

# Influence of life-history variation on the genetic structure of two sympatric salamander taxa

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## Abstract

Life-history characteristics are an important determinant of a species' dispersal abilities. We predict that variation in life history can influence population-level genetic patterns. To test this prediction, we estimate population-level genetic structure for two sympatric species of stream-breeding salamander. The Cope's giant salamander (*Dicamptodon copei*) rarely metamorphoses into a terrestrial adult, thereby limiting overland dispersal and potentially gene flow. In contrast, the Pacific giant salamander (*D. tenebrosus*) commonly metamorphoses, which is expected to facilitate overland dispersal and gene flow. Three sets of analyses based on microsatellite data support these hypotheses, showing that *D. tenebrosus* displays minimal population-level genetic structuring and no pattern of isolation by distance, whereas *D. copei* displays a high degree of population-level genetic structure and significant isolation by distance. Specifically, nearly all pairwise  $F_{ST}$  values were significantly different from 0 between populations of *D. copei*, with fewer than half the pairwise  $F_{ST}$  values significant from 0 in *D. tenebrosus*. Additionally, STRUCTURE analyses indicated eight genetic clusters for *D. copei* but only one genetic cluster for *D. tenebrosus*. Finally, Mantel tests showed significant correlations between stream and overland distance with genetic distance for *D. copei* but no significant correlations of either landscape feature for *D. tenebrosus* at the scale of the study. These results provide a case study of the link between life-history variation and population genetic patterns while controlling for phylogeny and environmental variation.

**Keywords:** comparative gene flow, *Dicamptodon copei*, *Dicamptodon tenebrosus*, dispersal, life-history variation, population structure

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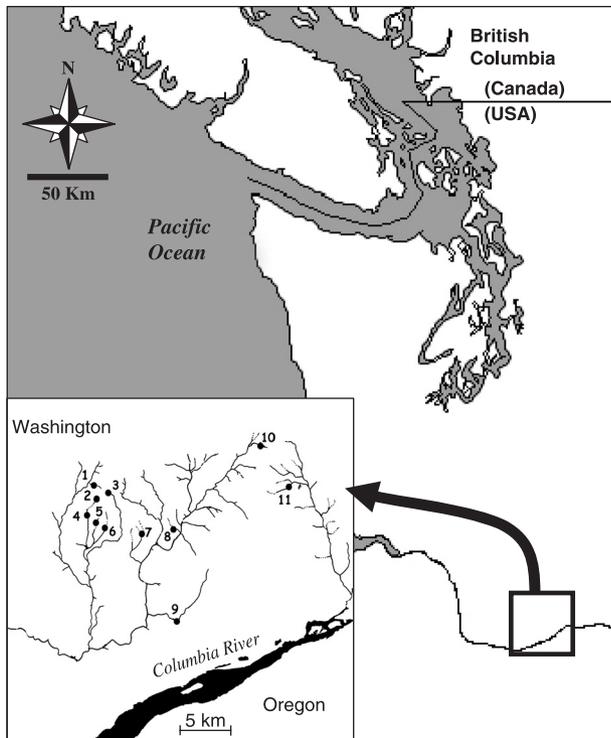
## Introduction

Understanding how variation of life-history traits affects evolutionary patterns and processes within and among populations is a central theme in evolutionary biology (Newman 1992; Roff 1992; Stearns 1992). One critical component of life history is variation in dispersal ability, which is a key factor influencing genetic structuring among populations (Bohonak 1999) and helps explain limits to species' geographical ranges (Reaka 1980; Kunin & Gaston 1993; Brown *et al.* 1996; Holt 2003). A general relationship between dispersal ability and genetic population structure has been demonstrated, whereby

organisms with high dispersal ability tend to display increased gene flow and lower levels of population differentiation relative to organisms with low dispersal abilities (Peterson & Denna 1997; Bohonak 1999; Lourie *et al.* 2005).

To properly evaluate the effect of dispersal ability on genetic structuring, researchers need to simultaneously compare genetic structure of organisms with limited vs. greater dispersal abilities. Ideally, such studies should be performed in a common environment on closely related species with clear differences in dispersal capabilities. Failure to control for phylogenetic relationships when comparing unrelated sympatric species can confound life-history characteristics associated with different evolutionary histories. Similarly, comparing allopatric species that are phylogenetically similar can confound the effects

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**Fig. 1** Regional map of study area. Inset shows sampled localities where *Dicamptodon tenebrosus* and *D. copei* are sympatric. Sample sizes are as follows for each locality (with *D. copei* occurring first and *D. tenebrosus* second): locality 1: 26, 12; locality 2: 19, 5; locality 3: 27, 17; locality 4: 7, 16; locality 5: 20, 18; locality 6: 14, 18; locality 7: 25, 20; locality 8: 23, 21; locality 9: 26, 7; locality 10: 27, 13; locality 11: 21; 10.

of variation in life-history characteristics with influences of dissimilar environments or environmental histories. Because of these restrictions, there are few study organisms that meet the necessary criteria. As a result, studies that compare gene flow in closely related species with clear differences in dispersal capabilities in a common environment are rare (e.g. King & Lawson 2001; Dawson *et al.* 2002).

Two species that meet these requirements are sympatric members of the genus *Dicamptodon*: the Cope's giant salamander (*D. copei*) and the Pacific giant salamander (*D. tenebrosus*). These two stream-breeding salamander species are closely related (Steele *et al.* 2005), have partially overlapping distributions in portions of the Pacific Northwest of the USA, and can be found in close sympatry, thereby providing an opportunity to compare gene flow rates in each species within a common environment.

These two species also have clear differences in metamorphosis frequency that should affect their dispersal ability and population genetic structure. *D. copei* is an almost exclusively neotenic species that retains larval characteristics (e.g. gills) throughout its life and matures sexually in the larval form (Nussbaum 1970, 1976), thereby limiting

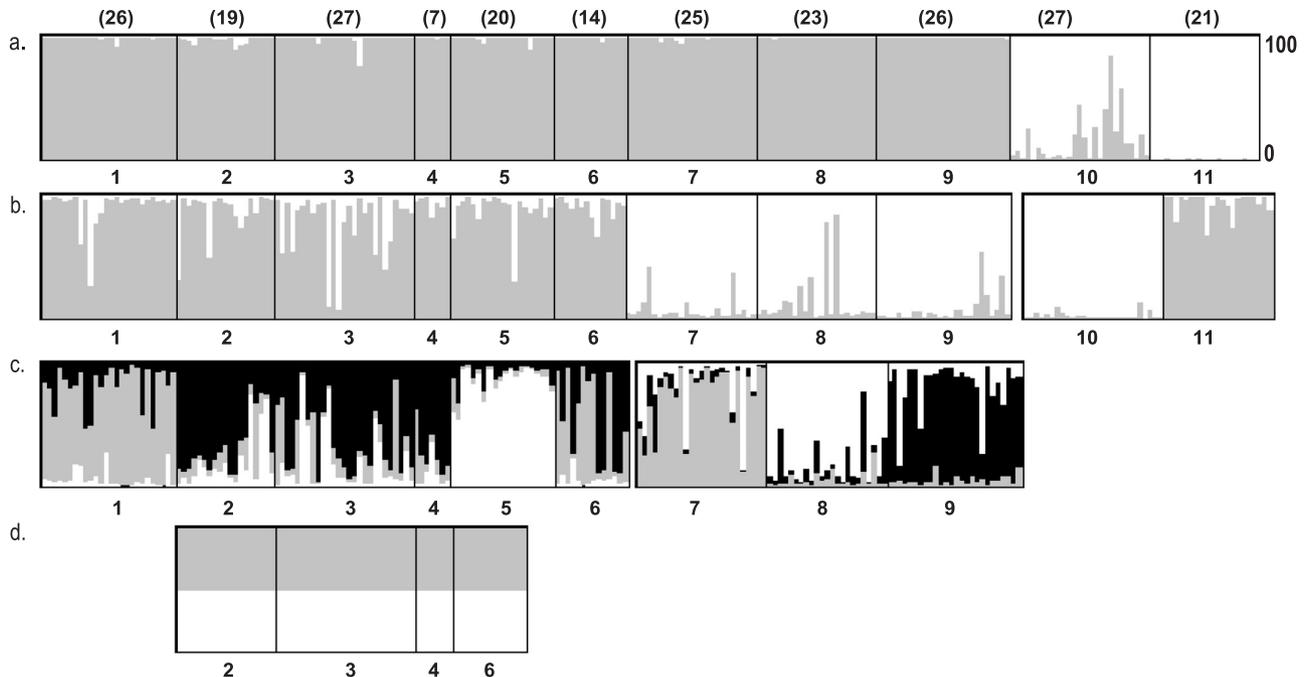
overland dispersal among streams and watersheds. Metamorphosed individuals of *D. copei* are extremely rare, with just six terrestrial specimens documented (Nussbaum 1976; Jones & Corn 1989; Loafman & Jones 1996), none of which have been reported in the area studied herein. Because of its aquatic nature, gene flow should be correlated with stream distance between localities rather than overland distance. In contrast, *D. tenebrosus* regularly metamorphoses into a terrestrial adult, allowing for both overland and in-stream dispersal. Consequently, an overall lower degree of genetic differentiation among populations is predicted for *D. tenebrosus*.

In this study, we examine the effect of dispersal ability on the genetic structure of populations and predict an inverse relationship between dispersal ability and genetic structure. We predict higher gene flow and lower population structure within *D. tenebrosus* than in *D. copei* because the former commonly metamorphoses and the latter has never been known to metamorphose within our study area. Data from microsatellites are used to infer gene flow and population-level genetic structuring for each species at the population level. Our results provide insight into how life-history traits affect gene flow while controlling for the potentially confounding influences of variation in phylogenetic history and environment.

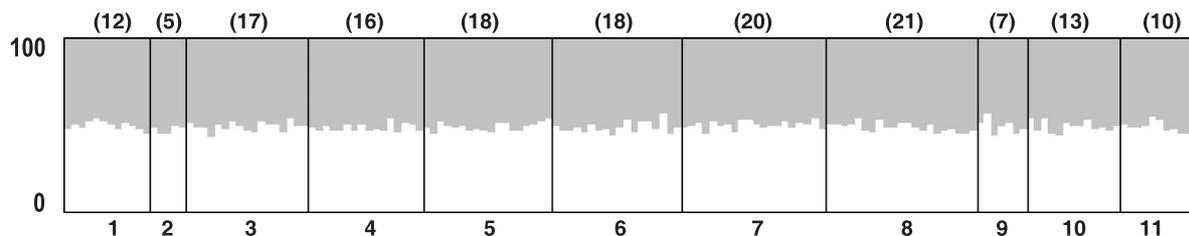
## Materials and methods

### Sample collection and DNA amplification

Tissue samples from sympatric populations of *Dicamptodon copei* and *D. tenebrosus* were obtained from 11 localities in the Cascade Mountains of Washington State (Fig. 1; Appendix I). Sites were selected such that pairwise distances between localities spanned a range of geographical distances, thereby allowing predictions about the relationship between differences in dispersal ability and genetic population structure to be tested. Localities 1 through 10 occur within the Washougal River drainage, while locality 11 is in the nearby Hamilton Creek drainage (Fig. 1). Locality 11 was chosen specifically as a test whether *D. tenebrosus* gene flow occurs over land because localities 1–10 drain into the Columbia River and dispersal via an aquatic route would thus be virtually impossible for this species. Individuals were sampled from ~100-m stretches of stream at each site. Sampled individuals of *D. copei* comprised neotenic adults and larvae, while samples of *D. tenebrosus* comprised aquatic larvae and some metamorphosed adults. Sample sizes from each locality are shown in Figs 2 and 3. Individuals were sampled in the field by clipping approximately 0.5 cm<sup>2</sup> of tail tissue, placing it in 95% EtOH, and storing it at –20 °C upon return to the laboratory. Subsequent DNA extractions were performed with a QIAGEN DNeasy Tissue kit (QIAGEN, Inc.).



**Fig. 2** Graphical output for STRUCTURE runs with highest  $\ln$  likelihood values from the five repetitions used to calculate  $\Delta K$  for *Dicamptodon copei*. Number of clusters in each analysis is based on calculation of  $\Delta K$  (Evanno *et al.* 2005). Columns represent sampled individuals, coloured proportions of columns represent probability of assignment to different clusters, values along  $x$ -axis represent locality number, and numbers in parentheses indicate sample size after removal of full siblings and individuals with missing data. (a) Initial clustering of all 11 localities into two groups. (b) Subsequent analysis of initial clusters reveals substructure in localities 1–6 and localities 7–9. Localities 10 and 11 cluster independently. (c) Analysis at the third level of population structuring reveals localities 1 and 5 to be distinct while localities 2, 3, 4, and 6 display genetic admixture. Localities 7–9 form independent clusters. (d) Final iteration for localities 2, 3, 4 and 6 indicate a lack of further substructure.



**Fig. 3** Graphical output from STRUCTURE runs for *Dicamptodon tenebrosus*. Columns represent sampled individuals, coloured proportions of columns represent probability of assignment to different clusters, values along  $x$ -axis represent locality number, and numbers in parentheses indicate sample size after removal of full siblings and individuals with missing data.

We developed 15 microsatellite markers in conjunction with Ecogenics GmbH for the Cope's giant salamander, 11 of which cross-amplified in *D. tenebrosus*. Polymerase chain reaction (PCR) conditions for microsatellite amplifications followed those of Steele *et al.* (2008). Samples were run on 96-well plates with negative and positive controls to ensure consistency in allele scoring. Forward PCR primers were fluorescently labelled with one of four different dye colours to allow for post-PCR multiplexing on an ABI 3730 automated sequencer (Applied Biosystems, Inc.). The LIZ 500 size standard was run concurrently with each sample and later used for comparison scoring of microsatellite

alleles using the software GeneMapper version 3.7 (Applied Biosystems, Inc.).

#### Genetic analyses

Genotypic data were collected for both species at each locality. The program CONVERT (Glaubitz 2004) was used to format data sets for subsequent analyses. We initially screened for relatedness in each population using COLONY (Wang 2004). Hybridization is not assumed to be occurring between the two species and sampled individuals were screened for possible hybridization using NewHybrids

(Anderson & Thompson 2002). Each data set was then analysed with GENEPOP version 3.4 (Raymond & Rousset 1995) and GDA (Lewis & Zaykin 2001) to assess genetic variability within and among sampled localities, calculate numbers of alleles per locus, determine observed and expected heterozygosities and test for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between loci. A sequential Bonferroni correction (Rice 1989) was used to control for multiple comparisons. A global estimate of  $F_{ST}$ , with a 95% confidence interval, was estimated for each species from Weir & Cockerham's  $\theta$  (1984) using FSTAT version 2.9.3 (Goudet 2001). Pairwise  $F_{ST}$  values for each locality were then calculated for each species using ARLEQUIN version 3.01 (Excoffier *et al.* 2005).

To allow direct comparisons of genetic differentiation between the two species based on different numbers of loci, a standardized measure of genetic differentiation ( $G'_{ST}$ ) was also calculated for pairwise comparisons (Hedrick 2005). This standardized measure is appropriate for comparing levels of genetic differentiation among different organisms or among loci with different levels of variation. It also takes into account the high level of variability common in microsatellites, which can limit the upper bound of  $F_{ST}$  to be  $< 1$  (Hedrick 2005). Data were recoded with RecodeData version 0.1 (Meirmans 2006) and analysed in ARLEQUIN to generate maximum possible values of pairwise  $F_{ST}$  given the observed within-population variation. Original  $F_{ST}$  values were then divided by these maximized values to generate  $G'_{ST}$ .

The program STRUCTURE (Pritchard *et al.* 2000) was used to infer genetic structuring using a Bayesian clustering algorithm to assign individuals to a number of genetic clusters ( $K$ ). The determination of these genetic groupings is not based on  $F_{ST}$  values but rather on the assignment of multilocus genotypes into clusters that minimize deviation from HWE and linkage disequilibrium. Based on 11 sampled localities, we screened for values of  $K$  from 1 to 11 by running  $1.2 \times 10^5$  iterations, with the first  $2 \times 10^4$  iterations discarded as 'burn-in', for each probable value of  $K$ . Stationarity of the Markov chain before sampling was confirmed by viewing graphs of log-likelihood values plotted against iterations. Variance in log likelihood values from five repetitions run for each value of  $K$  was used to calculate the parameter  $\Delta K$ , which represents the number of genetically homogeneous clusters at the highest level of hierarchical population structuring (Evanno *et al.* 2005). This parameter ( $\Delta K$ ) is an *ad hoc* statistic that in simulation accurately identifies the true number of genetic clusters at the uppermost level of hierarchical structure. It provides an objective alternative to selecting  $K$  instead of simply choosing a  $K$  with highest log probability, which tends to overestimate  $K$  (Evanno *et al.* 2005). Because  $\Delta K$  represents the uppermost level of population structuring, there can

often be substantial substructuring of individuals within these initial groupings (Evanno *et al.* 2005). Therefore, we iteratively examined clusters for further substructuring until calculation of  $\Delta K$  revealed no further genetic population structure.

To estimate isolation-by-distance correlations, we examined two different measures of pairwise distance: minimum stream distance and straight-line topographic distance. Minimum stream distance was measured as the shortest stream distance between sampling localities barring any overland travel, whereas topographic distance between localities is a measure of the straight line overland distance between localities accounting for elevational changes. The software ArcGis version 8.2 (ESRI) was used to measure pairwise topographic distances from a digital elevation model of the area, while minimum stream distances were measured from a digital stream map of the area. Pairwise genetic distances were calculated using  $F_{ST}$  values. Mantel tests were conducted in ARLEQUIN to test for isolation by distance using both stream and overland topographic distances. Significance of correlations was determined through 100 000 random matrix permutations. In cases where Mantel tests indicated significant correlation with both stream and topographic distances, Akaike weights (Akaike 1969), which indicate the probability of a model being the best among a set of candidate models, were also calculated to provide probabilities for stream vs. topographic distance as the more likely model for correlation of isolation by distance.

To account for potential bias introduced by unequal sample sizes between the two study species (see Fig. 1), we conducted two sets of analyses. First, we jackknifed and bootstrapped over all loci and populations to determine if overall  $F$ -statistics varied between the two study species. Second, we randomly chose a sample of *D. copei* to match the sample size of *D. tenebrosus* for each study locality and reran analyses.

## Results

### *Tests of Hardy–Weinberg and linkage disequilibrium*

One locus in *Dicamptodon copei* (D07) and two loci in *D. tenebrosus* (D04 and D05) were significantly out of HWE across all populations (Appendix II) and were removed from the respective data sets to meet the assumptions of the program Structure. No loci were in linkage disequilibrium in either species after correcting for multiple comparisons. In an effort to minimize any effect of missing data, we screened for and removed individuals with an excessive percentage of missing genotypes ( $> 30\%$ ) resulting in a final data set with 4% missing data for *D. copei* and 3% missing data for *D. tenebrosus*. Final sample sizes from each locality for each species are shown in Fig. 1.

**Table 1** Below diagonal are pairwise  $F_{ST}$  values for *Dicamptodon tenebrosus*, above the diagonal are values for *D. copei*. Values significantly different from zero are indicated with asterisks. Locality numbers (1–11) correspond to those in Fig. 1

	1	2	3	4	5	6	7	8	9	10	11
1	—	0.029*	0.031*	0.039*	0.043*	0.021*	0.074*	0.057*	0.066*	0.074*	0.155*
2	0.000	—	0.009*	0.021*	0.032*	0.023*	0.040*	0.041*	0.058*	0.046*	0.115*
3	0.046*	0.024	—	0.014	0.035*	0.017*	0.044*	0.042*	0.057*	0.057*	0.132*
4	0.027*	0.000	0.035*	—	0.037*	0.011	0.058*	0.071*	0.074*	0.073*	0.138*
5	0.044*	0.000	0.04*	0.029*	—	0.041*	0.045*	0.058*	0.068*	0.066*	0.158*
6	0.02	0.000	0.019*	0.039*	0.016	—	0.05*	0.056*	0.066*	0.067*	0.140*
7	0.000	0.000	0.000	0.000	0.001	0.000	—	0.05*	0.057*	0.058*	0.152*
8	0.014	0.013	0.063*	0.033*	0.027*	0.02*	0.000	—	0.039*	0.064*	0.147*
9	0.000	0.000	0.052*	0.023	0.026	0.014	0.014	0.000	—	0.065*	0.178*
10	0.001	0.000	0.037*	0.024*	0.032*	0.018	0.000	0.016	0.002	—	0.105*
11	0.013	0.036	0.104*	0.08*	0.063*	0.061*	0.042*	0.033*	0.063*	0.026	—

**Table 2** Below diagonal are pairwise  $G'_{ST}$  values for *Dicamptodon tenebrosus*, above the diagonal are values for *D. copei*.  $G'_{ST}$  is a standardized measure of genetic differentiation (Hedrick 2005) that provides direct comparison of values between species. Locality numbers (1–11) correspond to those in Fig. 1

	1	2	3	4	5	6	7	8	9	10	11
1	—	0.151	0.165	0.194	0.216	0.106	0.354	0.276	0.280	0.383	0.673
2	0.000	—	0.051	0.106	0.164	0.12	0.198	0.207	0.254	0.248	0.509
3	0.090	0.05	—	0.071	0.184	0.093	0.218	0.212	0.254	0.309	0.593
4	0.052	0.000	0.068	—	0.176	0.056	0.270	0.336	0.308	0.376	0.579
5	0.092	0.000	0.081	0.059	—	0.205	0.214	0.281	0.29	0.346	0.681
6	0.039	0.000	0.038	0.076	0.033	—	0.241	0.277	0.284	0.36	0.614
7	0.000	0.000	0.000	0.000	0.002	0.000	—	0.227	0.231	0.280	0.622
8	0.027	0.026	0.123	0.063	0.056	0.0395	0.000	—	0.159	0.319	0.608
9	0.000	0.000	0.108	0.046	0.057	0.029	0.029	0.001	—	0.284	0.663
10	0.001	0.000	0.077	0.05	0.069	0.038	0.000	0.032	0.005	—	0.463
11	0.027	0.074	0.209	0.158	0.135	0.124	0.081	0.067	0.128	0.055	—

### Relatedness and hybridization

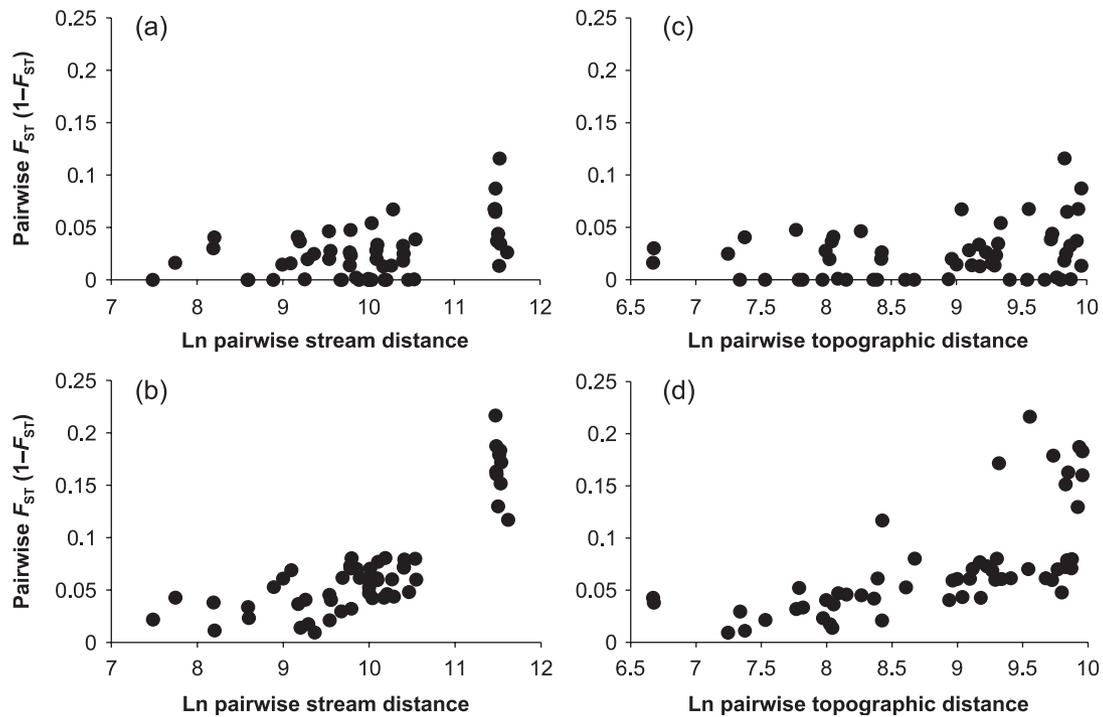
Results from COLONY designated several individuals from each locality as full siblings. In each case, we retained only one of the sampled full siblings in the data set and removed all others to eliminate any bias (e.g. deviations from HWE due to underestimations of observed heterozygosity) caused by these relationships. There were no differences between the two study species as to the number of full-sibs detected when averaged across sites. Tests for possible hybridization revealed one sample to be a backcrossed individual, which was excluded from data analysis. All other *D. tenebrosus* samples showed > 95% assignment to their species type and all *D. copei* samples showed > 99% assignment to their respective species type.

### Population structure and isolation by distance

Global values of  $\theta$  and 95% confidence intervals indicated a significantly higher overall degree of population genetic

structuring in *D. copei* ( $\theta = 0.079 \pm 0.013$ ) than in *D. tenebrosus* ( $\theta = 0.031 \pm 0.008$ ). Except for two comparisons (localities 3 vs. 4 and localities 4 vs. 6), pairwise  $F_{ST}$  values for *D. copei* were all significantly different from zero ( $P < 0.001$ ) and ranged from low (0.009) to relatively high (0.178) levels of divergence (Table 1). Pairwise  $F_{ST}$  values for *D. tenebrosus* were considerably lower, with a range of 0–0.08, and over half of the pairwise comparisons were not significantly different from zero. Pairwise  $G'_{ST}$  values were higher for *D. copei* than *D. tenebrosus* in all cases, except in the comparison of locality 4 vs. 6 (Table 2).

Graphical results from the program STRUCTURE revealed a high degree of population substructure across the study area for *D. copei* (Fig. 2) and no genetic population structure for *D. tenebrosus* (Fig. 3). Iterative calculations indicated hierarchical population structuring for *D. copei* and identified most sampled localities as distinct genetic units (Fig. 2a–d) and a final estimate of  $K = 8$ . The final round of iterations could not assign individuals from the nearby localities of 2, 3, 4 and 6 (Fig. 1) to more than one genetic cluster. Localities



**Fig. 4** Plots of isolation by distance based on pairwise stream distances of samples localities for *Dicamptodon tenebrosus* (a) and *D. copei* (b) and pairwise topographic distances of sampled localities for *D. tenebrosus* (c) and *D. copei* (d).

**Table 3** Comparison of summary statistics for *Dicamptodon copei* and *D. tenebrosus*. Values for *D. copei* are given at the full sample size and resampled to adjust sample sizes at each locality to that of *D. tenebrosus*. Data were estimated using *FSTAT* 2.93 (Goudet 2001).  $F_{ST}$  was estimated as in Weir & Cockerham (1984), with confidence intervals determined via bootstrapping over loci.  $R_{ST}$  was estimated as in Rousset (1996).  $F_{IS}$  and  $F_{ST}$  were estimated as in Weir & Cockerham (1984) with standard errors (in parenthesis) determined by jackknifing over loci

Species	$F_{ST}$ 95% lower CL	$F_{ST}$ 95% upper CL	$R_{ST}$	Overall $F_{IS}$	Overall $F_{IT}$	Structure $K$ value
<i>D. tenebrosus</i>	-0.037	0.061	0.024	-0.015 (0.024)	0.008 (0.027)	1
<i>D. copei</i>	0.077	0.142	0.237	0.037 (0.020)	0.105 (0.018)	8
<i>D. copei</i> (resampled)	0.067	0.145	0.223	0.036 (0.023)	0.102 (0.021)	7

3, 4, and 6, which were also involved in nonsignificant pairwise  $F_{ST}$  values, belonged to this final genetic cluster (Fig. 2d). In contrast, individuals of *D. tenebrosus* could not be assigned to more than one genetic cluster even at the highest level of hierarchical population structure (i.e.  $K = 1$ ) (Fig. 3).

Results of Mantel tests indicated no significant correlations of genetic distances for *D. tenebrosus* with topographic ( $r = 0.315$ ,  $P = 0.09$ ) or stream distances ( $r = 0.505$ ,  $P = 0.06$ ). For *D. copei*, genetic distances were strongly correlated with both stream ( $r = 0.912$ ,  $P < 0.001$ ) and topographic distances ( $r = 0.706$ ,  $P < 0.001$ ). To determine which dispersal model (stream or topographic) best explains the patterns observed for *D. copei*, Akaike weights were calculated and indicated the likelihood of a stream-based dispersal model as 100% and topographic-based dispersal model as 91%.

An examination of isolation-by-distance plots (Fig. 4) indicates that pairwise stream distances of locality 11 form an outlying cloud of points that may be driving significance of the Mantel test. Re-analysis of the data excluding locality 11 indicates that genetic distances for *D. copei* remain strongly correlated with stream ( $r = 0.55$ ,  $P = 0.005$ ) and topographic ( $r = 0.687$ ,  $P \leq 0.001$ ) distances. Genetic distances for *D. tenebrosus* also remained nonsignificant for both stream ( $r = 0.022$ ,  $P = 0.507$ ) and topographic ( $r = 0.004$ ,  $P = 0.566$ ) distances.

Despite differences in samples sizes between the two study species, 95% confidence intervals for  $F$ -statistics failed to overlap for both the total data sets and the pruned *D. copei* data set when compared with the full *D. tenebrosus* data set (Table 3). Overall, there were few differences between the full and pruned *D. copei* data sets, and heterozygosity

changed little. With the pruned data set and iterative analyses, Structure recovered seven genetic clusters ( $K = 7$ ) as opposed to eight with the full data set, with the main difference being that locality 1 grouped with localities 2, 3, 4 and 6 in the final iteration (data not shown).

## Discussion

Based on differences in life-history traits that affect dispersal ability, we predicted patterns of population-level structuring of two closely related salamander species in sympatry. Such comparative studies can be confounded by either comparing genetic structure of allopatric or phylogenetically distant species. However, by examining closely related species in a common environment, we provide evidence that the pattern of population-level genetic structuring exhibited by each species correlates with species-specific life-history traits influencing dispersal capabilities.

The nonmetamorphosing, low-dispersing species (*Dicamptodon copei*) showed high levels of genetic structure while the metamorphosing and high-dispersing species (*D. tenebrosus*) displayed low levels of genetic structuring. Sample sizes at two localities for each species were small (Figs 2 and 3) and allele frequency distributions may not have been well characterized for these localities, but this is unlikely to be a serious issue because clear differences in genetic structure were observed between species. Whereas *D. tenebrosus* displayed no population-level structure across the study area and no pattern of isolation by distance, the patterns displayed by *D. copei* were consistent with the relative geographical proximity of samples. The first division of population structure within *D. copei* grouped localities 10 and 11 together, which are somewhat removed from the remaining localities that comprise the other genetic cluster. While these two localities are in different watersheds (Fig. 1), they are in close proximity; the initial grouping of the localities may indicate previous physical connection between the two watersheds, perhaps in the form of stream capture, or that overland movement of *D. copei* occurs more frequently than previously thought. The results from the second iteration of STRUCTURE indicate that substructure is partitioned by watershed, with localities 1 to 6 distinct from 7 to 9; localities 10 and 11 each form independent clusters. The third iteration shows localities 1 and 5 as distinct from localities 2, 3, 4 and 6; localities 7 to 9 each form distinct genetic clusters. The final iteration confirms that localities 2, 3, 4 and 6 lack any further genetic structure. It is unclear why the nearby locality 5 would not also share the genetic profile of these closely surrounding localities but it may indicate some barrier to gene flow that was not readily apparent. The correspondence of population structure with the proximity of sampled localities for *D. copei* indicates minimal movement among

localities and that *D. copei* may require substantially more time to colonize suitable habitat than *D. tenebrosus*. Such patterns suggest that life-history traits influencing species' dispersal ability can shape patterns of gene flow.

Due to its restricted range and localized abundance, *D. copei* is listed as a species of conservation concern in Washington and Oregon. While limited empirical evidence on the effect of forest management upon this species is available, *D. copei* is thought to be susceptible to some forest management strategies (Bull & Wales 2001). The apparently limited dispersal ability may place it at risk if timber harvest makes its current habitat unsuitable. On the other hand, *D. tenebrosus* is not a species of conservation concern due to its large distribution and high densities of larval occurrence (Murphy & Hall 1981; Murphy *et al.* 1981; Hawkins *et al.* 1983).

Detection of hybridization was unexpected, although the presence of a single backcrossed individual suggests that it may be occurring at relatively low levels in the study area. Hybridization may be more common in areas where degraded habitat limits abundance of *D. copei*, forcing it to hybridize with its more common congener. Additional studies will be useful in determining whether there is hybridization in other regions where the two species are sympatric and to test the hypothesis that hybridization may occur in areas with degraded habitat.

### Linking dispersal ability and population structure

Low rates of metamorphosis are likely responsible for the substantial degree of genetic population structure in *D. copei*. Consistent with its aquatic nature, dispersal routes appear to occur primarily along streams, and genetic patterns indicate a maximum distance of approximately 5 km (i.e. among the genetically homogeneous localities of 2, 3, 4, and 6). In contrast, regular metamorphosis of *D. tenebrosus* into terrestrial adults likely facilitates dispersal among localities and diminishes genetic structuring of populations. Genetic homogeneity of *D. tenebrosus* encompassed the extent of the study area, which is approximately 20 km. Although *D. tenebrosus* is capable of dispersing overland and along stream corridors, neither topographic nor stream distance was significantly correlated with genetic patterns, suggesting that *D. tenebrosus* is dispersing among localities regardless of physical or topographic features and is doing so to a degree sufficient enough to cause a high degree of genetic admixture across the study area. Differences in effective population sizes of the two species may also explain a higher degree of population in *D. copei* relative to *D. tenebrosus*. However, based on our surveys of the two species across their respective ranges, there appeared to be no differences in number of animals caught per unit effort (S. Spear and J. Baumsteiger, unpublished).

Dicamptodontids were once classified within the family Ambystomatidae (Tihen 1958) and the pattern of weak population structuring within *D. tenebrosus* and strong structure within *D. copei* is consistent with patterns observed between metamorphosing and nonmetamorphosing populations of ambystomatid salamanders (Shaffer 1984). Our results are also consistent with the general notion that a lack of long-distance dispersal in salamanders is associated with high levels of genetic population structure (Larson *et al.* 1984). Such patterns reinforce the idea that sexual reproduction via maturation in the larval form in salamanders leads to increased genetic divergence due to restricted gene flow between nonmetamorphosing populations.

Associations between dispersal ability and population-level genetic structure or phylogeographical patterns have been established for a variety of species (Chenoweth *et al.* 1998; Bohonak 1999; Dawson *et al.* 2002; Kelly & Eernisse 2007). The patterns observed in this study are consistent with the prediction that species with low dispersal rates and reduced gene flow display high degrees of genetic population structure (Slatkin 1987; Bohonak 1999). The species-specific patterns of population-level genetic structure observed in this study also correlate to the phylogeographical patterns exhibited by each species, suggesting a correlation between life-history variation, localized dispersal ability and overall geographical distribution. The nonmetamorphosing, low dispersing *D. copei* not only displays high levels of genetic structure at the population level, but also displays strong phylogeographical structure across its relatively small and fragmented distribution (Steele & Storfer 2007). In contrast, the metamorphosing and high dispersing *D. tenebrosus* displays low levels of genetic structuring at the population level, shallow phylogeographical structuring, and evidence of long-distance migration within its larger and more contiguous geographical distribution (Steele & Storfer 2006).

We recognize two potential shortcomings of our study. First, although we control for confounding effects of evolutionary history by comparing two sister taxa that occur sympatrically, our study is unreplicated in terms of spatial area. There may be characteristics about the landscape surrounding our study sites that uniquely affects the relative rates of gene flow between the two study species. Thus, a spatially replicated study would strengthen our overall results. Second, despite 2 years of sampling, we obtained unequal sample sizes for the two study species, which could bias results. However, both bootstrap and jackknife tests and resampling *D. copei* to match sample sizes of *D. tenebrosus* did not change our overall results. That is, *D. copei* remained significantly more structured genetically regardless of whether a full data set or a data set pruned to match the sample sizes of *D. tenebrosus* was used.

Because this study system is highly suitable for examining the effects of dispersal ability on population structure

(both species are closely related, have clear differences in dispersal ability and are sympatric), the connection between dispersal ability, population-level structure, and range-wide phylogeographical pattern seems straightforward and demonstrating a scaling-up pattern in other study systems may be difficult if the study organisms do not meet these requirements. Whereas this generalization is based on observed patterns within a single region, and different conclusions may be reached if rates of metamorphosis for either species vary across regions, it provides a working hypothesis that life-history traits, which influence a species' dispersal ability, can shape patterns of gene flow at local levels and also influence species' distributions at larger scales.

## Conclusion

Dispersal is a driving force that shapes genetic population structure in a variety of organisms (Doherty *et al.* 1995; McDonald *et al.* 1999; King & Lawson 2001; Dawson *et al.* 2002; Whiteley *et al.* 2004), and species with high dispersal capabilities tend to display less genetic structure than low dispersing species (Bohonak 1999). In turn, dispersal abilities can be influenced by species-specific life-history traits. Understanding how life-history traits ultimately influence genetic structuring requires comparative studies in common environments. The results of this comparative study provide insight into the role of life-history variation in genetically structuring populations while controlling for phylogeny and environment.

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This research was conducted as part of Craig A. Steele's PhD dissertation at Washington State University. His main research interests include determining the evolutionary or ecological processes that lead to fine scale genetic and phylogeographic structuring of populations. Jason Baumsteiger is interested in conservation and population genetics of amphibians and fishes. Andrew Storfer studies limits to species' ranges and uses population and landscape genetics as tools to understand the factors that shape distributions of species. He is also interested in host-pathogen co-evolution and conservation of amphibians.

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## Appendix I

Geographical locations and sample size for each locality

Locality	Sample size		Coordinates		
	<i>Dicamptodon copei</i>	<i>Dicamptodon tenebrosus</i>	Latitude	Longitude	Altitude (m a.s.l.)
1	26	12	45°42'23.5"	-122°12'58.1"	418
2	19	5	45°42'00.6"	-122°12'30.9"	536
3	27	17	45°42'06.8"	-122°11'48.5"	607
4	7	16	45°41'15.0"	-122°13'02.0"	419
5	20	18	45°40'56.9"	-122°12'41.8"	419
6	14	18	45°40'49.9"	-122°12'11.5"	419
7	25	20	45°40'43.8"	-122°10'40.6"	422
8	23	21	45°41'00.9"	-122°08'01.7"	254
9	26	7	45°37'43.7"	-122°08'29.5"	402
10	27	13	45°43'42.4"	-122°03'29.6"	690
11	21	10	45°42'10.9"	-122°02'04.2"	641

m a.s.l., metres above sea level.

## Appendix II

Table of generalized genetic statistics from the 15 microsatellite markers used in the study

Locus	<i>Dicamptodon copei</i>				<i>Dicamptodon tenebrosus</i>			
	No. of alleles	Range	$H_O^*$	$H_E^*$	No. of alleles	Range	$H_O^*$	$H_E^*$
D04	25	106–210	0.829	0.854	10	125–184	0.388	0.625
D05	17	143–191	0.761	0.792	9	147–179	0.231	0.771
D06	10	193–229	0.760	0.806	8	185–229	0.488	0.575
D07	22	180–266	0.642	0.920	4	271–315	0.576	0.553
D08	12	107–153	0.789	0.794	—	—	—	—
D13	16	104–166	0.796	0.826	16	107–187	0.854	0.867
D14	12	87–131	0.734	0.799	10	137–173	0.773	0.751
D15	13	189–237	0.614	0.642	—	—	—	—
D17	13	152–200	0.726	0.726	7	176–200	0.638	0.652
D18	16	132–200	0.796	0.813	9	214–250	0.837	0.797
D20	32	158–294	0.772	0.795	—	—	—	—
D22	10	91–127	0.795	0.810	—	—	—	—
D23	15	100–174	0.799	0.805	1	105	—	—
D24	15	116–182	0.896	0.920	5	120–136	0.180	0.272
D25	9	81–113	0.784	0.741	7	85–113	0.250	0.298

\*values are averages over all 11 populations;  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity.