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Gene flow and melanism in garter snakes revisited: a comparison of molecular markers and island vs. coalescent models

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Within populations, the stochastic effect of genetic drift and deterministic effect of natural selection are potentially weakened or altered by gene flow among populations. The influence of gene flow on Lake Erie populations of the common garter snake has been of particular interest because of a discontinuous colour pattern polymorphism (striped vs. melanistic) that is a target of natural selection. We reassessed the relative contributions of gene flow and genetic drift using genetic data and population size estimates. We compared all combinations of two marker systems and two analytical approaches to the estimation of gene flow rates: allozymes (data previously published), microsatellite DNA (new data), the island model (F_{ST}-based approach), and a coalescence-based approach. For the coalescence approach, mutation rates and sampling effects were also investigated. While the two markers produced similar results, gene flow based on F_{ST} was considerably higher ($Nm > 4$) than that from the coalescence-based method ($Nm < 1$). Estimates of gene flow are likely to be inflated by lack of migration-drift equilibrium and changing population size. Potentially low rates of gene flow ($Nm < 1$), small population size at some sites, and positive correlations of number of microsatellite DNA alleles and island size and between *M*, mean ratio of number of alleles to range in allele size, and island size suggest that in addition to selection, random genetic drift may influence colour pattern frequencies.

ADDITIONAL KEYWORDS: allozymes – coalescent theory – F_{ST} – genetic drift – Lake Erie – microsatellite DNA – migration – mutation – selection – *Thamnophis sirtalis*.

INTRODUCTION

Gene flow can be a powerful force maintaining genetic polymorphisms in the face of selection (e.g. Bossart & Scriber, 1995; King & Lawson, 1995; Ross & Keller, 1995) or constraining differentiation and local adaptation of subpopulations (e.g. Thompson, 1990; Daly, 1993; Riechert, 1993; Storfer *et al*., 1999; Storfer, 1999). However, rates of gene flow can be difficult to measure. Logistical problems often limit direct measures of gene flow and problems of interpretation (e.g. distinguishing present from past gene flow) can confound indirect measures. Estimates of gene flow among polymorphic populations of the garter snake (*Thamnophis sirtalis sirtalis* Linneaus, Serpentes: Colubridae) on the islands and mainland associated with Lake Erie suggest that rates of gene flow (Nm = number of migrants per generation) are high even between sites separated by a water barrier (Nm = 2.7–37.6 among island and mainland sites separated by up to 108 km) and long distances (Nm = 1–4.8 among sites separated by >1000 km; Lawson & King, 1996). These populations contain jet-black melanistic garter snakes in frequencies ranging from 0 to 0.49 among populations (King, 1988; table 4 in Lawson & King, 1996). Melanism is inherited as a Mendelian recessive trait (Blanchard & Blanchard, 1940; King, in press, but see Zweifel, 1998). The fact that melanism is more common in island and peninsular populations suggests isolation and/or climate may play a role in the origin and maintenance of high levels of melanism (fig. 1 in King, 1988). In particular, higher equilibrium body temperatures attained by

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melanistic adults may provide a fitness advantage in cool lakeshore habitats (Gibson & Falls, 1979; Bittner, King, & Kerfin, 2002).

Accurate estimation of gene flow rates is critical to understanding the roles of natural selection and genetic drift in these polymorphic populations. The previous analysis was based on 12 allozyme loci (see table 2 in Lawson & King, 1996). However, because allozyme frequencies can be affected by selection (Mitton, 1997), rates of gene flow may have been overestimated. Compared to allozymes, microsatellite DNA loci have faster mutation rates and higher levels of polymorphism, and variation at microsatellite DNA loci is presumably selectively neutral (Bruford & Wayne, 1993; Queller, Strassman & Hughes, 1993; Jarne & Lagoda, 1996). However, we are unaware of any studies reporting substantial differences in estimates of F_{ST} generated from allozyme vs. microsatellite DNA data (e.g. Lehmann *et al*., 1996; Sanchez *et al*., 1996; Estoup *et al*., 1998; see also table 1 in Bossart & Prowell, 1998). These observations led us to generate new microsatellite DNA data and reanalyse the previous allozyme data (reported in Lawson & King, 1996) for the same individuals and populations.

Typically, estimates of gene flow are based on F_{ST} using Wright's (1931) island model, which assumes n subpopulations of constant, equal size exchanging migrants at an equal rate m. Despite regular violations of assumptions of this model, researchers continue to use this indirect method to estimate gene flow, probably because 'direct' methods also have limitations (Beerli, 1998; Whitlock & McCauley, 1999). For example, the secretive nature and small size of garter snakes makes direct measurement of gene flow over a spatial scale of 100 km almost impossible. As an alternative to F_{ST}-based estimates of gene flow, a maximum likelihood method and computer program based on coalescent theory have been developed (Beerli, 1997, 1998; Beerli & Felsenstein, 1999, 2001). The computer program MIGRATE (Beerli, 1997) jointly estimates population sizes and migration rates. The program creates an initial gene genealogy from the data using UPGMA and parsimony, with migration events connecting the sampled alleles. Small adjustments result in the addition of trees when the probability of the data given the genealogy is greater than or equal to the original tree. Because it is impossible to sum over all conceivable allele genealogies, a statistical approximation technique is used to find population parameter estimates at the maximum of an approximate likelihood function, ignoring genealogies that contribute very little to the final likelihood (Beerli, 1997). Importantly, the coalescence-based method allows for unequal population sizes and asymmetric gene flow, although it does assume that population sizes remain constant over time and are in migration–mutation equilibrium (Beerli, 1998).

Here, we make use of two molecular genetic marker systems (allozymes and microsatellite DNA) and two analytical approaches (traditional F_{ST} analysis and coalescence theory) to estimate gene flow among polymorphic populations of garter snakes at Lake Erie. We then integrate estimates of gene flow with information on population size and history to evaluate alternative hypotheses for the maintenance of a colour pattern polymorphism in these populations.

METHODS

Microsatellite DNA analysis

Whole blood was collected in 1989–92 from ten populations (see table 1 and fig. 2 in Lawson & King, 1996). DNA was extracted using a standard phenol/chloroform protocol from approximately 25 individuals per population (range 23–26; total 252). Four polymorphic microsatellite DNA loci were assayed. Loci Nsμ3 and Nsμ9 were discovered in a closely related species, the Northern water snake *Nerodia sipedon* (Prosser, Gibbs, & Weatherhead, 1999). Loci Ts1 and Ts2 were designed for *Thamnophis sirtalis* (McCracken, Burghardt & Houts, 1999). Loci Ts1 and Ts2 are trinucleotide repeats; loci Nsμ3 and Nsμ9 are compound di- and tetranuclear repeats. Microsatellite DNA loci were amplified using PCR with the addition of α-^{32}P dATP for visualization of product (1.75 mM $MgCl_2$; 0.5 mM each forward and reverse primer; 0.2 mM each dATP, dCTP, dGTP, and dTTP; 5 ng μL^{-1} template DNA; 0.05 U μL^{-1} Fisher Taq; and 0.2 $\mu Ci\ \mu L^{-1}$ α-^{32}P dATP). 10 μL reactions were used with temperature profiles similar to those published (McCracken *et al*., 1999; Prosser *et al*., 1999). 3 μL of product were run on 8.3 M urea denaturing 6% sequencing gels for 2.5 h at 60 Watts. The sequence of M13mp18 phage DNA (Sequenase 2.0, USB Corp.) was also loaded on all gels as a size standard. Bands were visualized by autoradiography with X-ray film; exposure time was variable (1–3 days) depending upon the counts per minute on the gel. Alleles were scored according to exact size in base pairs by comparison against the size standard; each gel was scored independently by both authors.

It seems likely that modern settlement of the region has contributed to snake population declines through habitat loss and outright persecution (King *et al*., 1997; King & Oldham, in press). Therefore, for microsatellite DNA loci, we calculated M, the mean ratio of the number of alleles to the range in allele size. A reduction in M corresponds to a reduction in population size (Garza & Williamson, 2001). We used Spearman rank correlation to test for an

association between island size and mean number of alleles at allozyme loci, mean number of alleles at microsatellite DNA loci, and mean *M* (the four mainland populations were assigned the maximum rank for size).

F_{ST}-BASED ANALYSES

We reanalysed the allozyme data of Lawson & King (1996; see Table 4) using the subset of individuals assayed for microsatellite DNA loci to allow direct comparison of the two marker systems. Data for the following 11 allozyme loci were reanalysed: lactate dehydrogenase-A (LDH), phosphogluconate dehydrogenase (PGDH), glucose-6-phosphate dehydrogenase (G6PDH), superoxide dismutase-1 (SOD), creatine kinase-2 (CK), adenylate kinase (AK), tripeptidase-1 (TRIP), mannose-6-phosphate isomerase (MPI), glucose-6-phosphate isomerase (GPI), phosphoglucomutase (PGM), and transferrin (TRF). The fumarate hydratase locus was omitted because it has sex-linked inheritance (King & Lawson, 1996). For both microsatellite DNA and allozyme markers we used GENEPOP (Raymond & Rousset, 1995) to test for linkage disequilibrium among loci, Hardy–Weinberg equilibrium within loci and subpopulations, subpopulation differentiation, and isolation by distance. When multiple tests were conducted, we performed Bonferroni adjustment of the rejection zone from a base $\alpha = 0.05$. We also calculated a posteriori maximum likelihood estimates of null allele frequencies for microsatellite DNA loci.

For each data set, we generated *F*-statistics between population pairs over all loci. Jackknifing across loci was used to calculate F_{ST}*, a less-biased estimate of F_{ST}, and to estimate the variance in F_{ST}* (Weir & Cockerham, 1984). We calculated pairwise Nm using the relationship $F_{ST}^* \approx 1/(4Nm + 1)$ (Wright, 1943, 1969) and 95% confidence intervals around Nm by substituting $F_{ST}^* \pm 1.96$ SE. into this relationship. We then used the graphical method of Hutchinson & Templeton (1999) to visualize the regional importance of gene flow and drift.

Because F_{ST} assumes an infinite alleles mutation model which may be inappropriate for microsatellite DNA, we calculated R_{ST} as recommended by Slatkin (1995). It appears that F_{ST} will underestimate genetic differentiation among populations when used on microsatellite DNA data (Slatkin, 1995). However, compound microsatellite DNA loci do not mutate by a constant increment, as assumed by the program R_{ST} Calc (Goodman, 1997). Since two of our loci are compound di- and tetranucleotide repeats, the validity of the results is unknown. In a similar study of microsatellite DNA variation in the black rat snake (*Elaphe obsoleta*), pairwise estimates of F_{ST} and R_{ST} were highly correlated and qualitatively similar (Lougheed *et al*., 1999).

COALESCENCE-BASED ANALYSIS

We used the computer program MIGRATE to estimate all immigration and emigration rates between the populations jointly. We used the stepwise mutation model for microsatellite DNA loci and the infinite alleles model for allozyme loci. We used the default settings (ten short chains with 10 000 sampled and 500 recorded, three long chains with 100 000 sampled and 5000 recorded) and assumed equal mutation rate among loci. Examination of likelihood ratios as analyses proceeded suggested that modifications of these settings (e.g. increasing the number of short or long chains) was not necessary. The gene flow output parameter is 4Nm, so we divided results by 4 to get Nm. For each data set, we ran the analysis four times, then calculated averages and confidence intervals from all four runs. To test for isolation by distance, we averaged immigration and emigration for each pair of populations before using GENEPOP to perform Mantel tests with 10 000 permutations.

The coalescence-based analysis is sensitive to the effect of unsampled populations because coalescence events may take place outside sampled populations. Because it is impossible to sample every relevant population, a 'ghost' population containing no data may improve estimates (P. Beerli, pers. comm.). We explored the effect of unsampled populations using reduced data sets and ghost populations. We ran analyses with two populations (Middle Island and Pelee), five populations, and all ten populations, then repeated the process with the addition of one ghost population. We then graphed the relationship of Nm and number of populations sampled for the population pair (Middle Island and Pelee Island) common to all three analyses.

RESULTS

PATTERNS OF VARIATION IN MICROSATELLITE DNA AND ALLOZYME LOCI

For 11 allozyme loci, we found between 2–10 alleles per locus with direct-count heterozygosity over all loci of 0.27. The four microsatellite DNA loci had 21–36 alleles per locus; heterozygosity over all loci was 0.70. For allozymes, island size and mean number of alleles were uncorrelated (Table 1; Spearman rank correlation = 0.22, $P = 0.54$; the four mainland sites were ranked as tied for maximum island size in this analysis). For microsatellite DNA loci, large mainland populations had more alleles than populations on small islands (Table 1; Spearman rank correlation = 0.72,

Table 1. Area, sample size (*N*) for allozymes/microsatellites, estimated population size, θ = 4Nμ (mutation rate over all loci), mean number of alleles per locus, and mean *M* across loci in each population. *M* = number of alleles/(range in allele size/repeat unit size). Island populations are listed in order of increasing island area; mainland populations are listed in alphabetical order

Population	Area (ha)	*N*	Pop. size* (SE)	θ = 4Nμ Allozymes (SE)	θ = 4Nμ Microsatellites (SE)	Mean number of alleles/locus Allozymes	Mean number of alleles/locus Microsatellites	Mean *M*
Rattlesnake Is.	20	25/25	186 (98.0)	0.977 (0.0087)	0.914 (0.0979)	2.09	11.25	0.52
Middle Is.	23	25/25	475 (266.9)	1.103 (0.0226)	1.134 (0.1313)	2.55	12.75	0.58
West Sister Is.	32	23/23	147 (43.9)	0.960 (0.0712)	1.459 (0.2775)	2.18	11.50	0.50
North Bass Is.	285	24/24	156 (86.2)	1.066 (0.0587)	1.293 (0.1434)	2.27	14.00	0.56
Middle Bass Is.	312	25/26	281 (152.1)	1.001 (0.0258)	1.558 (0.0934)	2.27	12.25	0.56
Pelee Is.	4261	26/26	360 (203.2)	1.149 (0.0323)	1.870 (0.4914)	2.27	15.50	0.68
East Harbor	Mainland	26/26	558 (243.1)	1.023 (0.0345)	0.950 (0.1130)	2.00	12.50	0.58
Hillman Marsh	Mainland	25/25	67 (32.1)	1.146 (0.0192)	1.231 (0. 1764)	2.73	15.50	0.62
St. Clair Marsh	Mainland	26/26	na	1.168 (0.0364)	1.653 (0.1613)	2.45	18.25	0.76
Winous Point	Mainland	26/26	105 (48.6)	1.040 (0.0430)	1.005 (0.0899)	2.27	16.00	0.64

*The maximum estimated population size when multiple estimates were performed (Bittner, 2000).

$P = 0.018$). Mean *M* ratios for microsatellite DNA were positively correlated with island size (Table 1; Spearman rank correlation = 0.73, $P = 0.016$).

Loci of both data sets were independent by the linkage disequilibrium test (for allozymes, $P > 0.05$ for all locus pairs; for microsatellite DNA, $P > 0.60$ for all locus pairs). Allozyme allele frequencies differed significantly among populations for eight of 11 individual loci and across all loci combined ($P > 0.001$ for individual loci SOD, GPI, and AK). For microsatellite DNA, allele frequencies differed among populations ($P < 0.001$) for each locus and across all loci combined.

Tests for heterozygote deficiency revealed no deviation from Hardy–Weinberg proportions for allozymes. However, for microsatellite DNA loci, significant heterozygote deficiency was found in 13 of 40 locus-by-population combinations (in three of four loci) after adjusting α for 40 tests. Putative null homozygotes were present for Nsμ9 (one individual), Ts1 (four individuals), and Ts2 (six individuals); these 11 PCR reactions (1.1% of 1008 reactions) failed to amplify although the other three loci amplified successfully in each snake. Using GENEPOP, we performed a posteriori maximum likelihood estimates of null allele frequencies, assuming deviation from Hardy–Weinberg equilibrium at a locus is the result of one null allele. Putative null allele frequencies ranged from 0.033 to 0.454 (Table 2). Note that of these frequencies, the highest 20% occur on small islands. However, the three microsatellite DNA loci with putative null alleles (Nsμ9, Ts1, and Ts2) do not appear to differ in F_{ST} from the other sampled loci (see Table 4).

Table 2. Predicted frequency of a null allele at each locus, assuming heterozygote deficiency is due to a single null allele. (Locus Nsμ3 had no putative null homozygotes and only one significant rejection of HWE.) Bold indicates lack of Hardy–Weinberg equilibrium at $\alpha = 0.00125$ (adjustment for 40 tests). The highest 20% are also underlined to highlight the fact that they occur on islands

Population	Nsμ9	Ts1	Ts2
Rattlesnake Is.	**0.146**	0.180	0.212
Middle Is.	**0.297**	0.092	0.139
West Sister Is.	**0.139**	**0.297**	**0.454**
North Bass Is.	**0.151**	0.037	0.158
Middle Bass Is.	0.181	**0.215**	**0.215**
Pelee Is.	0.075	0.040	0.084
East Harbor	0.115	0.066	**0.192**
Hillman Marsh	0.126	**0.177**	0.147
St. Clair Marsh	**0.169**	**0.122**	0.064
Winous Point	0.033	0.066	0.049

F_{ST}-BASED ESTIMATES OF GENE FLOW

Pairwise estimates of F_{ST}* were generally low and uncorrelated with distance between sites, corresponding to a Case II non-equilibrium pattern in which gene flow is more influential than drift (Fig. 1, see also figs 1 and 3 in Hutchinson & Templeton, 1999). Estimates of Nm between pairs of populations ranged from 2.9 to 27.0 migrants per generation for microsatellite DNA loci (Fig. 2A) and 1.7–82.3 (one pair had negative F_{ST} so Nm was unquantifiable) for allozymes (Fig. 2B). We found no pattern of isolation by distance

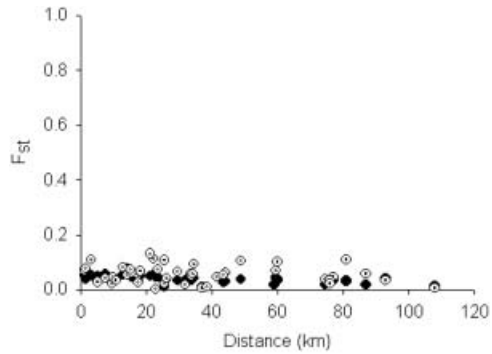

Figure 1. Relationship between pairwise F_{ST}* and geographical distance for both allozymes (closed circles) and microsatellites (open circles). Graph scaled to match figures in Hutchinson & Templeton (1999).

in estimates of gene flow from microsatellite DNA loci (Mantel test, $t = -0.01$, $P > 0.99$, Fig. 2A) or allozyme loci ($t = -0.01$, $P = 0.72$, Fig. 2B).

For clarity, R_{ST}-based estimates are not shown. Simulations suggest that under sampling conditions similar to ours, F_{ST} is a more conservative Nm estimator than R_{ST} (Gaggiotti *et al*., 1999). R_{ST}-based pairwise estimates of gene flow were much more variable than F_{ST}-based estimates from either allozymes or microsatellite DNA, ranging from 0.8 to 451.4, and six pairwise estimates of Nm were so high as to be unquantifiable (Goodman, 1997). There was no geographical pattern for Nm based upon R_{ST}.

Coalescence-based estimates of gene flow

Coalescence-based pairwise estimates of Nm were considerably smaller than the F_{ST} -based estimates (in Fig. 2, compare A vs. C and B vs. D). However, summing immigration into each population from the other nine populations suggests higher gene flow rates than the pairwise exchanges (Table 3). Coalescence-based estimates of Nm were slightly lower and more variable for microsatellite DNA markers than for allozyme markers (Fig. 2C vs. D, Table 3). We found no evidence of isolation by distance in the coalescence-based estimates of gene flow (Mantel test, microsatellite DNA: $t = 0.06$, $P = 0.81$, Fig. 2C; allozymes: $t = -0.01$, $P = 0.49$, Fig. 2D).

MIGRATE provides estimates of θ (= 4Nμ), the product of population size and mutation rate. Mean 4Nμ (averaged across populations) was similar among 13 of 15 loci, ranging from 0.806–1.797 (Table 4). In contrast, CK and G6PDH had markedly lower values for 4Nμ (<0.04, Table 4). However, because population size is included in the mutation estimates, we also examined 4Nμ among populations (Table 1). Among-population variation in 4Nμ was not correlated with island area (microsatellites: Spearman Rank Correlation = 0.16, $P = 0.65$; allozymes: Spearman Rank Correlation = 0.49, $P = 0.15$). Allozyme and microsatellite DNA loci did not differ in mean 4Nμ despite the fact that mutation rates are typically 2–3 orders of magnitude higher for microsatellite DNA loci (Jarne & Lagoda, 1996; Mann–Whitney $U = 30.0$, $P = 0.14$).

Our exploration of the performance of MIGRATE when the number of populations sampled was varied reveals that in general, sampling fewer populations resulted in higher estimates of Nm between populations. The addition of a ghost population ameliorated population undersampling somewhat, especially when the number of populations sampled was small (Fig. 3). However, the addition of a ghost population had little effect on estimates of Nm when all ten populations were included, suggesting that unsampled populations probably had little effect on our results.

DISCUSSION

Patterns of variation in microsatellite DNA and allozyme loci

As expected, we found high levels of polymorphism and heterozygosity in microsatellite DNA loci compared to allozyme loci. There were heterozygote deficiencies in some microsatellite DNA locus-by-population combinations (13 of 40), indicating the presence of at least one null allele at each of three loci. Other possible explanations for heterozygote deficiency include sex-linked inheritance and inbreeding. We ruled out sex linkage for each locus by confirming the presence of heterozygotes in females; in snakes, females are the heterogametic sex. We ruled out inbreeding because it would affect all loci, not just microsatellite DNA loci. Estimates of the maximum frequency of each null allele were high (Table 2), but these estimates are based on the assumption that all deviation from Hardy–Weinberg proportions at a locus is due to the presence of a single null allele. In fact, two or more null alleles may be present at lower frequencies. F_{ST} estimates for microsatellite DNA loci with putative null alleles were similar to those for other loci (Table 4), suggesting that the presence of null alleles should not severely affect the estimates of gene flow. Although null alleles are encountered with regularity in microsatellite DNA research (e.g. Pemberton *et al*., 1995; Gibbs *et al*., 1997; Becher & Griffiths, 1998; Van Treuren, 1998; Prosser *et al*., 1999), we are unaware of other studies in which null allele frequencies are as high as those estimated here.

Estimates of gene flow

Microsatellite DNA loci and allozymes produced very similar gene flow results within each analysis, sug-

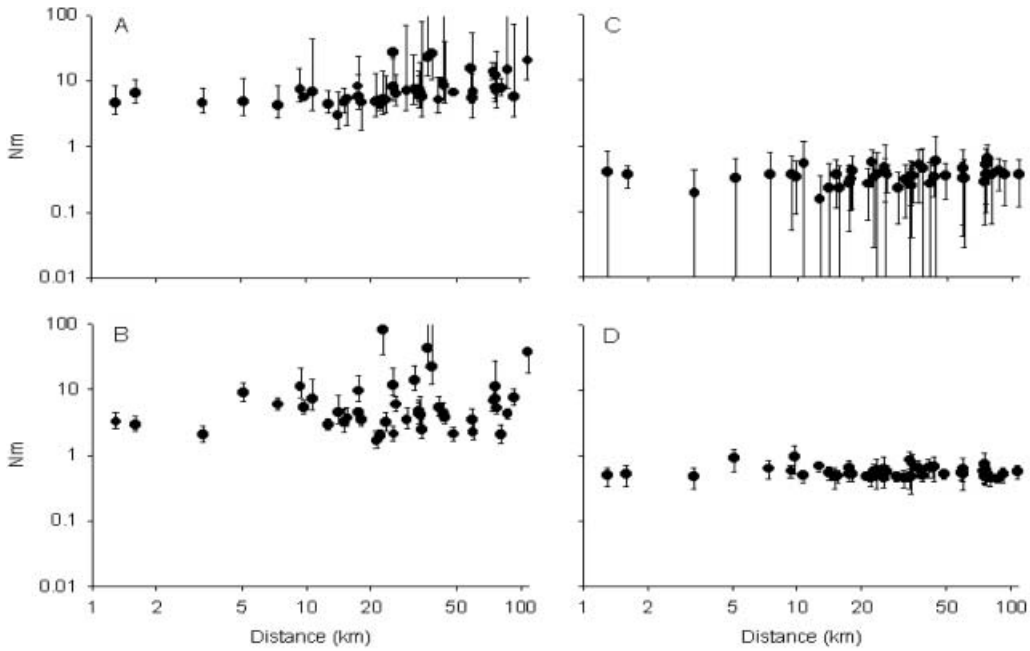

Figure 2. Relationship between estimated rate of gene flow, Nm (95% confidence limits), and geographical distance between pairs of populations in *Thamnophis sirtalis*. $N = 23$–26 individuals per population. (A) F_{ST} –based estimates of Nm from four microsatellite loci. For each pair of populations, jackknifing across loci was used to calculate F_{ST}* and to estimate the variance in F_{ST}*. Nm was calculated from the relationship $F_{ST}^* \approx 1/(4Nm + 1)$; 95% confidence intervals around Nm were estimated by substituting $F_{ST}^* \pm 1.96$ SE into this relationship. ($N = 252$). (B) F_{ST}-based estimates of Nm from 11 allozyme loci. Nm and confidence limits were calculated as in (A). ($N = 251$). (C) Coalescence-based estimates of Nm from four microsatellite loci. Mean rate of gene flow in each direction (and 95% confidence limits) were obtained from four runs of MIGRATE. Because rates of gene flow were similar in each direction for any given pair of populations, they (and their confidence intervals) were averaged for this graph. Confidence intervals extending down to the *X*-axis included zero. ($N = 252$). (D) Coalescence-based estimates of Nm from 11 allozyme loci. Means and confidence intervals were obtained from four runs of MIGRATE and averaged as in (C). ($N = 251$).

Table 3. Coalescence-based estimates of Nm into ten garter snake populations. Mean (min–max) and sum of immigration into each population from the other nine populations is based on the average of four runs of MIGRATE for each marker type

	Allozymes		Microsatellites	
Population	Mean (Min–Max)	Sum	Mean (Min–Max)	Sum
Rattlesnake Is.	0.46 (0.41–0.67)	4.14	0.29 (0.21–0.40)	2.57
Middle Is.	0.76 (0.68–0.92)	6.80	0.31 (0.08–0.43)	2.83
West Sister Is.	0.53 (0.43–0.84)	4.77	0.35 (0.22–0.49)	3.11
North Bass Is.	0.56 (0.46–1.03)	5.03	0.38 (0.15–0.63)	3.38
Middle Bass Is.	0.63 (0.55–0.70)	5.70	0.45 (0.33–0.63)	4.05
Pelee Is.	0.56 (0.48–0.97)	5.06	0.45 (0.26–0.58)	4.02
East Harbor	0.51 (0.45–0.63)	4.57	0.24 (0.11–0.33)	2.12
Hillman Marsh	0.67 (0.55–0.78)	5.99	0.40 (0.25–0.57)	3.56
St. Clair Marsh	0.57 (0.45–0.79)	5.09	0.42 (0.29–0.63)	4.15
Winous Point	0.56 (0.47–0.96)	5.04	0.32 (0.17–0.41)	2.35

gesting the effect of null alleles on the microsatellite DNA analysis may be minimal. Previous comparisons of F_{ST} have also shown similar results between markers (Lehmann *et al*., 1996; Sanchez *et al*., 1996; Estoup *et al*., 1998).

Wright's island model of gene flow makes four simplifying assumptions which are problematical for most population genetics studies (Whitlock & McCauley, 1999), including this one. First, Wright's model assumes that mutation rate is negligibly small rela-

Table 4. Locus-specific F_{ST} and coalescence-based estimates of mutation ($\theta = 4N\mu$, 95% confidence interval) across all ten populations. Means and confidence limits of $4N\mu$ based on four runs of MIGRATE

Marker Type	Locus	F_{ST}	Mean $4N\mu$ (95% CI)
Allozymes[1]	TRF	0.0510	0.806 (0.351–1.261)
	SOD	0.0304	1.308 (1.201–1.414)
	LDH	0.0467	1.347 (1.253–1.440)
	PGM	0.0582	1.357 (1.288–1.425)
	CK	0.0451	6.600×10^{-4} (6.160×10^{-4}–7.040×10^{-4})
	PGDH	0.0443	1.358 (1.308–1.408)
	TRIP	0.0827	1.343 (1.232–1.453)
	MPI	0.0677	1.354 (1.237–1.472)
	G6PDH	0.0664	0.038 (0.034–0.042)
	GPI	0.0108	1.342 (1.285–1.400)
	AK	0.0418	1.341 (1.301–1.381)
	Overall	0.0543	1.063 (1.031–1.095)
Microsatellites	Nsμ3	0.0345	1.776 (1.203–2.349)
	Nsμ9	0.0435	1.797 (1.548–2.045)
	Ts1	0.0349	1.541 (1.038–2.043)
	Ts2	0.0339	1.168 (0.875–1.461)
	Overall	0.0367	1.307 (1.152–1.462)

[1]TRF = transferrin, SOD = superoxide dismutase-1, LDH = lactate dehydrogenase-A, PGM = phosphoglucomutase, CK = creatine kinase-2, PGDH = phosphogluconate dehydrogenase, TRIP = tripeptidase-1, MPI = mannose-6-phosphate isomerase, G6PDH = glucose-6-phosphate dehydrogenase, GPI = glucose-6-phosphate isomerase, AK = adenylate kinase.

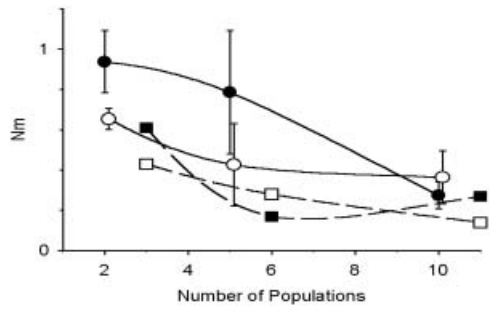

Figure 3. Effect of number of populations on estimated rate of gene flow. Shown is the rate of gene flow from Pelee Island to Middle Island (filled symbols) and from Middle Island to Pelee Island (open symbols) in *Thamnophis sirtalis* obtained using data for two, five, or ten populations (circles and solid lines) or for two, five, or ten populations plus a 'ghost' population (squares and dashed lines). Estimates for two, five, or ten populations represent means (±1 standard error) obtained from four runs of MIGRATE. Estimates from analyses that include a ghost population are each based on a single run of MIGRATE.

tive to migration rate. Microsatellite DNA loci have relatively high mutation rates, but our results suggest that snake migration rates are also high.

Second, Wright's model assumes constant and equal population sizes. It seems likely that garter snake population size is lower on small islands (*c.* 20 ha) than on large islands (up to 4260 ha) and at mainland sites. Furthermore, island populations may be less stable ecologically and may have experienced greater fluctuations in population size over time. Small values of *M* (Table 1) lend support to existing evidence of population size reductions (King *et al.*, 1997; King & Oldham, in press). Although our estimates of *M* may be inflated by small sample size, they suggest dramatic population size reductions, especially on the smallest islands. Our *M*-ratios ranged from 0.50 to 0.76 (Table 1). These values are smaller than those calculated for species in which population size has remained stable ($M = 0.823$–0.926 in 12 species) but are comparable to species that have suffered recent population reductions ($M = 0.599$–0.693 in eight species; table 2 in Garza & Williamson, 2001).

Third, Wright's model assumes that each population exchanges migrants with every other population at an equal rate (i.e. migration is random with respect to geographical distance). For overall estimates of F_{ST}, this assumption is difficult to satisfy given the spatial scale of our sampling (it is highly unlikely that two distant island populations exchange the same number of migrants as two populations on the Ohio mainland). However, when estimating pairwise rates of gene flow, we assume only that migration is symmetrical between population pairs. While this assumption is less restrictive, it is unlikely to hold between populations which differ greatly in size.

Finally, Wright's model assumes that populations are in migration–drift equilibrium. If insufficient time has passed since the last perturbation of equilibrium, migration estimates will be biased toward the previous conditions (Whitlock & McCauley, 1999). A pattern of isolation by distance suggests that equilibrium has been attained (Hutchinson & Templeton, 1999). However, our results indicate a lack of equilibrium with a spread of F_{ST}* corresponding to a Case II pattern with gene flow more influential than drift (Fig. 1; see also figs 1 and 3 in Hutchinson & Templeton, 1999). In contrast, significant or nearly significant patterns of isolation by distance were found in sympatric water snakes (*Nerodia sipedon insularum*) and brown snakes (*Storeria dekayi*; King & Lawson, 2001). Water snakes are larger and more aquatic than garter snakes whereas brown snakes are smaller and presumably less vagile than garter snakes. A possible explanation is that brown and water snakes both approached equilibrium faster than garter snakes, but for different reasons: small population size in brown snakes and high gene flow rates in water snakes.

The variance in F_{ST} among loci can be used to test hypotheses about genetic structure (Robertson, 1975; Latta & Mitton, 1999). We tested whether the presence of a water barrier between populations results in greater genetic structure by comparing the among-locus variance of F_{ST} in island populations vs. mainland populations. There was no difference in variance of F_{ST} between populations separated by a water barrier and those not separated by a water barrier (two-tailed $F = 2.13$, $0.10 < P < 0.20$; power = 0.74), suggesting that water is not an effective barrier to gene flow or that variance in F_{ST} reflects historical (not contemporary) gene flow. Other studies have also encountered the problem of distinguishing historical and contemporary gene flow (e.g. animals: Baughman *et al*., 1990; plants: Comes & Abbott, 1998; Ruckelshaus, 1998; Latta & Mitton, 1999; Sork *et al*., 1999). Unfortunately, a theoretical framework for estimating gene flow under non-equilibrium conditions is lacking. There is general agreement that traditional F_{ST} analyses are inadequate for estimating gene flow in these circumstances. However, the fact that F_{ST}-based estimates of Nm do correlate with life history variables affecting dispersal demonstrates their utility (Turner & Trexler, 1998; Bohonak, 1999).

Coalescence-based estimates of Nm were lower than those obtained using F_{ST}. The majority of coalescence-based Nm values were below 1 and many had 95% confidence intervals that included zero, whereas F_{ST}-based estimates typically exceeded 4 and the lower 95% confidence limit exceeded 1 (Fig. 1). Given the geographical distance (up to 108 km) and water barrier separating most populations, we feel that the lower migration rates generated by the coalescence-based analysis may be a more reasonable estimate of gene flow. F_{ST}-based estimates of Nm between pairs of populations may be inflated by gene flow occurring indirectly through a third population whereas coalescence-based estimates are not affected in this way and so may give a more accurate estimate of gene flow between population pairs. Interestingly, summing coalescence-based immigration rates into each population (Table 3) gives a value comparable in magnitude to F_{ST}-based estimates of Nm.

Microevolution in polymorphic garter snake populations

In the absence of gene flow, either directional selection or genetic drift alone should eventually drive one colour pattern allele to fixation in each population. Of the ten populations studied, only two are likely to be fixed at the colour pattern locus (mainland populations in Ontario; see table 4 in Lawson & King, 1996), with the remainder showing a range of colour pattern frequencies. In the presence of gene flow, morph frequencies should reach an equilibrium that depends on the combined effects of gene flow, drift and selection. The postglacial history of Lake Erie (Calkin & Feenstra, 1985) suggests a garter snake range expansion 8000 ybp followed by formation of Lake Erie and isolation of island snake populations 4000 ybp. Provided population sizes have remained relatively constant, this should be adequate time for populations to approach equilibrium (see fig. 3 in Whitlock & McCauley, 1999). Changes in population size (e.g. recent population declines due to human activities) may disrupt this equilibrium. However, since 1980, frequencies of melanism in these populations have remained stable (R.B. King & T.D. Bittner, unpubl. data).

Lincoln–Petersen estimates of garter snake population size (Table 1; see also table 29 in Bittner, 2000) indicate that, at least in some populations, random genetic drift might affect morph frequencies. Population estimates for West Sister, Rattlesnake and Middle Island (small islands that were sampled in their entirety) range from 58 to 475 adults. Population estimates for larger islands and mainland populations range from 46 to 558 adults but these populations were sampled incompletely.

Gene flow interacts with population size to limit the effects of drift; $Nm < 1$ allows fixation and loss of alleles due to drift, whereas $Nm > 4$ prevents fixation and loss of alleles (Slatkin, 1987; Hartl & Clark, 1989). Although patterns of genetic variation were generally congruent between marker types, the two methods of

analysis lead to conflicting conclusions. The F_{ST}-based analysis leaves little role for genetic drift, as most pairwise estimates exceed 4. The coalescence-based analysis allows the possibility of significant genetic drift because no pairwise estimates exceed 1. The extent to which these rates may be overestimates of contemporary gene flow resulting from violation of assumptions, weak selection on allozymes, non-equilibrium F_{ST}, inappropriate mutation model (for F_{ST} from microsatellite DNA), and recent changes in population size, is unknown.

Both indirect and experimental evidence suggest that selection acts on colour pattern in these populations. As an indirect test for natural selection on colour pattern, Lawson & King (1996) computed F_{ST} for colour pattern based on assumptions of Mendelian inheritance and Hardy–Weinberg equilibrium. If natural selection on colour pattern varies among populations, then estimates of F_{ST} based on colour pattern would overestimate population subdivision relative to molecular (neutral) estimates of F_{ST}. Estimates of F_{ST} for colour pattern exceeded those based on allozymes by an order of magnitude, suggesting that selection on colour pattern does influence the frequency of melanism.

Experimental evidence suggests that selection may favour melanistic snakes. A thermal advantage for melanism was supported by Gibson & Falls (1979) and Bittner *et al*. (2002). A thermoregulatory advantage for melanistics could lead to higher growth rates, larger body sizes, and greater reproductive success, although no evidence has been found for these effects in natural populations. The presumed disadvantage of melanism, greater visibility to predators (Gibson, 1978), was not supported by a predation study using clay models; there was no difference in predation between striped and melanistic models (Bittner, 2003).

Like Lake Erie populations of the common garter snake, sympatric populations of the Lake Erie water snake (*N. s. insularum*) are also polymorphic for colour pattern (King, 1987). Selection favours reduced banding pattern in island water snake populations (King, 1993). While genetic drift was probably important in the early establishment of the water snake polymorphism, it is currently maintained by an equilibrium between selection and gene flow (King & Lawson, 1995). In contrast, gene flow is significantly lower in garter snakes compared to water snakes (King & Lawson, 2001), and genetic drift probably has greater current importance in determining morph frequencies in garter snake populations. The current frequency distribution of melanism in Lake Erie garter snake populations is a result of the complex interaction of gene flow, genetic drift and selection. Importantly, the microsatellite DNA loci analysed here provide more evidence of drift than do allozyme loci; number of microsatellite alleles and *M* are positively correlated with island size in garter snakes.

Future work should include application of the coalescence-based analyses to allozyme data from sympatric water snake (*N s. insularum*) and brown snake (*S. dekayi*) populations for comparison with gene flow in garter snakes. For all three species, we recommend initiating or expanding analyses of microsatellite DNA variation. Additional tissue sampling would provide direct estimates of genetic drift and indirect estimates of effective population size based on changes in allele frequency over a span of a decade or more (Waples, 1989). In addition, we suggest calculating *M*-ratios (Garza & Williamson, 2001) and testing for correlation of *M*-ratios and number of alleles with island size (as in this paper). Analysis of microclimate conditions should offer insight into selection for thermoregulatory ability (T.D. Bittner, unpubl. data), while studying the reproductive success of melanistic vs. striped females would aid in evaluating the biological significance of thermal differences between morphs. Finally, large-scale selection experiments in the field could be used to measure the strength of selection in some garter snake populations (e.g. Arnold, 1983; King, 1987, 1993; Brodie, 1992).

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