

The Influence of Ecology and Genetics on Behavioral Variation in Salamander Populations across the Eastern Continental Divide

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ABSTRACT: Understanding the unique contributions of ecology and history to the distribution of species within communities requires an integrative approach. The Eastern Continental Divide in southwestern Virginia separates river drainages that differ in species composition: the more aquatic, predatory *Desmognathus quadramaculatus* is present only in the New River drainage (which drains to the Gulf of Mexico), while *Desmognathus monticola* is present in both the New River drainage and the James River drainage (which drains to the Atlantic Ocean). We investigated natural distributions, behavioral variation in experimental mesocosms, population genetic, and phylogenetic implications of community structure. The presence of *D. quadramaculatus* increased the terrestriality of *D. monticola* in natural and experimental situations but to different degrees in allopatric and sympatric populations. Our ecological data suggest that the degree of terrestriality in *D. monticola* is a result of a balance between the optimal aquatic habitat and risks of predation. Our genetic analyses suggest that *D. monticola* has experienced a recent range expansion and has only a recent history of association with *D. quadramaculatus* in Virginia. This is surprising given the strong behavioral variation that exists in populations experiencing unique community compositions over a scale of meters. This study demonstrates the need to combine both ecology and genetics toward an understanding of the factors affecting species distributions, behavioral variation between populations, and patterns of genetic variation across a landscape.

Keywords: phylogenetics, behavioral ecology, ecological interactions, salamanders, southern Appalachians, *Desmognathus*.

For community ecologists, there are two dominant paradigms concerning the distribution of organisms in a community: the niche assembly and dispersal assembly perspectives (Hubbell 2001). The niche assembly perspective emphasizes the force of species interactions (e.g., competition, predation) in structuring distribution and abundance patterns within a community. Often, community structure that arises from species interactions is then implicated as a factor influencing the evolutionary divergence of lineages (e.g., Hutchinson 1957, 1959). For example, divergence of resource use is a common behavioral consequence of species interactions that may lead to character displacement and eventually speciation (e.g., Losos 1994; Losos et al. 1998; Magurran 1999). The dispersal assembly perspective emphasizes the influence of history (e.g., migration routes, dispersal barriers) in structuring communities. In addition, the emerging field of community genetics (see Neuhauser et al. 2003; Whitham et al. 2003) emphasizes the importance of synthesizing community ecology and population genetics to gain fresh insights into the consequences of species interactions (Antonovics 1976, 2003). Reconciling the unique contribution of ecological and historical impacts on communities therefore requires an examination of both ecology and genetics (Losos 1996; Schluter 1996, 1998; Foster and Endler 1999; Hatfield and Schluter 1999).

There has been a long history of research into how species interactions may influence community structure in the salamander genus *Desmognathus* (Caudata: Plethodontidae) and the genetic and evolutionary consequences of that structure (e.g., Dunn 1917, 1926; Wilder and Dunn 1920; Hairston 1987; Titus and Larson 1996). Special emphasis has been placed on how competition and predatory interactions affect spatial distributions within communities, which in turn may influence broader patterns of speciation (Dunn 1917; Wilder and Dunn 1920; Hairston

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1949; Tilley 1968; Titus and Larson 1996). The genus *Desmognathus* is unique in that the spatial distribution of species is highly structured along the stream to forest floor ecotone, with large aquatic taxa displacing smaller taxa into more terrestrial, less optimal sites (Hairston 1987). The idea was that the ancestral taxa inhabited streams, with derived taxa becoming less aquatic as a consequence of being ecologically driven to exploit more terrestrial habitats. Molecular data, however, point to the opposite sequence of events; terrestrial, direct-developing species of the genus are basal, and the more aquatic species are derived (Titus and Larson 1996; Rissler and Taylor 2003). These examples illustrate the danger of extrapolating current ecological forces as causal factors in evolution and highlight the interpretative power of applying a historical approach in community ecology.

In this article, we examine whether species interactions influence the evolution of behavior at a finer spatial scale, focusing on a single species with populations that experience interactions with different species over a scale of meters. *Desmognathus monticola*, the seal salamander, is a semiaquatic species that is endemic to, and broadly distributed throughout, the Appalachian mountains of the eastern United States. At roughly the center of this geographic distribution (in southwestern Virginia, Giles County), local populations of *D. monticola* occur on both sides of the Eastern Continental Divide (ECD). The ECD separates the New River drainage (which flows to the Mississippi River and the Gulf of Mexico) from the James River drainage (which flows to the Atlantic Ocean). *Desmognathus monticola* populations in the headwater streams on different sides of the continental divide are often within meters of one another via terrestrial routes of dispersal but completely isolated via aquatic routes (fig. 1).

An important feature of the ECD is that *Desmognathus quadramaculatus*, the black-bellied salamander, a large aquatic salamander that is known to interact with *D. monticola* through competition and predation, only inhabits streams in the New River drainage. The difference in distribution of *D. monticola* and *D. quadramaculatus* across the ECD creates a unique natural experiment to examine whether variation in community composition and therefore species interactions promotes evolutionary differences in behavior and habitat use over a small spatial scale. Specifically, *D. quadramaculatus* has been shown to displace *D. monticola* away from the stream edge, with *D. monticola* in turn displacing smaller species farther into the forest. This pattern is thought to be important for the evolution of taxa that exploit terrestrial habitats (Dunn 1917; reviewed in Titus and Larson 1996) and for influencing rates of gene flow via aquatic and terrestrial routes (Tilley 1997).

We investigated whether species interactions have led to

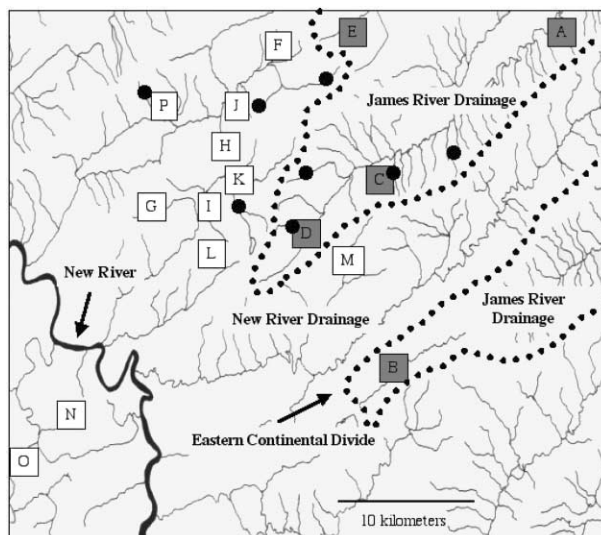


Figure 1: Distribution of sampling for *Desmognathus monticola* around the Eastern Continental Divide in Virginia. Circles are regions where stream measurements occurred for the natural distribution analyses. Gray squares are regions in the James River drainage, and white squares are regions in the New River drainage where salamanders were collected for DNA analyses. Letters correspond to collection localities (fig. 4; table A1 in the online edition of the *American Naturalist*).

local divergence in behavior, and we explored the possible evolutionary consequences of that divergence. One possibility is that the ECD is an important zoogeographic boundary that has permitted *D. monticola* to diverge genetically as well as in their behavioral responses to *D. quadramaculatus* in sympatric and allopatric areas. If this were true, we would expect there to be genetic divergence as well as ecological divergence across the ECD. However, if we find ecological divergence and a lack of genetic divergence, this would point to rapid behavioral divergence either in the face of ongoing gene flow or following a recent population expansion event.

Local divergence in aquatic versus terrestrial habitat use may also have genetic consequences if the use of aquatic habitats enhances among-population gene flow via aquatic corridors. Tilley (1997) suggested that the larger, more aquatic congeners, *D. monticola* and *Desmognathus fuscus*, force the smaller *Desmognathus ochrophaeus* into even more terrestrial sites that are less interconnected by gene flow. Similarly, variation in the use of aquatic habitats by *D. monticola* could influence the distribution of genetic variance on either side of the ECD if the presence or absence of *D. quadramaculatus* influences its use of aquatic versus terrestrial habitats. The prediction would be that sympatric populations show increased terrestriality due to

species interactions and therefore suffer reduced levels of genetic variation because of restricted gene flow.

To examine the complex interplay between species interactions, behavior, and population genetic structure, we studied the spatial distribution of the species in natural communities and used experimental analyses of species interactions to determine what factors influence habitat choice and distribution patterns. We also used population genetic and phylogenetic analyses to provide a historical component to the interpretation of behavioral and ecological variation to investigate how habitat use has influenced genetic variation within and among populations that differ in community composition.

Methods

Study System

The genus *Desmognathus* is a diverse group of lungless salamanders in the Appalachian Highlands encompassing the most extensive range of habitats used by the family Plethodontidae (Hairston 1986). The species of *Desmognathus* near the Mountain Lake Biological Station, Virginia, form a continuum from a predominately terrestrial to a more aquatic life history and include *Desmognathus ochrophaeus* (most terrestrial), *Desmognathus fuscus*, *Desmognathus monticola*, and *Desmognathus quadramaculatus* (most aquatic). Experimental studies have shown that more aquatic species can influence the activity and spatial distribution patterns of more terrestrial ones, forcing them into drier, more terrestrial sites (e.g., Hairston 1980, 1986; Carr and Taylor 1985; Southerland 1986a, 1986b; Roubush and Taylor 1987), but none has focused on geographic variation in behavior within a species or on the genetic consequences of that behavior.

Natural Distributions across the Eastern Continental Divide

To determine whether spatial distributions of *D. monticola* differed on either side of the ECD, we randomly chose eight streams, four from each drainage, for extensive sampling (fig. 1). We used two transects (located 25–50 m apart) at each stream to compare spatial distributions during the day and night. The exact location of each transect was chosen to minimize environmental variation between streams. Each transect had two plots located on opposite banks of the stream for a total of four plots per stream. Each plot (5 m wide × 25 m long) was perpendicular to the streambed and traversed the stream to forest floor ecotone. The two paired plots at one site were surveyed on a single census date; therefore, one plot in each pair was surveyed during the day and the other at night.

We surveyed the plots in random order between June 23 and July 30. A night survey consisted of collecting all visually detected metamorphosed salamanders and placing a numbered flag at the location of capture. We also recorded the percent of the body exposed of each salamander. During a day survey, all cover objects in and out of the stream were turned over and replaced. A plastic hand rake was used to search the leaf litter for salamanders, and all metamorphosed salamanders were captured and a numbered flag was placed at the location of capture. Salamanders were transported to the laboratory where measurements were taken, including snout to vent length (tip of snout to posterior angle of the vent), mass, tail length, and sex. Tail percentage was used as a measure of condition (percent of tail relative to total body length). The following day, we returned salamanders to their point of capture and measured the distance from the stream edge to each flag.

To determine whether major environmental variables relevant to salamander biology (e.g., amount of cover; Kleeberger 1985) differed in the two drainages separated by the ECD, we set up four 1-m² plots in each of four streams per drainage to estimate the percent of sand, rocks >12 cm², boulders, logs, and leaf packs per drainage. We also noted whether *Salvinella fontinalis*, brook trout, was present.

Interactions among Species in Mesocosms

To investigate whether habitat use (the degree of terrestriality) of *D. monticola* is influenced by the presence of *D. quadramaculatus*, we compared the behavior of *D. monticola* individuals from populations separated by the ECD in experimental situations with and without *D. quadramaculatus*. *Desmognathus monticola* populations in the New River drainage are sympatric with, and therefore have prior exposure (either personal or evolutionary) to, *D. quadramaculatus*; *D. monticola* populations in the James River drainage are allopatric with *D. quadramaculatus*. Therefore, the experimental design was fully factorial with two factors and four total treatments (*D. monticola* with no prior experience [James River drainage] alone, *D. monticola* with prior experience [New River drainage] alone, *D. monticola* with no prior experience and *D. quadramaculatus*, and *D. monticola* with prior experience and *D. quadramaculatus*), each replicated nine times.

Nongravid adults of *D. monticola* were captured from populations in tributaries of the New River and James River surrounding the Mountain Lake Biological Station. Nongravid adults of *D. quadramaculatus* were captured from a more southern Virginia population surrounding Whitetop Mountain, Grayson County. All salamanders were housed in separate containers in a laboratory with a 12L : 12D photoperiod at 18°–19°C until the experiment

was initiated; at the end of the experiment, all animals were returned to their point of capture. Before the experiment, all individuals were weighed and measured for snout to vent length so that no disparity in body size occurred between treatments that might have inadvertently affected interpretation of treatment effects. All salamanders of each species were randomly assigned to experimental mesocosms across treatments. One individual of each focal species was present in an experimental mesocosm. No significant differences in salamander size occurred across treatments (see "Results"). Both animals were allowed to habituate for 3 days before the initiation of behavioral observations. In addition, in treatments where *D. quadramaculatus* was present, individuals were placed in the units an additional 3 days before the addition of *D. monticola*. This was done to simulate natural conditions where *D. quadramaculatus* is always near the edge of streams (residents), and *D. monticola* is most likely to interact with *D. quadramaculatus* when *D. monticola* returns to the stream to deposit eggs.

Experimental mesocosms were used to simulate the stream to forest ecotone (following Resetarits and Fauth 1998). In this experiment, each unit (3.33 m long \times 0.45 m wide \times 0.36 m deep cattle feed bunk) was positioned to maintain a slope of 13°–15° from the high terrestrial end to the low aquatic end. Water was pumped from a pond into the low end of each unit so that each maintained an independent water supply with individually controlled flow. Water entered each unit at a rate of 2.0 L/min and pooled at a depth of 5 cm. Water cascaded over two large (approximately 17.5 cm \times 17.5 cm) rocks (splashrock) before exiting through a drain. We simulated a streambed to forest floor habitat gradient by positioning cobbles (1.5 cm \times 1.5 cm), sand, and soil with leaf litter into the units. Each substrate (water, cobbles, sand) occupied an area approximately 0.5 m long \times 0.45 m wide within each of the 36 total units except for the leaf litter, which occupied an area 1.8 m long \times 0.45 m wide. A small rock (approximately 10 cm \times 10 cm; sandrock) was placed on the sand substrate and a log (12 cm long \times 6 cm wide \times 6 cm high; log) was placed in the leaf litter substrate to serve as potential cover objects. An overhang of duct tape was placed on the top edge of each unit to prevent escape. This allowed the units to be open to the environment, ensuring access by natural invertebrate prey and possible avian predators.

We examined salamander behavior during 20 nights and 14 days between June 26 and July 27, 1998. To ensure similar environmental conditions across dates, we supplemented the units with 1.90 L of water sprinkled over the entire unit every 2 days if a rain had not occurred. On sampling nights, we nondestructively searched all units by passing a flashlight over the entire unit to determine where

actively foraging salamanders were located. During the sampling days, we carefully lifted all cover objects (splashrock, sandrock, log) to determine where salamanders were located. During each census, we recorded the exact location of each salamander as the distance (cm) from the aquatic portion of the unit; the substrate was also noted. In addition, the percent of the body exposed of each salamander was estimated. This measure has been suggested to correlate negatively with the perceived threat of predation (reviewed in Sih 1987). If an animal was found under a cover object, percent body exposure was 0%; if an animal was out foraging on the sand and completely exposed, then percent body exposure was 100%.

We analyzed salamander size, distance to water, and percent body exposure by ANOVAs. All variables were tested for normality and homogeneity of variances. Percent body exposure was arcsine and square root transformed preceding analysis. We used date, treatment, and unit nested within treatment (replicates) as factors in the analysis for distance to water and percent body exposure. When replicates were not significant, the remaining factors were tested over the residual error. This procedure is equivalent to a repeated measures ANOVA (SAS Institute 1996). We also analyzed the same data in a more conservative fashion by taking the mean of a unit over all dates and using this as an observation. Salamander size was calculated as the difference in initial length (snout to vent) and mass from the final length and mass. Differences in substrate use across treatments were analyzed by χ^2 . All statistical analyses were performed using SAS 6.11 (SAS Institute 1996).

Molecular Population Genetics

We sampled 71 total individuals from Virginia, North Carolina, Georgia, Alabama, Kentucky, Tennessee, and West Virginia as well as the New River and James River drainages (table A1 in the online edition of the *American Naturalist*). Individuals were generally collected within 15 m of a streambed. Rather than focusing on large numbers of individuals from a few streams, we maximized the number of streams by sampling one to eight individuals per stream in each drainage. Individuals from outside of Virginia were used to place the genetic data into a broader geographic context. Tail tissue obtained from live or frozen (–80°C) animals was used to extract genomic DNA. Extraction was completed by either a standard phenol:chloroform extraction (Hillis et al. 1996) or the DNeasy tissue kit protocol (Qiagen, Valencia, Calif.).

Amplification of about 550 bp of the cytochrome oxidase I (CO I) gene and about 650 bp of a variable portion of the 12S rRNA gene was completed on all individuals by using primers COX1F (5'-GGTATTGAGTTTCGGTCTG-

3') with COX1R (5'-CTTAGTCTCTTAATTCGAGC-3') and VALB (5'-AAACTGGGATTAGATACCCCACTA-3') with VALG (5'-AGGTTTTCTGTGCGCCCTTAC-3'; Titus and Larson 1996; Rissler and Taylor 2003). The amplification profile entailed 35 cycles of denaturation at 94°C for 60 s, annealing at 52°C for 60 s, and extension at 68°C for 120 s on a Perkin-Elmer DNA thermocycler 480. Polymerase chain reaction (PCR) products were then purified by either a QIAquick PCR purification kit (Qiagen, Valencia, Calif.) or Centricon centrifugal filter devices YM-100 (Millipore, Bedford, Mass.). Purified PCR products were cycle sequenced in both forward and reverse directions using dideoxy terminator reactions (Applied Biosystems, Foster City, Calif.) that were purified by Centriseq spin columns (Princeton Separations, Adelphia, N.J.) and visualized using an ABI 377 automated DNA sequencer.

Forward and reverse sequences were assembled into contigs in Sequencher 3.0. We treated the mitochondrial genome as a single locus (see Rissler and Taylor 2003), appending data from the two separate mitochondrial genes from each individual. Sequences were first aligned using PILEUP (GCG, Wisconsin Package Version 10.0, Genetics Computer Group, Madison, Wis.), and the alignments were corrected manually. GenBank accession numbers are given in table A2 in the online edition of the *American Naturalist*.

Phylogenetic analyses were performed using neighbor joining to get the initial tree with tree bisection-reconnection swapping under the minimum evolution criterion. We also performed 1,000 bootstrap replicates on the neighbor-joining tree with distances calculated under the maximum likelihood model of evolution and on a parsimony tree in PAUP*4.0b (Swofford 1998). The model of evolution was chosen by ModelTest 3.04 (Posada and Crandall 1998).

Population genetic analyses were done in DnaSP 3.14 (Rozas and Rozas 1999). To detect population expansion events, we used F_s (Fu 1997), which tests for an excess of low-frequency alleles relative to the neutral expectation (Excoffier and Schneider 1999; Fedorov and Stenseth 2001; Joseph et al. 2002). We also used coalescent theory to examine the shapes of the gene trees. Tajima's D (Tajima 1989) and Fu and Li's (1993) D^* and F^* statistics were used to detect departures from a neutral coalescent process. These statistics, as originally constructed, were used to infer selection but are now commonly used to detect population expansion events (Knowles et al. 1999; Joseph et al. 2002; Crespi et al. 2003). Values are expected to be 0 under a neutral model of evolution with constant effective population size, negative under a model of population expansion (Slatkin and Hudson 1991; Fu 1997; Knowles et al. 1999) or a selective sweep (Maruyama and Birky 1991; Fu 1997; Filatov et al. 2000), and positive

under a model of population subdivision where long-term geographic subdivision enhances the accumulation of mutational differences between populations (Slatkin and Hudson 1991; Rogers and Harpending 1992; Marjoram and Donnelly 1994; Fu 1997; Wakeley and Hey 1997).

To examine the spatial structure of genetic variation across the ECD, we used only individuals from the New River and James River drainages. We used AMOVA 1.5 (Excoffier et al. 1992), an ANOVA procedure that partitions molecular variance according to sampling design. We compared the within and among genetic variance from populations separated by the ECD. If species interactions affected habitat use and the degree of terrestriality, we expected that genetic structure may also be different in sympatric and allopatric populations. We also examined whether the ECD served as a barrier to genetic exchange for *D. monticola*. AMOVA produces ϕ statistics that are analogous to Wright's F statistics (1951, 1965). The input data were the pairwise genetic distances (calculated under the maximum likelihood generated model of evolution) between all individuals from streams that had at least two collected individuals. All ϕ statistics were tested for significance using 1,000 random permutations. We also used a χ^2 test and a Z^* test with 1,000 permutations (Hudson et al. 1992) to compare the genetic differentiation between individuals from the New River and James River drainages.

Results

Natural Distributions across the Eastern Continental Divide

The natural distribution of *Desmognathus monticola* differed in the two drainages separated by the ECD. As expected, *D. monticola* was found significantly farther from the water in the region where it is sympatric with *Desmognathus quadramaculatus* (New River drainage; $F = 5.12$, $df = 1, 118$, $P = .026$; fig. 2). Trout influenced the spatial distribution of *D. monticola* differently in the two drainages, with *D. monticola* being found farthest from streams in the New River drainage where trout were present and in streams in the James River drainage where trout were absent (drainage \times trout: $F = 4.76$, $df = 1, 118$, $P = .031$). Interestingly, the condition of *D. monticola* was highest in the areas where the species inhabits the most aquatic habitats: in the New River drainage where trout were absent and in the James River drainage where trout were present ($F = 4.24$, $df = 1, 76$, $P = .043$).

Snout to vent length ($F = 1.28$, $df = 3, 76$, $P = .286$) and mass ($F = 0.86$, $df = 3, 71$, $P = .464$) did not differ across the ECD. None of the environmental variables that are thought to be important for salamanders varied across the ECD: sand ($T = -0.110$, $N = 16$ plots per drainage,

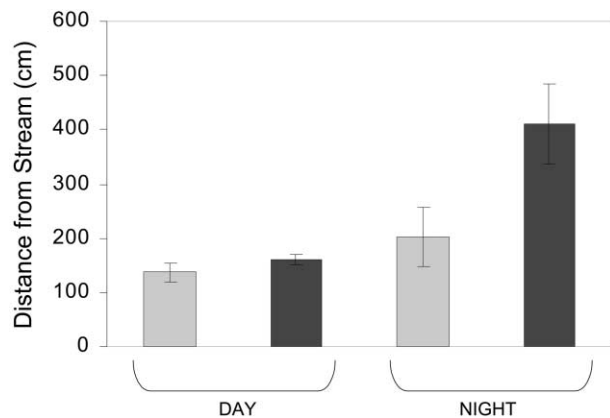


Figure 2: Distance from the stream of natural populations of *Desmognathus monticola* in the James River and New River drainages (fig. 1) in Giles and Craig Counties, Virginia. *Desmognathus monticola* is sympatric with *Desmognathus quadramaculatus* in the New River drainage. Means with standard errors are given (cm). Gray, James River populations. Black, New River populations.

$P = .914$), rocks $>12 \text{ cm}^2$ ($T = -0.312$, $N = 16$, $P = .757$), boulders ($T = -0.092$, $N = 16$, $P = .927$), logs ($T = -0.591$, $N = 16$, $P = .561$), and leaf packs ($T = 0.835$, $N = 16$, $P = .413$).

Interactions among Species in Mesocosms

Individuals of *D. monticola* altered their substrate use and became more terrestrial when in the presence of *D. quadramaculatus*. During the day, *D. monticola* altered substrate use in every treatment when in the presence of *D. quadramaculatus* ($\chi^2 = 43.41$, $df = 6$, $P = .001$; *D. monticola* was not found in the sand or sandrock areas during the day; therefore, this category was deleted from the analysis). *Desmognathus monticola* was always found in more aquatic habitats when *D. quadramaculatus* was absent (James River drainage: $\chi^2 = 24.19$, $df = 2$, $P = .001$; New River drainage: $\chi^2 = 10.91$, $df = 2$, $P = .004$).

During the night, *D. monticola* with prior exposure to *D. quadramaculatus* (i.e., those from the New River drainage) were found more often in more terrestrial sites, and animals with no prior exposure to *D. quadramaculatus* (i.e., those from the James River drainage) were found in more aquatic sites ($\chi^2 = 8.44$, $df = 3$, $P = .038$). When each population (sympatric or allopatric) was compared in the two situations (absence or presence of *D. quadramaculatus*), *D. monticola* was always found in more terrestrial habitats in the presence of *D. quadramaculatus* (allopatric: $\chi^2 = 12.71$, $df = 3$, $P = .005$; sympatric: $\chi^2 = 9.74$, $df = 3$, $P = .021$). In the absence of *D. quadramaculatus*, animals with prior exposure to *D. quadra-*

maculatus (sympatric) were in the aquatic region less often than expected by chance, while animals with no prior exposure to *D. quadramaculatus* (allopatric) were in the aquatic region more often than expected by chance. Prior exposure did not affect substrate use of *D. monticola* in the presence of *D. quadramaculatus* ($\chi^2 = 6.22$, $df = 3$, $P = .101$).

Distance to water also differed between individuals of *D. monticola* captured from tributaries of the New and James River drainages. Animals that had no prior exposure to *D. quadramaculatus* reacted more strongly to the presence of *D. quadramaculatus* than individuals with prior exposure (sympatric; fig. 3). Allopatric individuals differed in distance to water ($F = 15.80$, $df = 1, 16$, $P = .001$) when in the absence or presence of *D. quadramaculatus*. Sympatric individuals were farther from the water in the presence of *D. quadramaculatus*, but the effect was not significant ($F = 1.18$, $df = 1, 16$, $P = .705$). Therefore, those populations that had prior experience behaved as if *D. quadramaculatus* was present by being farther from water even when *D. quadramaculatus* was experimentally absent. When we analyzed the same data but used the mean of distance to water for each experimental mesocosm over the 23 observation dates, animals without prior experience (allopatric) were significantly farther from water than animals with prior experience (sympatric) when *D. quadramaculatus* was present ($F = 3.83$, $df = 3, 63$, $P = .014$). In all cases, percent body exposure was significantly higher during the night, when foraging occurs, than during the day as expected (data not shown). All other comparisons of percent body exposure between treatments were not significant.

We predicted that an increased terrestrial habitat in the presence of *D. quadramaculatus* would negatively affect the condition of *D. monticola*. All *D. monticola* were distributed randomly with respect to treatment, and there was no difference in length and mass before the experiment began (table 1). Although the mesocosm experiments were probably too short to detect treatment effects on growth in body length, body mass was significantly affected by treatment (table 1). *Desmognathus monticola* from the allopatric population did not gain mass in the presence of *D. quadramaculatus*; this is the treatment where individuals of *D. monticola* were most terrestrial. Also, in the absence of *D. quadramaculatus*, individuals from the sympatric population were not as heavy as allopatric *D. monticola*; this is the treatment where sympatric salamanders, even in the absence of *D. quadramaculatus*, were found in more terrestrial sites compared with their allopatric counterparts.

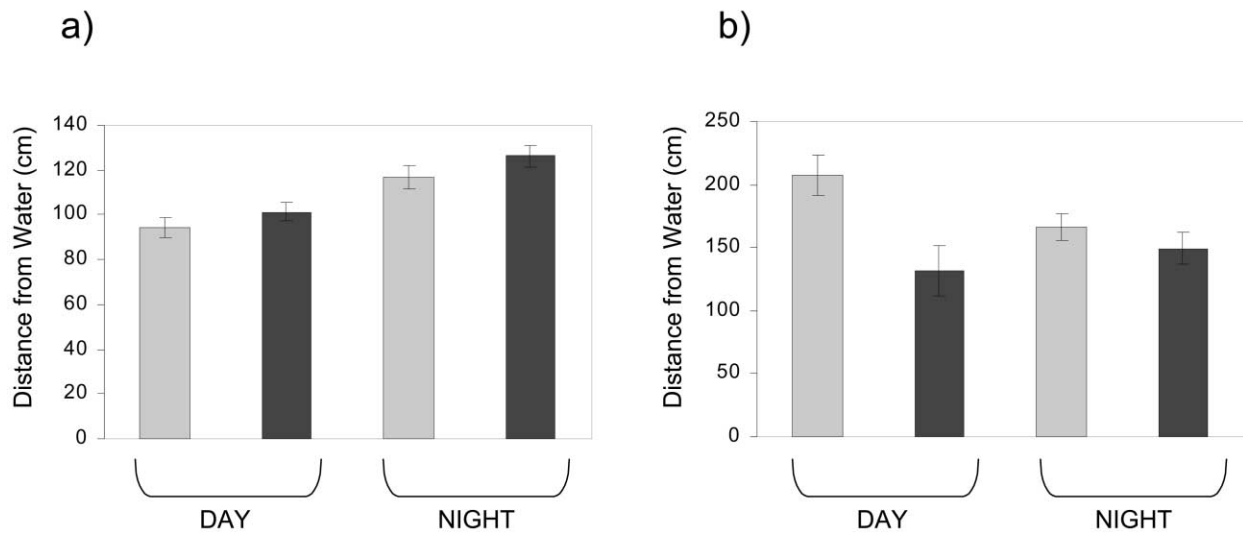


Figure 3: Variation in distance from the water source of *Desmognathus monticola* from allopatric (gray) populations and sympatric (black) populations in experimental mesocosms. *a*, In the absence of *Desmognathus quadramaculatus*. *b*, In the presence of *D. quadramaculatus*. Means with standard errors are given.

Molecular Population Genetics

The sequence data included 1,299 characters and 360 parsimony informative sites. The HKY + Γ model of evolution was chosen with base frequencies of A = 0.3687, C = 0.1749, G = 0.1818, and T = 0.2746; transition transversion ratio = 2.6329; among-site rate variation = 0; and Γ shape parameter = 0.3673. There was little overall genetic diversity among haplotypes from a fairly broad distribution including samples from North Carolina, Kentucky, Tennessee, West Virginia, and Virginia (fig. 4). Haplotypes from northern Alabama, however, were distinct (fig. 4). The southern Alabama haplotypes formed an even more distinct clade that was basal to the major *D. monticola* clade (Rissler and Taylor 2003). Quantitative estimates of haplotype and nucleotide diversity showed little genetic variation between samples collected from Virginia, North Carolina, Kentucky, Tennessee, and West Virginia (table 2).

A significantly negative F_s statistic (-10.622 ; $P < .000$) is consistent with population growth under an expanding population model. Tajima's D (-2.843 ; $P < .001$) and Fu and Li's D^* (-6.582 ; $P < .020$) and F^* (-6.095 ; $P < .020$) statistics were also significantly negative. Therefore, the shape of the phylogeny, based on nucleotide differences between individuals within a clade, had shallower branches than expected under the neutral model of evolution. The tree, therefore, has an overabundance of relatively young alleles, which is consistent with a model of population expansion (Tajima 1989; Maruyama and Birky 1991; Slat-

kin and Hudson 1991; Fu 1997; Knowles et al. 1999; Filatov et al. 2000).

Less than 1% of the genetic variation was distributed between the New River and James River drainages, while the rest of the genetic variation was distributed within streams (table 3). In fact, a negative variance component resulted for the streams within drainage component. This is common in situations where there is a lack of genetic structure and occurs because the estimates are approximated by subtraction of other components of variance from the overall variance observed (Excoffier et al. 1992). In addition, separate nonparametric analyses of the genetic differentiation between the populations separated by the ECD were not significant ($\chi^2 = 18.05$, $df = 19$, $P = .515$; $Z^* = 6.59$, $P = .727$; Hudson et al. 1992).

Discussion

Recent studies suggest that there is much to be gained from an integrative approach to community ecology, in which phylogenetic history is considered with current ecological interactions as potential mechanisms affecting the distribution and abundance of taxa within communities (Travis 1996; Schluter 1998; McPeck 1999; Hubbell 2001; Neuhauser et al. 2003; Whitham et al. 2003; Cavender-Bares et al. 2004). Our data indicate that behavioral variation occurs in populations of *Desmognathus monticola* separated by the ECD, so much so that the behavior of individuals from sympatric populations differs from that

Table 1: Variation in mass of *Desmognathus monticola* across treatments within experimental mesocosms

Drainage and congener present	Treatment	Treatment comparison	Difference in final and initial mass (g) ^a	F	df	P
James:						
No	1	1 vs. 2	.06 ± .15	6.15	1, 14	.027
Yes	3	3 vs. 4	-.71 ± .27	.08	1, 15	.778
New:						
No	2	1 vs. 3	-.36 ± .12	4.77	1, 15	.045
Yes	4	2 vs. 4	-.45 ± .28	.46	1, 14	.510

Note: Congener refers to the presence or absence of *Desmognathus quadramaculatus*. See text for details on the treatments. *Desmognathus monticola* did not vary in length ($\bar{X} \pm SE = 58.29 \pm 1.19$ cm; $F = 1.13$, $df = 3, 29$, $P = .355$) or mass ($\bar{X} \pm SE = 4.32 \pm 0.26$ g; $F = 1.81$, $df = 3, 29$, $P = .167$) across treatments before the experiment began.

^a Means of the nine replicates per treatment \pm SE. A positive value means the animal gained mass, and a negative value means the animal lost mass.

of individuals from allopatric populations even in the experimental absence of *Desmognathus quadramaculatus*. This variation in nature occurs over a small spatial scale (fig. 1).

The different patterns of behavior in *D. monticola* across the ECD and the fact that the ECD is one important biogeographic barrier for *D. quadramaculatus* raise the possibility that *D. monticola* populations on either side of the ECD have differentiated genetically as well. However, our population genetic and phylogenetic analyses suggest that the behavioral divergence is a relatively recent phenomenon and not a result of *D. monticola* having evolved in situ with *D. quadramaculatus*.

The behavioral divergence and patterns of terrestriality across the ECD suggest a complex behavioral response to variation in species interactions across a small spatial scale. In the mesocosms, naive animals from the James River drainage (currently allopatric populations) are more aquatic than *D. monticola* from the New River drainage (currently sympatric populations), but they react strongly by shifting habitat use into highly terrestrial sites (and subsequently suffer by a reduction in mass) when *D. quadramaculatus* is presented to them. Taken together, the mesocosm experiments and natural distribution data suggest that the degree of terrestriality in *D. monticola* is a result of a balance between the optimal aquatic habitat and risks of predation. This lability, in terms of the degree of terrestriality, permits coexistence of congeners and suggests that species interactions have been a mechanism generating community structure in the genus (Losos et al. 2003; Cavender-Bares et al. 2004). However, our data do not allow us to differentiate between genetic versus learned causes of the behavioral differences.

Biogeographic differences in the composition of communities can profoundly affect the behavior of individuals and the niches they exploit (Kurzava and Morin 1994;

Fauth 1998; Losos et al. 1998; Schluter 2000a, 2000b). These ecological differences across populations may have evolutionary consequences (Morin 2003). Analyses in other species, especially fishes such as Trinidadian guppies (*Poecilia reticulata*), European minnows (*Phoxinus phoxinus*), and Canadian three-spined sticklebacks (*Gasterosteus aculeatus*), have demonstrated that population variation in foraging, mating preferences, and antipredatory behavior is a direct consequence of selection exerted by the presence of predators (Endler 1980; Reznick et al. 1990; Magurran et al. 1992; Foster 1999; Magurran 1999). Our data on the *Desmognathus* community are interesting because behavior varies over a scale of meters by land. In our case, geology (ECD) and evolutionary history play an important but only indirect role in affecting behavioral differences across populations of *D. monticola* by serving as a barrier to *D. quadramaculatus*.

Previous ecological research has clearly demonstrated the importance of competition and predation in structuring communities of Desmognathine salamanders (Carr and Taylor 1985; Kleeberger 1985; Southerland 1986a, 1986b; Roudebush and Taylor 1987). Grover (2000) and Grover and Wilbur (2002) show that aquatic habitats are preferred in many plethodontid species and that habitat choice can change depending on the presence of congeners. Our current research is the first to examine the prediction that *D. monticola* populations experiencing unique community compositions show differentiation in habitat choice and the degree of terrestriality.

Interactions at the stream edge may be intensified when brook trout are present (Resetarits 1991, 1995). The presence of trout may shift *D. quadramaculatus* into more terrestrial sites. *Desmognathus monticola* would then be expected to shift into even more terrestrial sites, as is seen in streams of the New River drainage where trout are present. In the James River drainage, *D. quadramaculatus*

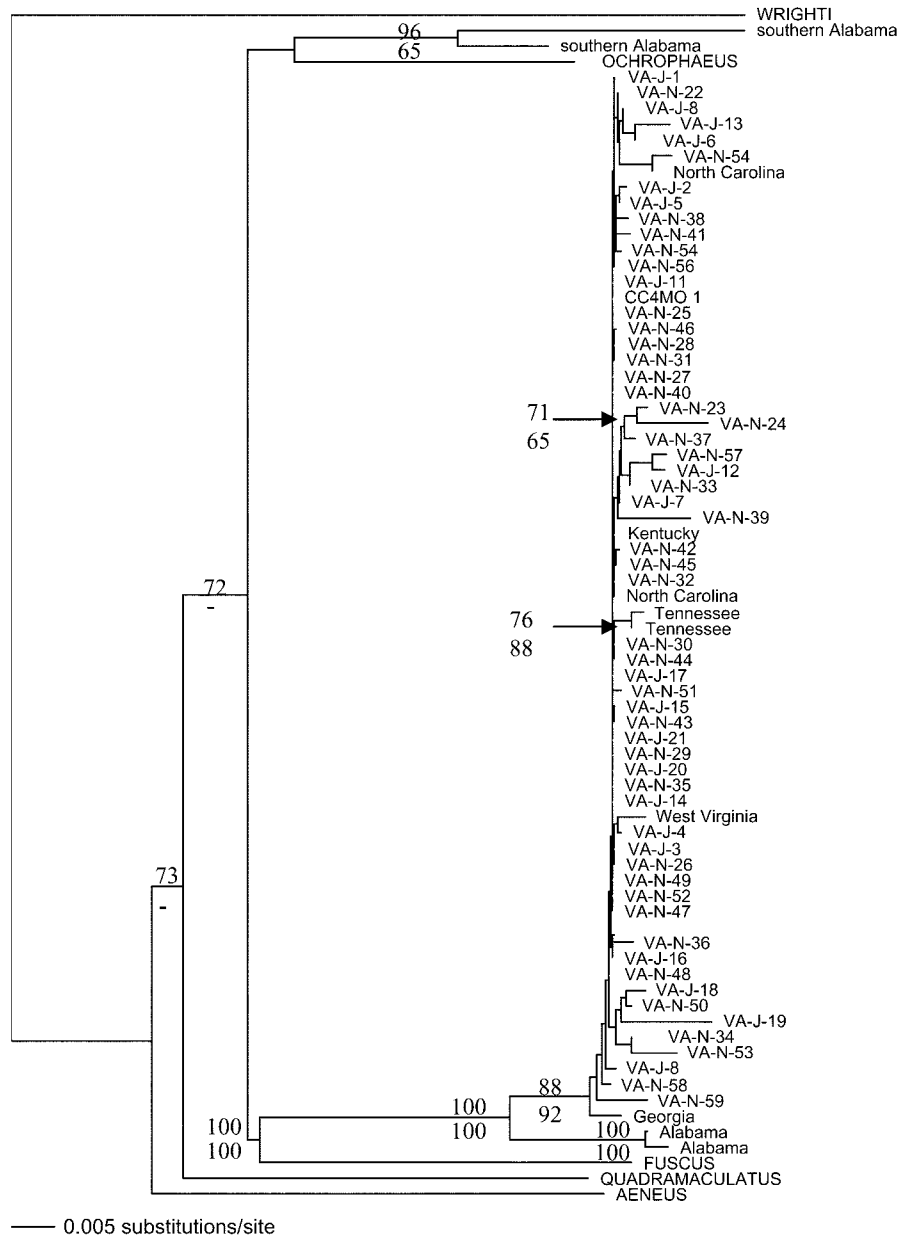


Figure 4: Topology with branch lengths based on a neighbor-joining analysis (with the distances corrected under the maximum likelihood generated model of evolution) for the 12S rRNA and CO I mitochondrial genes of *Desmognathus monticola* and rooted with *Desmognathus wrighti*. Bootstraps of 1,000 replicates on nodes with >60% support. Upper bootstrap value from a parsimony search; lower bootstrap values from the neighbor-joining search. Samples from the geographic region of intensive study are coded with state (VA = Virginia), drainage (J = James or N = New), and stream (number; refer to table A1 in the online edition of the *American Naturalist*).

is absent, and therefore there are no obvious negative consequences of trout presence; in fact, *D. monticola* is most aquatic in streams of the James River drainage where brook trout occur.

Ecological interactions play an important role in structuring the behavior and spatial distribution of multiple

species within the *Desmognathus* salamander community. What are the evolutionary consequences of these interactions? The population genetic data suggest that there is not enough overall population structure in the mitochondrial genome within *D. monticola* to conclusively determine the effect that variation in resource use on either

Table 2: Estimates of haplotype and nucleotide diversity for the specimens of *Desmognathus monticola*

	Haplotype diversity (SD)	π (SD) ^a	K^b	Eta^c	θ^d
Entire clade ^e	.604 (.072)	.004 (.000)	3.060	76	.020
TN, VA, WV, KY	.566 (.076)	.002 (.000)	1.750	54	.014
James and New River	.544 (.081)	.002 (.000)	1.761	51	.013
James ($n = 21$)	.500 (.133)	.002 (.001)	1.714	18	.006
New ($n = 38$)	.573 (.098)	.002 (.001)	1.997	37	.010

Note: TN = Tennessee, VA = Virginia, WV = West Virginia, KY = Kentucky.

^a Nucleotide diversity.

^b Average number of nucleotide differences.

^c Total number of mutations.

^d Number of mutations per site calculated from *Eta*.

^e Does not include samples from southern Alabama (Monroe County); see figure 4.

side of the ECD might have on among-population divergence. Previous studies have also shown relatively little genetic diversity within *D. monticola* (Tilley 1997). This has been interpreted as a result of an aquatic lifestyle because populations are homogenized by frequent gene flow via aquatic corridors. However, the relationship between the use of aquatic habitats and among-population dispersal is not well established. It seems more likely that overall genetic diversity is more strongly influenced by past migration events than current levels of gene flow. For example, of the four sympatric species in our study area, it is only the most aquatic species (*D. quadramaculatus*) that has never traversed the ECD. It seems just as likely, therefore, that the aquatic habitat restricts rather than enhances among-population dispersal. In fact, in a comparative analysis of phylogeographic structure in *Desmognathus ochrophaeus*, *Desmognathus fuscus*, *D. monticola*, and *D. quadramaculatus* (Rissler and Taylor 2003), *D. quadramaculatus*, the most aquatic species, and *D. ochrophaeus*, the most terrestrial species, had the most population genetic structure. Overall, the differences between the species in population structure, genetic diversity, and tree shape showed no tendency to covary with whether the species are predominately aquatic or terrestrial.

The significantly negative Tajima's *D* and Fu and Li statistics suggest that the low overall genetic diversity in

D. monticola is due to a recent range expansion. Nuclear loci (allozymes) show the same pattern of low variation (Tilley 1997), suggesting that a demographic change that influenced all loci is responsible, rather than a selective sweep within the mitochondrial genome. The phylogeographic data (Rissler and Taylor 2003; this study) also show a clear pattern of northward migration. The northward range expansion of *D. monticola*, with the northern Alabama populations being basal followed by Georgia, is similar to other patterns of range expansion in other *Desmognathus* species (Rissler and Taylor 2003), amphibians (Shaffer and McKnight 1996; Tilley and Mahoney 1996), reptiles (Burbrink et al. 2000), and crayfish (Crandall and Templeton 1999). Taken together, the phylogenetic data suggest that the genetic diversity within *D. monticola* is strongly influenced by its recent migration history, and though we cannot discount the possibility of ongoing high levels of gene flow, it is not necessary to explain the data.

In conclusion, our results provide novel insights into the ecology and genetics of *Desmognathus* salamanders. For these two salamander species, the determinants of community structure are a combination of processes that fit the dispersal assembly and niche assembly views of the ecological spectrum (Hubbell 2001). Habitat use and the behavior of species are strongly influenced by current ecological interactions, but those interactions are in turn determined by historical contingencies of migration that determine the composition of the communities. Interestingly, the same two perspectives can be applied to the genetics of these populations, but for the genetics, it is the historical processes that provide the most interpretive power in explaining levels of diversity. In other words, our population genetic and phylogenetic perspective suggests that *D. monticola* has only a recent history of association with *D. quadramaculatus*. This is unexpected given the ecology; strong behavioral variation exists in populations experiencing unique community compositions and thus species inter-

Table 3: Summary of ϕ statistics generated from AMOVA (Excoffier et al. 1992)

	ϕ	Percent	<i>P</i>
Among drainages (ϕ_{CT})	.007	.71	.223
Among streams within drainage (ϕ_{SC})	-.048	-4.72	.672
Within streams (ϕ_{ST})	-.040	104	.733

Note: *P* values were determined from a random permutation test with 1,000 replicates.

actions over a scale of meters. In communities such as these, molecular population genetic methods provide an important historical perspective to understanding the relative roles of ecology and history in shaping patterns of biodiversity (Cavender-Bares and Wilczek 2003).

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