

COLOR-PATTERN VARIATION IN LAKE ERIE WATER SNAKES: THE ROLE OF GENE FLOW

RICHARD B. KING¹ AND ROBIN LAWSON²

¹Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115

²Osher Foundation Laboratory for Molecular Systematics, California Academy of Sciences, Golden Gate Park, San Francisco, California 94118, and

Leeds City Museum, Natural History Department, Municipal Buildings, Calverley Street, Leeds LS1 3AA, United Kingdom

Abstract.—In an effort to clarify the evolutionary processes influencing color-pattern variation in Lake Erie island water snake (*Nerodia sipedon*) populations, rates of gene flow among island and mainland populations were estimated from patterns of allozymic variation detected using electrophoresis. Rates of gene flow were high with Nm , the number of migrants per generation, averaging 25.5 among island sites, 9.2 between the Ontario mainland and the islands, and 3.6 between the Ohio mainland and the islands. Based on estimates of current population size from mark-recapture work and of past population size extrapolated from the extent of shoreline habitat, values of m between island and mainland populations ranged from 0.0008–0.01. Synthesis of estimates of the rate of gene flow with information on inheritance of color pattern, the strength of natural selection, and population history supports the hypothesis that color-pattern variation in island populations results from a balance between gene flow and natural selection. However, depending on the mode of inheritance of color pattern, stochastic processes such as drift may have been important in the initial stages of differentiation between island and mainland populations.

Key words.—Allozymes, gene flow, natural selection, *Nerodia sipedon*, snakes.

Received January 3, 1994. Accepted August 2, 1994.

Understanding the role of gene flow in population differentiation and speciation is central to the study of evolution (Ehrlich and Raven 1969; Endler 1973; Slatkin 1987). Although estimates of gene flow are now available for a wide variety of organisms (Wright 1978; Slatkin 1981; Larson et al. 1984; Caccone 1985; Waples 1987; Liebherr 1988), few examples exist for which such estimates can be combined with information on other evolutionary processes (natural selection, genetic drift, historical events) to illustrate how the patterns of differentiation seen in nature arise (but see McNeilly 1968; Eastale 1986; Riechert 1993). Here, we examine the role of gene flow in color-pattern differentiation among Lake Erie island and mainland populations of water snakes (*Nerodia sipedon*). Specifically, we estimate rates of gene flow among island and mainland populations from patterns of allozymic variation. We then combine these estimates with information on natural selection, color-pattern inheritance, and population history to provide an unusually complete analysis of microevolution.

Lake Erie island water snake populations exhibit continuous variation in color pattern, ranging from regularly banded morphs to uniformly gray unbanded morphs. In contrast, nearby mainland populations consist solely of banded morphs (see figures in Conant and Clay 1937; Camin and Ehrlich 1958; King 1987). Color-pattern differences between island and mainland populations have resulted in recognition of island populations as *N. s. insularum* (vs. mainland *N. s. sipedon*; Conant and Clay 1937, 1963; Cliburn 1961). The high frequency of snakes with reduced patterns (smaller and fewer dorsal and lateral bands) in island populations is attributed to natural selection by visual predators and the persistence of regularly banded morphs to gene flow from the mainland (Camin et al. 1954; Camin and Ehrlich 1958; Ehrlich and Camin 1960). This balance between selection and gene flow is often cited in evolutionary biology texts (e.g., Ford 1975; Stebbins 1977; Berry 1982; Hedrick 1983), and an exhibit illustrating it is found in the British Museum,

Natural History. However, empirical work has focused primarily on the influence of natural selection (Camin et al. 1954; Camin and Ehrlich 1958; Ehrlich and Camin 1960; King 1987, 1992, 1993b); relatively little is known about the role of gene flow.

On islands, water snakes inhabit exposed rocky shorelines whereas on the mainland they inhabit heavily vegetated marshland. Because of this habitat difference, selection imposed by visual predators apparently operates in opposite directions in island and mainland habitats. Young snakes with reduced patterns are more cryptic against island backgrounds than are fully patterned snakes (King 1992). Furthermore, on islands, young snakes with reduced patterns experience higher survivorship than do fully patterned snakes (King 1992, 1993b). In contrast, snakes with reduced patterns are less cryptic against mainland backgrounds than are fully patterned snakes (King 1992) and presumably experience reduced survivorship on the mainland. (Among older snakes, fully patterned individuals are more cryptic than are individuals with reduced patterns against island and mainland backgrounds [King 1992], but no difference in survivorship is evident, perhaps because older snakes are too large to be subdued by most predators [King 1993b].)

Evidence regarding gene flow is more speculative. Observations of water snakes “swimming several miles from the nearest shore” (Camin and Ehrlich 1958, p. 507) suggest that high levels of gene flow between mainland and island populations are possible. However, adult water snakes exhibit high site specificity (Blanchard and Finster 1933; Brown 1940; King 1987) and home rapidly following displacement (Fraker 1970). In Lake Erie island populations, fewer than 3% of adult snakes are estimated to move between islands or between localities within islands each year: marked snakes were never observed to move among study sites separated by as little as 1.3 km (King 1987).

Direct estimates of gene flow such as these, based on observed movements of known individuals, are subject to sev-

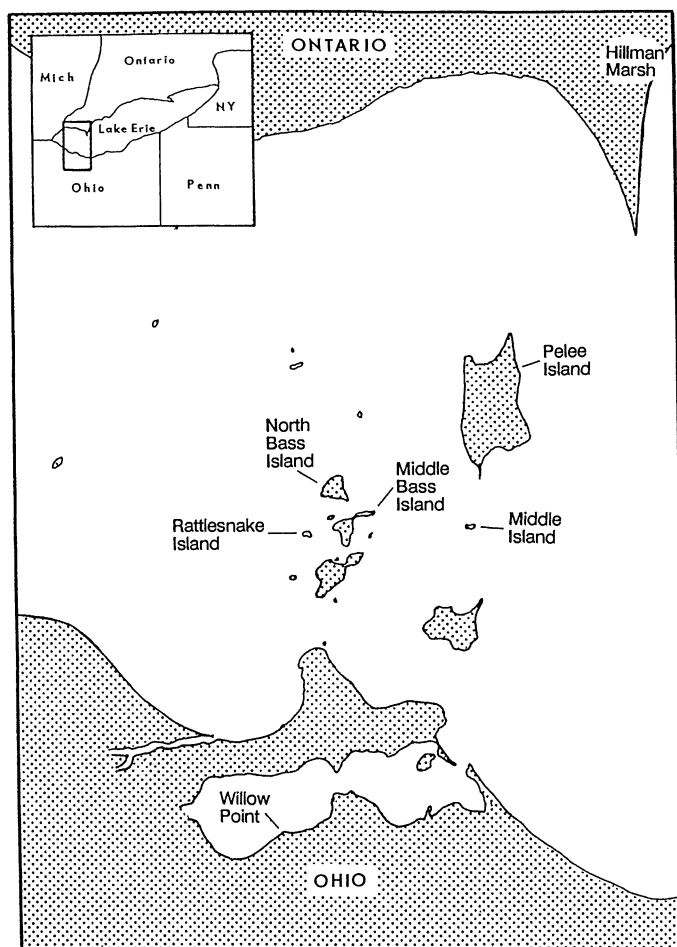


FIG. 1. The island region of western Lake Erie, showing the five island sites, the Ontario mainland site (Hillman Marsh), and the Ohio mainland site (Willow Point) included in this study.

eral sources of error (Slatkin 1987). In species in which it is difficult to mark and recapture large numbers of individuals of all age classes (e.g., snakes), it may be impossible to assess movement patterns with confidence. Dispersing individuals may be less likely to reproduce than nondispersers, resulting in overestimates of gene flow (Endler 1977), and rare or episodic dispersal may go undetected, resulting in underestimates of gene flow. Indirect estimates of gene flow, such as we use in this study, avoid these shortcomings by assessing the genetic consequences of gene flow (Slatkin 1987). These methods typically use information on the distribution of genetic markers within and among populations to estimate Nm , the number of migrants per generation (where N is effective population size and m is rate of gene flow). Estimates of Nm are of value in determining whether random genetic drift could plausibly account for population differentiation ($Nm < 1$) or whether some other process, for example, natural selection, is required ($Nm > 1$) (Slatkin 1987).

Importantly, because we have independent information on adult population size (King 1986), we are able to estimate the rate of gene flow, m , from Nm . This allows us to synthesize information on gene flow, selection, color-pattern inheritance, and population history to assess whether a balance between

TABLE 1. Geographic distance (kilometers) between Lake Erie island and mainland study sites (shortest straight-line distance determined from National Oceanic and Atmospheric Administration chart #14830).

	Pelee Island	North Bass Island	Middle Bass Island	Rattlesnake Island	Middle Island	Ohio mainland
Ontario mainland	25.5	43.5	44.2	48.9	41.5	73.0
Pelee Island		10.7	9.4	15.1	5.1	36.3
North Bass Island			1.3	3.3	9.8	29.9
Middle Bass Island				1.6	7.4	25.9
Rattlesnake Island					12.7	31.4
Middle Island						26.2

gene flow and selection provides a plausible explanation for color-pattern differences between island and mainland water snake populations. Our synthesis suggests that in nature, inheritance and population history may constrain selection and gene-flow coefficients to a narrower range than might be predicted from equilibrium models of population differentiation. Furthermore, even within this range of selection and gene-flow coefficients, stochastic processes such as random genetic drift may be necessary during the initial stages of population differentiation.

METHODS

Study Sites and Tissue Collection

Blood and muscle samples were collected nondestructively from live water snakes captured by hand at five island sites and two mainland sites, one on the Ohio mainland and one on the Ontario mainland (fig. 1). The five islands from which tissue samples were collected varied in size from 20 ha (Rattlesnake Island) to more than 4000 ha (Pelee Island), spanning nearly the full range of island sizes in Lake Erie. Islands were 9–19 km from the nearest Ohio mainland point, 13.5–33 km from the nearest Ontario mainland, and were separated from each other by 1.3–15.1 km (table 1). The two mainland sites were close to the Lake Erie shore adjacent to the island area and were 25.5–48.9 km from individual islands (table 1).

Blood samples were collected from vessels in the tail using a heparinized hypodermic syringe (Bush and 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, Wyo.). 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. Water snakes were measured to obtain snout-vent length, classified by sex and color pattern, and individually marked by scale clipping (Brown and 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 and Bennett 1989) or adult survivorship (Willis et al. 1982).

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

Protein	Locus	Tissue source	Buffer system*
Isocitrate dehydrogenase (E.C. 1.1.1.42)	<i>Idh-1</i>	rbc	A
6-Phosphogluconate dehydrogenase (E.C. 1.1.1.49)	<i>G-6-pgdh</i>	rbc	A
Adenylate kinase (E.C. 2.7.4.3)	<i>Ak</i>	muscle	A
Tripeptidase (leu-gly-gly) (E.C. 3.4.11.4)	<i>Trip-2</i>	rbc	A
Fumarate hydratase (E.C. 4.2.1.2)	<i>Fum</i>	muscle, rbc	A
Glucose-6-phosphate isomerase (E.C. 5.3.1.9)	<i>Gpi</i>	muscle	A
Transferrin	<i>Trf</i>	plasma	B

* Buffer systems: A. Electrode buffer; 0.04 M citric acid adjusted to pH 7.8 with N-(3-aminopropyl)-morpholine (Sigma A-9028), and used once only. Gel buffer; a 1:20 dilution of this electrode buffer (modified from Clayton and Tretiak 1972). B. The discontinuous TRIS-citrate buffer system of Poulik (1957).

Electrophoresis

Electrophoresis was performed 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 water snakes (Lawson 1985, 1987 and table 2 herein). Analyses focused on seven proteins found to be polymorphic in pilot surveys or known to be polymorphic in populations elsewhere. Proteins assayed were isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, adenylate kinase, tripeptidase, fumarate hydratase, glucose-6-phosphate isomerase, and transferrin (table 2). At the fumarate hydratase locus, alternative alleles are inherited in sex-linked fashion (unpubl. data); thus, for this locus we included only males in our analyses (because natricine snakes have ZW sex chromosomes [Cundall 1970], females are hemizygous at this locus and heterozygote frequencies can be determined only for males).

Analyses

Genotypic frequencies at each locus were tested for deviations from Hardy-Weinberg equilibrium conditions (Weir 1990). We computed F_{st} for each locus for all possible pairs of sites using the method of Nei and Chesser (1983) as modified by Van Den Bussche et al. (1986). Jackknifing across loci was used to generate F_{st}^* , a less biased estimate of F_{st} , and to estimate the variance in F_{st}^* (Weir and 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 $F_{st}^* \pm 1.96$ SE into this relationship (for an alternative method, see Johnson et al. 1988).

Other methods for estimating F_{st} (Nei and Chesser 1983; Weir and Cockerham 1984) gave results similar to the method of Van Den Bussche et al. (1986). We present results from the latter method because it avoids undefined, negative values for F_{st} and because it includes a correction for sample size. The lack of rare alleles in our samples precludes estimating gene flow by Slatkin's rare-allele method (Slatkin 1981, 1985a, 1985b, 1987). However, estimates of Nm based on Slatkin's method are generally similar to those based on F_{st} (Slatkin and Barton 1989).

We computed F_{st} and Nm between pairs of populations because the relationship between pairwise estimates and geographic distance provides a simple test for isolation by distance (Slatkin 1993). Statistical significance of the relationship between pairwise estimates of Nm and geographic dis-

tance was tested using the Mantel test (Douglas and Endler 1982). The form of this relationship was characterized using linear regression (Slatkin 1993).

Patterns of gene flow 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 and Selander 1981). Tree lengths were compared using FREQPARS (Swofford and Berlocher 1987). When applied to populations linked by gene flow, such tree diagrams reflect levels of gene flow rather than phylogenetic history: populations that exchange migrants only rarely are placed on more distant branches than are populations that exchange migrants more frequently.

RESULTS

The seven polymorphic loci assayed were represented by two to three alleles each in the populations sampled (tables 2, 3). Most alleles were present in all populations although allelic frequencies at some loci were quite variable (table 3). Within populations, genotypic frequencies did not deviate significantly from Hardy-Weinberg expectations ($P > 0.05$).

F_{st}^* varied from 0.005 to 0.093, and Nm varied from 2.4 to 53.1 between pairs of sites (table 4). Confidence intervals around these estimates are large because when F_{st}^* is small, as is the case here, small changes in F_{st}^* result in large changes of Nm . Rates of gene flow (Nm) decreased with increasing distance between sites (Mantel test of $\log(\text{distance})$ versus $\log(Nm)$, $t = -2.15$, $P < 0.05$, fig. 2) as expected under a model of isolation by distance (Slatkin 1993). Nm was greater between island-island pairs than between island-mainland pairs. Nm was lower between the Ohio mainland and the islands than between the Ontario mainland and the islands.

Estimates of gene flow based on patterns of genetic variation may fail to distinguish historical patterns of gene exchange from ongoing gene flow (Larson et al. 1984; Caccone 1985). For Lake Erie water snake populations this is a problem of distinguishing gene flow that occurred before the isolation of island populations from gene flow that has occurred since isolation (ca. 4000 ybp). Two lines of evidence suggest our estimates reflect ongoing gene flow. First, in the absence of selection, F_{st} (and hence Nm) approaches equilibrium rapidly (Hartl and Clark 1989). Second, no relationship between distance and Nm is seen among Lake Erie populations of

TABLE 3. Sample size (N), allelic frequency, and heterozygote frequency (H) for protein loci in Lake Erie island and mainland water-snake (*Nerodia sipedon*) populations.

Ontario mainland	Pelee Island	North Bass Island	Middle Bass Island	Rattle- snake Island	Middle Island	Ohio mainland
Isocitrate dehydrogenase-1						
N 27	108	103	44	42	25	87
A	0.037	0.019	0.049	0.011	0.059	0.000
B	0.963	0.981	0.951	0.989	0.941	1.000
H	0.074	0.037	0.097	0.023	0.119	0.000
6-Phosphogluconate dehydrogenase						
N 27	108	103	44	42	25	89
A	0.185	0.171	0.117	0.148	0.119	0.180
B	0.667	0.667	0.772	0.727	0.714	0.800
C	0.148	0.162	0.112	0.125	0.167	0.020
H	0.519	0.491	0.408	0.523	0.452	0.360
Adenylate kinase						
N 26	108	100	44	42	25	88
A	1.000	0.977	0.920	0.966	0.976	0.920
B	0.000	0.023	0.080	0.034	0.024	0.080
H	0.000	0.046	0.160	0.068	0.048	0.160
Tripeptidase-2						
N 27	106	100	44	42	25	88
A	0.296	0.518	0.415	0.500	0.440	0.600
B	0.704	0.482	0.585	0.500	0.560	0.400
H	0.444	0.453	0.470	0.455	0.357	0.480
Fumarate hydratase						
N 10	54	42	29	12	18	44
A	0.300	0.148	0.083	0.034	0.042	0.056
B	0.700	0.852	0.917	0.966	0.958	0.944
H	0.400	0.296	0.167	0.069	0.083	0.111
Glucose-6-Phosphate isomerase						
N 26	108	103	44	42	25	89
A	0.000	0.014	0.005	0.011	0.000	0.020
B	0.019	0.042	0.019	0.000	0.000	0.060
C	0.981	0.944	0.976	0.989	1.000	0.920
H	0.038	0.111	0.049	0.023	0.000	0.016
Transferrin						
N 28	110	103	45	42	24	90
A	0.500	0.545	0.631	0.667	0.488	0.563
B	0.500	0.450	0.369	0.333	0.512	0.437
C	0.000	0.005	0.000	0.000	0.000	0.000
H	0.429	0.482	0.408	0.533	0.595	0.375

garter snakes (*Thamnophis sirtalis*) or brown snakes (*Storeria dekayi*) (Lawson and King in press; King and Lawson unpubl. data), suggesting that 4000 yr has been enough time for the relationship between distance and Nm to break down, at least in some species. (Island garter snakes and brown snakes have a history of isolation similar to that of water snakes but, because they are smaller in body size and more terrestrial, garter snakes and brown snakes are expected to have lower rates of gene flow.)

Dendrograms generated using Nei's unbiased genetic identities, Cavalli-Sforza and Edward's chord distances, and modified Rogers' distances were similar in topology in that the five island sites consistently grouped together, the Ontario mainland site was on the next closest branch, and the Ohio mainland site was on the most distant branch. The tree based on modified Rogers' distances (fig. 3) was shorter (length =

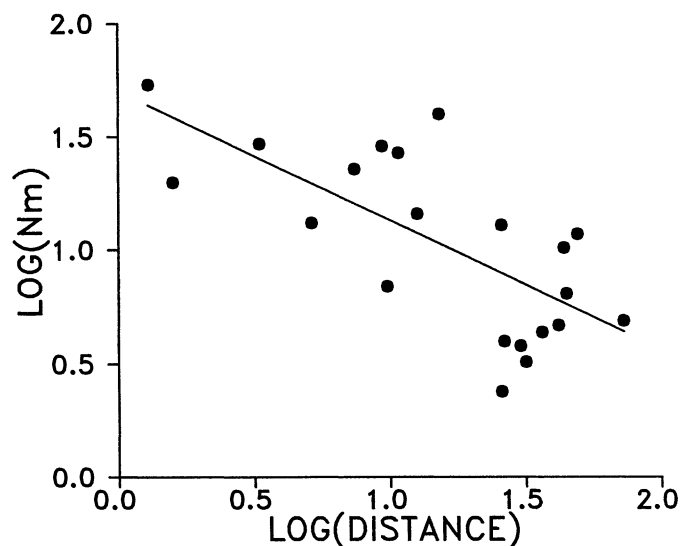


FIG. 2. Relationship between rate of gene flow and geographic distance between pairs of study sites. The regression line, $\log(Nm) = 1.70 - 0.57 \cdot \log(\text{distance})$, is included for heuristic purposes, not for significance testing (see text).

5.01) than trees based on Nei's unbiased genetic identities (length = 5.21) or Cavalli-Sforza and Edward's chord distances (length = 5.11).

SYNTHESIS

To evaluate whether gene flow and natural selection might balance each other to produce the pattern of variation seen in Lake Erie water snake color patterns, four kinds of information are required: (1) mode of inheritance, (2) strength of natural selection, (3) rate of gene flow, and (4) population history. Available information of each kind is summarized below and then used to address the question of whether observed combinations of selection and gene flow might reasonably be expected to produce the patterns of variation seen in island water snake populations. Because the direction and strength of selection is expected to be similar among the islands but to differ between islands and mainlands, and because rates of interisland gene flow are high (table 4), a simple island-continent model, in which islands are treated as a single genetic unit, is used in this synthesis.

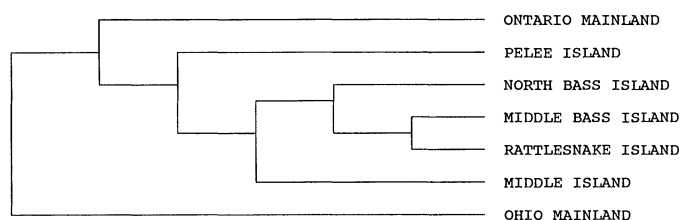


FIG. 3. Dendrogram generated using the distance-Wagner method with modified Rogers' distances, illustrating patterns of gene flow among Lake Erie island and mainland water snake populations.

TABLE 4. Estimates of F_{st}^* (standard deviation) (above diagonal) and Nm (95% confidence interval) (below diagonal) between pairs of study sites.

	Ontario mainland	Pelee Island	North Bass Island	Middle Bass Island	Rattlesnake Island	Middle Island	Ohio mainland
Ontario mainland		0.019 (0.013)	0.024 (0.011)	0.038 (0.019)	0.021 (0.016)	0.050 (0.023)	0.049 (0.028)
Pelee Island	12.8 (8.6, 25.3)		0.009 (0.001)	0.009 (0.005)	0.006 (0.003)	0.018 (0.008)	0.054 (0.032)
North Bass Island	10.3 (7.7, 15.6)	26.8 (24.9, 28.9)		0.005 (0.002)	0.008 (0.005)	0.035 (0.021)	0.062 (0.029)
Middle Bass Island	6.4 (4.6, 10.2)	29.0 (19.9, 53.1)	53.1 (41.3, 74.4)		0.012 (0.009)	0.011 (0.001)	0.093 (0.038)
Rattlesnake Island	11.7 (7.3, 27.9)	39.7 (30.6, 56.5)	29.4 (19.8, 57.1)	19.8 (12.6, 45.3)		0.017 (0.006)	0.072 (0.040)
Middle Island	4.7 (3.5, 7.2)	13.3 (9.9, 20.3)	6.9 (4.6, 12.8)	22.8 (21.4, 24.3)	14.5 (11.64, 19.3)		0.058 (0.034)
Ohio mainland	4.9 (3.4, 8.6)	4.4 (3.0, 8.0)	3.8 (2.7, 5.9)	2.4 (1.8, 3.6)	3.2 (2.2, 5.7)	4.0 (2.7, 7.2)	

Inheritance

Island water snake populations exhibit continuous variation from regularly banded to uniformly gray and unbanded, suggesting that color pattern may be inherited as a quantitative trait influenced by alleles at many loci. With this pattern of inheritance in mind, heritability of individual color-pattern components (DB, the number of dorsal blotches; LB, the number of lateral blotches; ROWS, the height of lateral blotches in scale rows; and VEXT, the extent of ventral pigmentation) was estimated as 0.20 to 0.79 (table 1 in King 1993a). However, correlations between within-family means and variances and the results of two laboratory crosses suggest that color pattern is controlled in part by a single major locus with alleles for a reduced pattern recessive to alleles for a regular (mainland) pattern (King 1993a). Given these results, we take two approaches in synthesizing the effects of gene flow and selection on island water snake color patterns. One is to assume that inheritance is Mendelian with the allele for a reduced pattern fully recessive to the allele for a regular pattern; the other is to assume that inheritance is quantitative. As will be seen below, these approaches represent opposite extremes with respect to the range of conditions under which population differentiation can occur.

Strength of Natural Selection

Estimates of the strength of natural selection operating on color pattern come from a longitudinal study in which neonates born to wild-caught females were scored for color pattern, marked, released on a single island, and resampled the following year to determine color-pattern distributions among survivors (King 1987, 1993b). Being longitudinal, this data set requires fewer assumptions (Lande and Arnold 1983) than do available cross-sectional data sets (e.g., Camin et al. 1954; Camin and Ehrlich 1958; Ehrlich and Camin 1960). It is also the largest data set (317 released, 54 recaptured) and thus should provide the most accurate estimates of selection. Finally, because snakes were scored for individual color-pattern components and as regular or reduced in pattern, this data set provides estimates of selection for both Mendelian and quantitative models of inheritance.

For the Mendelian-inheritance model, relative fitness of

regular and reduced patterns were estimated based on their frequency among released and recaptured snakes. Snakes were classified as regular or reduced by three methods, all of which gave similar results. (1) Banded morphs were classified as regular, unbanded and intermediate morphs were classified as reduced (morph criteria in King 1987). (2) Snakes falling within the ranges of DB, LB, ROWS, and VEXT scores of 145 mainland snakes were classified as regular, other snakes were scored as reduced. (3) Snakes were classified as mainland (regular) or island (reduced) using discriminant functions generated from DB, LB, ROWS, and VEXT scores of 1560 island and 145 mainland snakes. (Snakes in the mass-release experiment were not used in generating discriminant functions. Discriminant functions correctly classified 79% of island snakes and 96% of mainland snakes by site.) Relative fitnesses were computed as number recaptured/number released. These were standardized to a maximum of 1, giving relative fitness of 1 for the reduced pattern and 0.78–0.90 for the regular pattern (depending on method of classification). This is equivalent to a selection coefficient, s of 0.1–0.22.

For the quantitative-inheritance model, directional-selection differentials (in SD units) were -0.35 for DB, -0.25 for LB, -0.03 for ROWS, and -0.03 for VEXT; directional selection gradients (also in SD units) were -0.30 for DB, -0.27 for LB, 0.01 for ROWS, and 0.16 for VEXT (table 4.A in King 1993b).

Rate of Gene Flow

For the island-continent model, an estimate of gene flow, m , between the mainland and the islands is needed. In generating such an estimate, we assume that values of Nm between mainland-island pairs (table 4) reflect the movement of individuals between that mainland and the islands as a group, rather than between that mainland and the specific island in question. Our reasoning is that because interisland gene flow is common (table 4), estimates of the number of migrants moving between any mainland-island pair includes contributions from movement between that mainland and the other islands. We therefore averaged Nm between the Ohio mainland and the islands ($= 3.6$) and between the Ontario

mainland and the islands (= 9.2) and summed these values to obtain an overall estimate of Nm between the mainland and the islands of 12.8 individuals per generation. (Hierarchical F_{st} values [Weir and Cockerham 1984], generated using BIOSYS-1, gave an estimate of Nm between island and mainland regions of 14.5 individuals per generation, supporting our assumption that values of Nm between mainland-island pairs reflect the movement of individuals between that mainland and the islands as a group.)

Next, to estimate m from Nm , an estimate of current adult water snake population size of the island region ($N = 1262$; table 2 in King 1986) was used, giving $m = 0.01$. However, because confidence limits on this estimate of population size are large (523–4064; King 1986), because some water snakes occur at localities not included in this estimate (pers. obs.), and because current population sizes are markedly smaller than in the recent past (King 1986), this value may overestimate the rate of gene flow occurring before the middle of this century.

To estimate m before recent population declines, we first estimated past population size. We measured the extent of island shoreline (water snakes are restricted to shoreline habitats in the island region; King 1986) from topographical maps using a computerized digitizer and SigmaScan software (Jandel Scientific). We included all 19 islands in our analysis because historically, water snakes were found on all 19 (King unpubl. data). However, the five largest islands (Pelee, Kelleys, South Bass, Middle Bass, and North Bass) account for 80% of island shoreline. We then multiplied the extent of island shoreline (114.4 km) by 140, the mean number of adult water snakes per kilometer of shoreline observed at study sites where density remains high (range = 22–381 adults/km; table 1 in King 1986). This gave an estimate for N across all islands of 16,016 (estimates for individual islands ranged from 42 on Starve Island to 4760 on Pelee Island) and an estimate for m of 0.0008.

Population History

The geological history of the island region provides a time frame for differentiation between island and mainland populations. Islands in western Lake Erie were formed by rising lake levels following the retreat of the Wisconsin glaciation and consequent uplift of the eastern outlet of Lake Erie (Calkin and Feenstra 1985). Although glaciers retreated about 12,000 ybp, uplift sufficient to flood the shallow island region of western Lake Erie did not occur until about 4000 ybp (Calkin and Feenstra 1985). Presumably, the island region was inhabited by water snakes before island formation: isotope studies indicate that present climatic conditions date from 7000–8000 ybp (Fritz et al. 1975) and river drainages dissecting the area (e.g., Detroit, Maumee, Portage, and Sandusky rivers) would have provided typical mainland water snake habitat. Thus, island water snake populations likely originated as rising lake levels fragmented a formerly continuous population (King 1987). Before isolation, all water snake habitats were presumably similar to present-day mainland habitats and snakes with reduced patterns did not gain a selective advantage until after rising lake levels exposed the limestone and dolomite, which now comprise the island

shorelines. Using age at first reproduction (2 yr in males, 3 yr in females; Feaver 1977; King 1986) as an estimate of generation time, 4000 yr represents roughly 1000–2000 water snake generations over which selective differences between island and mainland habitats may have operated.

Combinations of Selection and Gene Flow Producing Patterns of Variation Seen in Island Water Snake Populations

Mendelian Inheritance.—Assume that (1) color pattern is inherited in Mendelian fashion with reduced pattern fully recessive to regular pattern; (2) on islands, snakes with reduced patterns have fitness $1 - h$ ($h < 0$) and snakes with regular patterns have fitness 1; (3) mainland populations are fixed for the regular pattern allele; and (4) gene flow occurs from the mainlands to the islands at rate m . Then the change in the frequency of the reduced pattern allele (q) in the island population each generation is

$$\Delta q = \left[\frac{q - hq^2}{1 - hq^2} - q \right] - mq,$$

and at equilibrium, allele frequency in the island population is

$$\hat{q} = \frac{h \pm [h^2 + 4mh(m + 1)]^{1/2}}{2(m + 1)h}$$

(after Hartl and Clark 1989, p. 310).

Replacing h with the more familiar selection coefficient, $s = 1 - [1/(1 - h)]$, snakes with regular patterns have fitness $1 - s$, snakes with reduced patterns have fitness 1, and $0 < s < 1$. Using values of 0.1–0.22 for s and values of 0.0008–0.01 for m (based on past and current population estimates), equilibrium frequencies of the reduced allele in island populations are expected to exceed 90% (fig. 4A). Present-day allele frequencies appear somewhat lower than this. Frequencies of snakes with reduced patterns (scoring unbanded and intermediate morphs as reduced and banded morphs as regular, morph criteria in King 1987) average 54% (range = 29%–74%) across nine islands (table 5A) (using color-pattern scores of mainland snakes or discriminant functions as criteria for classifying snakes as regular or reduced gives similar frequencies). Assuming Hardy-Weinberg conditions, this gives an average allelic frequency of 73% (range = 53%–86%) (table 5A). (Because selection and gene flow result in deviations from Hardy-Weinberg expectations, estimates of allelic frequency are only approximate.) However, before the 1950s, frequencies of the reduced allele (estimated from the literature and from museum specimens; table 5 in King 1987) consistently exceeded 90% (range = 92%–100%, $n = 5$ islands) (table 5B, C). Changes in the frequency of snakes with reduced patterns over time may reflect the increased effect of gene flow (increased m) as population sizes have declined (King 1987).

Considering equilibrium allele frequencies gives an incomplete picture of color-pattern evolution in Lake Erie water snake populations. This is because under the island-continent model, each combination of m and s also has a second, unstable equilibrium below which the favored allele decreases in frequency (Hartl and Clark 1989, p. 311). Thus, this un-

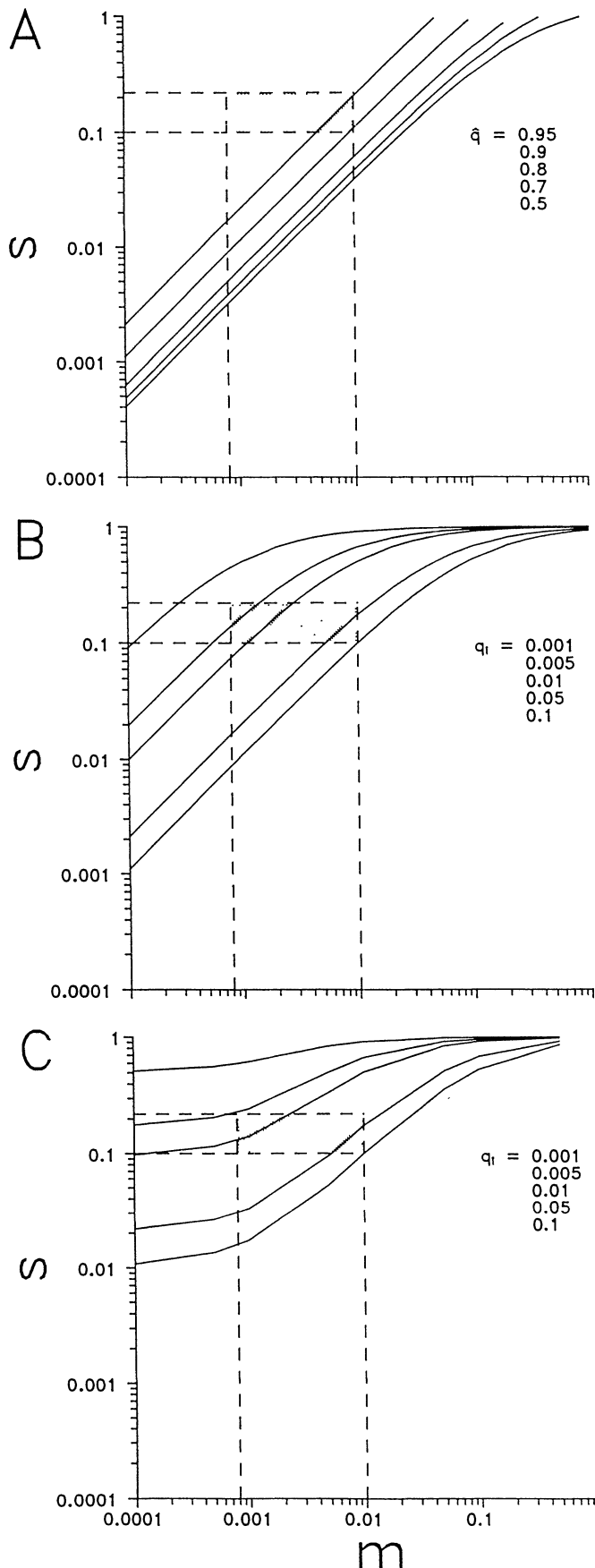


TABLE 5. Frequency of the reduced-pattern phenotype and estimated frequency of the reduced pattern allele in current and past Lake Erie water-snake populations. Estimates for current populations (A) are based on field work conducted 1980–1992. Estimates for past populations are based on published accounts (B) and museum specimens (C) as described in King (1987).

Island	N	Phenotypic frequency	Allelic frequency
A. Current populations			
East Sister Island	12	0.42	0.65
Johnsons Island	28	0.29	0.53
Kelleys Island	75	0.71	0.84
Middle Island	44	0.40	0.63
Middle Bass Island	353	0.65	0.81
North Bass Island	392	0.74	0.86
Pellee Island	600	0.48	0.69
Rattlesnake Island	45	0.56	0.75
Sugar Island	11	0.60	0.77
B. Past populations, published accounts			
Kelleys Island, pre-1937	16	0.88	0.94
Middle Island, 1949	131	0.93	0.97
Middle Bass Island, pre-1937	51	0.90	0.95
Pellee Island, pre-1937	36	1.00	1.00
C. Past populations, museum collections			
Middle Island, 1945–1958	16	0.93	0.97
Pellee Island, 1933–1937	21	0.86	0.93
1947–1950	33	0.85	0.92
South Bass Island, 1930–1940	21	0.95	0.98
1948–1953	19	0.95	0.97

stable equilibrium represents an initial allele frequency, q_i , which must be exceeded before a given combination of m and s can result in an increase in allele frequency. Using the estimates of s and m described above, values for q_i necessary to achieve equilibrium allele frequencies of greater than 50% fall between 0.001 and 0.1 (fig. 4B).

In the case of Lake Erie island water snake populations, initial allele frequencies are further constrained by the time available for differentiation to occur (ca. 1000 generations). Using the estimates of s and m described above, values of q_i necessary to achieve $q > 50\%$ within 1000 generations fall between 0.005 and 0.1 (fig. 4C). (Increasing the number of generations [e.g., to 1500] or increasing final allele frequency [e.g., to 75%] has little effect on q_i for the range of s and m used here.)

Quantitative Inheritance.—Assume (1) that inheritance is quantitative, (2) that heritability is constant through time and identical in island and mainland populations, (3) that the directional selection differential (in SD units) in island popula-

←

FIG. 4. A. Combinations of gene flow (m) and selection coefficients (s) giving stable equilibrium frequencies ($\hat{q}$) for the reduced-pattern allele of 50% or greater, based on an island-continent model in which inheritance is Mendelian and reduced pattern is fully recessive to regular pattern. B. Combinations of m and s giving stable equilibrium frequencies for the reduced pattern allele of 50%, given different initial allele frequencies (q_i). C. Combinations of m and s giving frequencies of the favored allele in island populations of 50% within 1000 generations, given different initial allele frequencies (q_i). In each panel, likely combinations of m and s in Lake Erie water snakes are shown by the shaded area.

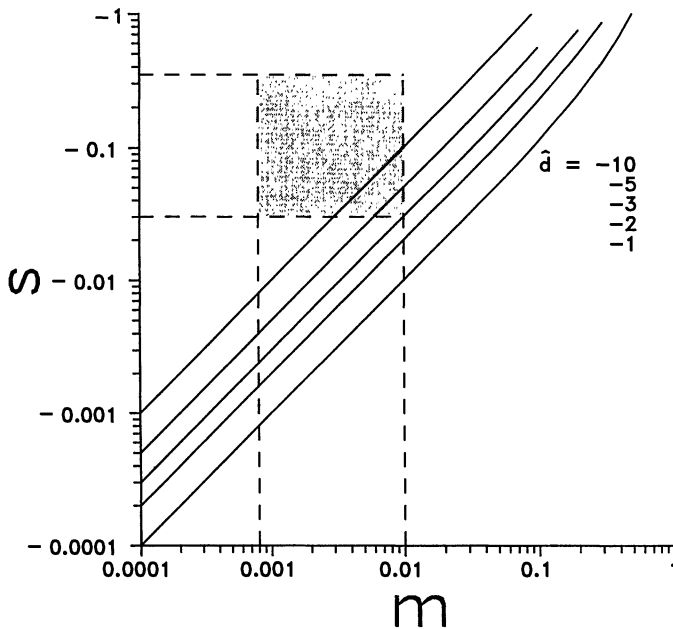


FIG. 5. Combinations of gene flow (m) and selection differentials (s) giving equilibrium differences (d) in mean color-pattern scores between island and mainland populations of 1 SD or greater based on an island-continent model with quantitative-inheritance. Likely combinations of m and s in Lake Erie water snakes are shown by the shaded area.

tions is s , (4) that gene flow occurs from mainland to island populations at rate m , and (5) that the difference in phenotypic mean between mainland and island populations (in SD units) is d . Then the equation for between-generation response to directional selection on a single phenotypic character ($r = h^2 s$) can be extended to include gene flow as

$$r = h^2[s(1 - m) + dm],$$

and the equilibrium difference between island and mainland populations is

$$\hat{d} = s(1/m - m).$$

Note that, whereas the between-generation response to selection and gene flow depends on heritability, the equilibrium difference between populations does not.

In the present case, in which directional selection differentials range from -0.03 – -0.35 among color-pattern components and m is estimated at 0.0008 – 0.01 , equilibrium color-pattern scores in island populations are expected to fall three or more standard deviations below those in mainland populations (fig. 5). Observed color-pattern scores in nine island populations average 0.8 SD (range = 0.0 – 1.7) lower than in the mainland population for DB, 0.9 SD (0.0 – 2.2) lower for LB, 2.8 SD (1.2 – 3.8) lower for ROWS, and 5.2 SD (0.8 – 6.7) lower for VEXT (where SD refers to standard deviation of mainland snakes). Furthermore, differentiation should arise rapidly; differences in mean color-pattern score of at least 1 SD are expected within 100 generations provided h^2 is at least 0.1.

This model overlooks two potentially important aspects of color-pattern evolution in Lake Erie water snakes. One is that

color-pattern components are genetically correlated so selection on one should result in correlated responses in the others (King 1993a). The other is that in addition to directional selection, quadratic selection (also termed curvilinear or variance selection) also influences water snake color patterns (King 1993b). The first of these problems can be overcome by rewriting the model as

$$\Delta \mathbf{z} = \mathbf{G}[\boldsymbol{\beta}(1 - m) + \mathbf{d}m],$$

as in multivariate selection models (Lande and Arnold 1983). Here, $\mathbf{z}$ is the vector of changes in trait means, $\mathbf{G}$ is the genetic variance-covariance matrix, $\boldsymbol{\beta}$ is the vector of standardized selection gradients, m is rate of gene flow, and $\mathbf{d}$ is the vector of trait differences between island and mainland populations. At equilibrium,

$$\hat{\mathbf{d}} = \boldsymbol{\beta}(1/m - m).$$

In the present case,

$$\mathbf{G} = \begin{bmatrix} 0.17 & 0.19 & 0.10 & 0.12 \\ 0.19 & 0.32 & 0.23 & 0.19 \\ 0.10 & 0.23 & 0.37 & 0.25 \\ 0.12 & 0.19 & 0.25 & 0.40 \end{bmatrix}$$

(computed from the full-sib analysis of King [1993a] after standardizing color-pattern scores to mean of 0 and variance of 1)

and

$$\boldsymbol{\beta} = \begin{bmatrix} -0.30 \\ -0.27 \\ 0.01 \\ 0.16 \end{bmatrix}$$

(from top to bottom, vector entries refer to DB, LB, ROWS, and VEXT; from King 1993b). This gives

$$\hat{\mathbf{d}} = \begin{bmatrix} -30.0 \\ -27.0 \\ 1.0 \\ 16.0 \end{bmatrix} \quad \text{and} \quad \begin{bmatrix} -374.7 \\ -337.2 \\ 12.5 \\ 199.8 \end{bmatrix}$$

(in SD units) for $m = 0.01$ and 0.0008 , respectively. Equilibrium differences differ from observed differences between island and mainland populations in magnitude (DB and LB) and direction (ROWS and VEXT). However, the approach to equilibrium is slow especially when m is small, and before reaching equilibrium, character means are strongly affected by correlated selection (fig. 6). For example, after 1000 generations (approximately the time for which island populations have been isolated) and with $m = 0.0008$, island water snakes are expected to have lower means than mainland water snakes for all four color-pattern components, even though, at equilibrium, means for VEXT and ROWS in island populations are expected to exceed those in mainland populations (fig. 6C). Although the direction of these nonequilibrium differences is consistent with that observed between island and mainland populations, the magnitude of these differences is unrealistically large.

Quadratic selection is not so easily incorporated into a model involving gene flow. This is because unlike the mean, the variance of a composite population (consisting of fractions m and $1 - m$ of two other populations) is not a simple function of the variances of the source populations. This dif-

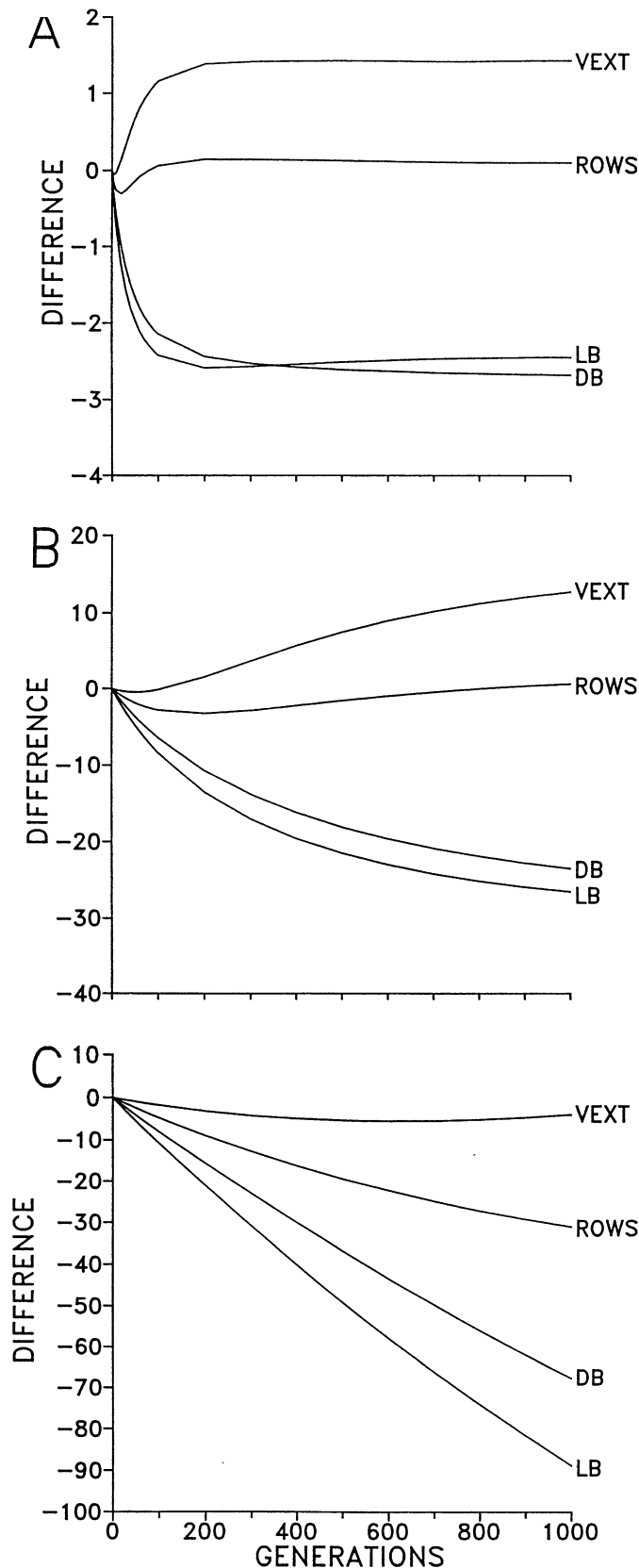


FIG. 6. Correlated evolution of differences in color-pattern components between island and mainland water snake populations (in SD units). Components include number of dorsal blotches (DB),

difficulty is unfortunate because differences in variances between island and mainland populations generally exceed differences in means. Variances in nine island populations average 12.3 (range = 2.2–18.3) times greater than on the mainland for DB, 4.1 (1.6–7.4) times greater for LB, 6.1 (3.1–8.2) times greater for ROWS, and 18.8 (5.0–24.8) times greater for VEXT. Thus, although the quantitative-inheritance model suggests that estimated selection differentials and gene-flow coefficients could give rise to differences in mean color-pattern scores that are similar in direction to those observed between island and mainland populations, model and observation differ with respect to the magnitude of these differences. Furthermore, the model fails to address differences in variance between island and mainland populations.

DISCUSSION

Estimated rates of gene flow among Lake Erie water snake populations are high, averaging 25.5 individuals per generation among islands, 9.2 individuals per generation between the Ontario mainland and the islands, and 3.6 individuals per generation between the Ohio mainland and the islands. That gene flow occurs more commonly between the Ontario mainland and the islands than between the Ohio mainland and the islands is somewhat surprising given that the Ohio site is closer to all islands but Pelee (table 1). One reason for this may be that the Catawba/Marblehead peninsula represents a barrier to gene flow from our Ohio mainland site. Regardless, estimated rates of gene flow generally decrease with increasing distance between sites (fig. 2).

High rates of gene flow preclude random genetic drift as the sole process responsible for color-pattern differences between island and mainland populations, lending credence to the hypothesis that color-pattern variation within island populations represents a balance between natural selection and gene flow. Furthermore, observed differences between island and mainland populations are in general agreement with differences predicted from our synthesis of available information on gene flow, selection, color-pattern inheritance, and population history. However, our synthesis also suggests that selection and gene flow may not have been the only processes contributing to differentiation between island and mainland populations: if reduction in color pattern is a recessive Mendelian trait, then stochastic processes (random genetic drift, inbreeding, and founder events) during the initial stages of differentiation may have been necessary to achieve allele frequencies sufficient for selection to become effective. Other models of inheritance make no such requirement on initial allele frequencies, thus further information on color-pattern inheritance would be useful.

Our synthesis is limited by the accuracy with which pop-

number of lateral blotches (LB), height of lateral blotches in scale rows (ROWS), and extent of ventral pigmentation (VEXT). Rates of gene flow are $m = 0.1$ (A), 0.01 (B), and 0.0008 (C). A is included to show the approach to equilibrium; B and C correspond to the range of gene-flow rates estimated here (note: scale of y-axis differs among panels). Lower rates of gene flow result in greater differences between populations and a slower approach to equilibrium.

ulation size, number of migrants, and selection are estimated. For example, our estimate of past population size ignores the fact that some areas of shoreline may be unsuitable as water snake habitat. Thus, we may have overestimated past population size and hence underestimated past rates of gene flow. Our synthesis is also limited by the validity of underlying assumptions. For example, levels of differentiation predicted under the quantitative-inheritance model are generally greater than is currently observed between island and mainland water snake populations. However, this model assumes that genetic variances and covariances are similar in island and mainland populations and have remained constant through time, that color pattern has been subject only to directional selection, and that G and β have been estimated with reasonable accuracy. Although these assumptions simplify the quantitative-inheritance model, possible errors in estimating G , variable evidence for directional selection (King 1987, 1993b), and the possibility that quadratic selection affects color pattern (King 1993b) suggest that results from this model be interpreted with caution.

Despite these limitations, our synthesis is useful in illustrating the range of conditions under which differentiation between island and mainland populations might occur. The two alternative models of inheritance described here represent opposite extremes in this regard. Under the Mendelian-inheritance model, selection coefficients must exceed gene flow by an order of magnitude or more in order for the reduced allele to reach a frequency of at least 50% (roughly the minimum frequency observed in any present island population, table 5) in approximately 1000 generations (fig. 4C). Additionally, given our estimates of s and m , an initial allele frequency of 0.005 must be achieved before differentiation can proceed (fig. 4C). In contrast, under the quantitative-inheritance model, differentiation of at least 1 SD (roughly the minimum difference between mainland and island color-pattern scores) will occur provided the selection differentials exceed gene flow only slightly (fig. 5). Furthermore, differentiation will proceed even if populations are initially identical.

The Mendelian-inheritance model is of further interest in that it provides a possible explanation for why pattern reduction has not arisen in other water snake populations having selective regimes that might be similar to that of the Lake Erie islands (e.g., on limestone and dolomite shores elsewhere in the Great Lakes; King 1993a): perhaps the initial allele frequency required before differentiation can proceed has not been achieved elsewhere. For a recessive allele to reach frequencies of 0.005 or greater, as may be necessary in order for selection to become effective (fig. 4C), requires a population structure in which random genetic drift, inbreeding, or founder events are likely. The degree of population subdivision imposed by island formation in Lake Erie may have been important in the initial stages of population differentiation of island and mainland water snakes.

Because other modes of inheritance do not have this initial allele-frequency requirement, it is perhaps surprising that the allele for reduced color pattern appears to be recessive. However, because recessive alleles are functionally neutral when rare, they can persist at higher frequencies than can disadvantageous dominant alleles. Thus, before the change in se-

lective regime to one favoring pattern reduction (brought about by Lake Erie island formation), recessive alleles for pattern reduction are more likely to have been present in ancestral populations than are dominant alleles. Interestingly, melanism in garter snakes (*Thamnophis sirtalis*), a trait known to be inherited as a Mendelian recessive (Blanchard and Blanchard 1940), also occurs at high frequencies in the Lake Erie area (King 1988). Melanism may provide a thermoregulatory advantage and thus be favored by natural selection in cool, lakeshore habitats (Gibson and Falls 1979, 1988). However, as suggested here for pattern reduction in island water snake populations, initial increases in the frequency of melanism in Lake Erie garter snakes may have required genetic drift, inbreeding, or founder events, and may have been facilitated by population subdivision.

Our suggestion that population subdivision was important in the initial stage of differentiation between Lake Erie island and mainland water snake populations and that natural selection has subsequently carried island populations toward a new adaptive peak is not unlike the processes envisioned in Wright's shifting-balance theory (Wright 1931, 1988).

ACKNOWLEDGMENTS

Funding for this project was provided by the National Science Foundation (DEB-9107382), the Ontario Ministry of Natural Resources, the Ohio Department of Natural Resources, and the California Academy of Sciences. Permits were provided by the Ontario Ministry of Natural Resources, the Canadian Wildlife Service, the Essex Region Conservation Authority, the Ohio Department of Natural Resources, the Ottawa National Wildlife Refuge, the United States Department of Agriculture, and the United States Fish and Wildlife Service. Research protocols were approved by the Indiana University Bloomington Animal Resources Committee, Northern Illinois University Institutional Animal Care and Use Committee, and the Ontario Ministry of Natural Resources Animal Care Committee. Logistical support was provided by the Ohio State University F. T. Stone Laboratory. We thank M. Slatkin and D. Swofford for copies of computer programs; J. Hageman and D. Insley for ably piloting us among the islands; the McTavish family for their hospitality; T. Near for assistance in the field; G. Anderson, M. Crowe, M. Johns, B. King, C. Lynch, C. Trombino, and two anonymous reviewers for comments on the manuscript; and our wives for tolerating yet another tissue-collecting trip.

LITERATURE CITED

- Ayala, F. J., J. R. Powell, M. L. Tracy, C. A. Mourao, and S. Perez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics* 70:113-139.
- Berry, R. J. 1982. Neo-Darwinism. Arnold, London.
- Blanchard, F., and F. Blanchard. 1940. The inheritance of melanism in the garter snake *Thamnophis sirtalis sirtalis* (Linnaeus) and some further evidence of effective autumn mating. *Papers of the Michigan Academy of Sciences, Arts and Letters* 26:177-193.
- Blanchard, R. N., and E. B. Finster. 1933. A method of marking live snakes for future recognition with a discussion of some problems and results. *Ecology* 14:334-347.
- Brown, E. E. 1940. Life history and habits of the northern water snake, *Natrix sipedon sipedon* Linne. Ph.D. diss. Cornell University, Ithaca, N.Y.

- Brown, W. S., and W. S. Parker. 1976. A ventral scale clipping system for permanently marking snakes (Reptilia, Serpentes). *Journal of Herpetology* 10:247–249.
- Bush, M., and J. Smeller. 1978. Blood collection and injection techniques in snakes. *Veterinary Medicine/Small Animal Clinician* 73:211–214.
- Caccone, A. 1985. Gene flow in cave arthropods: a qualitative and quantitative approach. *Evolution* 39:1223–1235.
- Calkin, P. E., and B. H. Feenstra. 1985. Evolution of the Erie-basin Great Lakes, Pp. 149–170 in P. F. Karrow and P. E. Calkin, eds. *Quaternary evolution of the great lakes*. Geological Association of Canada, St. Johns, Newfoundland.
- Camin, J. H., and P. R. Ehrlich. 1958. Natural selection in water snakes (*Natrix sipedon* L.) on islands in Lake Erie. *Evolution* 12:504–511.
- Camin, J. H., C. Triplehorn, and H. Walter. 1954. Some indications of survival value in the type “A” pattern of the island water snakes in Lake Erie. Chicago Academy of Sciences, Natural History Miscellanea 131:1–3.
- Clayton, J. W., and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* 29:1169–1172.
- Cliburn, J. 1961. The taxonomic position of *Natrix sipedon insularum* Conant and Clay. *Herpetologica* 17:166–168.
- Conant, R., and W. Clay. 1937. A new subspecies of watersnake from the islands in Lake Erie. University of Michigan Occasional Papers of the Museum of Zoology 346:1–9.
- . 1963. A reassessment of the taxonomic status of the Lake Erie water snake. *Herpetologica* 19:179–184.
- Cundall, D. L. 1970. Chromosomal analysis of three North American species of water snakes (genus *Natrix*). M.S. thesis, University of Arkansas, Fayetteville.
- Douglas, M. E., and J. A. Endler. 1982. Quantitative matrix comparisons in ecological and evolutionary investigations. *Journal of Theoretical Biology* 99:777–795.
- Easteal, S. 1986. The ecological genetics of introduced populations of the Giant Toad, *Bufo marinus*. IV. Gene flow estimated from admixture in Australian populations. *Heredity* 56:145–156.
- Ehrlich, P. R., and J. Camin. 1960. Natural selection in Middle Island water snakes (*Natrix sipedon* L.). *Evolution* 14:136.
- Ehrlich, P. R., and P. H. Raven. 1969. Differentiation of populations. *Science* 165:1228–1232.
- Endler, J. A. 1973. Gene flow and population differentiation. *Science* 179:243–250.
- . 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, N.J.
- Feaver, P. E. 1977. The demography of a Michigan population of *Natrix sipedon* with discussions of ophidian growth and reproduction. Ph.D. diss. University of Michigan, Ann Arbor.
- Ford, E. 1975. Ecological genetics. Academic Press, New York.
- Fraker, M. A. 1970. Home range and homing in the water snake, *Natrix sipedon sipedon*. *Copeia* 1970:665–673.
- Fritz, P., T. W. Anderson, and C. F. M. Lewis. 1975. Late-quaternary climatic trends and history of Lake Erie from stable isotope studies. *Science* 190:267–269.
- Gibson, A. R., and J. B. Falls. 1979. Thermal biology of the common garter snake *Thamnophis sirtalis* (L.) II. The effects of melanism. *Oecologia* 43:99–109.
- . Melanism in the common garter snake: a Lake Erie phenomenon. Pp. 223–245 in J. F. Downhower, ed. *The biogeography of the island region of western Lake Erie*. Ohio State University Press, Columbus.
- Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics, 2d ed. Sinauer, Sunderland, Mass.
- Hedrick, P. W. 1983. Genetics of populations. Science Books International, Boston.
- Jayne, B. C., and A. F. Bennett. 1989. The effect of tail morphology on locomotor performance of snakes: comparison of experimental and correlative methods. *Journal of Experimental Zoology* 252:126–133.
- Johnson, M. S., B. Clarke, and J. Murray. 1988. Discrepancies in the estimation of gene flow in *Partula*. *Genetics* 120:233–238.
- King, R. B. 1986. Population ecology of the Lake Erie water snake, *Nerodia sipedon insularum*. *Copeia* 1986:757–772.
- . 1987. Color pattern polymorphism in the Lake Erie water snake, *Nerodia sipedon insularum*. *Evolution* 41:241–255.
- . 1988. Polymorphic populations of the garter snake *Thamnophis sirtalis* near Lake Erie. *Herpetologica* 44:451–458.
- . 1992. Morph and age specific crypsis in Lake Erie water snakes. *Evolutionary Ecology* 6:115–124.
- . 1993a. Color pattern variation in Lake Erie water snakes: inheritance. *Canadian Journal of Zoology* 71:1985–1990.
- . 1993b. Color pattern variation in Lake Erie water snakes: prediction and measurement of natural selection. *Evolution* 47:1819–1833.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Larson, A., D. B. Wake, and K. P. Yaney. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics* 106:293–308.
- Lawson, R. 1985. Molecular studies of the thamnophiine snakes. Ph.D. diss. Louisiana State University, New Orleans.
- . 1987. Molecular studies of thamnophiine snakes. I. The phylogeny of the genus *Nerodia*. *Journal of Herpetology* 21:140–157.
- Lawson, R., and R. B. King. In press. Gene flow and melanism in Lake Erie garter snake populations. *Biological Journal of the Linnean Society*.
- Liebherr, J. K. 1988. Gene flow in ground beetles (Coleoptera: Carabidae) of differing habitat preference and flight-wing development. *Evolution* 42:129–137.
- McNeilly, T. 1968. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. *Heredity* 23:99–108.
- Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1990. Proteins I. Isozyme electrophoresis. Pp. 45–126 in D. M. Hillis and C. Moritz, eds. *Molecular systematics*. Sinauer, Sunderland, Mass.
- Nei, M., and R. K. Chesser. 1983. Estimation of fixation indices and gene diversities. *Annals of Human Genetics* 47:253–259.
- Poulik, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature* 180:1477–1479.
- Riechert, S. A. 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. *Behavioral Ecology and Sociobiology* 32:355–363.
- Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics VI*. University of Texas Publications 7103:49–90.
- Slatkin, M. 1981. Estimating levels of gene flow in natural populations. *Genetics* 99:323–335.
- . 1985. Rare alleles as indicators of gene flow. *Evolution* 39:53–65.
- . 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16:393–430.
- . 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787–792.
- . 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264–279.
- Slatkin, M., and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43:1349–1368.
- Stebbins, G. 1977. Processes of organic evolution. Prentice-Hall, Englewood Cliffs, N.J.
- Swofford, D. L., and S. H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. *Systematic Zoology* 36:293–325.
- Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72:281–283.
- Van Den Bussche, R. A., M. J. Hamilton, and R. K. Chesser. 1986. Problems of estimating gene diversity among populations. *Texas Journal of Science* 38:281–287.

- Waples, R. S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* 41:385–400.
- Weir, B. S. 1990. *Genetic data analysis*. Sinauer, Sunderland, Mass.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F -statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- Willis, L., S. T. Threlkeld, and C. C. Carpenter. 1982. Tail loss patterns in *Thamnophis* (Reptilia: Colubridae) and the probable fate of injured individuals. *Copeia* 1982:98–101.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics* 16: 97–159.
- . 1943. Isolation by distance. *Genetics* 28:114–138.
- . 1969. *Evolution and genetics of populations*, Vol. 2. The analysis of gene frequencies. University of Chicago Press, Chicago.
- . 1978. *Evolution and the genetics of populations*, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.
- . 1988. Surfaces of selective value revisited. *American Naturalist* 131:115–123.

Corresponding Editor: T. J. Hilbish