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ORIGINAL ARTICLE

Patterns of genetic differentiation in *Thamnophis* and *Taricha* from the Pacific Northwest

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ABSTRACT

Aim The co-evolutionary interaction between the common garter snake, *Thamnophis sirtalis*, and the rough-skinned newt, *Taricha granulosa*, takes place throughout much of the Pacific Northwest (North America). The biogeography of the Pacific Northwest has been heavily influenced by the last Pleistocene glaciation, which reached a maximum as late as 14,000 yr BP. We researched: (1) what type of population structure is present for garter snakes and newts, (2) whether the population structure of these species is consistent with a Pleistocene glaciation hypothesis, and (3) how population structure and migration possibly affect co-evolution between these species.

Location The Pacific Northwest of North America, specifically northern California, Oregon and Washington in the USA.

Methods We sampled approximately 20 populations for each species from three different transects. Using microsatellite markers and tissue samples from both species, we quantified the population structure for both species. Individual-based assignment tests were used to estimate contemporary migration rates.

Results Both *Th. sirtalis* and *Ta. granulosa* exhibited little genetic differentiation among our study sites, even among those separated by large distances. Significant population structure was detected on multiple geographic scales. Differences in population structure were observed among transects and between garter snake and newt transects. Contemporary migration rate estimates indicate high levels of genetic exchange between populations.

Main conclusions Prior to this study, little was known about the fine-scale population structure of either species in this region. Patterns of population structure for garter snakes and newts reflect a shared biogeographical history affected by the Pleistocene glaciation in the Pacific Northwest. Both species apparently migrate frequently between populations, thus potentially retarding the process of adaptive co-evolution. We find that populations from a northern coastal transect (Washington) are most likely to be locally adapted.

Keywords

Biogeography, co-evolution, garter snake, gene flow, North America, phylogeography, Pleistocene glaciation, population structure, *Taricha granulosa*, *Thamnophis sirtalis*.

INTRODUCTION

Genetic subdivision among populations plays a central role in the geographic mosaic theory of co-evolution (Thompson, 1994, 1999a,b; Burdon & Thrall, 1999; Nuismer *et al.*, 1999; Gomulkiewicz *et al.*, 2000). Co-evolution is a process that occurs across a complex geographic landscape, and co-evolutionary interactions are not equivalent across the

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range of an interaction. Genetic subdivision allows populations to follow separate evolutionary paths so that populations across a species' range may exhibit a diversity of phenotypic values. Populations in which reciprocal selection is strong act as sources of adaptive change for traits at the phenotypic interface of co-evolution (Brodie & Ridenhour, 2003). Depending upon the rate of gene exchange between populations, maladaptation may occur in populations intermediate to the hot and cold spots (Nuismer *et al.*, 1999; Gomulkiewicz *et al.*, 2000). In particular, Gomulkiewicz *et al.* (2000) showed that asymmetries in gene flow in two interacting species can increase the chance of maladaptation at a stable equilibrium. The term maladaptation will be used herein in the sense that a population is not at its fitness optimum (i.e. not local adaptation *sensu* Kawecki & Ebert, 2004).

The theoretical predictions related to the geographic mosaic remain largely untested in natural populations. Relatively few empirical studies have examined genetic subdivision within co-evolutionary systems (some recent examples include Dybdahl & Lively, 1996; Althoff & Thompson, 2001; Mutikainen & Koskela, 2002; Leebens-Mack & Pellmyr, 2004; Sullivan & Faeth, 2004). In a host–parasite study of the stinging nettle, *Urtica dioica* and the greater dodder, *Cuscuta europaea* (Mutikainen & Koskela, 2002), selection was found to be strong enough to prevent maladaptation within the system. Similarly, Dybdahl & Lively (1996) found high levels of gene flow in a freshwater snail, but found evidence of strong local adaptation of the snail to its trematode parasite. In contrast, Sullivan & Faeth (2004) found the opposite scenario – asymmetric population structuring in the endophytic fungus *Neotyphodium* and its host grass *Festuca arizonica* led to maladaptation. While no formal quantification of gene flow was made in the wild parsnip *Pastinaca sativa*–parsnip webworm *Depressaria pastinacella* system, physical transfer of individuals by 30 m from their source population significantly increased mortality, leading to the conclusion that gene flow is most probably maladaptive in this interaction (Zangerl & Berenbaum, 2003). Further empirical studies of gene flow in co-evolutionary systems are needed in order to gain an understanding of the causes of local (mal)adaptation.

The effect of migration on adaptive dynamics in co-evolutionary interactions depends heavily on the genetic control of traits at the co-evolutionary phenotypic interface. Some theoretical work has demonstrated that increased migration can be adaptive in major-gene co-evolution (Gandon *et al.*, 1996; Gandon & Michalakis, 2002). Increased genetic variance arising from migration and resulting in a quicker response to selection explains this result. The important factor is actually gene flow *relative* to the interacting species' gene flow (Gandon & Michalakis, 2002). Empirical evidence for this phenomenon exists in the anther-smut fungus *Microbotryum violaceum* and its host plant *Silene latifolia* (Kaltz *et al.*, 1999), and to a lesser degree in a *Pseudomonas fluorescens* bacterium–bacteriophage system (Morgan *et al.*, 2005). However, the role of gene flow as a facilitator of adaptation relies on genes-of-major-effect underlying the traits that mediate ecological interactions. In other cases, where traits are polygenic and interactions lead to escalatory co-evolutionary dynamics, migration among populations is predicted to lead to local maladaption (Ridenhour & Nuismer, in press).

The interaction between the common garter snake and the rough-skinned newt is mediated by polygenic traits and exhibits the selection mosaic pattern predicted by the geographic mosaic theory of co-evolution (Thompson, 1994; Brodie *et al.*, 2002). *Taricha granulosa* uses the potent neurotoxin tetrodotoxin (TTX) as a chemical defence that results in the death of most would-be predators (Brodie, 1968). *Thamnophis sirtalis* has evolved resistance to the effects of TTX, thus allowing this snake to exploit newts as prey items (Brodie & Brodie, 1990, 1991). Garter snake resistance levels are spatially variable and point towards the presence of at least two hot spots: one in central Oregon and one in the San Francisco Bay area (Brodie *et al.*, 2002). *Taricha granulosa* toxicity levels have been less extensively studied but are spatially variable as well, and also point to at least one hot spot in central Oregon (Hanifin *et al.*, 1999).

Little is known about the population structure of *Ta. granulosa* and *Th. sirtalis* in the Pacific Northwest. A large-scale phylogeography of *Ta. granulosa* indicates that rough-skinned newts in California are more divergent than and distantly related to newts in Washington and Oregon (Kuchta & Tan, 2005). Work on other taxa lends some insight into the genetic subdivision of newts. Twitty *et al.* (1964) showed that the red-bellied newt, *Ta. rivularis*, of northern coastal California demonstrates a high degree of breeding-site fidelity using mark–recapture studies: a large fraction of the newts displaced several kilometres and across river drainages would return to their original breeding site within a year. From a transect no greater than 26 km in length and using allozymes, Hedgecock (1978) reported a F_{st} value of 0.062 for *Ta. rivularis*, suggesting a surprisingly high degree of population subdivision for such a short distance. This result is somewhat unexpected, given that: (1) the deme size of *Ta. rivularis* was estimated to be upwards of 10,000 (Hedgecock, 1978), (2) the longevity of newts is unknown, but marked newts of 17–18 years of age were recaptured by Twitty, (3) females lay large numbers of eggs, and (4) newts are capable of long-distance overland migration (Twitty *et al.*, 1964). A more recent study on *Ta. granulosa* using microsatellites estimated $F_{st} = 0.005$ for two populations separated by 16 km in central Oregon (Jones *et al.*, 2001); this value is approximately 12 times lower than that reported by Hedgecock (1978). The large difference in F_{st} between these two studies may in part arise from the use of allozymes vs. microsatellites.

Knowledge of the population structure of *Th. sirtalis* in the Pacific Northwest is scant as well. A phylogeography of *Th. sirtalis* in the Pacific Northwest revealed three distinct clades (Janzen *et al.*, 2002). These lineages were similar to those observed in *Ta. granulosa*: a California clade, a northwest coastal clade, and an intermountain clade. No fine-scale studies have been performed in the Pacific Northwest, but

studies of Lake Erie garter snakes found that there is no isolation by distance between populations of *Th. sirtalis* (King & Lawson, 2001; Bittner & King, 2003). In contrast, water snakes (*Nerodia sipedon*) and brown snakes (*Storeria dekayi*) from the same area show a negative relationship between gene flow and distance (King & Lawson, 2001). Populations of *Th. sirtalis* that are 100 km apart show approximately the same number of migrants as those just a few kilometres distant (Bittner & King, 2003). Gregory & Stewart (1975) measured movements of 4.3–17.7 km between feeding grounds and hibernation areas in Manitoba. The lack of isolation by distance and the observed long-distance migration suggest that *Th. sirtalis* is capable of long-distance dispersal and may have little genetic subdivision.

The biogeography of the Pacific Northwest of North America has been heavily influenced by Pleistocene glaciation. A glacial maximum was reached between 17,000 and 14,000 yr BP (Thackray *et al.*, 2004); glaciers had receded from the region by *c.* 11,500 yr BP (Doerner & Carrara, 1999). Studies of plants, mammals, insects, fish, and crustaceans (Conroy & Cook, 2000; Althoff & Thompson, 2001; Arbogast *et al.*, 2001; Edmands, 2001; Ettl & Peterson, 2001; Ritland *et al.*, 2001; Smith *et al.*, 2001; Small *et al.*, 2003) have all found patterns consistent with recolonization during the glacial recession and periodic flooding that occurred. Low levels of genetic variation often found in the Pacific Northwest are attributed to range expansion after the glacial recession (Brown *et al.*, 1997; Althoff & Thompson, 2001; Edmands, 2001). Studies of some amphibians (the Del Norte salamander, *Plethodon elongatus* and the spotted frog, *Rana pretiosa*) revealed population structure congruent with glacial recolonization (Green *et al.*, 1996; Mahoney, 2004). There is a paucity of biogeographical studies of snake species in the Pacific Northwest, although a study on the population structure of the rubber boa, *Charina bottae*, from the California Floristic Province demonstrates that climatic fluctuation events shape population structure for snake species as well (Calsbeek *et al.*, 2003).

We sampled *Th. sirtalis* and *Ta. granulosa* in a broad area in the Pacific Northwest in order to assess the fine-scale population genetics of these two co-evolving species. We estimated genetic differentiation, population structure, and gene flow using microsatellite loci in order to improve understanding of the biogeographical history of these species and their co-evolutionary past and future. Furthermore, such estimates give us insight into the potential for local adaptation within this predator–prey system.

METHODS

We collected *Th. sirtalis* and *Ta. granulosa* from 19 and 22 populations, respectively, from northern California, Oregon, and Washington (Fig. 1). We targetted collection sites to be *c.* 32 km apart (see Supplementary Material Tables S1.1 and S1.3 for separation distances, and Table S1.5 for site locations). Sample localities that have the same name (e.g. Hunter Creek) may be several kilometres apart for snakes and newts (Supplementary Material Table S1.5). Selecting short inter-population distances reduces the effect of history on population structure and leads to better estimates of contemporary gene flow (Slatkin, 1993). We calculated the linear distance between populations based upon latitude and longitude coordinates.

We collected samples from three transects – referred to as the 'northern coast', the 'southern coast', and 'inland' (Fig. 1) – which have variable terrain types that could be described, respectively, as: along a river drainage, across multiple river drainages, and mountainous. The inland transect begins at the crest of the Cascade mountains, ends at the Pacific coast at the Tenmile Creek population, and follows the North Santiam and Alsea river drainages. The southern coast transect begins at the Tenmile Creek population on the central Oregon coast, follows the coast southwards until reaching Orick, CA, and crosses multiple river drainages. The northern coast transect begins just south of the mouth of the Columbia River at Warrenton, OR, proceeds due north until reaching the Strait of San Juan, and goes through the mountainous western third of Washington.

These three transects were picked for two reasons. First, the three transects varied greatly in garter snake phenotypic differences between the endpoints (Brodie *et al.*, 2002). At one end of each transect, garter snake resistance to TTX was high, while at the other end resistance was low. If genetic divergence parallels phenotypic divergence, these transects should provide good opportunity to observe population structure in *Th. sirtalis* and *Ta. granulosa*. This provides us with the greatest inference into the co-evolutionary process. Second, we wished to have some level of replication at the transect level. By taking several transects across different terrain types, we hope that, on average, our study will provide better insight into the comparative population structure and gene flow of *Th. sirtalis* and *Ta. granulosa*.

Sample collection and genotyping

We collected garter snakes and newts from mid-May to the beginning of July from each population. We attempted to capture 20 individuals per population. Tail clips from both species were stored in individually labelled microcentrifuge tubes containing 90% ethanol. The number of individuals genotyped in each population ranged from 3 to 24 (Table 1).

We isolated whole genomic DNA using Qiagen DNeasy 96 Tissue kits. Isolated DNA concentrations measured using a Molecular Devices SpectraMAX 190 microplate spectrophotometer were diluted using ddH_2O to a standard concentration for use in polymerase chain reactions (PCR). Diluted DNA was frozen and stored at −20°C.

We genotyped individuals using four microsatellite loci for both *Th. sirtalis* and *Ta. granulosa* (see Supplementary Material Appendix S1 for PCR conditions). For *Th. sirtalis*, microsatellite loci Nsμ2, Nsμ3, and Nsμ10 described in Prosser *et al.* (1999) and locus Ts1 of McCracken *et al.* (1999) were used for analysis. For *T. granulosa*, microsatellite loci Tgr01, Tgr04,

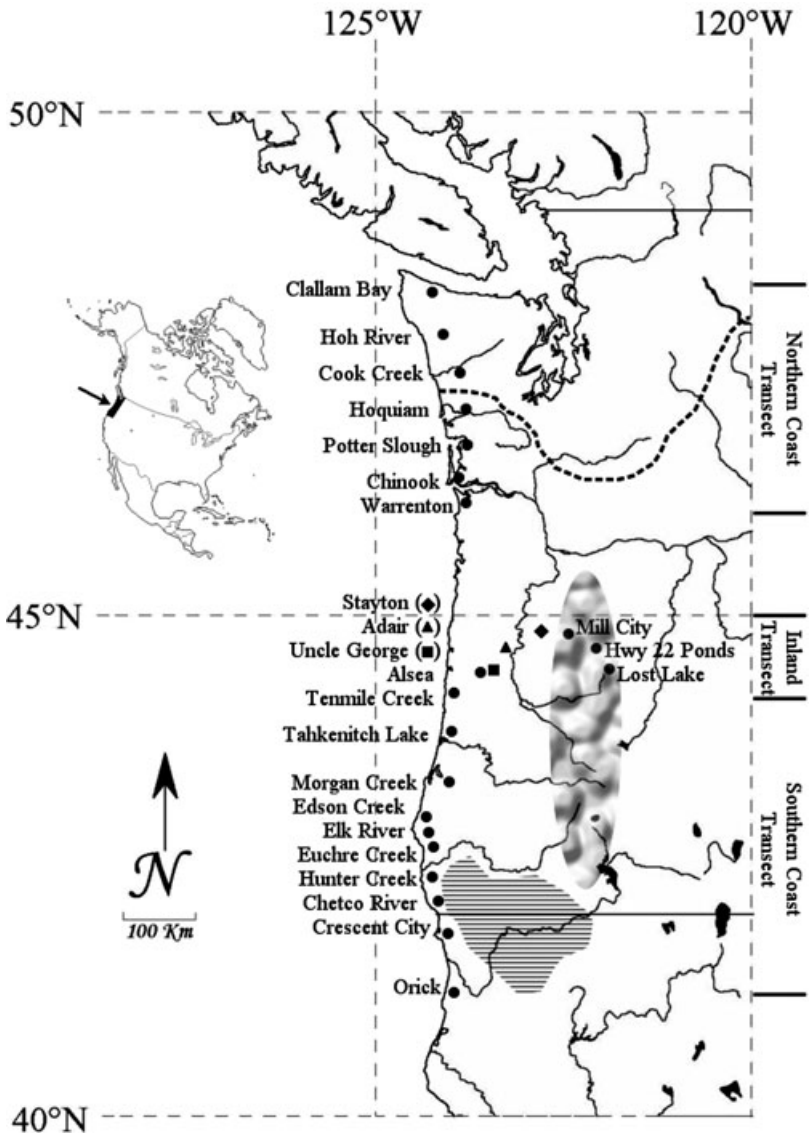

Figure 1 Locations of sample populations in the Pacific Northwest. The dashed line indicates the approximate southern extent of the last glacial maximum. The shaded region indicates the area of glaciation in the Oregon Cascades (Wright & Frey, 1965). The horizontal bars indicate the portion of the Klamath-Siskiyou Mountains where glaciation occurred at higher altitudes. The transect designation for the populations is on the right; Tenmile Creek was included in both the inland and southern coast transects.

Tgr10, and Tgr14 described in Jones *et al.* (2001) were used. Several other loci were screened for both species; the utilized microsatellites were chosen because of their high levels of polymorphism and reliable amplification across all samples. We visualized PCR products using an Applied Biosystems 3730 DNA analyser (a high-throughput capillary sequencer). We scored genotypes using Applied Biosystems GeneMapper software.

Statistical analyses

We measured genetic differentiation using the parameter θ (Weir & Cockerham, 1984) (denoted as θ_{st} in this paper to distinguish it from other θ parameters used in evolutionary genetics). Because microsatellites fit stepwise mutation models, estimates of θ_{st} may be low and underestimate the degree of population structure (Gaggiotti *et al.*, 1999). Slatkin (1995) developed R_{st} – another measure of population structure – to account for the effect of a stepwise mutation process. We calculated R_{st} using the method of Rousset (1996) at the whole-population level for comparison with θ_{st} values. Sometimes R_{st} may be misleading owing to potentially higher variance in estimates (Balloux & Goudet, 2002); we therefore used θ_{st} for all subsequent analyses.

We used fstat (Goudet, 2001) to estimate θ_{st} for three different levels: across the entire sampling region, across individual transects, and pairwise between all sample populations. For all pairwise population comparisons of genetic differentiation, the hypothesis H_1: $\theta_{st} \neq 0$ was tested; significance was determined by fstat using randomization of individuals (not alleles) among populations ($n = 5060$). For pairwise comparisons across the entire set of populations, significance levels were corrected using the Bonferroni method; the Dunn-Šidák method was used for correcting significance levels when questions involved the significance within a transect (Sokal & Rohlf, 1995).

Table 1 Population abbreviations and sample sizes*

Locale name	Two-letter abbreviation	Newts sampled	Snakes sampled
Clallam Bay	Cl	20	16
Hoh River	HR	22	3
Cook Creek	Co	5	24
Hoquiam	Ho	20	15
Potter Slough	Po	24	20
Chinook	Ch	20	20
Warrenton	Wa	14	23
Lost Lake	Lo	23	17
Hwy 22 Ponds	Hw	20	0
Mill City	Mi	20	5
Stayton	St	10	12
Adair	Ad	20	21
Uncle George	Un	20	0
Alsea	Al	20	1
Tenmile Creek	Te	20	3
Tahkenitch Lake	Ta	20	22
Morgan Creek	Mo	20	0
Edson Creek	Ed	20	0
Elk River	El	20	19
Euchre Creek	Eu	20	2
Hunter Creek	Hu	20	12
Chetco River	Cc	20	0
Crescent City	Cr	0	6
Orick	Or	20	23

*Latitude and longitude for each population are given in Table S5.

We used ARLEQUIN (v. 3.01; Excoffier *et al.*, 2005) to perform AMOVA in order to test for structure within transects. Specifically, we tested for significant levels of variation among groups of populations within a sample (H_1: $\varphi_{CT} \neq 0$; where φ_{CT} is a hierarchical *F*-statistic). Significance of all estimates was based on randomization tests ($n = 1 \times 10^4$). AMOVA analysis for each transect consisted of choosing two groups of populations and examining φ_{CT}. We also estimated φ_{CT} for the entire study using transects as population groups.

We used restricted maximum likelihood (REML) to test for the presence of isolation by distance (IBD) following the methods of Yang (2004). The log of $1/4(1/\theta_{st}-1)$ was regressed on log distance (Slatkin, 1993), and non-independence of residuals was modelled using various covariance structures (see Yang, 2004 for details). REML tests of IBD yield better slope and significance estimates by accounting for autocorrelation among data, and are preferable to jackknife, bootstrap, and Mantel methods (Yang, 2004). The scale at which IBD occurs within *Th. sirtalis* and *Ta. granulosa* was estimated by subsetting the pairwise population data according to their distance apart; subsets were created on an increasing 50-km scale (i.e. subset 1 was all data from populations <50 km apart, subset 2 was all data from populations <100 km apart, etc.). Subsets were analysed as above for IBD; the smallest distance at which IBD was detected was then considered the scale for IBD for the species. We checked for IBD within each of the three transects as well. Similarly to the IBD analysis, we used the regression of θ_{st}' on the natural logarithm of distance [$\theta_{st}' = \theta_{st}/(1-\theta_{st})$; Rousset, 1997] to estimate the mean amount of migration between populations for each species (neighbourhood size, $4D\pi\sigma^2$; where D is the population density and σ^2 is the dispersal distance variance).

Non-equilibrium conditions and historical events confound estimates of gene flow. To disentangle contemporary gene flow from these factors, we used Bayesian assignment tests to estimate *contemporary* migration rates (m) (BAYESASS 1.3, Wilson & Rannala, 2003). Ongoing gene flow relates more directly to the question of adaptation than do historical events; contemporary gene flow acts to balance selective pressures in separate populations while historical events do not (i.e. historical event effects are only visible in neutral genetic variation). This analysis was carried out for 9×10^6 generations with the first third of the samples discarded as a 'burn-in'. The iterative changes in allele frequencies (p), inbreeding value (F), and migration rate (m) were adjusted so that roughly 40–60% of all changes in these parameters were accepted. Initially, five replicates were run for each species. After the first five replicates, it was apparent that some neighbouring populations behaved non-independently. Accordingly, these populations were combined and three more replicates were run.

RESULTS

All loci showed a great diversity of alleles (13 alleles minimum); *Th. sirtalis* loci tended to be slightly more diverse than those used for *Ta. granulosa* analysis. Allele counts for garter snakes were 27, 13, 34, and 20 for locus Ts1, Nsμ2, Nsμ3, and Nsμ10, respectively. Allele counts in newts were 15, 13, 18, and 30 for locus Tgr1, Tgr04, Tgr10, and Tgr14, respectively. Hardy-Weinberg expectations were met in nearly all populations sampled; newt populations from Orick, Hoquiam, and Threemile Creek exhibited a heterozygote deficiency owing to Tgr04, suggesting that there might be a null allele at this locus in these populations. All eight microsatellite loci amplified very reliably; of the 717 individuals genotyped, only 38 individuals failed to amplify at one locus, and only one individual failed at two loci. While a power analysis would be complicated given the number of pairwise comparisons performed (every comparison would require its own analysis), in many cases we detected significant θ_{st} values as small as 0.01 (Tables S1.2 and S1.4).

Genetic subdivision estimates are low for *Th. sirtalis* and *Ta. granulosa* in the study range ($\theta_{st} = 0.036$, 0.031, respectively). The assumption of a stepwise mutation model made little difference in the measured population structure for both garter snakes and newts ($R_{st} = 0.041$, 0.066 respectively). θ_{st} values did not differ significantly between transects for either species (*Th. sirtalis* θ_{st}: 0.032, 0.017, 0.028; *Ta. granulosa* θ_{st}: 0.009, 0.025, 0.014; values given are for the inland, northern coast, and southern coast transects, respectively). Pairwise values of θ_{st} were low in general, regardless of the geographic distance separating the populations or the transect of origin (Tables S1.1–S1.4).

Transects were significantly genetically differentiated from one another for both garter snakes and newts ($\varphi_{CT} = 0.015$ and 0.021, respectively; $P < 0.001$ for both). Furthermore, AMOVA detected significant among-group variance for almost all transects. For the northern coast, significant subdivision was detected between populations north of Potter Slough and those populations south of and including Potter Slough in *Th. sirtalis* ($\varphi_{CT} = 0.014$, $P = 0.013$). Newt population structure showed a similar pattern in this region, but population subdivision was found between populations north of Hoquaim and those populations south of and including Hoquiam ($\varphi_{CT} = 0.029$, $P = 0.023$). For the southern coast, garter snakes were significantly subdivided, with a distinct group consisting of the southern populations of Crescent City and Orick ($\varphi_{CT} = 0.008$, $P = 0.024$). *Taricha granulosa* was again similar in structure, but with the distinct southern group consisting only of the Orick population ($\varphi_{CT} = 0.037$, $P = 0.002$). There was no significant among-group variance for *Th. sirtalis* populations along the inland transect. In contrast, *Ta. granulosa* populations exhibit significant structure between populations lying to the east of Adair and those populations west of and including Adair ($\varphi_{CT} = 0.010$, $P = 0.001$).

The scale at which IBD first occurred differed for garter snakes and newts. IBD in *Th. sirtalis* was detected at the 400-km level ($\chi^2 = 6.86$, d.f. = 2, $P = 0.0324$), while in *Ta. granulosa* IBD was detected at the 200-km scale ($\chi^2 = 6.75$, d.f. = 2, $P = 0.0342$). The effect of IBD was greater in newts ($\beta = -0.5302 \pm 0.1554$) than in garter snakes ($\beta = -0.2823 \pm 0.0964$). The *Ta. granulosa* northern coast transect was the only transect in which IBD was evident ($\chi^2 = 6.24$, d.f. = 2, $P = 0.0441$, $\beta = -1.9184 \pm 0.0993$).

The average pairwise θ_{st} values for *Th. sirtalis* were 0.0452, 0.0088, and 0.0344 for the inland, southern coast, and northern coast transects, respectively. The average pairwise θ_{st} for the southern coast was significantly smaller than that for the other two transects (bootstrapped *t*-tests with 1.0×10^6 samples: $P < 0.001$ for both), and the difference between the inland and northern transects was marginally significant ($P = 0.07$). The average pairwise θ_{st} values for *Ta. granulosa* were 0.0097, 0.0226, and 0.0096 for the inland, southern coast, and northern coast transects, respectively. The average pairwise θ_{st} value for the southern coast transect was significantly larger than the inland and northern coast values ($P < 0.001$, $P = 0.005$, respectively). In comparison, for garter snakes our estimate for the southern coast was significantly smaller than the inland and northern coast transect estimates.

Indirect estimates of gene flow based on population structure were higher in garter snakes than in newts. Analysis showed that neighbourhood size ($4D\pi\sigma^2$) for garter snakes was 65.4 individuals ($\beta = 0.0153$, $P = 0.0001$, $R^2 = 0.0938$, $n = 153$), while newt neighbourhood size was 47.5 individuals ($\beta = 0.0210$, $P < 0.0001$, $R^2 = 0.3442$, $n = 253$). Neighbourhood size is the number of breeding individuals falling within the potential range (characterized by the dispersal standard deviation, σ) of a breeding adult. The neighbourhood size estimates for both *Th. sirtalis* and for *Ta. granulosa* are relatively small compared with most estimates (see Waser & Elliott, 1991; Peterson, 1996; Rousset, 2000; Antolin *et al.*, 2001; Giokas & Mylonas, 2004).

Contemporary immigration rate estimates indicate that migration between populations is strong (Table 2). Several populations acted non-independently in the sample. For the

Table 2 Estimates of contemporary gene flow using BAYESASS for (a) *Taricha granulosa* and (b)*Thamnophis sirtalis**

Population	Transect	*m*	Migrant source
(a)			
Cl	N	0.5 (0.009)	HR
HR	N	0.5 (0.009)	Cl
Co	N	0.282	Cl/HR
Ho	N	0.294	Cl/HR
Po	N	0.281	Cl/HR
Ch	N	0.269	Cl/HR
Wa	N	0.306	Cl/HR
Lo	I	0.055	Te (Cl/HR)
Mi	I	0.296	Lo
St	I	0.266	Lo
Ad	I	0.280	Lo
Te	I,S	0.294	Lo, Or
Ta	S	0.246	Hu (Lo)
El	S	0.177	Cc
Eu	S	0.302	El (Po)
Hu	S	0.140	Cc (Cl/HR)
Cc	S	0.216	El
Or	S	0.030	Hu (Ad)
(b)			
Cl	N	0.308	Co
HR	N	0.261	Ch/Po
Co	N	0.017	Ch/Po
Ho	N	0.308	Co
Po	N	0.5 (0.020)	Ch
Ch	N	0.5 (0.020)	Po
Wa	N	0.305	Ch/Po
Lo	I	0.023	Mi (Ch/Po)
Mi	I	0.273	Lo (Hu/El)
St	I	0.299	Lo (Hu/El)
Ad	I	0.295	Lo
Te	I,S	0.266	Lo, Hu/El (Co)
Ta	S	0.298	Hu/El
El	S	0.5 (0.092)	Hu
Eu	S	0.248	Hu/El
Hu	S	0.5 (0.092)	El
Cr	S	0.289	Or
Or	S	0.062	Hu/El (Ch/Po)

*Migration rates (*m*) for *Taricha granulosa* (a) and *Thamnophis sirtalis* (b) were high for most populations (population abbreviations are listed in Table 1; for the transect column, I denotes inland, N denotes northern coast, and S denotes southern coast). Parenthetical values of *m* show migration rates after merging the original populations into one (e.g. Cl and HR became Cl/HR for newts). The 'migrant source' column shows the population on the same transect from which most immigrants originated; parenthetical values are given if a study population from a different transect composed a majority of the immigrants.

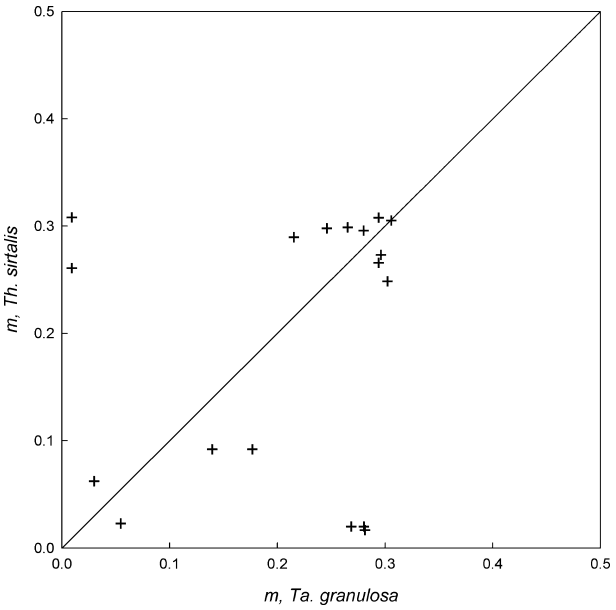

Figure 2 A comparison of *Taricha granulosa* and *Thamnophis sirtalis* immigration rates (m). Each point represents the average of five replicate runs; the diagonal line indicates a one-to-one relationship. Points lying above the diagonal indicate a population in which gene flow is lower for newts than it is for snakes, and thus newts are predicted to be better defended against garter snake predation; vice versa for points that lie below the diagonal. Only a few points are relatively distant from the line; these points come from populations within the northern transect.

newts, Clallam Bay and Hoh River seem to act as one population; Chinook and Potter Slough as well as Hunter Creek and Elk River are linked for garter snakes. After combining these linked populations, repeatability of m among replicate runs was high ($\rho^2 = 0.97$, 0.99 for newts and snakes, respectively). Only a few populations showed low migration rates ($m < 0.10$). Interestingly, in both species Lost Lake and Orick have little immigration. Furthermore, for both species the northern transect has low immigration populations (*Ta. granulosa*: Clallam Bay/Hoh River combined population; *Th. sirtalis*: Cook Creek, Chinook/Potter Slough combined population). Migration rates were asymmetric, with the low-immigration-rate populations often acting as immigrant 'sources' to other populations on the same transect (Table 2). Newt and snake immigration rates were relatively similar for most populations, with notable exceptions occurring within the northern coast transect (Fig. 2).

DISCUSSION

Little genetic differentiation is apparent for both the garter snake *Th. sirtalis* and the rough-skinned newt *Ta. granulosa* in the Pacific Northwest ($\theta_{st} = 0.036$, 0.031, respectively). Estimates of R_{st} (Slatkin, 1995) were not much different from θ_{st} ($R_{st} = 0.041$, 0.066 for garter snakes and newts, respectively), despite the tendency for microsatellite-based estimates of θ_{st} to overestimate genetic homogeneity (Gaggiotti *et al.*, 1999). The largest θ_{st} values for pairwise comparisons of 0.1628 (garter snakes) and 0.1357 (newts) represent relatively little genetic differentiation when considering the distance separating the populations (368.1 and 783.3 km, respectively).

Despite the lack of genetic differentiation within this region for garter snakes and newts, significant population structure was detected using hierarchical F-statistics. For both species there was significant structure among the three study transects ($\varphi_{CT} = 0.015$, 0.020 for garter snakes and newts, respectively). Furthermore, within each transect significant levels of structure were detected for the study organisms (see Genetic Divisions section below). Only the inland transect for garter snakes lacked any detectable levels of population structure using this method.

No significant difference was found when θ_{st} values for transects were compared with each other (e.g. the inland transect vs. the southern coast transect), implying that genetic differentiation within the transects was similar. However, the average pairwise θ_{st} estimates implied that genetic differentiation between populations along the southern coast was highest on average for newts and lowest on average for garter snakes. In contrast, genetic differentiation between populations along the inland transect was highest on average for garter snakes and lowest on average for newts.

Several findings suggest that *Ta. granulosa* is a more limited disperser than *Th. sirtalis*. The scale of IBD for newts was half that for garter snakes (200 km vs. 400 km). Neighbourhood size ($4D\pi\sigma^2$), an estimate of the effective population size and migration, was found to be 65.4 individuals for garter snakes and 47.5 individuals for newts. Newts had higher allelic richness scores than garter snakes as well. The fact that newts exhibit more population structure than garter snakes is not surprising, given their smaller size, restrictive habitat requirements, and the generally poor dispersal capabilities of amphibians (Rothermel & Semlitsch, 2002).

Estimating gene flow indirectly (i.e. estimation via population structure as done here) can be potentially misleading for many reasons (Whitlock & McCauley, 1999). Nonetheless, Bayesian assignment analysis corroborated the interpretation that contemporary migration rates are high. Other methods of estimating gene flow based on coalescence theory might yield lower estimates than the frequency-based approaches used here. In a study of Lake Erie *Th. sirtalis* using the same number of microsatellite loci (and two of the same loci) as used here, Bittner & King (2003) found that the number of migrants per generation estimated using coalescent techniques was typically around one, while F_{st} approaches yielded estimates of greater than four. However, the same study also found that the *net* immigration rate from all other populations calculated using coalescent techniques was approximately the migration rate estimated using F_{st}.

Isolation by distance

Thamnophis sirtalis and *Ta. granulosa* populations each showed patterns of IBD at relatively large scales (400 and 200 km, respectively). Demonstration of IBD is important because this

potentially demonstrates a stepping-stone model of migration. Knowing that garter snakes and newts fit this underlying model implies that co-evolution may occur in the manner described by the geographic mosaic theory of co-evolution. Furthermore, the calculation of dispersal distances and neighbourhood sizes are appropriate for species that exhibit IBD, but not appropriate for populations that fit other models (e.g. the island model; Wright, 1931). The fact that garter snake and newt populations are more isolated as geographic distance increases is of particular importance to the independent evolution of populations (by allowing for the formation of phenotypic mosaics via geographically variable selection).

King & Lawson (2001) also found no IBD in the fine-scale genetic subdivision of Lake Erie *Th. sirtalis* within a transect. Lake Erie garter snake populations were sampled at a similar scale to our sample populations (pairwise population distances of 1–100 km). As for *Th. sirtalis* in the Pacific Northwest, Lake Erie garter snakes recolonized that region after the Pleistocene glaciation, so the findings of this study parallel those of King & Lawson (2001). This lack of IBD for both garter snakes and newts at distances of less than 400 km/200 km indicates that, for populations within our experimental transects, (co-)evolution is probably not occurring independently. The northern newt transect may be distinct in this regard from our other transects because of IBD within that particular transect. If selection mosaics exist within the transects as we would expect based on our transect locations (Brodie *et al.*, 2002), then, as predicted by the geographic mosaic theory, we expect maladaptation to be present because of differential gene flow in these co-evolving species.

There could be multiple explanations for a lack of IBD at fine-scale distances. If populations are extremely subdivided at the scale of observation, then θ_{st} should be large and relatively invariable, resulting in no covariance between distance and genetic relatedness. Conversely, if there is a lack of subdivision at the scale of observation, then θ_{st} should be small and relatively invariable, again resulting in no covariance between distance and genetic relatedness. Non-equilibrium conditions also affect observed patterns of IBD (Slatkin, 1993), and could potentially lead to a lack of IBD. Another possibility is that landscape features (ridges, river drainage, rainfall, etc.) may lead to differential migration, and straight-line distance may not be the most appropriate measure of distance (Funk *et al.*, 2005; Guillot *et al.*, 2005). However, the general lack of variation in θ_{st} within our transects makes it unlikely that landscape features would play a role in the lack of IBD (i.e. no or little covariance should exist, regardless of the distance measure). Within the sampling transects and across fine-scale distances, it seems as if either non-equilibrium conditions or high levels of ongoing gene flow are the most likely explanations for the lack of IBD, given the nature of our data.

Genetic divisions

The gross patterns of population differentiation in *Ta. granulosa* and *Th. sirtalis* seem consistent with a shared historical biogeography. Populations exhibited a high degree of genetic relatedness for both species, probably reflecting the recent glaciation of the region *c.* 14,000 yr BP (Thackray *et al.*, 2004) in conjunction with ongoing gene flow. Studies across a broad range of taxa support the impact of glaciation on the biogeography of the Pacific Northwest. Hypothesized range expansions after the glacial recession have led to low levels of genetic variation in the region (Brown *et al.*, 1997; Althoff & Thompson, 2001; Edmands, 2001). The fact that neighbourhood size estimates were small and consistent with those of other species that have low vagility (such as kangaroo rats and sedentary lycaenid butterflies; Waser & Elliott, 1991; Peterson, 1996; Rousset, 2000) reinforces that the observed relatedness among populations is at least in part the result of recent recolonization of the region and not entirely the result of contemporary gene flow. Furthermore, some populations show relatively low θ_{st} values but are not well connected by contemporary migration (e.g. Orick and Lost Lake). The combination of all these studies suggests that garter snake and newt population structure as well as their co-evolution have been shaped by climatological shifts occurring in the past 14,000 years in western North America.

For *Ta. granulosa*, there is a clear break in the northern transect in terms of population structure. The northern populations of Clallam Bay, Hoh River, and Cook Creek are genetically differentiated from the southern populations on this transect ($\varphi_{CT} = 0.029$, $P = 0.023$). Bayesian assignment identified Clallam Bay and Hoh river as a single population that acts as a source of migrants for populations further south in Washington (Table 2). In addition, the presence of a north-south break at approximately the Siskiyou Mountains can be seen in our AMOVA analysis ($\varphi_{CT} = 0.037$, $P = 0.002$) and the pairwise comparisons of Orick with other populations along the southern coast. Orick is clearly distinct and currently isolated from the rest of the populations studied, as expected based on previous phylogeographic analyses (Kuchta & Tan, 2005). The inland transect showed significant east–west genetic division between the Stayton and Adair populations ($\varphi_{CT} = 0.010$, $P = 0.001$). Lost Lake in central Oregon looks to be a source of migrants to the inland transect and is genetically distinct from the surrounding populations (Table 2); directional gene flow out of Lost Lake could be facilitated by the altitudinal gradient from the Cascade mountains to the coast (Funk *et al.*, 2005).

The results for population structure in *Th. sirtalis* are consistent with the phylogeography of Janzen *et al.* (2002) and similar to those observed in *Ta. granulosa*. As was evident in newts, snake populations in northern and southern Washington exhibit a genetic divide ($\varphi_{CT} = 0.014$, $P = 0.012$). However, for garter snakes the southern extent of the northern group seems to reach to Hoquiam (i.e. slightly further south than for newts). Cook Creek and the Chinook/Potter Slough populations acted as migrant sources for the northern and southern Washington groups, respectively (Table 2). The California populations of Orick and Crescent City formed a distinct genetic group ($\varphi_{CT} = 0.008$, $P = 0.024$) from the rest

of the populations along the transect, supporting the hypothesis of the north-south biogeographical split at approximately the California/Oregon border. Furthermore, Orick and Crescent City seem to be relatively isolated from ongoing migration (Table 2). Similarly to the case for *Ta. granulosa*, garter snakes from the Cascades population of Lost Lake are isolated and act as a source of migrants. The fact that Lost Lake garter snakes are isolated may be evidence of the hypothesized intermountain *Th. sirtalis* clade (Janzen *et al.*, 2002); however, no evidence of significant genetic division was found along this transect (i.e. no significant φ_{CT}).

Implications for co-evolution

The process of co-evolution between garter snakes and newts must be occurring at a very quick pace (far outpacing mutation at neutral molecular sites), given the large phenotypic differences observed between some populations (Hanifin *et al.*, 1999; Brodie *et al.*, 2002). The apparent lack of genetic differentiation, owing to historical and contemporary gene flow, certainly presents the opportunity for maladaptation within the system, particularly if there is a geographic mosaic of selection pressures occurring on a finer scale than genetic subdivision. Those populations in which asymmetries in species' migration rates are the greatest should be the most susceptible to maladaptation (Fig. 2).

It is difficult to tell whether immigrant counts (as estimated by BayesAss) or neighbourhood sizes are more relevant to the study of local adaptation. Two different paradigms of local adaptation are the 'local vs. foreign' concept and the 'home vs. away' concept (Kawecki & Ebert, 2004). When considering the 'local vs. foreign' framework, the number of immigrants (foreign individuals) would seem more important. Alternatively, under the 'home vs. away' framework, the transfer of individuals away from the home environment (neighbourhood size) is the unit of currency. Interestingly, these two frameworks yield mathematically identical local adaptation scores, despite the difference in underlying concepts; thus it is unclear which population structure metric should be used.

The scale at which garter snakes disperse seems to be considerably larger than that of newts (see above). In general, we expect stronger selection pressure on a prey item than on its generalist predator (life-dinner principle; Dawkins & Krebs, 1979), which could discourage gene flow among prey populations. The observed asymmetry in garter snake and newt population structure should favour local adaptation in *Ta. granulosa*, which is concordant with this expectation. However, as previously mentioned, some of the difference in dispersal capabilities is probably the result of physical constraints.

Two key differences are apparent in garter snakes and newts from the southern coast transect. First, there is a disparity in the location of the genetic subdivision (the division occurs farther south for newts). Second, we observed the strongest differences in population structure in the southern coast transect, where newts show the greatest amount of genetic differentiation ($\theta_{st} = 0.025$; average θ_{st} significantly different from other transects). In contrast, garter snakes show the least amount of genetic subdivision on this transect ($\theta_{st} = 0.017$; average θ_{st} significantly different from other transects). From these results, we predict that newts are locally adapted along the southwest coast of Oregon, and garter snakes are not.

The inland transect exhibits the opposite pattern to the southern coast: garter snake populations are most genetically differentiated in this region ($\theta_{st} = 0.032$), and newts show the least genetic differentiation there ($\theta_{st} = 0.009$). Initially, we might expect *Th. sirtalis* to be more locally adapted than newts and to provide the impetus behind co-evolution along this transect. However, based upon our AMOVA results, the lack of any significant population structure in this region for garter snakes – whilst significant structure was detected in newts – might require us to reconsider this expectation.

The northern coast transect showed an intermediate level of genetic differentiation for both species, which may indicate that both species have the opportunity to adapt locally along this cline. It is also possible that interactions in this area may pre-date interactions in the rest of the study area if post-glacial recolonization occurred from a northern refugium, further favouring local adaptation in this region. However, at the population level several mismatches in contemporary migration rates (Fig. 2), the presence of IBD in newts, and the disparity in the location of genetic division (further north for *Ta. granulosa*) may lead to maladaptation. Further analysis of the phenotypic structure of *Th. sirtalis* and *Ta. granulosa* in the Pacific Northwest is necessary to assess the impact of population structure on the co-evolutionary process.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1. PCR conditions, distances, θ_{st}, and locales.

This material is available as part of the online article from http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2699.2006.01642.x (This link will take you to the article abstract).

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