et al. 1993). In addition, red-backed salamanders are known to be able distinguish neighbors from unknown individuals by smell (Jaeger 1981) and males from females from fecal pellets alone (Mathis 1990). Thus, kin recognition is at the very least a plausible mechanism for inbreeding avoidance in red-backed salamanders.

For this study, we selected an area with relatively homogeneous forest and no obvious barriers to dispersal. However, in the Eastern United States, it is almost impossible to select a site with little history of disturbance. Most of the Southern Appalachians region was deforested by the early 20th century (Yarnell 1998) and our study site appears to be no exception. Local oral history and the occurrence of Pitch Pine (*Pinus rigida*) and Black Locust (*Robinia pseudoacacia*) along the ridge suggest that the forest stand at our site is approximately 70-80 years old (HM Wilbur, personal communication). However, it should not be concluded that the red-backed salamander population in our study site is less than 70-80 years old. In general, red-backed

salamander populations are substantially reduced, but not completely absent from recent clearcuts where coarse woody debris remains (Knapp et al. 2003). It is possible that red-backed salamanders persisted in small numbers across the study site, perhaps underneath coarse woody debris or in small patches of remaining trees. This suggestion is certainly consistent with our finding of local differentiation and strong isolation by distance, though some scenarios involving recolonization cannot be ruled out. It would be interesting to know whether forest species survived deforestation during the late 19th/early 20th century by remaining at low densities over a broad area or by recolonizing from remaining forest patches. New methods for inferring population history from current genetic structure (e.g. Beaumont 1999; Fu & Li 1999; Estoup et al. 2004) could potentially answer this question for a range of taxa.

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Table 1 Genetic diversity of pooled samples, including observed and expected heterozygosities, and site-wide fixation indices (F_{ST}) for each locus. *<0.05, **<0.005, and ***<0.0005.

Locus	N	Alleles	H_O	H_E	F_{ST}
PcI16	368	10	0.32	0.30	0.001
PcLX16	384	10	0.72	0.73	0.031***
PcLX23	381	23	0.47	0.43	0.053***
PcJX06	376	19	0.61	0.62	0.020***
PcFX08	369	18	0.85	0.82	0.010**
PcDX23	319	10	0.78	0.82	0.007*

Table 2 Intra-plot demographic data and loci descriptors. A/L ± SE = average number of alleles per locus ± standard error. H_E ± SE = average expected heterozygosity ± standard error. H_O ± SE = average observed heterozygosity ± standard error.

Plot	Males	Females	Unsexed	A/L ± SE	H_E ± SE	H_O ± SE
S	17	17	14	7.333 ± 0.843	0.611 ± 0.080	0.607 ± 0.091
T	18	14	16	5.600± 1.661	0.512 ± 0.129	0.529 ± 0.127
U	25	13	10	8.200 ± 1.960	0.636 ± 0.078	0.638 ± 0.092
V	17	21	10	8.600 ± 1.568	0.601 ± 0.087	0.630 ± 0.097
W	21	20	7	8.400 ± 1.833	0.602 ± 0.090	0.588 ± 0.097
X	20	13	15	8.800 ± 1.497	0.627 ± 0.078	0.617 ± 0.091
Y	13	11	24	9.400 ± 1.364	0.609 ± 0.088	0.652 ± 0.109
Z	0	0	48	9.167 ± 1.352	0.704 ± 0.074	0.706 ± 0.070

Table 3 Pairwise F_{ST} values and significance (above diagonal, * <0.05, ** <0.005, *** < 0.0005) probability estimates bases on 10,100 haplotype permutations.

	S	T	U	V	W	X	Y	Z
S		**	ns	*	**	***	***	***
T	0.015		***	***	***	***	***	***
U	0.002	0.029		ns	*	*	***	**
V	0.010	0.026	0.005		ns	ns	***	***
W	0.013	0.035	0.008	-0.003		ns	***	**
X	0.017	0.028	0.010	0.003	0		***	***
Y	0.029	0.043	0.026	0.023	0.028	0.019		***
Z	0.016	0.061	0.011	0.017	0.014	0.019	0.033	

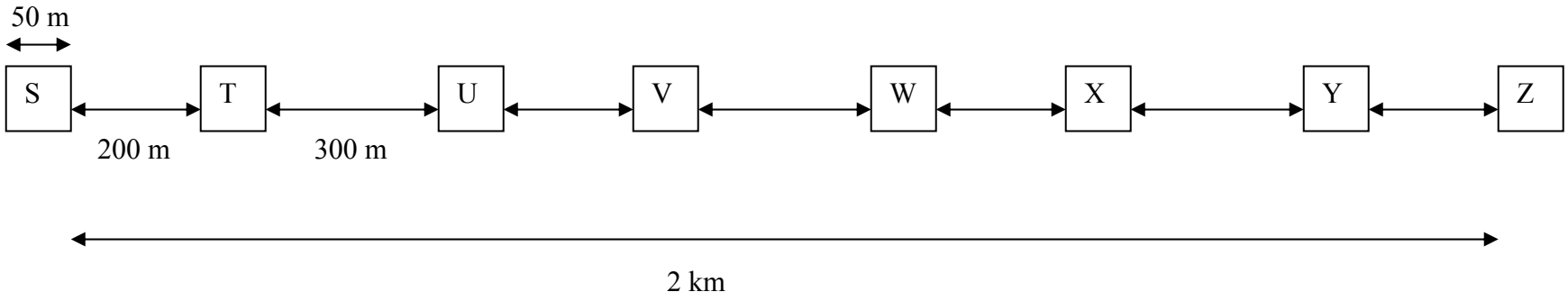
FIGURE LEGENDS

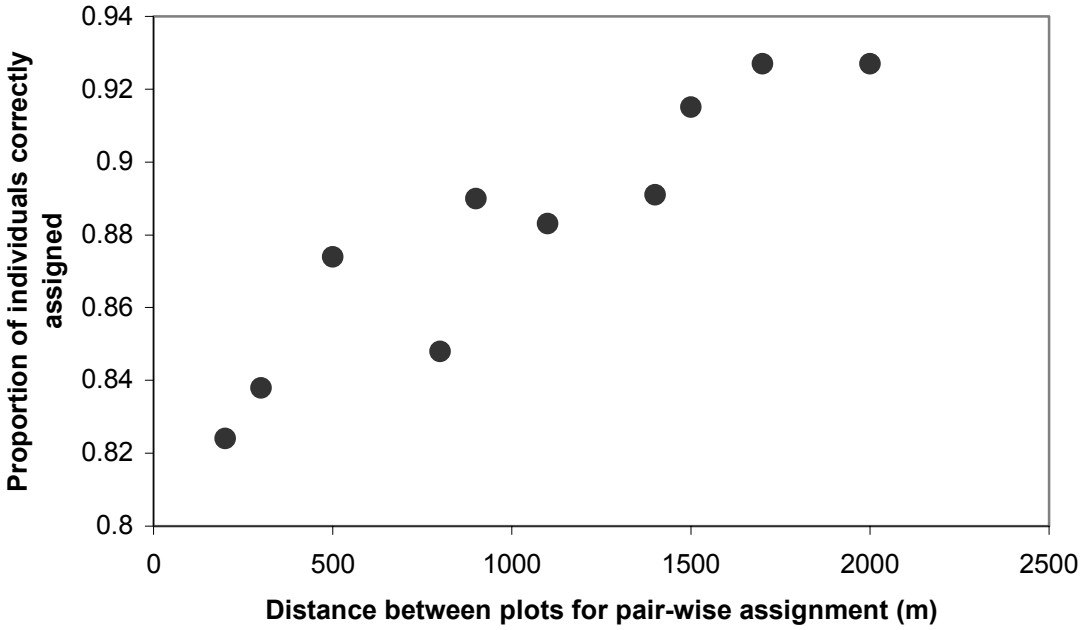
Figure 1. Diagram of study site. Forty-eight samples were collected from each of the eight plots labeled S through Z.

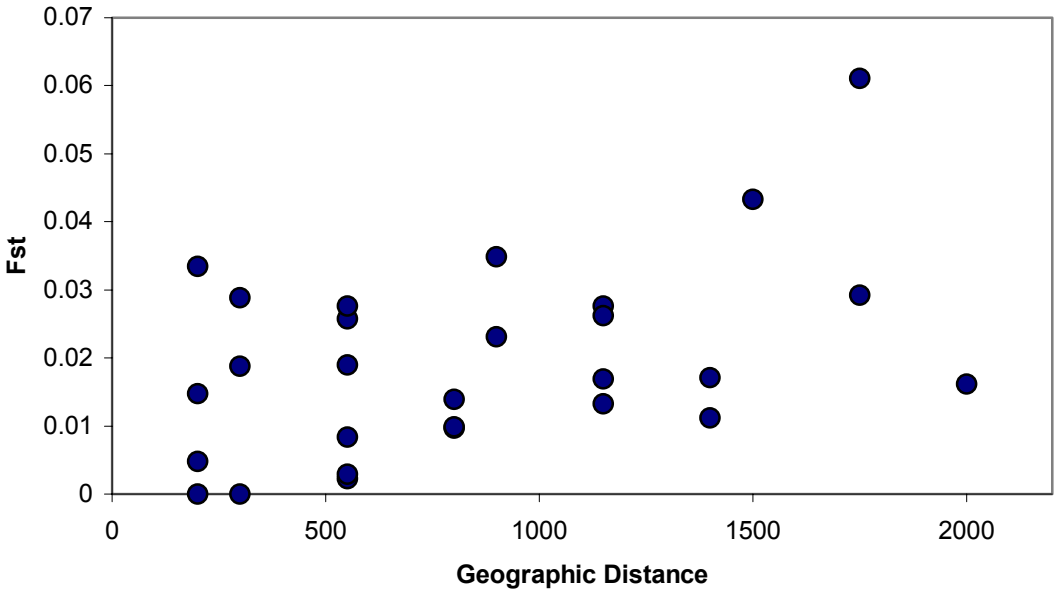
Figure 2. Results of Bayesian assignment tests for paired plots at each distance.

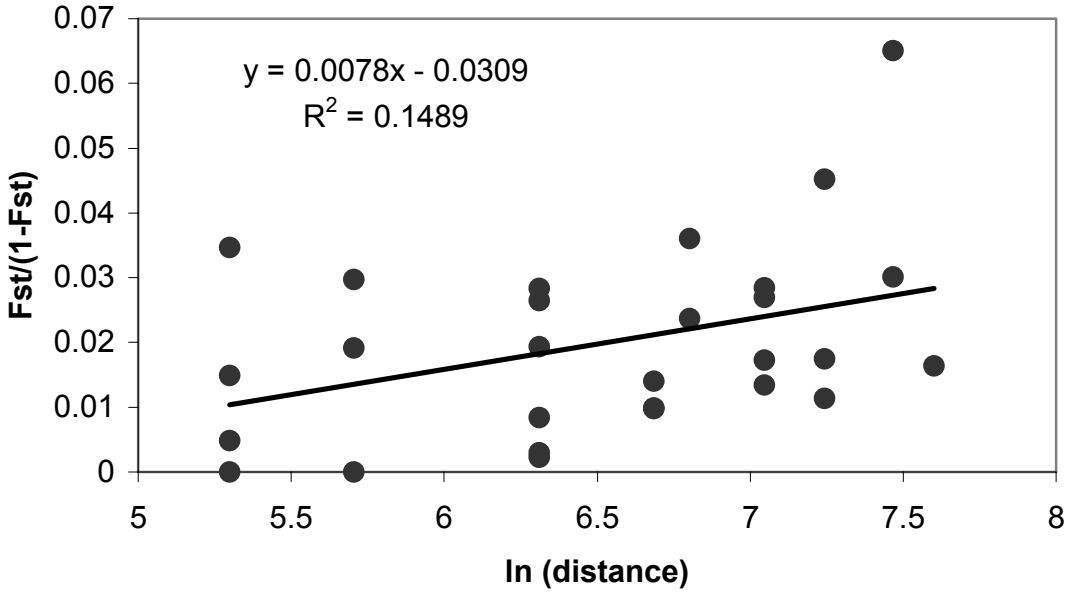
Figure 3. Relationship between F_{ST} and pairwise distance between plots along the transect.

Figure 4. Regression used to calculate the second moment of dispersal distance following Rousset (1997).


50 m
S
T
U
V
W
X
Y
Z
200 m
300 m
2 km


Proportion of individuals correctly assigned
0.94
0.92
0.9
0.88
0.86
0.84
0.82
0.8
0
500
1000
1500
2000
2500
Distance between plots for pair-wise assignment (m)


0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Fst
0
500
1000
1500
2000
Geographic Distance


y = 0.0078x - 0.0309
R2 = 0.1489
Fst/(1-Fst)
ln (distance)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
5
5.5
6
6.5
7
7.5
8

Author information

This research is part of a larger study of the effects of habitat fragmentation on the genetics and demography of terrestrial salamanders. Paul Cabe uses microsatellites to study population structure and mating systems in birds, trees, and salamanders. David Marsh studies the ecology and conservation biology of amphibians. Robert Page was a genetics technician on this project; he is now pursuing a PhD in salamander genetics. Teresa Hanlon is a laboratory instructor and Lisa Connors was a molecular technician at W & L. Mary Aldrich recently received her BS from W & L and is now pursuing a MD.