Ecological and genetic evidence that low-order streams inhibit dispersal by red-backed salamanders (*Plethodon cinereus*)

D.M. Marsh, R.B. Page, T.J. Hanlon, H. Bareke, R. Corritone, N. Jetter, N.G. Beckman, K. Gardner, D.E. Seifert, and P.R. Cabe

Abstract: While many studies have examined the barrier effects of large rivers on animal dispersal and gene flow, few studies have considered the barrier effects of small streams. We used displacement experiments and analyses of genetic population structure to examine the effects of first-order and second-order streams on the dispersal of terrestrial red-backed salamanders, *Plethodon cinereus* (Green, 1818). We marked red-backed salamanders from near the edges of one first-order stream and one second-order stream, and experimentally displaced them either across the stream or an equal distance farther into the forest. A comparison of return rates indicated that both streams were partial barriers to salamander movement, reducing return rates by approximately 50%. Analysis of six microsatellite loci from paired plots on the same side and on opposite sides of the second-order stream suggested that the stream did contribute to genetic differentiation of salamander populations. Collectively, our results imply that low-order streams do influence patterns of movement and gene flow in red-backed salamanders. We suggest that given the high density of first-order and second-order streams in most landscapes, these features may have important effects on species that, like red-backed salamanders, have limited dispersal and large geographic ranges.

Résumé : Alors que de nombreux travaux ont examiné l'effet de barrière des grandes rivières sur la dispersion et le flux génique chez les animaux, peu d'études se sont intéressées aux effets de barrière produits par les petits cours d'eau. Nous avons utilisé des expériences de déplacement et des analyses de structure génétique de population pour déterminer les effets de cours d'eau d'ordres 1 et 2 sur la dispersion de la salamandre rayée, *Plethodon cinereus* (Green, 1818), un amphibien terrestre. Nous avons marqué des salamandres rayées récoltées près des berges d'un cours d'eau d'ordre 1 et d'un autre d'ordre 2 et les avons déplacées expérimentalement ou bien sur la berge opposée ou alors à une distance équivalente vers l'intérieur de la forêt. Une comparaison des taux de retour indique que les deux cours d'eau constituent des barrières partielles au déplacement des salamandres, ce qui réduit les taux de retour de l'ordre de 50 %. L'analyse de six locus microsatellites dans des parcelles appariées sur la même berge ou alors sur des berges opposées du cours d'eau d'ordre 2 indique que le cours d'eau contribue à la différentiation génétique des populations de salamandres. Dans leur ensemble, nos résultats laissent croire que les cours d'eau d'ordres inférieurs influencent les patrons de déplacement et le flux génique chez la salamandre rayée. Nous croyons qu'étant donné la forte densité de cours d'eau d'ordres 1 et 2 dans la plupart des paysages, ces structures peuvent avoir un important effet sur les espèces qui, à l'instar des salamandres rayées, ont une dispersion réduite et une aire de répartition étendue.

[Traduit par la Rédaction]

Introduction

The hypothesis that major rivers can act as barriers to animal dispersal dates back at least to Alfred Russell Wallace's classic studies of Amazonian primates (Wallace 1852). Since Wallace's time, the "riverine barriers" hypothesis has been investigated for numerous taxa. Collectively, these studies suggest that the barrier effects of rivers often depend on the taxa being studied and on characteristics of the rivers themselves. For example, mark–recapture data suggest that large rivers such as the Mississippi may be barriers to black bear (*Ursus americanus* Pallas, 1780) movement, whereas smaller rivers such as Arkansas' White River are readily crossed by bears (White et al. 2000). Genetic evidence for riverine barriers has been found for Brazilian saddleback tamarins, *Saguinus fuscicollis* (Spix, 1823), (Peres et al. 1996) and for orangutans, *Pongo pygmaeus* (L., 1760), in Borneo (Goossens et al. 2005). Yet Lugon-Moulin et al.

Received 26 September 2006. Accepted 23 January 2007. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 13 March 2007.

D.M. Marsh,[1] R.B. Page,[2] T.J. Hanlon, H. Bareke, R. Corritone, N. Jetter, N.G. Beckman,[3] K. Gardner, D.E. Seifert, and P.R. Cabe. Department of Biology, Washington and Lee University, Lexington, VA 24450, USA.

[1]Corresponding author (e-mail: marshd@wlu.edu).
[2]Present address: BBSRB B306, Department of Biology, University of Kentucky, Lexington, KY 40508, USA.
[3]Present address: University of Minnesota, Ecology, Evolution, and Behavior, 100 Ecology Building, 1987 Upper Buford Circle, St. Paul, MN 55108, USA.

 doi:10.1139/Z07-008

(1999) found no evidence of riverine barriers to gene flow in common shrews (*Sorex araneus* L., 1758) and Patton et al. (1994) found evidence against the riverine barrier hypothesis for patterns of genetic differentiation in Amazon basin spiny rats (Echimyidae).

For reptiles, results have been largely positive with respect to the hypothesis that rivers reduce dispersal and gene flow. Rivers have been shown to affect the genetic structure of wood turtles (*Glyptemys insculpta* (Le Conte, 1830)) in Quebec (Tessier et al. 2005) and gekkos (*Gymnodactylus darwinii* (Gray, 1845)) in Brazil (Pellegrino et al. 2005), patterns of chromosomal variation in Andean lizards (*Liolaemus monticola* Müller and Hellmich, 1932) (Lamborot and Alvarez-Serrat 1993), and patterns of phenotypic differentiation in eastern fence lizards (*Sceloporus undulatus* (Bosc and Daudin in Sonnini and Latreille, 1801) (Pounds and Jackson 1981). However, for amphibians, the support for riverine barriers has been less consistent. Lampert et al. (2003) found evidence that the Chagres river is a barrier to gene flow for tungara frogs (*Physalaemus pustulosus* (Cope, 1864)) in Panama. In addition, Wagner et al. (2005) showed that the Columbia River differentiated discrete genetic units of the Larch Mountain salamander (*Plethodon larselli* Burns, 1954). But in Amazonian frog populations, no significant barrier effects on gene flow (Gascon et al. 1998; Lougheed et al. 1999) or on community similarity (Gascon et al. 2000) were detected in studies of multiple species.

While the barrier effects of large rivers have been studied intensively, the effects of smaller streams have been less thoroughly examined. This is unfortunate because first-order and second-order streams are much more common than rivers, both in terms of numbers and in terms of total linear distance (Selby 1985). For example, Australia's Acheron River is fed by more than 500 first-order streams, and the total length of these streams is more than 20 times that of the river (Gordon et al. 2004). Because low-order streams are so common, they will be encountered by dispersing animals much more often than will large rivers. Thus, many species may be better adapted for crossing streams than for crossing rivers. On the other hand, the great abundance of low-order streams means that even partial barrier effects could have a large cumulative influence on gene flow and dispersal.

One group that is potentially affected by low-order streams is terrestrial salamanders of the genus *Plethodon* Tschudi, 1838. Terrestrial salamanders are abundant and diverse in moist forests of eastern North America and are important components of these ecosystems (Burton and Likens 1975; Davic and Welsh 2004). The most common and best studied of the terrestrial plethodontids is the northern red-backed salamander, *Plethodon cinereus* (Green, 1818). Red-backed salamanders use terrestrial habitats in all phases of their life history, and they generally prefer upland areas to streamside habitats (Petranka 1998; Grover 2000; Grover and Wilbur 2002). When red-backed salamanders do colonize streamside habitats, they may be rapidly displaced by larger, more stream-adapted desmognathine salamanders (Grover and Wilbur 2002). Thus, both biotic and abiotic factors may result in stream habitats forming barriers to terrestrial salamander dispersal.

We used displacement experiments (Marsh et al. 2004, 2005) and analysis of microsatellite markers (Connors and Cabe 2003; Cabe et al. 2007) to investigate the barrier effects of low-order streams on red-backed salamanders. In the displacement experiments, we experimentally moved marked salamanders across one first-order and one second-order stream, as well as farther into the forest. We compared the recapture rates of these groups to ask whether having to cross a stream reduced the probability of successful return. In the genetic component of the study, we used six microsatellite loci from red-backed salamanders in paired plots on the same side and on opposite sides of a stream to analyze patterns of genetic differentiation. We asked whether salamanders from plots on opposite sides of the stream exhibited greater genetic differentiation than those from equidistant plots on the same side of the stream.

Materials and methods

Study site and species

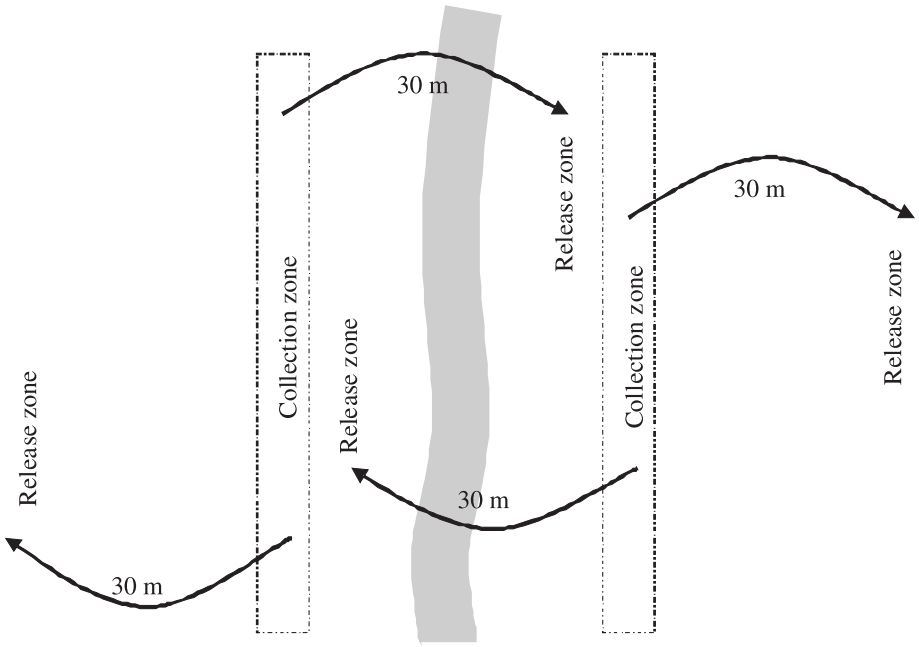
Our study used two streams that flowed through the Jefferson National Forest in Giles County, Virginia, USA. The larger stream, White Rocks Creek, is a forested, second-order stream with a mean width of 5–7 m and maximum depth of 0.5–1 m. During the study, water flow was continuous through the main channel with pools and eddies along the margin. The smaller stream was an unnamed first-order tributary of White Rocks Creek. This stream was approximately 2–3 m wide with a maximum depth of 0.1–0.3 m. Rocks and emergent vegetation protruded from the surface of the stream. Water was continuously present in the stream, but it flowed only during and immediately after rainfall events.

Red-backed salamanders inhabit moist, deciduous forest and reach high densities in mature forests throughout eastern North America (Petranka 1998). Their range extends from North Carolina to Nova Scotia and west to Minnesota. They are most commonly found under rocks and logs on the forest floor, though the majority of the population is usually underground at any given time (Test and Bingham 1948; Taub 1961). Red-backed salamanders lay eggs underneath rocks and logs or in the leaf litter, and hatchings emerge completely developed. These hatchlings reach sexual maturity at 2–3 years of age (Petranka 1998). Adults have small home ranges, on the order of 10–25 m^2 (Kleeberger and Werner 1982). While juveniles may engage in some longer distance dispersal, these movements appear to be on the order of tens of metres rather than hundreds of metres (Marsh et al. 2004). Both mark–recapture data (Gillette 2003) and analysis of genetic population structure (Cabe et al. 2007) suggest similarly limited dispersal. Male and female red-backed salamanders are seasonally territorial in Virginia (Mathis 1991), and displaced animals may home successfully to their territories up to distances of 50–90 m (Kleeberger and Werner 1982; Marsh et al. 2004). The mechanism involved in homing is not known, although it may involve olfaction, which is a common homing mechanism for other amphibians (Twitty 1966; Oldham 1967).

Displacement experiment

At each stream, we established two "collection zones," one on each side of the stream and 18–22 m from the

Fig. 1. Red-backed salamanders (*Plethodon cinereus*) were captured in collection zones and then moved either to release zones across the stream or to equidistant release zones farther into the forest. An additional control group (not shown) was re-released at the site of capture.



stream's center (Fig. 1). The length of each collection zone was approximately 100 m, although muddy patches at the second-order stream that were likely unsuitable for salamanders caused us to split the zone into three separate units. Within each collection zone, we placed one hundred 0.08 m^2 white oak cover boards flush with the soil. Cover boards were numbered consecutively and arranged within the collection zone to maintain about 2 m between the boards. Collection zones were established on 20–26 April 2004.

We allowed salamanders to colonize cover boards for approximately 3 weeks and then began to survey the collection zones on 14 May 2004. During surveys, we lifted each cover board and collected any red-backed salamanders found underneath. We brought the salamanders back to the laboratory (approximately 12 km away), where we batch-marked them with fluorescent elastomer tags (Davis and Ovaska 2001; Marsh et al. 2004) to indicate the side of the stream on which they were captured and their treatment assignment. Each mark was applied in duplicate (once on each side) to avoid any problems with the loss of single tags. We recorded the snout–vent length (SVL) and the board of original capture for each salamander. In total, we captured and marked 674 salamanders — 184 were small juveniles (SVL < 3.0 cm), 114 were large juveniles (3.0 cm < SVL < 3.5 cm), 233 were small adults (3.5 cm < SVL < 4.0 cm), 137 were large adults (SVL > 4.0 cm), and 6 were not measured.

Salamanders were randomly assigned to one of three treatments: release on the opposite side of the stream, release an equivalent distance into the forest on the same side of the stream, and a handling control in which salamanders were re-released at the original site of capture. Data from the handling controls were used for comparative purposes but were not incorporated into the main analysis, since it is trivial that recapture rates would be higher for this group. We assigned the three treatments at a ratio of four stream crossers to four forest releases to one handling control.

After salamanders had been marked and assigned to a treatment, we returned to the appropriate location to re-release the animals. Most salamanders were released on the morning following capture and no salamanders were held in the laboratory more than 48 h. Salamanders in the stream-crossing treatment were released underneath a cover board approximately 10 m across the stream and directly perpendicular to the board under which they were originally captured (Fig. 1). Thus, to home successfully, these salamanders had to travel 28–32 m and cross the stream. Salamanders in the forest treatment were released under a cover board 28–32 m farther into the forest on the same side of the stream and perpendicular to the cover board where they were originally captured. These salamanders had to move 28–32 m through forest to home successfully but did not have to cross a stream or any other obvious barrier to movement. Controls were replaced beneath the board where they were originally captured. All sites used were relatively flat with <10° slopes. Although very steep banks can be partial barriers to salamander movement, <30° slopes do not appear to affect homing behavior (Marsh et al. 2005). Thus, small differences in slopes between forest releases and stream releases were unlikely to have influenced our results.

We sampled the collection zones periodically from 14 May to 30 October 2004. The smaller stream and the larger stream were sampled alternately and each was checked 27 times during the study. When we encountered marked individuals, we captured and removed them from the study site to avoid double-counting. For each recapture, we recorded its site of origin, its treatment assignment, and its recapture date. All protocols for this experiment were carried out in accordance with the *Guidelines for the Use of Live Amphib-*

ians and Reptiles in Field and Laboratory Research (Herpetological Animal Care and Use Committee, American Society of Ichthyologists and Herpetologists) and were approved by the Institutional Animal Care and Use Committee at Washington and Lee University.

Data analysis for displacement experiment

We analyzed the effects of each stream on salamander return rates using logistic regression with successful return treated as a binary variable (i.e., each animal returned or did not return). We first used the release treatment by stream interaction to test whether the barrier effects differed between the second-order stream and the first-order stream. We then analyzed the main effect of the treatment to test whether return rate was affected by the presence of the stream. Finally, we used a two-factor ANOVA to analyze the effects of release treatment and stream on the SVL of salamanders that returned. This tested whether salamanders of specific sizes were particularly likely to cross the stream. A two-tailed α of 0.05 was used for each comparison and all calculations were performed in SYSTAT® version 10.2 (Systat Software Inc. 2002).

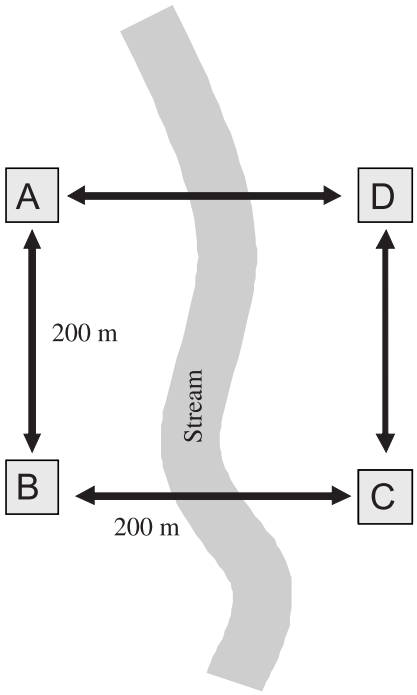
Genetic differentiation

We used dinucleotide microsatellites to examine whether White Rocks Creek, the second-order stream, appeared to contribute to genetic differentiation of red-backed salamanders. We established four plots of 50 m × 50 m at the vertices of a square with a length of 200 m (plots A, B, C, and D; Fig. 2). These plots were at least 100 m downstream and 80 m upland of the sites used in the displacement experiment, so it is very unlikely that any sampled animals had been experimentally displaced. Within each of the four plots, we collected tail tips (approximately 1 cm) from 48 red-backed salamanders; tail tips usually re-grow within 2 weeks. We placed tail tissue in a sterile 1.5 mL microtube containing collection buffer (10 mmol/L Tris, 10 mmol/L EDTA, pH 8) and then re-released salamanders at the point 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 min, treated with RNase at 37 °C for 25 min, 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 (10 mmol/L Tris, pH 8.0, 1 mmol/L EDTA). DNA samples were stored in a freezer and dilutions of this stock (1:4 or 1:9) were used as templates for the polymerase chain reaction (PCR).

We amplified five microsatellite loci (PcI16, PcLX16, PcLX23, PcJX06, and PcFX08) following the protocols detailed in Connors and Cabe (2003). Although the original protocols specified multiplex reactions, some loci were amplified independently to increase yields. A sixth locus, PcXD23 (primers HEX/GCAAAACAGCAACAAGACAAC, AACCTTGATGTTTGGCAAGG; GenBank accession No. AY151376) was amplified using similar protocols.

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® version 3.7 (Applied Biosystems 2001*a*). We determined genotypes in our laboratory using Genotyper® version 3.7 (Applied Biosystems 2001*b*). We manually inspected each allele call and binned alleles accordingly.

Fig. 2. DNA samples from 48 red-backed salamanders were collected at each of the four plots.



Data analysis for genetic differentiation

We used Microsatellite Analyzer (MSA) to obtain observed and expected heterozygosities, allele counts, and size ranges (Dieringer and Schlötterer 2003). We then used GenePop version 3.4 (Raymond and Rousset 1995) to test each locus for Hardy–Weinberg equilibrium. The probabilities from these tests were adjusted for an experimentwise 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.

We used several resampling methods (Manly 1997) to evaluate the null hypothesis that subpopulations on opposite sides of the stream (i.e., A–D and B–C in Fig. 2) will be no more genetically distinct than equidistant subpopulations on the same side of the stream (i.e., A–B and C–D). We based the first statistical test on measures of the pairwise genetic distance for each pair of plots. To ensure that our results did not depend on the chosen metric, we used three measures of pairwise genetic distance: F_{ST} (Weir and Cockerham 1984), Nei's genetic distance (Nei 1972), and Reynolds' genetic distance (Reynolds et al. 1983). For hypothesis testing, our summary test statistic was the mean genetic distance for pairs of plots on opposite sides of the stream minus the

mean genetic distance for pairs of plots on the same side of the stream (i.e., 0.5(AB + CD) – 0.5(AD + BC)). Thus, positive values of the test statistic indicate that across-stream plots are more distinct than the same-side plots, negative values indicate that same-side plots are more distinct, and zero is expected under the null hypothesis that the stream does not influence genetic differentiation. Genetic distance measures for plots diagonally across from one another (A–C and B–D) were not incorporated into the analysis.

We calculated the value of the test statistic from the actual data and bootstrapped multilocus genotypes within plots (i.e., resampled with replacement) to calculate a 95% confidence interval for the test statistic. We then determined whether the confidence interval overlapped zero, as would be expected under the null hypothesis that the stream did not influence genetic differentiation. We repeated this analysis for each distance measure. Because these different distance measures represent highly nonindependent checks for consistency rather than independent post hoc comparisons, we used an α of 0.05 for each distance measure. We note that our method of bootstrapping multilocus genotypes within plots is analogous to the determination of hierarchical F_{ST} values by existing statistical packages such as GDA (Lewis and Zaykin 2001), but with comparisons restricted to plots that are equidistant to one another. The use of equidistant plots also made our approach preferable to Mantel tests, which are commonly used to assess the relationship between genetic distance and geographic distance.

In the resampling analyses described above, we incorporated variation at the level of multilocus genotypes, as would result from random colonization and the sampling of variable individuals at each plot. It is also possible that variation could occur at the level of entire plots, owing to factors like simultaneous colonization by large numbers of individuals or a high degree of co-ancestry of sampled individuals. Although having only four plots does not permit extensive analysis of variation at the whole-plot level, we did carry out a set of additional tests that should be less sensitive to random variation among plots.

These latter hypothesis tests were based on a three-stage process. First, we bootstrapped all multilocus genotypes among the two plots on the same side of the stream (i.e., individuals from A and B combined and individuals from C and D combined). From the bootstrapped data, we calculated a genetic distance (F_{ST}, Nei's, or Reynold's) between the two pooled populations separated by the stream. In the second stage, we took the original data and bootstrapped multilocus genotypes between adjacent subpopulations across the stream from one another (i.e., A–D combined and B–C combined). We then calculated a genetic distance between these two pairs of pooled populations. In the third stage, we calculated a test statistic by subtracting the second genetic distance measure from the first (i.e., (AB to CD) – (AD to BC)). As in the previous tests, if subpopulations on opposite sides of the stream are more distinct from one another, values of the test statistic should be positive, whereas if the null hypothesis of no stream effect is true, the test statistic should be distributed around zero. By repeating this three-stage process 1000 times, we calculated 95% confidence intervals for all three measures of genetic distance and asked whether they overlapped zero. All resampling programs were implemented in Matlab® version 7.0.4 (MathWorks Inc. 2005) and the code is available from the senior author.

As a final component of our analysis, we used Arlequin version 2.0 (Schneider et al. 2000) to assess our ability to assign the multilocus genotypes of individual salamanders to the plots from which they came. The assignment procedure determines the log likelihood of observing each multilocus genotype in each of the study plots, where log likelihoods are calculated from the observed allele frequencies within each plot (Schneider et al. 2000). For individuals that were incorrectly assigned, we asked whether these misassignments were more likely for plots on the same side of the stream versus equidistant plots on the opposite side of the stream.

Results

Displacement experiment

At the second-order stream, 380 salamanders were released, of which 38 were recaptured (10.0%). Seventeen recaptures were controls (recapture rate 40.4%), 13 salamanders returned through the forest (recapture rate 7.7%), and 8 salamanders returned across the stream (recapture rate 4.7%; Fig. 3). At the first-order stream, 378 salamanders were released and 60 were recaptured (15.7%). Twenty-eight of the recaptures were replacement controls (recapture rate 63.3%), 22 returned though the forest (recapture rate 13.1%), and 10 returned across the stream (recapture rate 5.9%; Fig. 3).

The interaction between treatment and stream did not approach statistical significance (b = –0.13, t = –0.88, p = 0.38); therefore we pooled the data from the two streams. For the pooled data, return rate was significantly lower for salamanders returning across the stream versus salamanders returning through the forest (b = 0.37, t = 2.46, p = 0.014). This significant effect represented a 49% reduction in return rate. Had the data been analyzed separately for each stream (i.e., without pooling), conclusions would have been identical, although small sample sizes would have rendered the barrier effect of the large stream not significant at the 0.05 level.

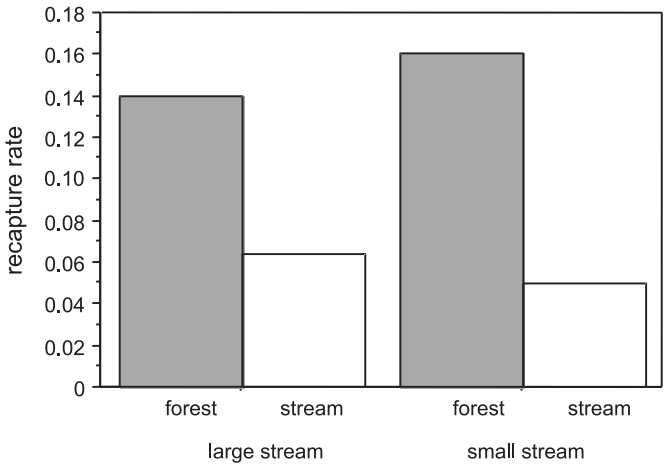
Salamanders returning across the stream were significantly larger than salamanders returning through the forest (4.07 ± 0.07 cm (mean ± SE) for stream crossers, 3.86 ± 0.08 cm for forest returns; $F_{[1,49]}$ = 4.44, p = 0.04). There were no significant differences in salamander size between the first-order stream site and the second-order stream site ($F_{[1,49]}$ = 1.58, p = 0.22), and there were no significant interactions between stream and treatment on salamander size that would indicate different effects between the two sites ($F_{[1,49]}$ = 0.71, p = 0.40).

Genetic differentiation

All loci within each sample plot were in Hardy–Weinberg equilibrium (for summary statistics see Table 1). There was some evidence for genetic disequilibrium for a single pair of loci: Pcl16 and PcJX06 showed significant disequilibrium at the 0.01 level. However, this result may be due to chance alone given that we evaluated disequilibrium for 15 pairs of loci. Furthermore, eliminating PcJX06 from the analysis did not substantively change any of our results.

Pairwise genetic distances between plots ranged from

Fig. 3. Recapture rates from red-backed salamanders displaced farther into the forest (shaded bars) and animals displaced across the stream (open bars) are shown for both the second-order (large) stream and the first-order (small) stream.



0.013 (F_{ST}) for plots A and B to 0.050 (F_{ST}) for plots B and C (Table 2). Based on bootstrapping of multilocus genotypes, there was small but statistically significant genetic differentiation across the stream (mean F_{ST} difference = 0.016, 95% confidence intervals = 0.005–0.030). Results were very similar using Nei's genetic distance (mean difference = 0.024, 95% confidence intervals = 0.005–0.043) and Reynold's distance (mean difference = 0.016, 95% confidence intervals = 0.005–0.030). Bootstrapping across pooled adjacent plots also suggested that the stream increased genetic differentiation. This was true for genetic distance based on F_{ST} (mean difference = 0.016, 95% confidence intervals = 0.005–0.028), Nei's genetic distance (mean difference = 0.025, 95% confidence intervals = 0.007–0.042), and Reynold's distance (mean difference = 0.015, 95% confidence intervals = 0.004–0.027).

Assignment of genotypes to plots based on maximum likelihood correctly classified 69.4% of individual salamanders. Of salamanders incorrectly assigned to plots 200 m away, 64.1% (25 of 39) were assigned to the plot on the same side of the stream, whereas 35.9% (14 of 39) were assigned to the plot across the stream. This difference is marginally different from the 50:50 null expectation ($\chi^2_{[1]} = 3.1$, $p = 0.08$), in a direction that is consistent with our previous results.

Discussion

Results for both the displacement experiment and the population genetic study suggest that streams act as partial barriers to dispersal and gene flow in red-backed salamanders. Streams reduced the return rates of salamanders and increased genetic differentiation among subpopulations. The displacement experiment suggested that the barrier effects of streams may be particularly strong for smaller salamanders; salamanders returning across the stream were significantly larger than salamanders returning through the forest.

Our results for the two streams can be compared with analogous results for the barrier effects of open fields and forest roads. Unlike streams, open fields had no apparent effects on red-backed salamander movement rate (Marsh et al. 2004). In contrast, forest roads reduced salamander movement by an average of 51% (Marsh et al. 2005), an effect similar to the 49% reduction observed here for streams. In both these previous experiments, we found no evidence that the barrier effects of open fields or roads depended on salamander size. Streams, on the other hand, appeared to be more of a barrier to smaller salamanders. It is possible that smaller salamanders are more likely to avoid streams because they make easier prey for aquatic salamanders or fish (e.g., Mathis et al. 2003; Lowe et al. 2004) or because they have generally poorer condition, which could make swimming against the current more difficult (see Lowe et al. 2006). Alternatively, it could be that the size effects that we observed are due more to the influence of experimental displacement on homing behavior than to any particular adaptive response.

Because we did not directly observe the movements of the salamanders, we have no information as to how salamanders actually crossed the streams. The first-order stream was broken by emergent vegetation and there were many places where salamanders could have crossed without encountering flowing water. The flow of the second-order stream was unbroken, although there was a fallen log spanning the stream at one end of the collection zones. That said, the observed returns across the second-order stream were not concentrated near the log and several of the returns were more than 200 m downstream from this point. This implies that at least some of the salamanders probably had to swim to home to their site of capture. Given this, it is surprising that we did not observe significant differences in the effects of the two streams, despite substantial differences in width, depth, and flow rate. If anything, return rate appeared to be lower across the first-order stream, which caused a 55% reduction in return rate compared with a 39% reduction at the

Table 1. Genetic diversity of pooled samples from red-backed salamanders, including sample size (N), number of alleles, observed and expected heterozygosities (H_o and H_e), and site-wide fixation indices (F_{ST}) for each locus.

Locus	N	Alleles	H_o	H_e	F_{ST}
PcI16a	174	6	0.26	0.29	0.063**
PcLX16a	180	7	0.55	0.61	0.058**
PcLX23a	182	14	0.51	0.52	0.016**
PcJX06a	185	10	0.41	0.43	0.010*
PcFX08a	179	14	0.81	0.86	0.004
PcXD23a	180	8	0.80	0.79	0.007

Note: *, $P < 0.05$; **, $P < 0.005$.

Table 2. Pairwise genetic distances of red-backed salamanders from among adjacent plots calculated using F_{ST}, Nei's genetic distance, and Reynolds' distance.

Plot pair	Bisected by road?	F_{ST}	Nei's	Reynolds'
AB	No	0.013	0.029	0.025
BC	Yes	0.050	0.083	0.060
CD	No	0.020	0.040	0.031
AD	Yes	0.014	0.034	0.026

large stream. This suggests that streams may present more of a behavioral barrier to salamander movement than an absolute physical barrier. Our previous study of the barrier effects of roads suggested that a substantial component of the observed reduction in dispersal results from salamanders that simply do not attempt to cross roads (Marsh et al. 2005).

Given that the second-order stream was only a partial barrier to salamander movement, it was interesting that we were able to detect a significant, albeit small, increase in genetic differentiation across the stream. This effect was primarily due to one pair of plots on opposite sides of the stream that were substantially different from one another (plots B–C, F_{ST} = 0.05). However, the high differentiation observed between these plots is unlikely to be due to chance. During the course of other research, we used identical methods to evaluate genetic differentiation among 19 pairs of red-backed salamander plots separated by 200 m of stream-free forest habitat (Cabe et al. 2007; P.R. Cabe, R.B. Page, and D.M. Marsh, unpublished data). For all 19 of these plot pairs, F_{ST} values were <0.035 (i.e., >30% lower than the value that we observed between stream plots B and C). Based on a regression of F_{ST} versus distance in continuous forest (Cabe et al. 2007), a F_{ST} of 0.05 is equivalent to the expected differentiation over a distance of about 2 km. It is also generally near the middle of the range of fine-scale (i.e., <16 km) F_{ST} values from microsatellite studies of amphibian populations reviewed by Newman and Squire (2001).

Quantitative estimates of dispersal rates from genetic and ecological methods often differ substantially (Koenig et al. 1996; Wilson et al. 2004; Riley et al. 2006). Some of these differences may arise from different assumptions of the two approaches. For example, genetic methods often assume an equilibrium between drift and gene flow, an island model of genetic exchange, and some known genetic effective population size (Whitlock and McCauley 1999). In contrast, ecological approaches such as mark–recapture usually assume that capture probability does not depend on dispersal distance and that no animals disperse off the study site (Koenig et al. 1996). Other differences between the results of the two approaches may relate to the fact that they are often estimating somewhat different quantities (Whitlock and McCauley 1999; Rousset 2001). Genetic methods usually estimate the long-term mean migration rate of individuals that go on to breed, whereas ecological approaches provide a short-term estimate of the numbers moving without regard to their fate after dispersal.

Given these discrepancies, it is encouraging that both the displacement experiments and the genetic approaches gave relatively similar impressions of the barrier effects of streams for red-backed salamanders. However, it should be noted that we only attempted to relate the two sets of results in a qualitative way. As of yet, there is no sure way of knowing what magnitude of reduction in dispersal observed in displacement experiments is consistent with any specific level of genetic differentiation, even with the assumption that gene flow and drift are at equilibrium. New approaches have begun to use maximum likelihood and Bayesian theory to better integrate direct estimates of dispersal with estimates from genetic population structure (e.g., Beerli and Felsenstein 1999, 2001; Wilson and Rannala 2003). Hopefully, by relating the results of displacement experiments to direct estimates of dispersal from mark–recapture, we will soon be able to integrate displacement experiments into this more unified framework for understanding dispersal.

What are the implications of the observed barrier effects for the ecology and evolution of red-backed salamanders? Obviously, given that streams appear to be only partial barriers to dispersal, it is unlikely that isolation by a single, small stream would lead to substantial genetic differentiation and local adaptation. Nevertheless, large streams, or multiple small streams, certainly do have the potential to lead to differentiation, particularly given the broad geographic range (Petranka 1998) and low dispersal rates (Cabe et al. 2007) of red-backed salamanders. Indeed, substantial variation in both physiological and behavioral characteristics is evident across the distribution of red-backed salamanders. Frequencies of the two primary color morphs (red-backed and lead-backed) vary widely over a scale of tens of kilometres (Angleberger and Chinnici 1975; Lotter and Scott 1977). In terms of behavioral variation, populations in Western Virginia have been reported to be highly territorial (Mathis 1991), whereas populations in Michigan appear to aggregate with conspecifics (Quinn and Graves 1999). Studies on other amphibian species have shown a surprising level of local adaptation over small spatial scales (Berven 1982; Freidenburg and Skelly 2004). Although red-backed salamanders exhibit some genetic differentiation over small spatial scales even in continuous forest, the cumulative barrier effects of streams and rivers would make it even easier for these kinds of interpopulation variations to develop.

Acknowledgements

We thank Mary Aldrich, Elizabeth Little, and Elizabeth Lyman for assistance with DNA extraction and PCR. Jesse Overcash and the US Forest Service provided permission to use the White Rocks Creek field site. Logistical support was provided by Mt. Lake Biological Station, and animal handling protocols were covered under Virginia State collecting permit No. 016783 and IACUC protocol DM-0204b. Earlier drafts of the manuscript were improved by comments from Pete Trenham and two anonymous reviewers. This research was supported by R.E. Lee Research Grants to N. Jetter and H. Bareke, a National Science Foundation (NSF) – Research Experience for Undergraduates grant to R. Corritone, and NSF – Division of Environmental Biology grant 0235695 to D.M. Marsh and P.R. Cabe.

References

Angleberger, M.P., and Chinnici, J.P. 1975. Dimorphism in the red-backed salamander *Plethodon cinereus* (Green) at Mountain Lake, Virginia. Va. J. Sci. **26**: 153–158.

Applied Biosystems. 2001*a*. GeneScan®. Version 3.7 [computer program]. Applied Biosystems, Foster City, Calif.

Applied Biosystems. 2001*b*. Genotyper®. Version 3.7 [computer program]. Applied Biosystems, Foster City, Calif.

Beerli, P., and Felsenstein, J. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics, **152**: 763–773. PMID:10353916.

Beerli, P., and Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n*

subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. U.S.A. **98**: 4563–4568. doi:10.1073/pnas.081068098. PMID:11287657.

Berven, K.A. 1982. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. II. An experimental analysis of larval development. Oecologia (Berl.), **52**: 360–369. doi:10.1007/BF00367960.

Burton, T.M., and Likens, G.E. 1975. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook experimental forest, New Hampshire. Ecology, **56**: 1068–1080. doi:10.2307/1936147.

Cabe, P.R., Page, R.B., Hanlon, T.J., Aldrich, M.E., Connors, L., and Marsh, D.M. 2007. Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. Heredity, **98**: 53–60. doi:10.1038/sj.hdy.6800905. PMID:17006531.

Connors, L.M., and Cabe, P.R. 2003. Isolation of dinucleotide microsatellite loci from red-backed salamanders (*Plethodon cinereus*). Mol. Ecol. Notes, **3**: 131–133. doi:10.1046/j.1471-8286.2003.00373.x.

Davic, R.D., and Welsh, H.H., Jr. 2004. On the ecological roles of salamanders. Annu. Rev. Ecol. Syst. **35**: 405–434.

Davis, T.M., and Ovaska, K. 2001. Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. J. Herpetol. **35**: 217–225. doi:10.2307/1566111.

Dieringer, D., and Schlötterer, C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Mol. Ecol. Notes, **3**: 167–169. doi:10.1046/j.1471-8286.2003.00351.x.

Freidenburg, L.K., and Skelly, D.K. 2004. Microgeographical variation in thermal preference by an amphibian. Ecol. Lett. **7**: 369–373. doi:10.1111/j.1461-0248.2004.00587.x.

Gascon, C., Lougheed, S.C., and Bogart, J.P. 1998. Patterns of genetic population differentiation in four species of Amazonian frogs: a test of the riverine barrier hypothesis. Biotropica, **30**: 104–119. doi:10.1111/j.1744-7429.1998.tb00373.x.

Gascon, C., Malcolm, J.R., Patton, J.L., da Silva, M.N.F., Bogart, J.P., Lougheed, S.C., Peres, C.A., Neckel, S., and Boag, P.T. 2000. Riverine barriers and the geographic distribution of Amazonian species. Proc. Natl. Acad. Sci. U.S.A. **97**: 13672–13677. doi:10.1073/pnas.230136397. PMID:11095705.

Gillette, J.R. 2003. Population ecology, social behavior, and intersexual differences in a natural population of red-backed salamanders: a long-term field study. Ph.D. dissertation, Department of Biology, University of Louisiana, Lafayette, La.

Goossens, B., Chikhi, L., Jalil, M.F., Ancrenaz, M., Lackman-Ancrenaz, I., Mohamed, M., Andau, P., and Bruford, M.W. 2005. Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. Mol. Ecol. **14**: 441–456. doi:10.1111/j.1365-294X.2004.02421.x. PMID:15660936.

Gordon, N.D., McMahon, T.A., Finlayson, B.L., and Gippel, C.J. 2004. Stream hydrology. Wiley, West Sussex, UK.

Grover, M.C. 2000. Determinants of salamander distributions along moisture gradients. Copeia, 2000: 156–168. doi:10.1643/0045-8511(2000)2000[0156:DOSDAM]2.0.CO;2.

Grover, M.C., and Wilbur, H.M. 2002. Ecology of ecotones: interactions between salamanders on a complex environmental gradient. Ecology, **83**: 2112–2123.

Kleeberger, S.R., and Werner, J.K. 1982. Home range and homing behavior of *Plethodon cinereus* in Northern Michigan. Copeia, 1982: 409–415. doi:10.2307/1444622.

Koenig, W.D., Van Vuren, D., and Hooge, P.N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol. Evol. **11**: 514–517. doi:10.1016/S0169-5347(96)20074-6.

Lamborot, M., and Alvarez-Serrat, E. 1993. Karyotypic variation within and between populations of *Liolaemus monticola* (Tropiduridae) separated by the Maipo River in the coastal range of central Chile. Herpetologica, **49**: 435–449.

Lampert, K.P., Rand, A.S., Mueller, U.G., and Ryan, M.J. 2003. Fine-scale genetic pattern and evidence for sex-biased dispersal in the tungara frog, *Physalaemus pustulosus*. Mol. Ecol. **12**: 3325–3334. doi:10.1046/j.1365-294X.2003.02016.x. PMID:14629349.

Lewis, P.O., and Zaykin, D. 2001. Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). Available from http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php [accessed 13 August 2004].

Lotter, F., and Scott, N.J., Jr. 1977. Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. Copeia, 1977: 681–690. doi:10.2307/1443166.

Lougheed, S.C., Gascon, C., Jones, D.A., Bogart, J.P., and Boag, P.T. 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis*). Proc. R. Soc. Lond. B Biol. Sci. **266**: 1829–1835.

Lowe, W.H., Nislow, K.H., and Bolger, D.T. 2004. Stage-specific and interactive effects of sedimentation and trout on a headwater stream salamander. Ecol. Appl. **14**: 164–172.

Lowe, W.H., Likens, G.E., and Cosentino, B.J. 2006. Self-organization in streams: the relationship between movement behaviour and body condition in a headwater salamander. Freshw. Biol. **51**: 2052–2062. doi:10.1111/j.1365-2427.2006.01635.x.

Lugon-Moulin, N., Brunner, H., Balloux, F., Hausser, J., and Goudet, J. 1999. Do riverine barriers, history or introgression shape the genetic structuring of a common shrew (*Sorex araneus*) population? Heredity, **83**: 155–161. doi:10.1038/sj.hdy.6885670. PMID:10469203.

Manly, B.F.J. 1997. Randomization, bootstrap and Monte Carlo methods in biology. Chapman and Hall, New York.

Marsh, D.M., Thakur, K.A., Bulka, K.C., and Clarke, L.B. 2004. Dispersal and colonization through open fields by a terrestrial, woodland salamander. Ecology, **85**: 3396–3405.

Marsh, D.M., Milam, G.S., Gorham, N.A., and Beckman, N.G. 2005. Forest roads are partial barriers to salamander movement. Conserv. Biol. **19**: 2004–2008. doi:10.1111/j.1523-1739.2005.00238.x.

Mathis, A. 1991. Territories of male and female terrestrial salamanders: costs, benefits, and intersexual spatial associations. Oecologia (Berl.), **86**: 433–440. doi:10.1007/BF00317613.

Mathis, A., Murray, K.L., and Hickman, C.R. 2003. Do experience and body size play a role in responses of larval ringed salamander, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. Ethology, **109**: 159–170. doi:10.1046/j.1439-0310.2003.00849.x.

MathWorks Inc. 2005. Matlab®. Version 7.0.4 [computer program]. The MathWorks Inc., Natick, Mass.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**: 283–292. doi:10.1086/282771.

Newman, R.A., and Squire, T. 2001. Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). Mol. Ecol. **10**: 1087–1100. doi:10.1046/j.1365-294X.2001.01255.x. PMID:11380868.

Oldham, R.S. 1967. Orienting mechanism of the green frog, *Rana clamitans*. Ecology, **48**: 477–491. doi:10.2307/1932683.

Patton, J.L., daSilva, M.N.F., and Malcolm, J.R. 1994. Gene genealogy and differentiation among arboreal spiny rats (Rodentia,

Echimyidae) of the Amazon basin — a test of the riverine barrier hypothesis. Evolution, **48**: 1314–1323. doi:10.2307/2410388.

Pellegrino, K.C.M., Rodrigues, M.I., Waite, A.N., Morando, M., Yassuda, Y.Y., and Sites, J.W. 2005. Phylogeography and species limits in the *Gymnodactylus darwinii* complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic Forest. Biol. J. Linn. Soc. **85**: 13–26. doi:10.1111/j.1095-8312.2005.00472.x.

Peres, C.A., Patton, J.L., and daSilva, M.N.F. 1996. Riverine barriers and gene flow in Amazonian saddle-back tamarins. Folia Primatol. (Basel), **67**: 113–124. PMID:9032947.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C.

Pounds, J.A., and Jackson, J.F. 1981. Riverine barriers to gene flow and differentiation of fence lizard populations. Evolution, **35**: 516–528. doi:10.2307/2408199.

Quinn, V.S., and Graves, B.M. 1999. Space use in response to conspecifics by the red-backed salamander (*Plethodon cinereus*, Plethodontidae, Caudata). Ethology, **105**: 993–1002. doi:10.1046/j.1439-0310.1999.00486.x.

Raymond, M., and Rousset, F. 1995. GenePop: population genetics software for exact tests and ecumemicism. J. Hered. **86**: 248–249.

Reynolds, J.B., Weir, B.S., and Cockerham, C.C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics, **105**: 767–779. PMID:17246175.

Riley, S.P.D., Pollinger, J.P., Sauvajot, R.M., York, E.C., Bromley, C., Fuller, T.K., and Wayne, R.K. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Mol. Ecol. **15**: 1733–1741. doi:10.1111/j.1365-294X.2006.02907.x. PMID:16689893.

Rousset, F. 2001. Genetic approaches to the estimation of dispersal rates. *In* Dispersal. *Edited by* J. Clobert, E. Danchin, A.A. Dhondt, and J.D. Nichols. Oxford University Press, Oxford, U.K. pp. 18–28.

Schneider, J., Roessli, D., and Excoffier, L. 2000. Arlequin: a software for population genetics data analysis. Version 2.000 [computer program]. Available from http://lgb.unige.ch/arlequin/software/ [accessed 30 May 2005].

Selby, M.J. 1985. Earth's changing surface: an introduction to geomorphology. Oxford University Press, Oxford, UK.

Systat Software Inc. 2002. SYSTAT®. Version 10.2 [computer program]. Systat Software Inc., Richmond, Calif.

Taub, F.B. 1961. The distribution of red-backed salamanders, *Plethodon c. cinereus*, within the soil. Ecology, **42**: 681–698. doi:10.2307/1933498.

Tessier, N., Paquette, S., and Lapointe, F.J. 2005. Conservation genetics of the wood turtle (*Glyptemys insculpta*) in Quebec, Canada. Can. J. Zool. **83**: 765–772.

Test, F.H., and Bingham, B.A. 1948. Censuses of a population of the red-backed salamander (*Plethodon cinereus*). Am. Midl. Nat. **39**: 362–372. doi:10.2307/2421590.

Twitty, V.C. 1966. Of scientists and salamanders. W.C. Freeman and Co., San Francisco, Calif.

Wagner, R.S., Miller, M.P., Crisafulli, C.M., and Haig, S.M. 2005. Geographic variation, genetic structure, and conservation unit designation in the Larch Mountain salamander (*Plethodon larselli*). Can. J. Zool. **83**: 396–406. doi:10.1139/z05-033.

Wallace, A.R. 1852. On monkeys of the Amazon. Proc. Zool. Soc. Lond. **20**: 107–110.

Weir, B.S. 1990. Genetic data analysis: methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, Mass..

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, **38**: 1358–1370. doi:10.2307/2408641.

White, T.H., Jr., Bowman, J.L., Leopold, B.D., Jacobson, H.A., Smith, W.P., and Vilella, F.J. 2000. Influence of Mississippi alluvial valley rivers on black bear movements and dispersal: implications for Louisiana black bear recovery. Biol. Conserv. **95**: 323–331. doi:10.1016/S0006-3207(00)00024-0.

Whitlock, M.C., and McCauley, D.E. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. Heredity, **82**: 117–125. doi:10.1038/sj.hdy.6884960. PMID:10098262.

Wilson, A.J., Hutchings, J.A., and Ferguson, M.M. 2004. Dispersal in a stream dwelling salmonid: inferences from tagging and microsatellite studies. Conserv. Genet. **5**: 25–37. doi:10.1023/B:COGE.0000014053.97782.79.

Wilson, G.A., and Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics, **163**: 1177–1191. PMID:12663554.