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Population genetic analysis identifies source–sink dynamics for two sympatric garter snake species (*Thamnophis elegans* and *Thamnophis sirtalis*)

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Abstract

Population genetic structure can be shaped by multiple ecological and evolutionary factors, but the genetic consequences of these factors for multiple species inhabiting the same environment remain unexplored. We used microsatellite markers to examine the population structures of two coexisting species of garter snake, *Thamnophis elegans* and *Thamnophis sirtalis*, to determine if shared landscape and biology imposed similar population genetic structures. These snakes inhabit a series of ponds, lakes and flooded meadows in northern California and tend to converge on prey type wherever they coexist. Both garter snakes had comparable effective population sizes and bidirectional migration rates (estimated using a maximum-likelihood method based on the coalescent) with low but significant levels of genetic differentiation (F_{ST} = 0.024 for *T. elegans* and 0.035 for *T. sirtalis*). Asymmetrical gene flow revealed large source populations for both species as well as potential sinks, suggesting frequent extinction–recolonization and metapopulation dynamics. In addition, we found a significant correlation between their genetic structures based on both pairwise F_{ST}s for shared populations (P = 0.009) and for bidirectional migration rates (P = 0.024). Possible ecological and evolutionary factors influencing similarities and differences in genetic structure for the two species are discussed. Genetic measures of effective population size and migration rates obtained in this study are also compared with estimates obtained from mark–recapture data.

Keywords: coalescent-based maximum likelihood, extinction–recolonization, F_{ST}, isolation by distance, metapopulation, microsatellites

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Introduction

Patchily distributed populations can become genetically distinct over time as a consequence of random genetic drift and response to locally varying selection. Gene flow counters these two processes and acts as a homogenizing force that opposes differentiation (Wright 1931). The net result of all three processes is some pattern of population genetic structure. The degree to which a population experiences drift, selection and gene flow depends on multiple ecological and evolutionary factors. While similar species inhabiting a common landscape may encounter comparable factors influencing population genetic differentiation, it is not known whether patterns of genetic structure tend to evolve in parallel.

Although recent studies have reported population genetic structures for multiple sympatric species (e.g. McMillen-Jackson & Bert 2003; Michels *et al*. 2003; Brede & Beebee 2004; Molbo *et al*. 2004; Zardoya *et al*. 2004), few studies have statistically compared genetic structures. Results for each species are usually reported separately without statistical comparison. This qualitative approach arises because multiple species are rarely sampled from the same sites (but see Rüber *et al*. 2001; Brede & Beebee 2004). A general exception comes from studies of symbionts (Anderson *et al*. 2004 and references cited therein), but in these cases, the comparison species are usually so phylogenetically divergent that perceptions of and responses to a common environment may be very different. Our research provides a statistical comparison of population genetic structures for two closely related species that coexist on the same landscape.

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In this study, we compare patterns of population genetic differentiation at microsatellite loci for two coexisting garter snake species, the terrestrial garter snake, *Thamnophis elegans*, and the common garter snake, *Thamnophis sirtalis*. The study area is located at and around Eagle Lake in Lassen County, California. The habitat is predominantly arid sagebrush–yellow pine forest dotted with numerous permanent and semipermanent lakes and ponds as well as meadows that flood with snowmelt in the spring. The study system is comprised of 22 such water bodies that vary in size, permanence and degree of isolation, all occurring within 1050 km^2 and ranging in elevation from 1500 to 2100 m. Both garter snakes have widespread distributions in the western United States and Canada (Rossman *et al*. 1996; Stebbins 2003) and are abundant at the study area. The water bodies provide habitat for the snakes' primary prey: amphibians, small fish and leeches (Kephart 1982; Kephart & Arnold 1982). Garter snakes in temperate climates hibernate during winter and emerge to mate in the immediate vicinity of those hibernacula in the spring (Aleksiuk & Gregory 1974; Moore & Lindzey 1992; Whittier & Tokarz 1992). Because garter snake hibernacula are found at many of our study sites (Kephart 1981), we expected garter snake populations to be genetically structured about the lakes, ponds and meadows that comprise our study system.

Populations of *T. elegans* in the study area are more common and tend to be larger than those of *T. sirtalis*. Of the 22 water bodies included in this study, *T. elegans* are found at 20, and *T. sirtalis* are found at 13. Overall, *T. elegans* outnumbers *T. sirtalis* at most sites in the study area. Half of the study sites support both species, and only two (Feather Lake and Gordon Lake) are dominated almost exclusively by *T. sirtalis*. We therefore expect larger effective population sizes for *T. elegans* and a higher degree of population differentiation for *T. sirtalis*. Because *T. sirtalis* occupies fewer sites, we also expect to find a greater degree of isolation by distance in this species.

The diets of *T. elegans* and *T. sirtalis* at the study area tend to converge at sites where they coexist in our study system (Kephart 1982; Kephart & Arnold 1982). Specifically, both species capitalize on the explosive breeding events of anurans, whose larvae and metamorphs are a primary prey source when abundant. Both species will also prey on fish (*Rhinichthys osculus*, *Richardsonius egregius*, *Gila bicolor* and *Castomus tahoensis*) and leeches (*Erpobdella* spp.), although *T. elegans* does so to a much greater degree. *T. elegans* are better at controlling their buoyancy in water and can therefore dive to actively hunt fish, whereas *T. sirtalis* can consume fish only when they are easily caught in drying pools (Kephart 1981). This difference in ability to catch fish may explain why *T. sirtalis* are rarely found along the shoreline of Eagle Lake, where fish are abundant, but amphibian breeding is inconsistent from year to year.

Recent phylogenetic reconstructions of garter snake relationships based on mitochondrial sequence data suggest that *T. elegans* and *T. sirtalis* diverged from a common ancestor in the Pliocene. Assuming a rate of sequence divergence of 1.3% per million years (Myr) based on agamid lizards (Macey *et al*. 1998), the two species diverged around 6.9 million years ago (Ma) (de Queiroz *et al*. 2002). The recent evolutionary history of *T. elegans* and *T. sirtalis* at the study area begins after the Pleistocene, when much of the currently suitable habitat in western North America was covered by glaciers (Barnosky *et al*. 1987; Janzen *et al*. 2002). Glacial recession at the end of the Pleistocene around 10 000 years ago allowed recolonization of parts of the western United States by *T. sirtalis* from multiple refugia. Lassen County populations of *T. sirtalis* probably originated from the Great Basin or southern California (Janzen *et al*. 2002). Overall, populations of *T. elegans* in western North America are much more divergent (0.3–7%; Bronikowski & Arnold 2001) than those of *T. sirtalis* (0.3–0.6%; Bronikowski & Arnold 2001; Janzen *et al*. 2002), suggesting that *T. sirtalis* is a relative newcomer to western North America.

The goal of this study was to use microsatellite markers to examine genetic differentiation in two closely related species inhabiting a common landscape. We investigated patterns of isolation by distance and estimated measures of genetic differentiation, including bidirectional migration rates and effective population sizes. We then estimated the correlation between species using two different measures of genetic structure. Previous research in the study system estimated effective population sizes for two *T. elegans* populations and two *T. sirtalis* populations based on mark–recapture data (Kephart 1981). Furthermore, only six dispersal events out of over 800 recaptures were documented over distances less than 5 km, leading to the conclusion that dispersal events were relatively rare. We qualitatively compared these mark–recapture estimates for effective population size and migration rate from Kephart (1981) to those based on microsatellite data in an effort to evaluate direct and indirect methods of estimating population genetic parameters.

Materials and methods

Sampling

We collected tissue samples from *Thamnophis elegans* and *Thamnophis sirtalis* in and around the Eagle Lake basin in Lassen County, California. A total of 858 *T. elegans* from 20 populations and 433 *T. sirtalis* from 13 populations were sampled (Table 1). Overall, 22 sites were included in the study, 12 of which support both species. For each snake, the tail tip or a 2 × 4-mm piece of ventral scale was taken and stored in Drierite®, an anhydrous calcium sulphate desiccant.

Table 1 Study site names, abbreviations, latitude and longitude (decimal degrees) and summary of genetic variability measures. H_O and H_E calculated without TS042 and TE051B for *Thamnophis elegans* and *Thamnophis sirtalis*, respectively

					Thamnophis elegans				*Thamnophis sirtalis*			
Site	Lat.	Long.	Elev. (m)	Abbrev.	*N*	H_O	H_E	N_a	*N*	H_O	H_E	N_a
Antelope Mtn. Pond*	40.614	–120.923	1920	AMP	30	0.50	0.53	5	19	0.61	0.64	5
Ashurst Lake	40.750	–120.965	1950	ASH	30	0.55	0.58	5	38	0.58	0.59	7
Blue Water	40.834	–120.919	1770	BLW	27	0.51	0.56	5				
Bullard Lake	40.775	–120.901	1860	BUL	67	0.51	0.52	6	35	0.55	0.57	7
Cleghorn Res.	40.777	–120.804	1880	CLG	22	0.55	0.58	5				
Colman Lake	40.516	–120.714	1965	COL	24	0.47	0.56	5	27	0.59	0.57	7
Deans Meadow	40.557	–120.719	1980	DNS	26	0.50	0.55	5	27	0.47	0.52	5
Feather Lake	40.542	–121.018	1740	FEA					24	0.53	0.63	7
Gallatin Shoreline*	40.562	–120.760	1575	GAL	56	0.47	0.51	5				
Gordon Lake	40.768	–120.882	1850	GOR					42	0.51	0.56	7
Jacks Lake	40.810	–121.025	1690	JKS	27	0.50	0.54	5				
Little Cleghorn Res.	40.787	–120.794	1880	LTC	40	0.46	0.53	5				
Mahogany Lake	40.534	–120.732	2065	MAH	91	0.52	0.53	5	26	0.53	0.60	7
McCoy Flat Reservoir	40.453	–120.940	1700	MCY	16	0.47	0.51	4				
Nameless Meadow*	40.524	–120.743	1915	NML	29	0.54	0.53	5	29	0.49	0.56	7
Papoose Meadows	40.528	–120.757	1645	PAP	140	0.51	0.54	6				
Pikes Point	40.557	–120.784	1555	PIK	48	0.52	0.54	4				
Pine Valley Meadow*	40.619	–120.969	1730	PVM	70	0.52	0.51	5	83	0.57	0.61	9
Rocky Point	40.684	–120.757	1580	RKY	19	0.47	0.58	5				
Roney Corral	40.511	–120.857	1825	RON	24	0.45	0.50	4	29	0.47	0.60	6
Camp Stanford	40.803	–120.932	1870	STF	45	0.54	0.54	6	32	0.50	0.59	7
Small Vernal Pools*†	40.507	–120.731	1890	SVP								
Summit Lake	40.766	–120.839	1890	SUM	27	0.50	0.52	5	22	0.58	0.61	5

N, sample size; H_O, average observed heterozygosity; H_E, average expected heterozygosity; N_a, average number of alleles per locus;
* informal names, not official geographical place names; † a study site from Kephart (1981) that was not sampled for this study.

Obtaining microsatellite data

A total of 11 primer sets for microsatellite markers were used, five obtained from the literature and three developed *de novo* (Table 2). An enriched library was prepared according to the method of Hamilton *et al*. (1999), and positive clones were screened based on the method of Hoffman *et al*. (2003). In summary, whole genomic DNA was digested with *Msp*I, size-selected, ligated to oligo linkers and amplified with linker primers. Four biotinylated oligo probes ($dGACA_4$, $dGATA_4$, $dGGAT_4$ and $dGGGA_4$) were hybridized to linker DNA and selected using streptavidin magnetic particles (Promega). Enriched DNA was then amplified using linker primers and purified using a polymerase chain reaction (PCR) purification kit (QIAGEN). Linkers were removed and DNA was ligated into pBluescript vector and transformed into Epicurian Coli XL1-Blue Supercompetent Cells (Stratagene). Positive colonies were picked into sterile water, boiled and sequenced at the Nevada Genomics Center at the University of Nevada at Reno, USA. Primers were designed using OLIGO version 6.0 (Rychlik 1998) and optimized with an MJ Research Peltier gradient thermocycler.

To conduct preliminary analyses of microsatellite polymorphism, 100 ng of DNA from four individuals of each species were amplified in 25-μL volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 0.48 μM forward (labelled with fluorescent ABI dye) and reverse primer, and 0.3 U *Taq* DNA polymerase. PCR profiles were 94 °C for 5 min followed by 36 cycles of 94 °C for 45 s, the optimized annealing temperature for 45 s and 72 °C for 1 min 30 s, ending with 72 °C for 10 min. PCR products were separated using an ABI 3100 capillary electrophoresis genetic analyser and data were visualized using GENOTYPER 3.7 (ABI PRISM). Table 3 shows the primer sequences, repeat sequences and optimal annealing temperatures of the three microsatellite markers cloned for this study.

DNA extraction, PCR, genotyping

Whole genomic DNA was extracted using sodium dodecyl sulphate-proteinase K digestion followed by a standard phenol–chloroform extraction, NaCl purification and isopropanol precipitation. For all species, 5–100 ng DNA was PCR amplified in a 12.5-μL reaction with the above reagent concentrations. PCR profiles consisted of 94 °C for 2 min

Table 2 Comparison of the genetic diversity found at all microsatellite loci for each species averaged over all populations

	Thamnophis elegans (20)						*Thamnophis sirtalis* (13)						
Locus	Total alleles	Allele size range (bp)	H_O	H_E	N_a	Pops. out of HWE	Total alleles	Allele size range (bp)	H_O	H_E	N_a	Pops. out of HWE	Reference
Nsμ10	12	120–144	0.53	0.58	5	0	13	141–168	0.69	0.74	8	0	Prosser *et al*. (1999)
Nsμ2	6	139–160	0.60	0.65	4	0	1	138	—	—	—	—	Prosser *et al*. (1999)
Nsμ3	19	129–193	0.84	0.88	11	0	20	145–195	0.73	0.81	11	0	Prosser *et al*. (1999)
Nsμ7	2	170–176	0.06	0.10	2	0	9	174–200	0.70	0.71	5	1	Prosser *et al*. (1999)
Nsμ8	5	129–161	0.42	0.41	3	0	2	120–128	0.30	0.39	2	0	Prosser *et al*. (1999)
TE051B	4	96–104	0.10	0.14	2	1	24	145–198	0.38	0.86	11	9	this study
TS010	15	108–146	0.78	0.83	9	0	4	108–124	0.30	0.29	3	0	this study
TS042	19	148–194	0.34	0.79	9	17	1	191	—	—	—	—	this study
Ts2	16	118–149	0.63	0.69	8	0	2	86–92	0.16	0.23	2	0	McCracken *et al*. (1999)
Ts3	4	92–129	0.48	0.49	2	0	13	141–168	0.69	0.74	8	0	McCracken *et al*. (1999)
3Ts	—	—	—	—	—	—	33	338–416	0.86	0.92	16	0	Garner *et al*. (2002)

H_O, average observed heterozygosity; H_E, average expected heterozygosity; N_a, average number of alleles per population. Numbers in parentheses: numbers of populations sampled.

Table 3 Genetic characteristics of three microsatellite primer sets developed for this study

Locus	Repeat motif	Primer sequences (5′–3′)	Species of origin	T_m (°C)
TE051B	$(TTCC)_3(TTCA)_2(TTCC)_3$	F: GATTCAAGGCAGTGAACATACC R: ACCACTGTCCCAAACCTACCTC	*Thamnophis elegans*	63
TS010	$(ATGG)_3,(ATGA)_6$	F: TGACTCAGATGCCCTCAGTCTA R: CGGACCAACCAGGAACAGAAAT	*Thamnophis sirtalis*	60
TS042	$(GA(CA)_4)_4$	F: TCAGGATACGGCAACCAGGCTT R: GCTCCCAAACCCATCACTCAG	*Thamnophis sirtalis*	68

F, forward primer; R, reverse primer; T_m, optimum annealing temperature.

followed by 36 cycles of 94 °C for 30 s, appropriate annealing temperature for 30 s and 72 °C for 30 s, ending with 72 °C for 2 min. PCR products were genotyped and analysed as above.

Data analysis

Exact tests for departure from Hardy–Weinberg equilibrium (HWE) were performed for each locus separately, and significance was evaluated using the Markov chain method (Guo & Thompson 1992; Markov chain parameters: 5000 dememorizations; 500 000 steps per chain). Tests for linkage disequilibrium were performed for each population and globally for each species using a likelihood-ratio test with level of significance determined by permutation (Slatkin & Excoffier 1996; Markov chain parameters: 5000 dememorizations, 1000 batches, 5000 iterations per batch). Levels of statistical significance were adjusted according to a sequential Bonferroni correction for multiple comparisons (Rice 1989). Genetic variability within each population was quantified by counting the number of alleles and determining observed and expected heterozygosities. Number of alleles per locus in each population and over all populations as well as measures of linkage disequilibrium were calculated in GENEPOP (Raymond & Rousset 1995). All other analyses were performed in ARLEQUIN version 2.000 (Schneider *et al*. 2000).

Overall and population pairwise estimates of F_{ST} were obtained using a hierarchical analysis of molecular variance, AMOVA (Excoffier *et al*. 1992), in ARLEQUIN version 2.000. This analysis makes the same assumptions as other methods for estimating F_{ST} under Wright's island model, namely an infinite number of populations with equal sizes that are constant over time, equal migration rates that are very low, no mutation and no selection (Wright 1931). Significance was assessed after 16 000 permutations for global estimates and 3000 permutations for pairwise estimates. *P* values were adjusted with the sequential Bonferroni correction.

In order to visualize the pattern of isolation by distance (Wright 1943), we regressed genetic distance, defined as $F_{ST}/(1 - F_{ST})$, on the logarithm of geographical distance, as

suggested by Rousset (1997). We then evaluated the relative roles of gene flow and random genetic drift using the pattern revealed by the scatterplot (Hutchison & Templeton 1999). The Pearson product–moment correlation coefficients between the genetic and geographical distance matrices were assessed using Mantel tests (Mantel 1967; Mantel & Valand 1970; Manly 1997), implemented in ARLEQUIN version 2.000. *P* values were obtained through 10 000 permutations.

MIGRATE version 1.7.6.1 (Beerli & Felsenstein 2001) was used to calculate effective population size as a function of mutation rate ($\Theta = 4N_e\mu$) as well as effective numbers of migrants ($4N_em$), where N_e is effective population size, μ is mutation rate and m is the rate of migration into the population. This analysis used the stepwise-mutation model (Ohta & Kimura 1973), which assumes that mutations occur in a stepwise fashion, with the addition or deletion of one repeat unit at a time, and that loci are neutral and unlinked. Assumptions of the maximum-likelihood approximation using the coalescent approach include diploid individuals reproducing according to a diffusion equation approximation of a Wright–Fisher model with constant population sizes, and constant migration and mutation rates (mutation–migration equilibrium; Beerli 1998; Beerli & Felsenstein 1999). F_{ST} estimates were used as starting values for the initial analysis. For all other analyses, ending parameters of the previous run were used as starting values for the next run until results equilibrated at approximately the same values. Ten short chains with 10 000 sampled genealogies each and two long chains with 100 000 sampled genealogies each were run for each analysis. One of every 20 constructed genealogies was sampled, and multiple long chains were combined for estimates. We used adaptive heating with temperature specifications of 1.0, 1.2, 1.5 and 3.0. Heating allows chains to be run at different temperatures, the highest of which explores the most genealogy space. Chains can swap based on an acceptance-rejection step so that colder chains explore peaks while hotter chains sample more widely. The temperature difference between chains can be adjusted based on rate of swapping. This method is based on the analysis of Geyer & Thompson (1994) and is called MC^3 or MCMCMC (Markov-coupled Markov chain Monte Carlo).

We evaluated the effect of unbalanced sample sizes on our results by repeating the MIGRATE analysis on a *T. elegans* data set with equalized sample sizes. Because the *T. elegans* data set has the most unbalanced sampling design (sample sizes ranged from 16 to 140), we expect any biases to be strongest with that data set. Populations with more than 30 individuals were randomly subsampled to a maximum sample size of 30. We conducted two replicate runs and compared the results to results using the entire sample. We found no effect of unbalanced sample sizes on the patterns of migration and population size.

The correlation between the genetic structures of *T. elegans* and *T. sirtalis* was assessed for sites in common using Mantel tests. Pairwise genetic distance matrices, using $F_{ST}/(1 - F_{ST})$, were compared in ARLEQUIN 2.000, and the asymmetric matrices of bidirectional migration rate were compared using a Mantel test implemented in CADM (congruence among distance matrices; Legendre 2001), a program that allowed statistical comparison of full matrices. Significance of all Mantel tests were assessed over 10 000 permutations. A Pearson correlation coefficient for effective population sizes between species was obtained in SAS (version 9.2; SAS Institute 2002).

Results

Tests of disequilibrium

Most microsatellite loci were in Hardy–Weinberg and linkage equilibrium in all populations. For *Thamnophis elegans*, the TS042 locus had a significant heterozygote deficit in 17 out of 19 genotyped populations after sequential Bonferroni correction and was excluded from further analysis. An additional locus was also out of HWE at one population. For *Thamnophis sirtalis*, the TE051B locus had a significant heterozygote deficit in 9 out of 12 populations genotyped and was excluded from further analysis. An additional locus was also out of HWE at one population. Linkage disequilibrium was found in the Feather Lake *T. sirtalis* population between Nsμ3 and 3Ts.

Allelic variation

Average observed and expected heterozygosities for all populations are shown in Table 1 and for all loci in Table 2. Overall observed and expected heterozygosities were calculated excluding both TS042 for *T. elegans* and TE051B for *T. sirtalis*. The total number of alleles at a locus varied from 2 to 19 for *T. elegans* and 1 to 33 for *T. sirtalis* (Table 2). The average number of alleles per locus within a population ranged from 4 to 6 for *T. elegans* and 5 to 9 for *T. sirtalis* (Table 1), while the average number of alleles per population for a locus ranged from 2 to 11 for *T. elegans* and 2 to 16 for *T. sirtalis* (Table 2).

Population structure

Global estimates of F_{ST} were relatively low but highly significant for both species. *T. sirtalis* had an F_{ST} of 0.035 ($P < 0.00001$), and that of *T. elegans* was slightly lower at 0.024 ($P < 0.00001$). Both garter snakes had approximately the same total variance in allele size (*T. elegans*: 25.11; *T. sirtalis*: 21.12). Pairwise estimates of F_{ST} are shown in Table S1 (Supplementary material) for *T. elegans* and Table S2 (Supplementary material) for *T. sirtalis*. Thirty out of 190 *T. elegans* population pairs were found to be significantly differentiated after sequential Bonferroni correction. Pikes

Point had the highest number of significant comparisons (10), while populations at Ashurst Lake, Nameless Meadow, and Rocky Point showed no significant differentiation from other populations. Rocky Point was remarkably undifferentiated; its most significant comparison was with Pikes Point with a P value of 0.20. This anomalous result for Rocky Point may be attributed to a high percentage of missing values for that population (27%, compared with 4% over the entire data set and 7% missing data for *T. sirtalis*). Among a total of 78 *T. sirtalis* population comparisons, 25 were statistically significant. Colman Lake had the most significant comparisons (seven), while Roney Corral had none.

Isolation by distance

A significant positive relationship was found between genetic and geographical distance for both *T. elegans* (slope = 0.006) and *T. sirtalis* (slope = 0.033; Fig. 1). Mantel tests for the geographical and genetic distance matrices showed that *T. elegans* and *T. sirtalis* had significant Pearson correlation coefficients ($r = 0.198$, $P = 0.0064$ for *T. elegans*; $r = 0.416$, $P < 0.0001$ for *T. sirtalis*). The slopes of the regressions for both species were significantly different ($P = 0.029$).

The pattern produced by the regression of genetic distance on geographical distance was similar for both species. The scatterplots were wedgelike, with the variance in genetic distance increasing with geographical distance. For *T. elegans*, the points most responsible for this effect had relatively high genetic distances in relation to geographical distance and were all associated with Pikes Point. The two most deviant points described distances from Pikes Point to Deans Meadow and Colman Lake (Fig. 1A). The most deviant point for *T. sirtalis* was associated with Deans Meadow and Nameless Meadow (Fig. 1B), which were relatively more differentiated given the geographical distance. A number of points for *T. elegans* also had relatively low genetic distances given the geographical distances. These points were largely due to distances from Rocky Point, which had many F_{ST}s of 0 (Table S1), suggesting recent colonization of that site.

Effective population size and migration rate

Estimates of effective population size (Θ) for *T. elegans* ranged from 0.060 to 0.306 with an average of 0.131 (Table S3, Supplementary material). Assuming a typical vertebrate microsatellite mutation rate of 10^{-4} per locus per generation (Dallas 1992; Edwards *et al*. 1992; Weber & Wong 1993; Banchs *et al*. 1994; Ellegren 1995), average Θ translates to an N_e of approximately 328. Papoose Meadows had the largest N_e (765) with Mahogany Lake, Bullard Lake and Pine Valley Meadow as runners-up with effective sizes of 571, 497 and 467, respectively. All of these populations were also the largest sources of migrants, even after taking their large

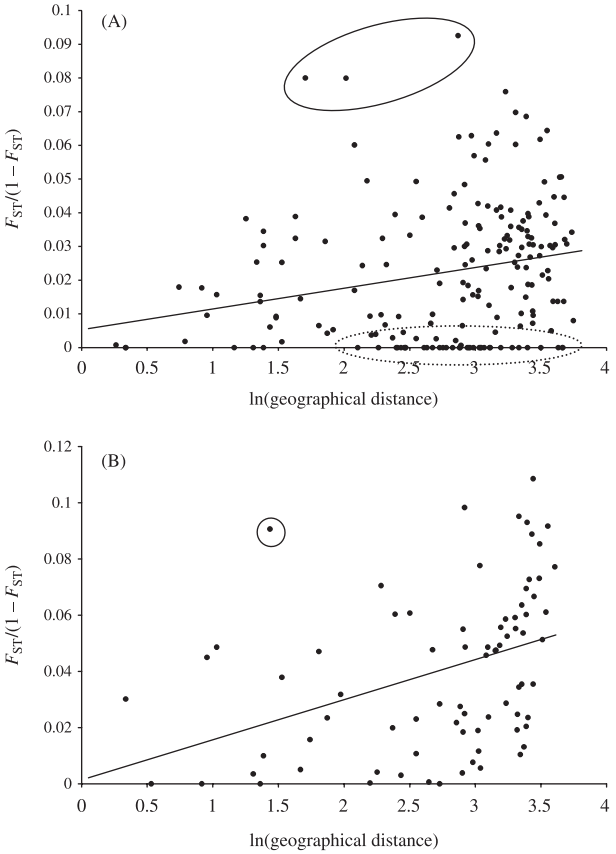

Fig. 1 Isolation-by-distance plots for (A) *Thamnophis elegans* and (B) *Thamnophis sirtalis*. Circled points refer to data causing deviations from a pattern of migration–drift equilibrium: solid circles — restricted gene flow relative to geographical distance, dotted circle — high gene flow relative to geographical distance.

effective sizes into account. Papoose Meadows was the most important source population for the entire study area (Fig. 2A) with a combined $4N_em$ of 107.5, which translates to a migration rate (m) of 0.086. In other words, 8.6% of individuals at all other sites originated from the Papoose Meadows population. The smallest populations, McCoy Flat Reservoir ($N_e = 151$) and Rocky Point ($N_e = 165$), also had the smallest rates of migration to other populations (0.007 for each). Cleghorn Reservoir had the highest total immigration rate from all other populations ($m = 0.029$), while Papoose Meadows received the fewest migrants ($m = 0.013$). Estimates of $4N_em$ for paired *T. elegans* populations were low, averaging 1.27, corresponding to an N_em of 0.32 with an average migration rate of 0.0012 migrants per generation (calculated after taking effective sizes for each population into account). As previously mentioned, Papoose Meadows had by far the highest net emigration rate, identifying that population as an important source of migrants for the region. Populations whose rates of immigration exceeded emigration (i.e. potential sink populations) included Cleghorn Reservoir, Rocky Point and

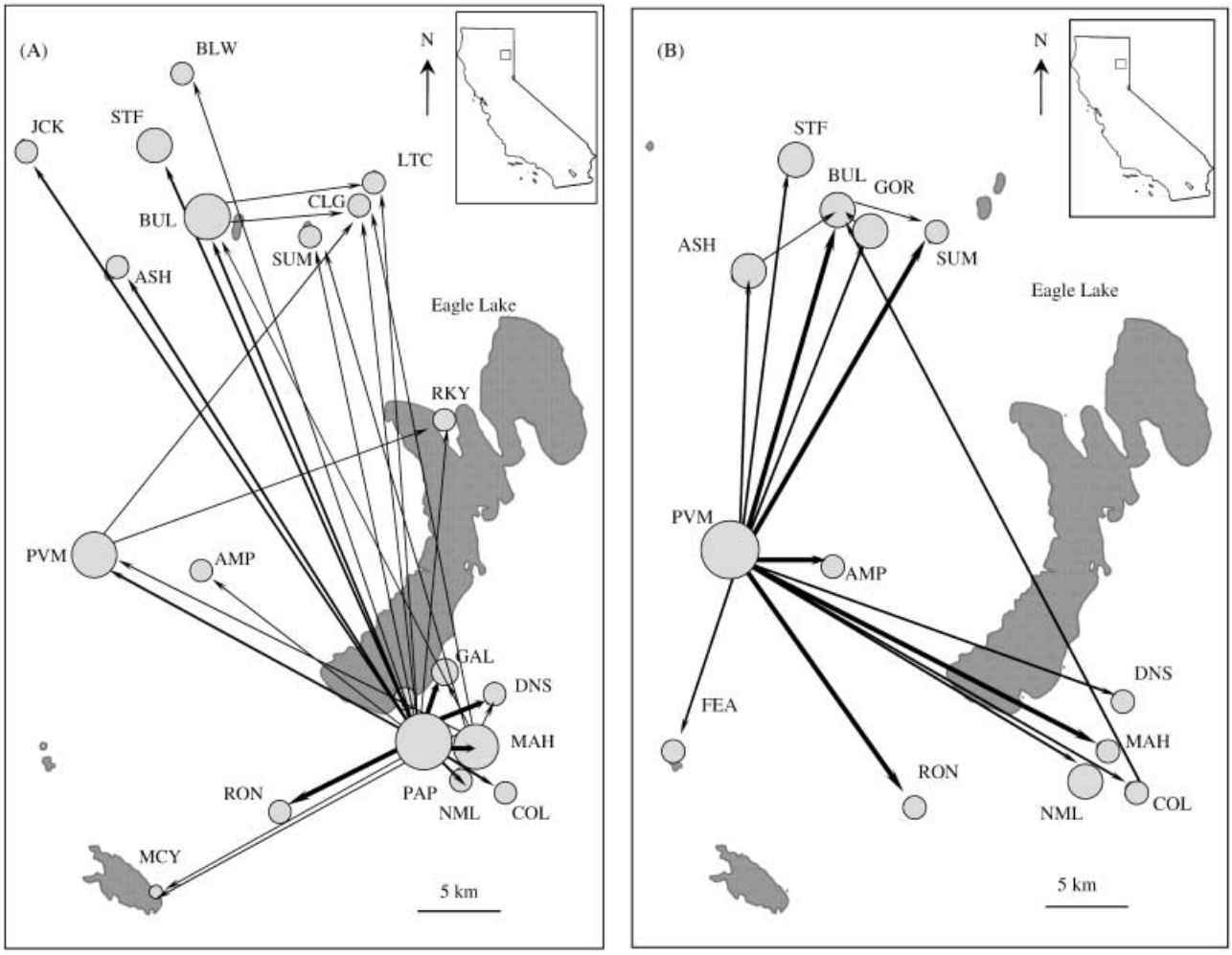

Fig. 2 Map of the study area showing relative effective population sizes and directions and rates of migration for *Thamnophis elegans* (A) and *Thamnophis sirtalis* (B). Circle size and arrow width are proportional to effective population size and migration rate, respectively. Each unit increase in circle diameter represents an increase of 153 effective individuals. Migration rates less than 0.27% for A and 0.33% for B are not shown. Above these values, each unit increase in arrow size represents an increase in migration rate of 0.13% for A and 0.16% for B.

McCoy Reservoir. Pikes Point had approximately equal rates of immigration and emigration.

Effective population sizes for *T. sirtalis* were comparable to but slightly smaller than those for *T. elegans*, with Θ ranging from 0.073 to 0.324 and averaging 0.130. Again, assuming a mutation rate of 10^{-4}, the average N_e was approximately 325 (Table S4, Supplementary material). Pine Valley Meadow had by far the largest N_e (810), followed by Gordon Lake (405). The smallest population was Antelope Mountain Pond with 180 effective individuals. Average $4N_em$ between population pairs was also low but slightly higher than for *T. elegans* at 2.14, which corresponds to an N_em of 0.54 and a migration rate of 0.0017. Pine Valley Meadow was the only major source of migrants in the study area, with a total $4N_em$ out of the population of 84.3 ($m = 0.076$). Bullard Lake, Feather Lake and Summit Lake were all primary recipients of migrants, most of which came from Pine Valley Meadow (Fig. 2B). However, Bullard Lake also received a substantial proportion of migrants from Colman Lake ($m = 0.006$) and Gordon Lake ($m = 0.004$), both of whose rates of emigration were highest for migrants to Bullard Lake. Colman Lake had approximately equal rates of immigration and emigration.

Overall, the population genetic structures of the two species at 11 shared sites were significantly correlated. Mantel tests comparing symmetric matrices of pairwise F_{ST} estimates revealed a correlation of 0.412 ($P = 0.009$). The same test comparing asymmetrical matrices of pairwise bidirectional migration rates (m) revealed a slightly lower but still significant correlation ($r = 0.297$; $P = 0.015$). Effective population sizes (N_e) for both species, however, were not significantly correlated ($r = -0.322$; $P = 0.335$).

Discussion

Predictions based on the relative abundances of each species were not supported by the microsatellite data. Although *Thamnophis elegans* was more abundant than *Thamnophis sirtalis*, effective population sizes for both species were virtually identical (*T. elegans* mean N_e = 328; *T. sirtalis* mean N_e = 325). However, more population differentiation was found for *T. sirtalis* (F_{ST} = 0.035), which has fewer populations distributed over the same area, than *T. elegans* (F_{ST} = 0.024). *T. sirtalis* also exhibited a stronger pattern of isolation by distance (r = 0.4164; P < 0.0001) than *T. elegans* (r = 0.1995; P = 0.0055), although their average migration rates were comparable (m = 0.0012 for *T. elegans*; m = 0.0017 for *T. sirtalis*). Overall patterns of population genetic structure for *T. elegans* and *T. sirtalis* estimated by two methods were found to be significantly correlated.

We found a moderate degree of population differentiation for the two garter snakes at a small spatial scale, even though they are locally abundant, have widespread distributions, and have the capacity to travel long distances. Several other studies have also found significant genetic differentiation in snakes over short geographical distances. Prior *et al*. (1997) obtained comparable levels of population differentiation at distances less than 50 km using RAPD (random amplified polymorphic DNA) markers in the black rat snake (*Elaphe obsoleta*; F_{ST} = 0.006–0.056), and Gibbs *et al*. (1997) found even higher levels of differentiation at microsatellite loci between populations of eastern massasauga rattlesnakes (*Sistrurus catenatus*). The latter populations were between 2 and 500 km apart, with F_{ST} values ranging from 0.085 to 0.261. Finally, Prosser *et al*. (1999) studied microsatellite variation among three populations of the northern water snake (*Nerodia s. sipedon*), each within 2 km of the others, and found a small but significant global F_{ST} (0.006, P = 0.009). Two other studies on genetic differentiation among Lake Erie *T. sirtalis* populations (Lawson & King 1996; Bittner & King 2003) found comparable levels of global F_{ST} for allozymes (0.01–0.08) and microsatellites (0.03–0.04) across populations separated by a maximum of 108 km, although significance levels of F_{ST} were not reported. Previous studies in high-latitude populations of *T. sirtalis* have documented seasonal migrations away from den sites at up to 18 km (Gregory & Stewart 1975; Gregory 1977), so it is surprising that both *T. elegans* and *T. sirtalis* populations are genetically structured on such a small spatial scale.

Both species showed patterns of isolation by distance that indicate lack of regional migration–drift equilibrium (case IV; Hutchison & Templeton 1999). Specifically, the relative importance of gene flow and drift varies depending on geographical distance. Smaller geographical distances were associated with smaller genetic distances that had less scatter, a pattern consistent with the homogenizing effect of gene flow over short distances. Larger geographical distances were associated with genetic distances that had more scatter, indicating little correlation between the two parameters. Such a pattern is consistent with more extreme genetic isolation, where drift is driving genetic differentiation among populations irrespective of geographical distance, and gene flow is minimal. For *T. elegans*, the distance at which the dominant evolutionary force shifts from gene flow to genetic drift is approximately 5.5 km, while for *T. sirtalis*, this distance is around 4 km.

Estimates of effective population size and bidirectional migration rates between population pairs identified important source populations for both species. For *T. elegans*, the source population was at Papoose Meadows, while the *T. sirtalis* source population was at Pine Valley Meadow. In these populations, total emigration exceeded immigration, while other populations received more total migrants than they exported. These two kinds of populations meet Pullium's (1988) criteria for source and sink populations, respectively. For both species, the source populations had much larger effective sizes and emigration rates than the other populations. In both cases, they are more than twice the average size of the other populations and contribute around four times the migrants. The observed source–sink patterns, in conjunction with a regional lack of migration–drift equilibrium as indicated by isolation-by-distance plots, suggest frequent extinction–recolonization events indicative of source–sink metapopulation dynamics (Hanski & Simberloff 1997; Stacey *et al*. 1997). This conclusion is also supported by field observations of massive snake die-offs and population crashes following drought years in the study system (Arnold, unpublished). Further research is needed to determine the relative frequency of extinction events by investigating stability of effective population sizes over time (Hoffman *et al*. 2004). To our knowledge, this is the first study to document source–sink metapopulation dynamics in a natural system using molecular markers. The ability to identify sources using neutral markers has important implications for conservation biology. Genetic analyses using microsatellite markers allow nonlethal sampling of DNA while being relatively easy and inexpensive. Maximum-likelihood analysis based on the coalescent allows bidirectional estimation of migration rates and subsequent identification of likely source populations that may be prioritized for conservation.

Sink populations, those with negative population growth, are more difficult to identify using these methods. The net removal of individuals from a population may be due to emigration or mortality. Migration data alone are not sufficient to determine if a population's size is decreasing due to a high mortality rate and would go extinct in the absence of immigration. Nevertheless, potential candidates for extinction can be identified as populations with small effective sizes and rates of immigration that exceed

emigration. Such populations can then be targeted for studies on direct measures of population growth using mark–recapture techniques to verify the molecular results. In this study, we found several potential *T. elegans* sinks in the Cleghorn Reservoir, Rocky Point and McCoy Reservoir populations. Rocky Point and McCoy Reservoir had the smallest effective sizes, and for all populations, total immigration far exceeded total emigration. Unusually low pairwise F_{ST} values between Rocky Point and other populations support a hypothesis of recent colonization from immigrants originating throughout the study area.

Because *T. elegans* and *T. sirtalis* tend to converge on amphibian prey wherever they coexist (Kephart 1982; Kephart & Arnold 1982), it is not surprising that they have different source localities. Kephart's (1982) observation that coexistence is more likely at sites with more species of breeding amphibians suggests that these two species are competitors. Although regional population dynamics for the two species are similar, their different source populations suggest that conservation strategies focusing on a single species may not be effective for other coexisting species, even a closely related congener. A management plan that focuses on one area may leave an important population for another species unprotected. It is important therefore for community-level conservation strategies to investigate population dynamics for all relevant species.

Although we attempted to sample every population of significance in the study area, some important populations were undoubtedly excluded. In particular, populations along the perimeter of the study area may receive migrants from unsampled sites. The effect of missing populations on estimates of population structure is discussed by Beerli (2004). In a two-population model, estimates of effective population size as measured by Θ ($4N_e\mu$), will be biased upward by 'ghost' populations, but estimates of M (m/μ) are robust to the presence of unsampled populations. Values for $4N_em$ (ΘM) therefore would also be biased upward. Sample size does not significantly affect these results, and the influence of ghost populations decreases as more populations are sampled (Bittner & King 2003; Beerli 2004). Because we sampled 20 populations of *T. elegans* and 13 of *T. sirtalis*, the adverse effects of missing populations should be minimal.

Direct measures of migration and effective population size for *T. elegans* and *T. sirtalis* at the study area using mark–recapture methods produced estimates (Kephart 1981) comparable to those found in this study. In Kephart's study, variance effective size was estimated as a function of census size and variance in reproductive success using the equation,

$$N_e = \frac{2N - 2}{1 + (V_k/\bar{k})}$$

(Wright 1931; Crow & Kimura 1971) where N represents population census size, V_k is the variance in reproductive success, and $\bar{k}$ is the average number of gametes each individual contributes to the next generation (assumed to equal 2; Crow & Norton 1955). Reproductive success in garter snakes was assessed by palpating pregnant females to count embryos, thereby obtaining an estimate of litter size (Fitch 1987; Farr & Gregory 1991). Sufficient data were available to estimate effective population size for *T. elegans* populations at Mahogany Lake and Pikes Point and *T. sirtalis* populations at Mahogany Lake and Roney Corral (Table 4). Three of the four comparisons showed underestimation of effective population size from mark–recapture methods, with only the *T. sirtalis* Roney Corral population having a larger estimate from mark–recapture, which was almost twice the value of the microsatellite estimate. The greatest difference was for the *T. elegans* Mahogany Lake population, for which the microsatellite estimate of effective size was more than double the mark–recapture estimate. Overall, however, the two methods produced roughly comparable estimates of effective population size.

Kephart (1981) documented six dispersal events from 823 recapture events at 21 study sites (Table 5), 12 of which were included in this study, all between sites less than 3 km apart. Four of these cases involved movement by *T. elegans* individuals, and two involved *T. sirtalis*. Of the *T. elegans* dispersal events, one was from a series of five vernal ponds not included in the present study (SVP, see Table 1) to Colman Lake (1.7 km), one was from SVP to Mahogany Lake (3.0 km), one was from Mahogany Lake to Nameless Meadow (1.4 km), and one was from Nameless Meadow to Mahogany Lake (1.4 km). Of the *T. sirtalis* dispersal events, both were between Colman Lake and SVP (1.7 km), one in each direction. From these data, Kephart (1981) concluded that interdemic migration was very low. We calculated the migration rates as the proportion of migrants in a population, using the number of individuals captured at each site as an estimate of population size. Indirect estimates of migration rate were calculated from estimates of Θ. Because SVP were not included as a population in the

Table 4 Comparison of mark–recapture and microsatellite estimates of effective population size

	Population	Mark–recapture	Microsatellite
Thamnophis elegans	Mahogany Lake	235	571
	Pikes Point	204	306
Thamnophis sirtalis	Mahogany Lake	148	251
	Roney Corral	511	283

Table 5 Comparison of mark–recapture and microsatellite estimates of migration rate (*m*)

	From	To	Census size	Mark–recapture	Microsatellite
Thamnophis elegans	SVP	Colman Lake	27	0.037	*0.001*
	SVP	Mahogany Lake	371	0.003	*0.001*
	Mahogany Lake	Nameless Mdw	111	0.009	0.002
	Nameless Mdw	Mahogany Lake	371	0.003	0.001
Thamnophis sirtalis	SVP	Colman Lake	74	0.014	*0.001*
	Colman Lake	SVP	9	0.111	—

Italicized values are the average migration rates; census sizes and mark–recapture *m* from Kephart (1981).

current study, the indirect estimate of migration from this site was replaced by the average migration rate into the receiving population. Migration rates obtained by direct observation of dispersal events always overestimated those obtained using maximum likelihood of molecular marker data. Direct estimates of migration were from a factor of three to an order of magnitude higher than indirect estimates. This discrepancy is probably because observed dispersals only represent movement per se and not gene flow. Of the individuals that disperse from their natal populations, only a fraction of those will succeed in contributing gametes to subsequent generations of the new population, thus allowing detection of those successful dispersals using molecular markers. Nevertheless, Kephart's (1981) conclusion that migration events are rare is supported by the molecular data.

For study sites supporting populations of both *T. elegans* and *T. sirtalis*, there was a weak but significant correlation between genetic structures of the two species. Such a relationship may be a function of a common environment, ecological similarity, evolutionary history, or some combination of these factors. The microgeographical scale of the study area points to a greater role for landscape and ecological factors rather than evolutionary processes in shaping the spatial partitioning of genetic variation in this system. Interestingly, garter snake populations were significantly structured around hibernacula associated with water bodies, even though water per se is not required for thermoregulation, courtship, parturition, or hibernation. The observed pattern appears to be a direct effect of the patchy distribution of their main prey items (amphibians, fish and leeches) for which water is an obvious necessity. The shared diet of both species and the patchy distribution of their prey may therefore be central to the population genetic dynamics of the garter snake community.

In contrast to the Lassen County study system, sympatric populations of *T. elegans* and *T. sirtalis* in coastal California and Washington have been shown to exploit terrestrial prey species (Arnold 1992). While Lassen County garter snakes converge on prey types where they coexist, *T. elegans* in both coastal systems specialize on slugs, whereas *T. sirtalis* consumes amphibians. Since slugs are distributed homogeneously across the landscape, as opposed to the discrete ponds inhabited by amphibians, one can hypothesize that *T. sirtalis* populations in the coastal systems will be more spatially structured than the sympatric *T. elegans* populations. Such a comparative approach to studies of systems with divergent species dynamics promises to provide further insight into how ecological processes shape community genetics.

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Supplementary material

The supplementary material is available from http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2734/MEC2734sm.htm

Table S1 Pairwise F_{ST} values for *Thamnophis elegans* populations (below diagonal) and associated *P* values (above diagonal), obtained after 3000 permutations.

Table S2 Pairwise F_{ST} values for *Thamnophis sirtalis* populations (below diagonal) and associated *P* values (above diagonal), obtained after 3000 permutations.

Table S3 Estimates of $4N_em$ (off-diagonal) and $4N_e\mu$ (diagonal, bold) for *Thamnophis elegans*. Gene flow occurs from columns to rows.

Table S4 Estimates of $4N_em$ (off-diagonal) and $4N_e\mu$ (diagonal, bold) for *Thamnophis sirtalis*. Gene flow occurs from columns to rows.

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