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Gene flow and melanism in Lake Erie garter snake populations

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Melanistic garter snakes (*Thamnophis sirtalis*) are unusually common near Lake Erie, apparently because selection for thermoregulatory ability in cool lake-shore habitats (which favours melanistic morphs) outweighs selection for crypsis (which favours striped morphs). However, morph frequencies are highly variable among sites, suggesting that random genetic drift also influences colour pattern. In an effort to better understand the evolutionary processes influencing garter snake colour patterns, we estimated F_{st} and Nm (the number of migrants per generation) among island and mainland populations from patterns of allozymic variation detected using electrophoresis. Estimates of Nm were high, ranging from 2.7 to 37.6 between pairs of study sites and making it unlikely that differences in morph frequencies among sites were solely the result of random genetic drift. Furthermore, differences in F_{st} estimates between colour pattern (a one-locus two-allele trait) and allozyme loci suggest that colour pattern alleles are not in Hardy-Weinberg equilibrium, most likely as a result of natural selection. Comparison of allozymic data from Lake Erie with those from more distant sites suggests that gene flow occurs over long distances in *T. sirtalis*.

ADDITIONAL KEY WORDS: — *Thamnophis sirtalis* – natural selection – random genetic drift – allozymes – colour pattern.

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INTRODUCTION

The presence of phenotypic variation within populations and differentiation among populations often provides an opportunity to directly observe micro-evolutionary processes (natural selection, gene flow, random genetic drift) in nature. An example is provided by populations of the garter snake (*Thamnophis sirtalis*, Serpentes: Colubridae) near Lake Erie (straddling the U.S.-Canadian border in North America) in which melanism is unusually common (King, 1988, and references therein). Melanistic morphs are jet-black with a small amount of white on the chin and throat and contrast strikingly with typical striped garter snakes (Fig. 1). Melanism is inherited in Mendelian fashion with melanism recessive to striped (Blanchard & Blanchard, 1940; Gibson & Falls, 1975).

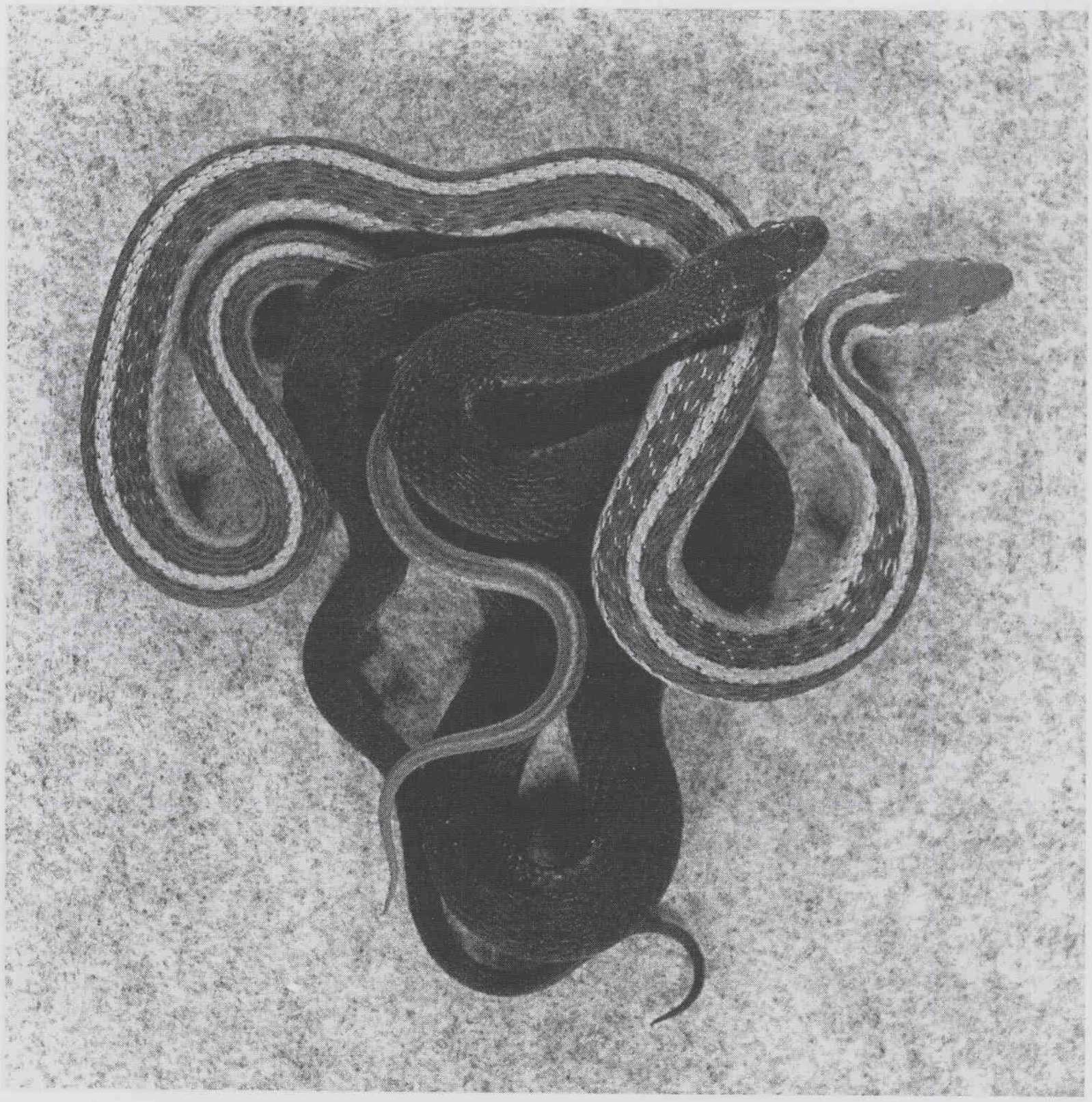

Figure 1. Striped and melanistic morph garter snakes from the island region of western Lake Erie.

Possible differences between striped and melanistic morphs in thermal biology and crypticity led Gibson & Falls (1979, 1988) to hypothesize that colour pattern was influenced by the joint effects of selection for thermoregulatory ability (favouring melanistic morphs) and selection for crypsis (favouring striped morphs); melanistic morphs are common in cool lake-shore habitats presumably because the advantages of increased thermoregulatory ability outweigh the increased risk of predation. Evidence that selection for thermoregulatory ability favours melanistic morphs includes the observations that (1) heat flow is greater through the excised skin of melanistic morphs than of striped morphs, (2) in matched pairs of intact animals, melanistic morphs heat more quickly than striped morphs, and (3) in the field, melanistic morphs have higher body temperatures during the colder part of the active season (Gibson & Falls, 1979, 1988). Evidence that selection for crypsis favours striped morphs is less direct but includes the observations that (1) in the field, melanistic morphs are more conspicuous than striped morphs to human observers and (2) melanistic morphs are less common at a site were predation is thought to be more intense than at a site were predation is less intense (Gibson & Falls, 1979, 1988). [In contrast, Sattler & Guttman (1976) suggested that melanistic morphs might be more cryptic than striped morphs but presented no supporting evidence]. However, frequencies of melanistic morphs are highly variable among sites, leading King (1988) to suggest that random genetic drift might also influence morph frequencies. The insular nature of many Lake Erie garter snake populations suggests that random genetic drift is possible. However, without data on rates of gene flow, it is impossible to evaluate whether populations are sufficiently isolated for random genetic drift to be important.

Here, we estimate the rate of gene flow among Lake Erie island and mainland garter snake populations, using allozyme data to compute F_{st} and Nm, the number of migrants per generation. Low gates of gene flow (< 1 individual per generation) would suggest that random genetic drift alone could produce large differences in morph frequency among sites; high rates of gene flow (> 1 individual per generation) would suggest that other evolutionary processes (e.g. natural selection) are operating (Slatkin, 1987). In addition, we provide an indirect test for natural selection on colour pattern by estimating F_{st} and Nm for the colour pattern locus and comparing these estimates with those derived from allozyme frequencies. To do this, we assume that colour pattern genotype frequencies meet Hardy-Weinberg expectations. If this assumption holds, F_{st} and Nm estimates should be similar for colour pattern and allozyme loci. However, if colour pattern genotype frequencies do not meet Hardy-Weinberg expectations (as would be the case if colour pattern were subject to natural selection), F_{st} and Nm estimates should differ between colour pattern and allozyme loci (e.g. table 1 in Slatkin, 1987; Cabe & Alstad, 1994). Finally, we analyse patterns of genetic differentiation and gene flow in *T. sirtalis* over a broader geographic range by including data from populations in New York, Quebec, and Wisconsin.

METHODS

Study sites and tissue collection

Blood and muscle samples were collected non-destructively from live garter snakes captured by hand in 1989–1992 at six island and four mainland sites. These included two sites on the Ohio mainland and two on the Ontario mainland (Fig. 2). The

islands from which tissue samples were collected ranged from 20 ha (Rattlesnake Island) to > 4000 ha (Pelee Island), spanning nearly the full range of island sizes in Lake Erie. Islands were 14.1–38.6 km from Ohio mainland sites, 25.5–87.0 km from Ontario mainland sites, and were separated from each other by 1.3–34.6 km (Table 1). Ohio mainland sites were 17.5 km apart; Ontario mainland sites were

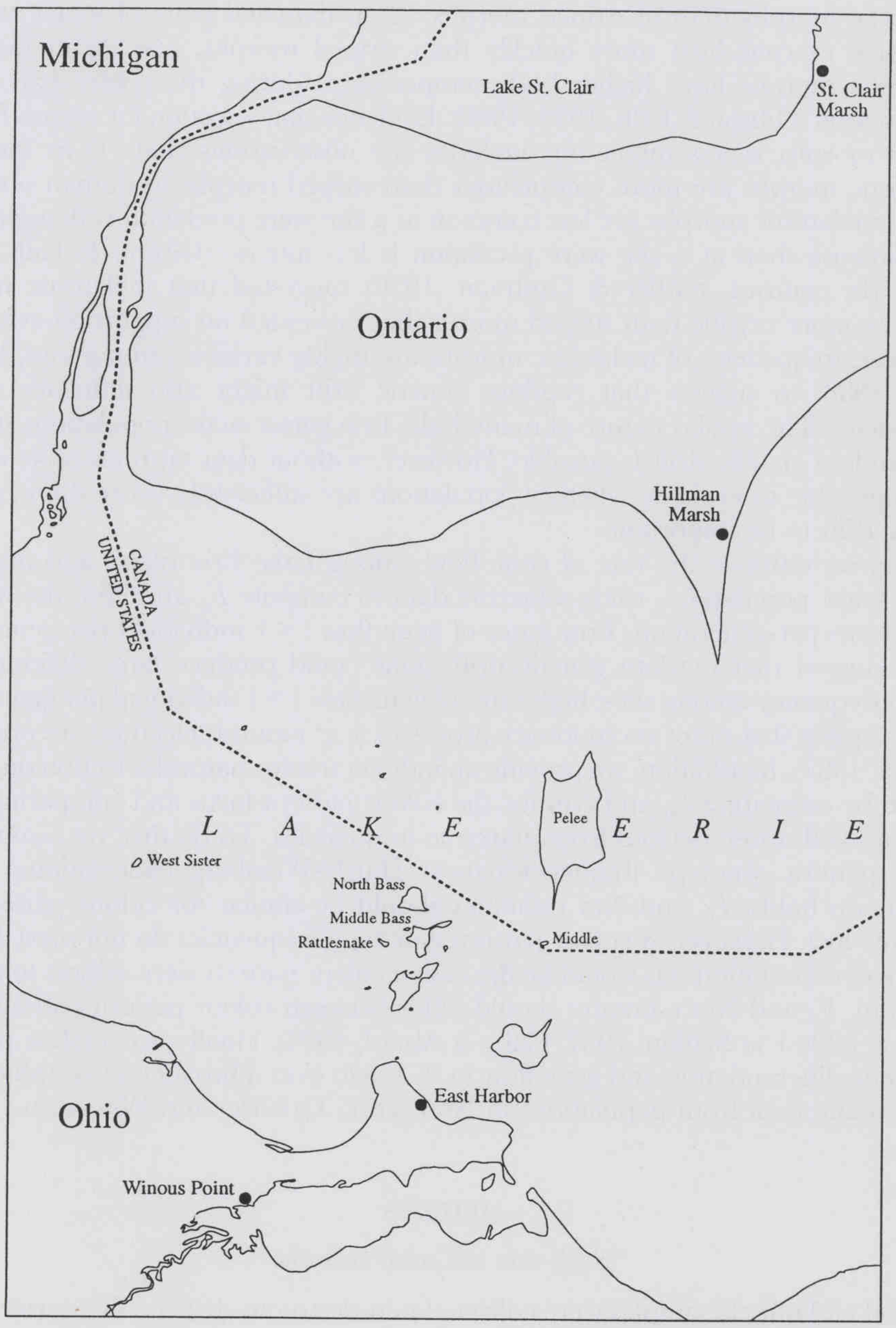

Figure 2. The island region of western Lake Erie showing six island and four mainland sampling sites included in this study.

TABLE 1. Geographic distances (km) between pairs of Lake Erie island and mainland study sites (shortest straight-line distance)

	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point
St. Clair Marsh	37.0	59.0	76.0	77.0	76.0	81.0	87.0	93.0	108.0
Hillman Marsh		25.5	43.5	44.2	41.5	48.9	60.0	59.6	74.5
Pelee Island			10.7	9.4	5.1	15.1	34.2	22.8	38.6
North Bass Island				1.3	9.8	3.3	22.2	18.1	29.5
Middle Bass Island					7.4	1.6	23.6	14.1	26.3
Middle Island						12.7	34.6	17.6	33.7
Rattlesnake Island							21.3	15.6	25.6
West Sister Island								33.6	31.9
East Harbor									17.5

37.0 km apart; Ohio mainland sites were 59.6–108.0 km from Ontario mainland sites (Table 1).

Blood samples were collected from vessels in the tail using a heparinized hypodermic syringe (Bush & Smeller, 1978); muscle samples were obtained by removing a small portion of the tail tip. The wound resulting from removal of the tail tip was protected with liquid bandage (New Skin, Inc., Cody, WY). Blood samples were separated into plasma and red blood cell fractions by centrifugation. Tissue samples were frozen in liquid nitrogen within 30 min of collection and stored at –80°C until used for electrophoresis. Snakes were measured to obtain snout-vent length, classified by sex and colour pattern (striped or melanistic), and individually marked by scale clipping (Brown & Parker, 1976). Following tissue collection, snakes were released at the site of capture. Long-term effects of tissue collection appeared to be minor: snakes recaptured following tissue sampling were in good health and tail loss in snakes has little effect on locomotion (Jayne & Bennett, 1989) or adult survivorship (Willis, Threlkeld, & Carpenter, 1982). Voucher specimens have been deposited at the California Academy of Sciences.

Tissue samples were collected sacrificially from garter snakes from near Altamont, New York; Touraine, Quebec; and Oshkosh, Wisconsin (Touraine and Oshkosh material was provided by F. Cook and G. Burghardt, respectively). These three sites were between 545 and 740 km from the East Harbor site near Lake Erie and between 340 and 1220 km from each other.

Electrophoresis

Electrophoresis was carried out at the California Academy of Sciences using standard methods (Selander *et al.*, 1971; Ayala *et al.*, 1972; Murphy *et al.*, 1990) adapted for use with natricine snakes (Lawson *et al*, 1991; King & Lawson, 1995; and Table 2 herein). The products of twelve polymorphic loci were assayed (Table 2). Alternative alleles for one locus, fumarate hydratase, are inherited in sex-linked fashion (King & Lawson, 1996) so for this locus we included only males in our analyses (natricine snakes have ZW sex chromosomes (Cundall 1970; Olmo 1986); hence, females are hemizygous at this locus and heterozygote frequencies can be

TABLE 2. Proteins, presumptive loci, tissue sources, and buffer systems used in electrophoresis

Protein	Locus	Tissue source*	Buffer system**
Lactate dehydrogenase-A (EC 1.1.1.27)	*Ldh–A*	M	1
Phosphogluconate dehydrogenase (EC 1.1.1.44)	*Pgdh*	M, RBC	1
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	*G-6-pdh*	M	1
Superoxide dismutase-1 (EC 1.15.1.1)	*Sod-1*	RBC	1
Creatine kinase-2 (EC 2.7.3.2)	*Ck-2*	M	1
Adenylate kinase (EC 2.7.4.3)	*Ak*	M	1
Tripeptidase-1 (leu-gly-gly) (EC 3.4.11.4)	*Trip-1*	M	1
Fumarate hydratase (EC 4.2.1.2)	*Fum*	M	1
Mannose-6-phosphate isomerase (EC 5.3.1.8)	*Mpi*	M, RBC	1
Glucose-6-phosphate isomerase (EC 5.3.1.9)	*Gpi*	M	1
Phosphoglucomutase (EC 5.4.2.2)	*Pgm*	M	1
Transferrin	*Trf*	P	2

*M=skeletal muscle, RBC=erythrocytes, P=plasma.
**1=N-(3-aminopropyl)-morpholine-citrate buffer of Clayton & Tretiak (1972) adjusted to pH 7.9. 2=The discontinuous buffer system of Poulik (1957).

determined only for males). Superoxide dismutase, fumarate hydratase, and transferrin were not analysed for snakes from New York, Quebec and Wisconsin.

Analyses

Genotypic frequencies at each locus were tested for deviations from Hardy-Weinberg equilibrium conditions using BIOSYS-1 (Swofford & Selander, 1981). We computed the fixation index F_{st} for each locus for all possible pairs of sites using the method of Nei & Chesser (1983) as modified by Van Den Bussche, Hamilton, & Chesser (1986). Jack-knifing across loci was used to generate F_{st}*, a less biased estimate of F_{st}, and to estimate the variance in F_{st}* (Weir & Cockerham, 1984). We estimated Nm based on the relationship $F_{st}^* = 1/(1 + 4Nm)$ (Wright, 1943, 1969). We estimated upper and lower 95% confidence limits on Nm by substituting $F_{st}^* \pm 1.96$ S.E. into this relationship. We computed F_{st} and Nm between pairs of populations because the relationship between pair-wise estimates and geographic distance provides a simple test for isolation by distance (Slatkin, 1993). Statistical significance of the relationship between pair-wise estimates of Nm and geographic distance was tested using the Mantel test (Douglas & Endler, 1982).

Patterns of gene flow among Lake Erie garter snake populations were investigated further by generating dendrograms by the UPGMA method with Nei's unbiased genetic identities and the distance-Wagner method with Cavalli-Sforza and Edward's chord distances and modified Rogers' distances using BIOSYS-1 (Swofford & Selander, 1981). Tree lengths were compared using FREQPARS (Swofford & Berlocher, 1987). When applied to populations linked by gene flow, such dendrograms reflect levels of gene flow rather than phylogenetic history: populations which exchange migrants only rarely are placed on more distant branches than are populations which exchange migrants more frequently.

A test for population subdivision by morph (striped vs. melanistic) was carried out using a hierarchical F_{st} analysis of morphs within sites for the three sites for which sample sizes of melanistic morphs were at least 15 (Pelee Island, Rattlesnake Island, Winous Point).

Observed frequencies of melanistic morphs were used to estimate frequencies of melanism alleles by assuming that colour pattern was in Hardy-Weinberg equilibrium, that is that the frequency of melanism alleles equalled the square-root of the frequency of melanistic morphs. These estimates were then used to compute pairwise F_{st} and Nm values for colour pattern. Means and variances in F_{st} were computed across the 45 pairwise combinations of sites for colour pattern, for individual allozyme loci, and for all allozyme loci combined. Estimates of melanism allele frequencies included data from King (1988); hence sample sizes were larger than those used to estimate allozyme frequencies for some sites.

To analyze patterns of genetic differentiation and gene flow over a broader geographic range we computed pairwise F_{st}* and Nm estimates using data from East Harbor (the Lake Erie site with the largest sample size), New York, Quebec, and Wisconsin.

RESULTS

The 12 allozyme loci were represented by 42 alleles, 2–10 alleles per locus, across the 10 Lake Erie populations (Appendix). Most alleles were found in most populations, though five alleles were unique, being found in only a single population. Of 120 possible tests for deviations from Hardy-Weinberg equilibrium genotype frequencies (12 loci at each of 10 sites), eight were significant at a testwise significance level of 0.05 (data not shown). When P values were adjusted for multiple tests (i.e. adjusted $P = 0.05/120 = 0.0004$), only one test (*Trf* on Middle Bass Island) showed a significant deviation from Hardy-Weinberg equilibrium expectations. Furthermore, jack-knifing across loci suggested that no one locus had a large effect on our analyses (data not shown, see also Table 6). Thus, subsequent analyses were carried out on the assumption that Hardy-Weinberg expectations were met for allozyme loci.

Within the Lake Erie area, estimates of F_{st}* ranged from 0.007 to 0.0.085 and estimates of Nm ranged from 2.7 to 37.6 individuals per generation between pairs of sites (Table 3). The relationship between log(Nm) and log(geographic distance) between pairs of sites was non-significant ($r = 0.111$, Mantel $t = 0.456$, $P = 0.676$) (Fig. 3A). Because gene flow might be higher between adjacent mainland sites (St Clair Marsh — Hillman Marsh, East Harbor — Winous Point) than between sites separated by a water barrier (island-island and island-mainland pairs), thus masking the relationship between Nm and distance, we repeated this analysis with mainland-mainland pairs omitted. The correlation between log(Nm) and log(geographic distance) remained small ($r = 0.0001$). Tree-building algorithms consistently grouped the two Ontario mainland sites (St. Clair Marsh and Hillman Marsh) together, but otherwise groupings varied depending on the method using (Fig. 4). Of the 10 sites included, Rattlesnake Island stands out in that rates of gene flow between it and the other sites are lower than among other pairs of sites (Table 3).

F_{st} for morphs within populations was small (0.007) relative to F_{st} among populations (0.029) suggesting that population subdivision by morph was negligible. Frequencies of melanistic morphs ranged from 0.000 to 0.491 and estimates of melanism allele frequencies ranged from 0.000–0.701 (Table 4). Pairwise estimates of F_{st} for colour pattern ranged from 0.000–0.538 (Table 5). Across the 45 pairwise combinations of sites, mean F_{st} was typically an order of magnitude larger, and

variance in F_{st} two orders of magnitude larger, for colour pattern than for allozymic loci (Table 6). No melanistic morphs were observed at St. Clair Marsh or at Hillman Marsh, but given that melanistic morphs are known from the Ontario mainland (King, 1988, and references therein), it is possible that melanism occurs at low frequencies at these sites and simply was not observed in our samples. This would make our estimates of melanism allele frequencies at these sites unrealistically low. Therefore, we repeated the analysis described above, assuming that the next snake caught at St. Clair Marsh and Hillman Marsh would be melanistic (giving estimated frequencies of the melanism allele of 0.131 and 0.128, respectively). Results were qualitatively similar to those described above: F_{st} for melanism averaged 0.099 (range = 0.000–0.0.413) and had a variance of 0.010 (cf. Table 6).

As with allozymes, estimates of Nm based on colour pattern showed no relationship to distance between pairs of sites (Fig. 3B; $r = -0.219$, Mantel $t = -1.458$, $P = 0.07$). The greater variance in Nm values for colour pattern relative to allozyme loci is evident by comparing Figure 3A and 3B.

Estimates of F_{st}* were higher and estimates of Nm were lower among pairs of

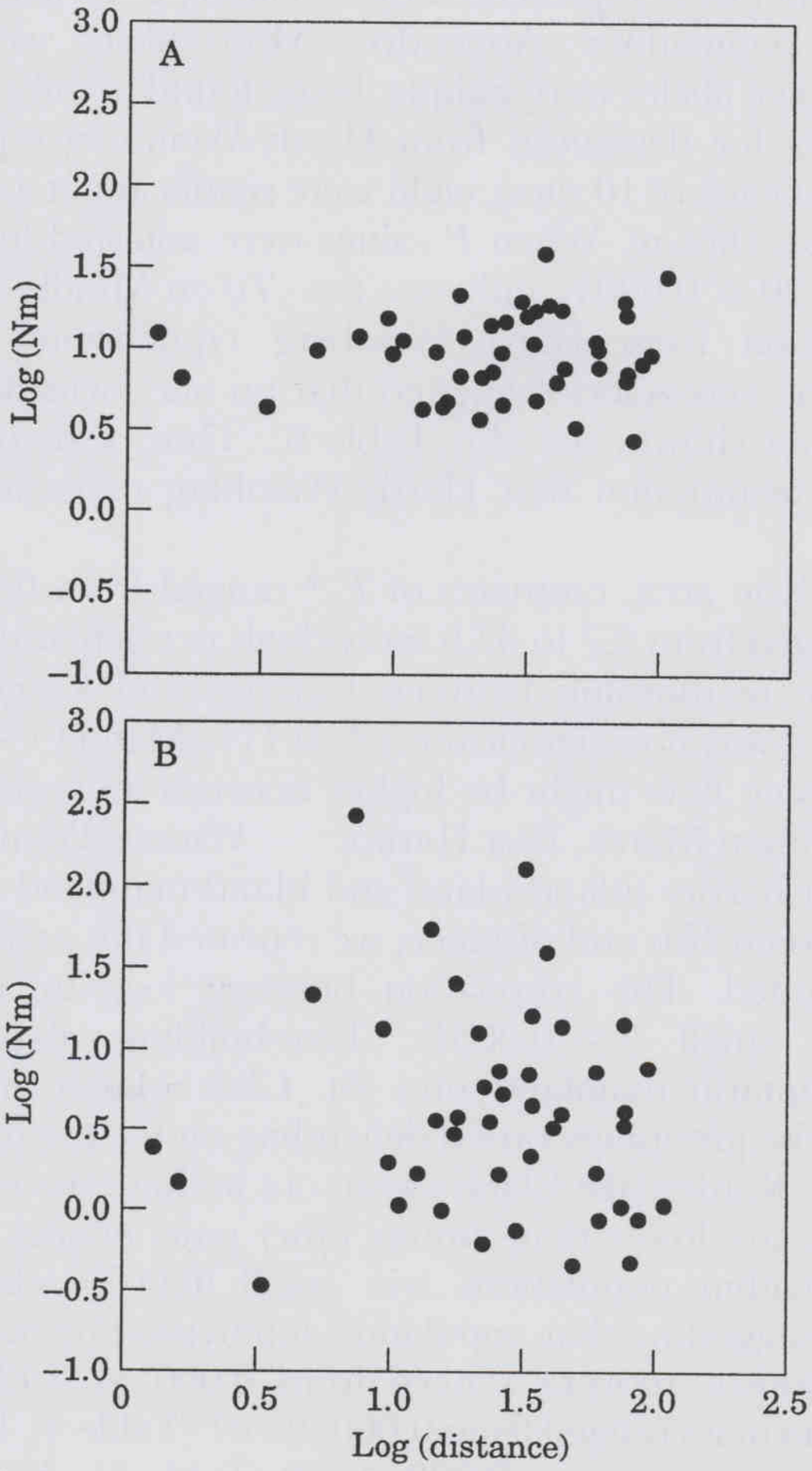

Figure 3. Relationship between Nm and geographic distance for (A) allozymic data and (B) colour pattern.

samples from East Harbor, New York, Quebec, and Wisconsin (separated by 340–1220 km) than among pairs of samples from the Lake Erie area (separated by 1.3–108 km) (compare Table 7 and Table 3). However, even over the longer distances separating the East Harbor, New York, Quebec, and Wisconsin sites, estimates of Nm equalled or exceeded one individual per generation (Table 7). Jackknifing identified two loci which had relatively large effects on estimates of F_{st}* and Nm. The B allele for Ldh-A was rare at the New York site but common elsewhere

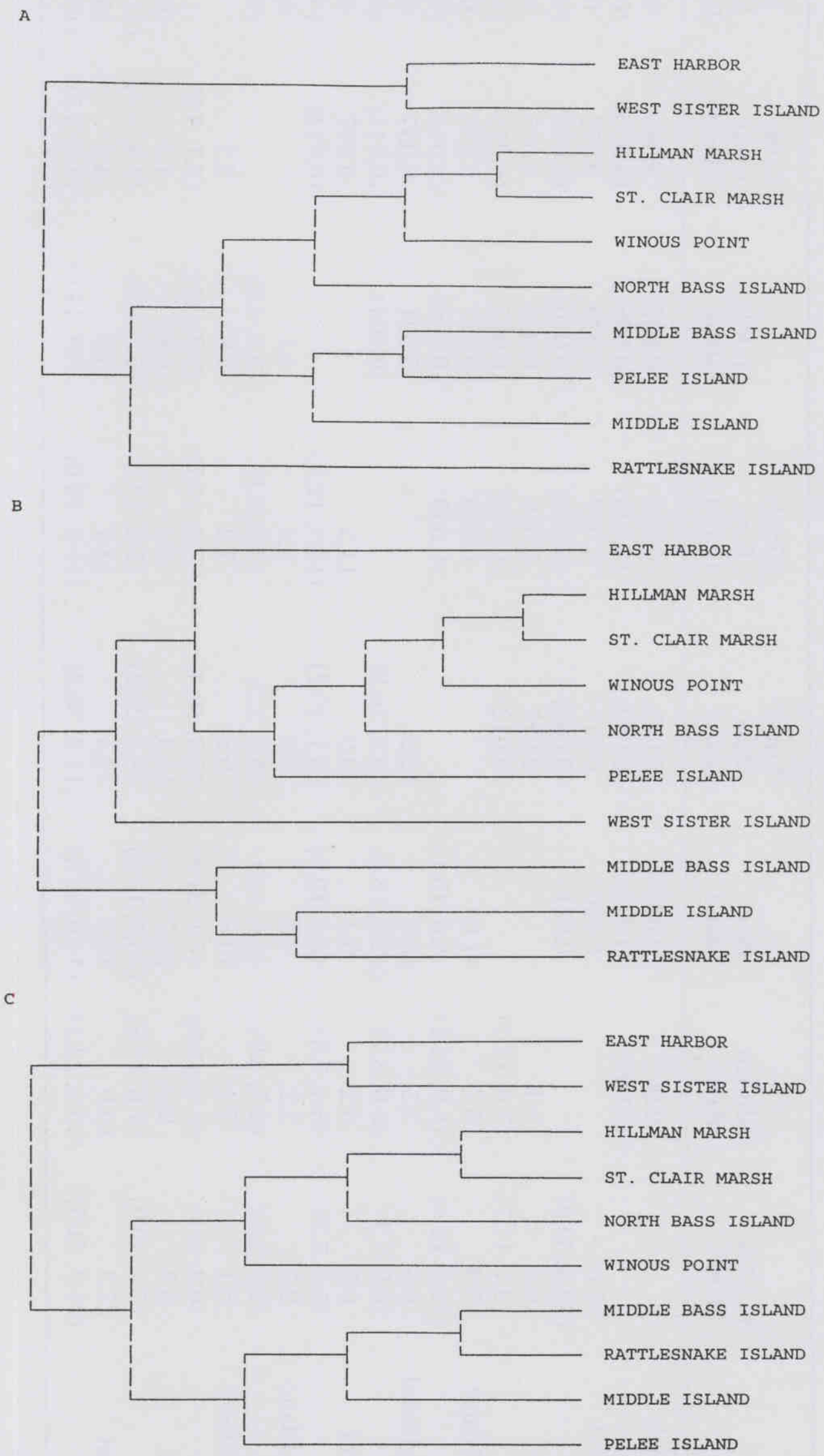

Figure 4. Dendrograms based on allozymic data using (A) UPGMA method with Nei's unbiased genetic identities, (B) distance-Wagner method with Cavalli-Sforza and Edward's chord distances, and (C) distance-Wagner method with modified Rogers' distances. Total tree lengths are 13.6, 14.3, and 13.9 for A, B, and C, respectively.

TABLE 3. Estimates of F_{st}* (standard deviation) (above diagonal) and Nm (95% confidence interval) (below diagonal) between pairs of study sites based on allozyme frequencies

	St. Clair Marsh	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point
St. Clair Marsh		0.007 (0.003)	0.023 (0.004)	0.015 (0.009)	0.036 (0.008)	0.037 (0.008)	0.085 (0.030)	0.030 (0.010)	0.028 (0.010)	0.009 (0.002)
Hillman Marsh	37.6 (29.8, 59.0)		0.026 (0.011)	0.015 (0.006)	0.032 (0.008)	0.039 (0.010)	0.072 (0.026)	0.032 (0.018)	0.025 (0.014)	0.013 (0.005)
Pelee Island	10.6 (9.7, 11.7)	9.4 (7.6, 12.1)		0.022 (0.012)	0.016 (0.006)	0.026 (0.006)	0.055 (0.018)	0.023 (0.022)	0.018 (0.009)	0.013 (0.006)
North Bass Island	15.9 (12.3, 22.4)	16.7 (13.9, 21.1)	11.0 (8.6, 15.1)		0.020 (0.009)	0.027 (0.006)	0.055 (0.018)	0.036 (0.023)	0.021 (0.016)	0.013 (0.009)
Middle Bass Island	6.8 (6.0, 7.7)	7.5 (6.6, 8.7)	15.0 (12.6, 18.6)	12.3 (9.9, 16.2)		0.021 (0.004)	0.037 (0.013)	0.034 (0.021)	0.026 (0.015)	0.017 (0.008)
Middle Island	6.5 (5.8, 7.3)	6.2 (5.4, 7.3)	9.4 (8.4, 10.6)	9.0 (8.1, 10.2)	11.5 (10.3, 12.9)		0.057 (0.014)	0.049 (0.022)	0.036 (0.012)	0.023 (0.009)
Rattlesnake Island	2.7 (2.2, 3.4)	3.2 (2.7, 4.0)	4.3 (3.6, 5.2)	4.3 (3.6, 5.3)	6.6 (5.5, 8.1)	4.1 (3.6, 4.8)		0.064 (0.021)	0.053 (0.017)	0.051 (0.015)
West Sister Island	8.1 (6.8, 9.9)	7.4 (5.7, 10.6)	10.8 (7.1, 21.8)	6.8 (5.0, 10.4)	7.2 (5.3, 10.7)	4.9 (3.9, 6.4)	3.7 (3.1, 4.5)		0.015 (0.012)	0.016 (0.006)
East Harbor	8.8 (7.4, 10.9)	9.8 (7.6, 13.8)	13.7 (10.7, 19.0)	11.9 (8.4, 20.2)	9.4 (7.2, 13.6)	6.8 (5.7, 8.2)	4.5 (3.8, 5.4)	16.5 (11.6, 28.2)		0.012 (0.007)
Winous Point	27.3 (24.0, 31.5)	19.0 (15.9, 23.5)	18.3 (14.6, 24.6)	19.5 (14.3, 30.4)	14.4 (11.6, 19.0)	10.8 (8.9, 13.6)	4.6 (4.0, 5.5)	15.6 (13.2, 19.2)	21.4 (16.3, 31.1)	

TABLE 4. Frequencies of melanistic morphs and estimated frequencies of the melanism allele in Lake Erie garter snake populations

	n	Proportion melanistic	Estimated frequency of melanism allele
St. Clair Marsh	57	0.000	0.000
Hillman Marsh	60	0.000	0.000
Pelee Island	307	0.202	0.449
North Bass Island	185	0.005	0.074
Middle Bass Island	218	0.101	0.318
Middle Island	325	0.120	0.346
Rattlesnake Island	108	0.491	0.701
West Sister Island	95	0.326	0.571
East Harbor	316	0.066	0.258
Winous Point	54	0.278	0.527

TABLE 5. Estimates of F_{st}* between pairs of study sites based on colour pattern frequencies

	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point
St. Clair Marsh	0.000	0.288	0.038	0.188	0.208	0.538	0.398	0.147	0.356
Hillman Marsh		0.289	0.038	0.188	0.208	0.538	0.398	0.148	0.356
Pelee Island			0.182	0.018	0.011	0.065	0.015	0.040	0.006
North Bass Island				0.094	0.111	0.413	0.282	0.061	0.243
Middle Bass Island					0.001	0.146	0.065	0.004	0.045
Middle Island						0.126	0.051	0.009	0.033
Rattlesnake Island							0.018	0.196	0.032
West Sister Island								0.101	0.002
East Harbor									0.076

TABLE 6. Comparison of F_{st} for individual allozyme loci, for all loci combined (F_{st}*), and for colour pattern. Shown are means, minima, maxima, and variances of F_{st} averaged across the 45 pairwise combination of study sites

Locus	Mean	Minimum	Maximum	Variance
Ldh-A	0.022	0.000	0.097	0.0006
Pgdh	0.030	0.001	0.082	0.0005
G-6-pdh	0.037	0.000	0.200	0.0017
Sod-1	0.017	0.000	0.072	0.0003
Ck-2	0.017	0.000	0.073	0.0004
Ak	0.004	0.000	0.015	<0.0001
Trip-1	0.028	0.000	0.094	0.0008
Fum	0.014	0.000	0.093	0.0003
Mpi	0.041	0.001	0.191	0.0018
Gpi	0.003	0.000	0.020	<0.0001
Pgm	0.021	0.000	0.078	0.0005
Trf	0.035	0.001	0.167	0.0015
F_{ST}*	0.031	0.007	0.085	0.0003
Colour pattern	0.150	0.000	0.538	0.0217

TABLE 7. Geographic distance, F_{st}* (Standard deviation), and Nm (95% confidence interval) among distantly separated sampling sites

Pair of sites	Geographical distance (km)	F_{ST}*	Nm
Quebec - New York	340	0.11 (0.073)	2.1 (1.4, 4.3)
Wisconsin - East Harbor	545	0.14 (0.105)	1.6 (0.9, 3.6)
Quebec - East Harbor	735	0.13 (0.079)	1.7 (1.1, 3.1)
New York - East Harbor	740	0.19 (0.085)	1.1 (0.8, 1.7)
Wisconsin - Quebec	1070	0.05 (0.019)	4.8 (3.7, 6.6)
Wisconsin - New York	1220	0.21 (0.122)	1.0 (0.6, 1.8)

and the C allele for *G-6-pdh* was common at the New York site but rare elsewhere (Appendix).

DISCUSSION

Estimated rates of gene flow ranged from 2.7 to 37.6 individuals per generation between pairs of garter snake study sites near Lake Erie. These rates are sufficiently high to preclude random genetic drift from resulting in fixation or near fixation of alternative alleles among populations. Thus, it appears unlikely that differences in morph frequencies (striped vs. melanistic) among sites is solely the result of random genetic drift. Furthermore, we found no evidence of population subdivision by morph, corroborating the results of Sattler & Guttman (1976) for a single Ohio mainland site, and suggesting that high frequencies of melanism in some populations are not the result of assortative mating.

Rates of gene flow were highest between the two pairs of adjacent mainland sites (Nm = 27.3 between East Harbor and Winous Point and 37.6 between St. Clair Marsh and Hillman Marsh). These values exceed those of island-mainland and island-island pairs separated by similar distances and suggest that the open water of Lake Erie has at least partially impeded gene flow over the 4000 years since rising lake levels isolated island populations (King & Lawson, 1995). Roughly comparable levels of gene flow (*c.* 8 individuals per generation) have been observed among Lake Michigan island and mainland garter snake populations (T. Grudzien, unpublished manuscript).

No evidence of isolation by distance was evident from our analyses; rates of gene flow were uncorrelated with distance between sites. This contrasts with the pattern seen in water snakes (*Nerodia sipedon*) from the same region in which rates of gene flow decrease with increasing distance (King & Lawson, 1995). One possible explanation is that garter snake populations are not in equilibrium, perhaps as a result of local extinction and recolonization events (Slatkin, 1993). However, why this would be true for garter snakes but not water snakes is unclear.

Differences in F_{st} estimates for colour pattern and allozyme loci suggest that colour pattern alleles are not in Hardy-Weinberg equilibrium, as assumed in estimating F_{st} for colour pattern. Because population size, rates of gene flow, and mating system are necessarily the same for allozymes and colour pattern, deviations from Hardy-Weinberg equilibrium for colour pattern are likely due to natural selection. Suppose melanistic morphs have a thermoregulatory advantage and striped morphs are more

cryptic to visual predators, and the net direction of selection on colour pattern resulting from these two processes varies among study sites. Then by assuming that the frequency of melanism alleles equals the square-root of the frequency of melanistic morphs, we are likely to have overestimated the frequency of melanism alleles at sites where melanism is favoured and underestimated the frequency of melanism alleles at sites where a striped pattern is favoured. And as a result, we will have overestimated F_{st} for colour pattern. Presumably, at sites where melanism is common, selection for thermoregulatory ability outweighs selection for crypsis, either because such sites are unusually cool or because they are relatively free of predation. Unfortunately, without independent data on variation in thermal regime and risk of predation, it is difficult to determine the relative importance of thermoregulation and crypsis in producing morph frequency differences among sites.

Population genetic models show that natural selection operating in opposite directions in two habitats coupled by gene flow can result in the maintenance of polymorphism in both niches (Karlin & McGregor, 1972). These models can be used to estimate the relative strength of selection and rate of gene flow necessary to produce differences in allele frequency of the magnitude seen in Lake Erie garter snake populations, provided some simplifying assumptions are made: (1) the strength of selection favouring striped morphs in one habitat equals that of selection favouring melanistic morphs in the other habitat, (2) population sizes are equal in the two habitats, and (3) gene flow occurs at equal rates in both directions. To produce equilibrium allele frequencies of about 60–70% (the maximum difference estimated here, Table 4), requires that the strength of selection (s) exceed the rate of gene flow (m) by about an order of magnitude. Unfortunately, without information on garter snake population size, it is not possible to estimate m from the values of Nm reported here. If local garter snake populations are relatively small (e.g. a few hundred adults as may be the case on some small islands, King, 1988), then selection coefficients of 0.1 or greater might be necessary to balance observed rates of gene flow; if populations are larger (as seems likely on larger islands and at mainland sites), weaker selection might be sufficient.

Given that *T. sirtalis* inhabits a wide range of thermal regimes, including areas considerably cooler than near Lake Erie (e.g. Gregory, 1977), it is perhaps surprising that melanism is not more wide-spread. Outside the Lake Erie area, reports of melanistic garter snakes phenotypically similar to those from the Lake Erie area are restricted to the Toronto islands in Lake Ontario, Ontario, Canada (L. Lowcock and R. MacCulloch, pers. comm.); a single individual from the Lake Michigan shoreline, Porter Co., Indiana (Field Museum of Natural History #249699); and islands in Lake Winnepegosis, Manitoba, Canada (Mason *et al.*, 1991). [See Peterson & Fabian (1984) and Catling & Freedman (1977) for descriptions of phenotypically similar *T. elegans* and *T. butleri*, respectively.] One possibility is that melanism might be favoured in other areas, but can become common only when certain population structure requirements are met. In particular, because melanism is recessive and hence effectively neutral when rare, a sub-divided population structure, in which stochastic processes (genetic drift, inbreeding, founder events) are more likely, may be necessary for melanism allele frequencies to increase to the point where selection becomes effective (King & Lawson, 1995). Small population size on some islands (just a few hundred adult garter snakes on small islands (King, 1988)) may increase the likelihood of such stochastic processes.

Gene flow over longer distances also appears to be relatively common in *T. sirtalis*,

with our estimates equaling or exceeding one individual per generation over distances as great as 1200 km. *T. sirtalis* is the most widely distributed snake in North America. Samples included in this study form an east-west transect through the range of the eastern subspecies, *T. s. sirtalis*. Greater genetic differentiation may occur within this subspecies to the north or south of our transect (as suggested by differences between populations on the Florida Peninsula and elsewhere (Dessauer, Cadle & Lawson, 1987)). Differentiation between this and other subspecies of *T. sirtalis* is also evident, both in allozymes (Dessauer *et al.*, 1987) and in life-history characters (Gregory & Larsen, 1993). However, our results suggest that high levels of gene flow and a lack of genetic differentiation appear to characterize large portions of this species' range.

Like colour pattern in Lake Erie garter snakes, colour pattern in Lake Erie water snakes (King & Lawson, 1995), behaviour in a desert spider (Riechert, 1993), and heavy-metal tolerance in a perennial grass (McNeilly, 1968) also appear to be influenced by the simultaneous effects of natural selection and gene flow. In all these cases, directional selection apparently favours alternate genotypes in different habitats. However, fixation or near fixation of favoured alleles is prevented by gene flow between habitats.

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APPENDIX. Sample sizes (n), allelic frequencies, and heterozygote frequencies (H) for protein loci in garter snake (*Thamnophis sirtalis*) populations from Lake Erie island and mainland sites and from New York, Quebec, and Wisconsin

	St. Clair Marsh	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point	New York	Quebec	Wisconsin
Lactate dehydrogenase-A													
(n)	56	59	103	103	108	55	98	24	130	55	35	29	41
A	0.196	0.212	0.398	0.136	0.375	0.209	0.270	0.417	0.273	0.327	0.986	0.345	0.171
B	0.804	0.788	0.602	0.864	0.625	0.791	0.730	0.583	0.727	0.673	0.014	0.655	0.829
(H)	0.393	0.356	0.466	0.252	0.546	0.382	0.398	0.500	0.392	0.509	0.029	0.345	0.293
Phosphogluconate dehydrogenase													
(n)	56	59	103	102	108	55	98	24	129	55	35	29	39
A	0.000	0.034	0.000	0.000	0.060	0.000	0.005	0.000	0.008	0.000	0.086	0.121	0.000
B	0.438	0.432	0.243	0.309	0.176	0.118	0.306	0.250	0.109	0.309	0.243	0.293	0.038
C	0.027	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.089	0.000	0.000	0.017	0.000
D	0.321	0.331	0.476	0.319	0.264	0.445	0.536	0.583	0.585	0.418	0.657	0.414	0.615
E	0.196	0.186	0.262	0.373	0.500	0.345	0.153	0.167	0.209	0.264	0.014	0.155	0.282
F	0.018	0.017	0.010	0.000	0.000	0.091	0.000	0.000	0.000	0.009	0.000	0.000	0.064
(H)	0.607	0.661	0.534	0.627	0.537	0.527	0.571	0.458	0.550	0.727	0.571	0.690	0.564
Glucose-6-phosphate dehydrogenase													
(n)	42	53	86	102	99	52	95	24	126	49	13	8	20
A	0.119	0.113	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.092	0.000	0.063	0.000
B	0.595	0.604	0.640	0.824	0.758	0.750	0.937	0.542	0.790	0.622	0.346	0.438	0.325
C	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.385	0.063	0.050
D	0.286	0.264	0.360	0.172	0.242	0.250	0.063	0.458	0.210	0.276	0.192	0.438	0.575
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.050
F	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(H)	0.643	0.453	0.419	0.255	0.323	0.269	0.105	0.417	0.325	0.673	0.462	0.625	0.700
Superoxide dismutase-1													
(n)	56	54	101	88	107	55	99	24	130	46			
A	0.116	0.111	0.094	0.205	0.061	0.182	0.076	0.063	0.031	0.174			
B	0.884	0.889	0.906	0.795	0.939	0.818	0.924	0.938	0.969	0.826			
(H)	0.196	0.222	0.168	0.273	0.121	0.218	0.091	0.125	0.046	0.348			
Creatine kinase-2													
(n)	56	59	103	103	108	55	98	24	130	55	35	30	41
A	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.009	0.025	0.024	0.005	0.000	0.136	0.020	0.000	0.004	0.055	0.400	0.317	0.000
C	0.964	0.975	0.976	0.995	1.000	0.864	0.980	1.000	0.996	0.945	0.571	0.683	1.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000
(H)	0.071	0.051	0.029	0.010	0.000	0.236	0.041	0.000	0.008	0.109	0.543	0.433	0.000

APPENDIX. (Continued)

	St. Clair Marsh	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point	New York	Quebec	Wisconsin
Adenylate kinase													
(*n*)	56	59	73	103	108	55	97	24	111	55	30	30	29
A	0.000	0.000	0.000	0.000	0.009	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	0.991	0.973	1.000	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.055	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tripeptidase-1													
(*n*)	56	60	103	103	108	55	98	22	130	55	35	25	41
A	0.036	0.083	0.053	0.228	0.074	0.018	0.122	0.091	0.000	0.091	0.000	0.000	0.000
B	0.955	0.917	0.893	0.757	0.903	0.955	0.679	0.909	0.908	0.909	1.000	1.000	0.902
C	0.009	0.000	0.053	0.015	0.023	0.027	0.199	0.000	0.092	0.000	0.000	0.000	0.098
(H)	0.089	0.133	0.194	0.311	0.194	0.091	0.500	0.182	0.123	0.182	0.000	0.000	0.195
Fumarate hydratase													
(*n*)	17	9	17	45	35	11	33	1	50	15			
A	0.471	0.333	0.647	0.478	0.486	0.545	0.394	0.500	0.480	0.467			
B	0.529	0.667	0.353	0.522	0.514	0.455	0.606	0.500	0.520	0.533			
(H)	0.471	0.222	0.353	0.378	0.457	0.545	0.364	1.000	0.480	0.667			
Mannose-6-phosphate isomerase													
(*n*)	56	60	103	103	108	55	98	24	130	55	35	30	41
A	0.250	0.167	0.107	0.257	0.218	0.136	0.255	0.542	0.354	0.327	0.114	0.100	0.220
B	0.625	0.775	0.782	0.723	0.782	0.864	0.745	0.417	0.512	0.545	0.886	0.717	0.780
C	0.125	0.058	0.112	0.019	0.000	0.000	0.000	0.042	0.135	0.127	0.000	0.183	0.000
(H)	0.500	0.383	0.369	0.398	0.361	0.236	0.408	0.583	0.638	0.655	0.229	0.333	0.293
Glucose-6-phosphate isomerase													
(*n*)	56	60	103	103	108	55	97	24	130	55	30	25	29
A	0.000	0.000	0.005	0.000	0.032	0.000	0.000	0.000	0.004	0.009	0.000	0.000	0.000
B	1.000	1.000	0.995	1.000	0.968	1.000	1.000	1.000	0.996	0.991	1.000	1.000	1.000
(H)	0.000	0.000	0.010	0.000	0.065	0.000	0.000	0.000	0.008	0.018	0.000	0.000	0.000
Phosphoglucomutase													
(*n*)	56	58	103	103	108	55	98	24	130	55	35	30	41
A	0.000	0.009	0.049	0.000	0.060	0.064	0.015	0.146	0.012	0.000	0.071	0.000	0.000
B	1.000	0.991	0.951	1.000	0.940	0.936	0.985	0.854	0.988	1.000	0.929	1.000	1.000
(H)	0.000	0.017	0.097	0.000	0.120	0.127	0.031	0.208	0.023	0.000	0.143	0.000	0.000
Transferrin													
(*n*)	56	60	102	102	104	54	97	24	130	54			
A	0.000	0.000	0.000	0.015	0.014	0.139	0.000	0.000	0.000	0.000			

APPENDIX. (Continued)

	St. Clair Marsh	Hillman Marsh	Pelee Island	North Bass Island	Middle Bass Island	Middle Island	Rattle-snake Island	West Sister Island	East Harbor	Winous Point	New York	Quebec	Wisconsin
Transferrin – continued													
B	0.009	0.025	0.010	0.010	0.029	0.139	0.005	0.042	0.015	0.046			
C	0.223	0.133	0.152	0.167	0.197	0.241	0.165	0.063	0.123	0.213			
D	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
E	0.205	0.258	0.382	0.314	0.452	0.250	0.696	0.396	0.358	0.306			
F	0.000	0.008	0.010	0.015	0.000	0.000	0.000	0.021	0.012	0.000			
G	0.563	0.542	0.446	0.480	0.308	0.222	0.134	0.479	0.492	0.417			
H	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009			
I	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
J	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.009			
(H)	0.518	0.533	0.608	0.708	0.538	0.870	0.412	0.417	0.562	0.648			