The temporal and spatial scale of microevolution: fine-scale colour pattern variation in the Lake Erie watersnake, *Nerodia sipedon insularum*

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ABSTRACT

Question: What is the temporal and spatial scale of microevolution?

Hypotheses: The combined effects of natural selection and gene flow result in variation in heritable traits on fine spatial and geographic scales.

Organism: The Lake Erie watersnake, *Nerodia sipedon insularum*.

Field site: US and Canadian islands in western Lake Erie.

Methods: We tested for variation in colour pattern frequency within islands, among islands, and over time using data from nearly annual censuses conducted since 1980, museum specimens, and published sources. We compared F_{ST} for a presumptive major colour pattern locus to F_{ST} for allozyme loci to determine whether spatial variation exceeded that expected by chance. We computed effective population size (N_e) based on temporal frequency changes in presumptive colour pattern alleles to determine whether temporal variation exceeded that expected by chance (N_e significantly less than ∞).

Conclusions: Morph frequencies did not differ significantly within islands or between islands separated by short distances. Morph frequencies did sometimes differ significantly among distant islands and among sampling periods from 1980 to the present, but no more than expected by chance. In contrast, a marked change in morph frequency occurred between historic (prior to 1961) and recent (1980–2003) samples. Possible mechanisms include changes in the strength of selection (due to changes in predator assemblages and visual environments) and rates of gene flow (due to changes in island watersnake population size).

Keywords: colour pattern, gene flow, islands, population genetics, selection, snakes.

INTRODUCTION

Evolution by natural selection can occur rapidly, on a fine spatial scale, and in the presence of significant gene flow (e.g. Bronikowski and Arnold, 1999; Hendry and Kinnison, 1999; Schluter, 2000; Pergrams and Ashley, 2001; Hendry *et al.*, 2002; Pfenning, 2003; Stockwell *et al.*, 2003; Verheyen *et al.*, 2003; Bell *et al.*, 2004;

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Blumstein *et al.*, 2004; Gilchrist *et al.*, 2004; Hargeby *et al.*, 2004; Yeh, 2004; Garant *et al.*, 2005; Postma and van Noordwijk, 2005). These are outcomes not anticipated by Darwin (1859) or widely appreciated by the framers of the evolutionary synthesis (Provine, 1971). Importantly, they have the potential to impinge on ecological processes in ways not previously recognized (Hairston *et al.*, 2005). As a consequence, case studies in which natural selection is thought to be operating only over long time periods and on coarse spatial scales warrant re-examination. Such analyses can provide information on the spatial and temporal constancy of microevolutionary processes, allow refined prediction of evolutionary outcomes, and guide management strategies for threatened and endangered species. In an effort to better understand the spatial and temporal scale of microevolution, we examine short-term fine-scale colour pattern variation in the Lake Erie watersnake, *Nerodia sipedon insularum*.

Restricted to islands of western Lake Erie, *N. s. insularum* is distinguished as a subspecies by a reduced and highly variable dorsal banding pattern (see Figure 2 in King, 1987; Figure 2 in King and Lawson, 1997) compared with mainland populations of Northern watersnakes (*N. s. sipedon*), all of which are regularly banded along their entire length (Conant and Clay, 1937). Studies of *N. s. insularum* have documented that colour pattern variation is heritable (King, 1993a), is a target of natural selection [visual predators overlook snakes with reduced patterns along exposed rocky island shorelines and snakes with regular patterns in more heavily vegetated mainland habitats (Camin *et al.*, 1954; Camin and Erhlich, 1958; Erhlich and Camin, 1960; King, 1992, 1993b)], and is influenced by gene flow between islands and the mainland (Camin and Ehrlich, 1958; King and Lawson, 1995; Hendry *et al.*, 2001). Moreover, random genetic drift may have played a role in the initial differentiation of island and mainland populations (King and Lawson, 1995). Thus, colour pattern variation in *N. s. insularum* serves as a textbook example of the combined effects of multiple microevolutionary processes (Freeman and Herron, 1998; Hedrick, 2000; Freeman, 2005). A restricted geographic distribution and declining population size have resulted in the listing of Lake Erie watersnakes as threatened in the United States and endangered in Canada (McKeating and Bowman, 1977; Fazio and Szymanski, 1999; US Fish and Wildlife Service, 2003).

Watersnake morph frequencies vary spatially and temporally within the island region (Conant and Clay, 1937; King, 1987), but neither the scale nor cause of this variation is well understood. Differences in morph frequency among islands have been attributed to differences in distance, and hence rate of gene flow, from mainland populations (Conant and Clay, 1937). However, the relationship between morph frequency and distance is not strong (King, 1987) and sites within islands and island groups have sometimes been pooled for analysis (Camin and Ehrlich, 1958). In snakes, significant genetic structure can occur over short distances (i.e. one to a few kilometres) (Gibbs *et al.*, 1997; King and Lawson, 2001), hence analysis of finer scale variation in colour pattern is warranted.

Evidence for temporal variation in watersnake morph frequency comes from comparisons of published data and museum collections from before 1961 with frequencies observed from 1980 to 1985 (King, 1987). These comparisons suggest that regularly patterned morphs have increased markedly in island populations since the early- to mid-1900s (King, 1987). Possible causes have been suggested [e.g. increased impact of mainland immigrants on declining island populations, lack of selection – gene flow equilibrium, change in selection regime (King, 1987; King and Lawson, 1995)], but remain untested. In this paper, we use a large (>6000 captures), long-term (1980–2003) data set to characterize spatial and temporal scales of colour pattern variation in Lake Erie watersnakes. We integrate this information with patterns of allozyme variation (King and Lawson, 1995, 2001) to test whether observed patterns deviate from those expected by chance.

METHODS

We collected data as described in King (1986) during four time periods (1980–1985, 1988–1992, 1996–1998, 2000–2003; hereafter referred to as Periods 1, 2, 3, and 4 respectively) spanning 24 years. Colour pattern was scored on a three-category ordinal scale [unbanded, intermediate, banded (defined in King, 1987)] and a two-category scale [regular vs. reduced (defined in King and Lawson, 1995)] based on evidence of a major gene effect with regular (banded) dominant to reduced (unbanded and intermediate) (King, 1993a). Results were qualitatively similar using the three- and two-category scales, so we only report the former here. Immature watersnakes were excluded because morph frequencies differ among age classes (King, 1993b); recaptures were excluded to meet the assumption of independence (watersnakes were individually marked by clipping ventral scales or through the use of PIT tags). During Periods 1 and 2, snakes were scored for colour pattern exclusively by one of the authors (R.B.K.). During Period 3 (1996–1998), most snakes were scored by A. Queral-Regil following field training by R.B.K.; the remainder were scored by R.B.K. During Period 4, most snakes were scored by R.B.K.; the remainder were scored by J.M.R., K. Stanford, M. Andre, or K. Bekker following field training by R.B.K.

Comparative data on morph frequency before 1980 came from published reports (Conant and Clay, 1937; Camin *et al.*, 1954) and examination of museum collections (raw data in King, 1985, 1987). Morph classification schemes in published reports were converted to the three-morph scheme as follows: types A and ab = unbanded, types B, bc, and C = intermediate, types cd and D = banded (King, 1987). Analysis of museum specimens was restricted to individuals older than 1 year [>270 mm snout-to-vent length (King, 1987)] from islands with sizeable samples (7–33 snakes) collected over relatively short time intervals (3–13 years).

Spatial variation in colour pattern

To determine the extent of spatial variation, watersnakes were sampled at multiple sites on the largest islands in Lake Erie (Kelleys, 5 sites; Middle Bass, 3 sites; Pelee, 3 sites; South Bass, 2 sites), a single site on North Bass Island, and on four small islands (Gibraltar, Middle, Rattlesnake, Sugar) (Fig. 1). Sites within islands were separated by 0.3–10.5 km, small islands were 0.2–5.1 km from the nearest large island, and large islands were 0.8–28.0 km from each other [distances determined from NOAA Chart #14830 or digital orthophoto quadrangles using ArcView; the locations of individual study sites are illustrated in Ray (2004)]. To maximize sample size and minimize possible confounding effects of temporal variation, tests for differences in morph frequency within and among islands used data from a single period (1980–1985 for Pelee Island, 2000–2003 for other islands). Independence of morph frequency among sites was tested within each island using Pearson's χ^2 statistic. When no differences in morph frequency were found (Results), sites within islands were pooled and χ^2 was used to determine whether morph frequency was independent of island within each period for those large islands for which data were available (not all islands were sampled in all periods). When significant differences were found among islands within a period, follow-up analyses were conducted to identify subsets of islands not differing in morph frequency. These involved comparisons among the three Bass Islands, between the Bass Islands and Pelee Island, between the Bass Islands and Kelleys Island, and between Pelee Island and Kelleys Island. Finally, χ^2 was used to test for independence of site and morph frequency for each small island and its nearest large-island neighbour.
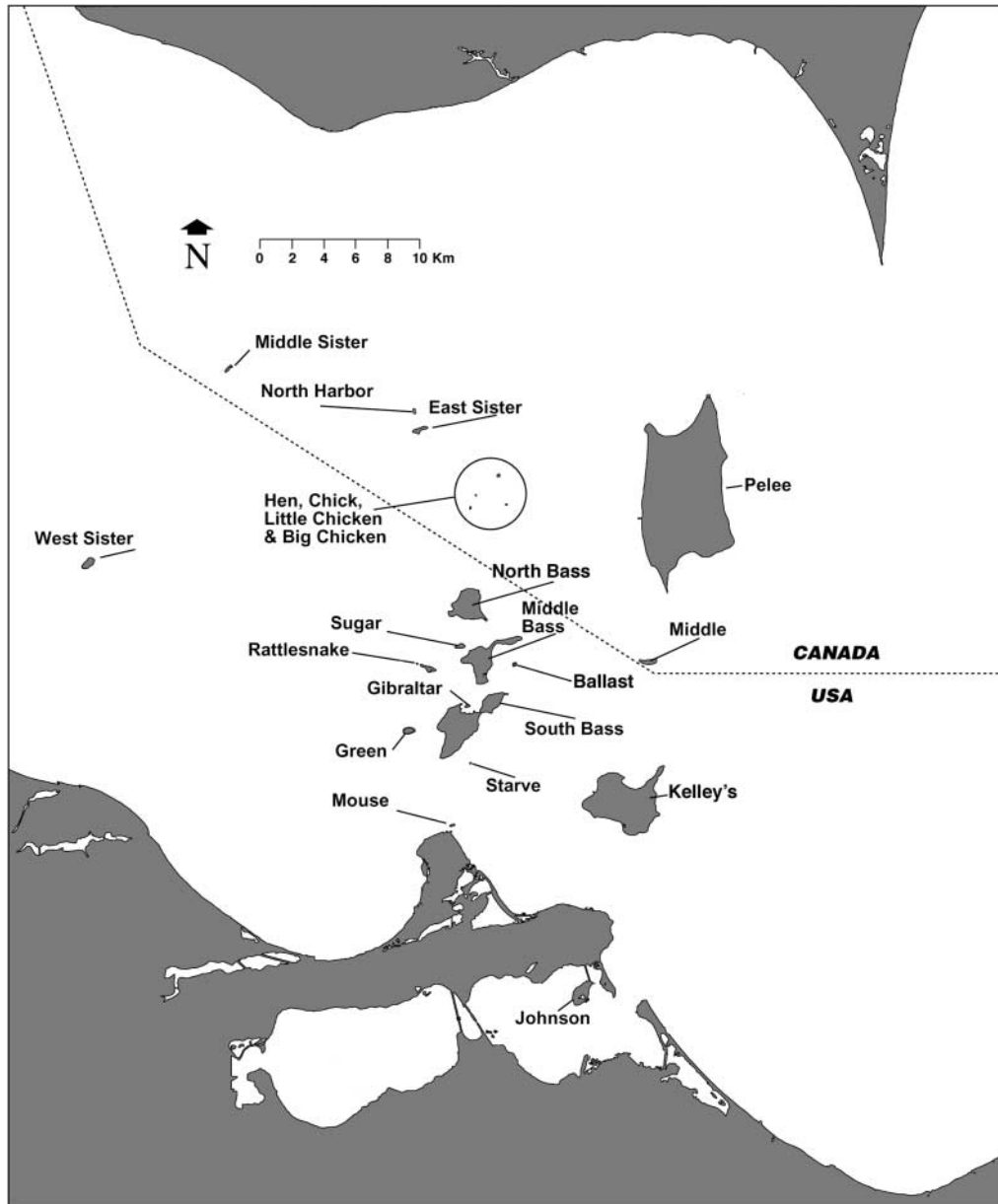
Fig. 1. The island region of western Lake Erie.

Temporal variation in colour pattern

Log-linear analysis was used to test for temporal variation in morph frequency on the six islands sampled in two or more periods (Kelleys, South Bass, Middle, Middle Bass, North Bass, Pelee). After confirming the absence of a morph-by-island-by-period interaction,

significance of the morph-by-period interaction was tested by comparing a model that included all two-way interactions to one that included only the morph-by-island and island-by-period interaction. [The morph-by-island interaction was expected to be significant *a priori* based on differences in morph frequency among islands found in this study and in King (1987). The island-by-period interaction was significant by design because not all islands were sampled equally in all periods.] Ordinal regression was used to test for temporal trends with morph as an ordered categorical dependent variable, island as a factor, and time period (1980–1985, 1988–1992, 1996–1998, 2000–2003) as a covariate. This analysis was repeated to include historical data (pre-1961) from Pelee, Kelleys, Middle, South Bass, and Middle Bass Islands. Historical data were treated as a single time period because sample sizes were sometimes small, some collection dates were only approximate, and morph frequencies among historical samples from a given island were not significantly different (data not shown).

RESULTS

Spatial variation in colour pattern

A total of 1965 adult snakes were analysed for differences in morph frequency among sites within islands (Pelee, $n = 189$, 43–88 per site; Kelleys, $n = 939$, 51–369 per site; Middle Bass, $n = 365$, 53–216 per site; South Bass, $n = 472$, 207–265 per site). Morph frequencies did not differ within islands (Table 1A). Small sample size at some sites meant that differences in morph frequency may have gone undetected (e.g. frequencies of banded morphs differed by as much as 19% among sites within an island). However, within-island comparisons for which sample sizes were large (>100 per site) revealed only small differences in morph frequency (e.g. frequencies of banded morphs differed by less than 2% among three sites on Kelleys Island and two sites on South Bass Island). Given these results, sites within islands were pooled for further analyses.

Altogether, 3628 adult snakes were analysed for differences in morph frequency among islands. In time Periods 1, 2, and 4, tests for differences in morph frequencies among large islands were significant (Table 1B). Follow-up analyses suggested that morph frequencies did not differ among the Bass Islands (South Bass, Middle Bass, North Bass) but did differ between these islands and Pelee Island. Morph frequencies on Kelleys Island were similar to those on the Bass Islands in Periods 1 and 3 and to those on Pelee Island in Period 4. Frequency of banded morphs was highest on Pelee Island (0.41–0.48), and lower on the other large islands (Kelleys, 0.25–0.33; South Bass, 0.28–0.34; Middle Bass, 0.32–0.39; North Bass, 0.24–0.28; Table 2). In general, morph frequencies on small islands did not differ significantly from those on the nearest large island (Table 1C); however, sample sizes for small islands were small ($n = 15$–43). Morph frequencies differed between Pelee and Middle Island in Period 1 but not Period 2 (Table 1C).

Temporal variation in colour pattern

A total of 3513 adult snakes were included in log-linear analysis of temporal variation in morph frequency from 1980 to 2003. The morph-by-island-by-period interaction was not significant ($G = 32.09$, d.f. = 30, $P = 0.364$) but the morph-by-period interaction was

Table 1. Tests for independence of watersnake morph frequency (A) among sites within islands, (B) among large islands, and (C) between small islands and the nearest large island

Time period	Islands	χ^2	d.f.	P
A. Among sites within islands				
Period 1	Pelee (3 sites, $n = 189$)	3.00	4	0.558
Period 4	Kelleys (5 sites, $n = 939$)	12.33	8	0.137
Period 4	Middle Bass (3 sites, $n = 365$)	5.39	4	0.249
Period 4	South Bass (2 sites, $n = 472$)	3.68	2	0.159
B. Among large islands				
Period 1	Pelee ($n = 195$), Kelleys ($n = 108$), North Bass ($n = 230$), Middle Bass ($n = 102$)	21.47	6	**0.002**
	North Bass, Middle Bass*	3.19	2	0.203
	North Bass, Middle Bass, Kelleys*	3.66	4	0.454
	North Bass, Middle Bass, Pelee	20.70	4	**<0.001**
	Pelee, Kelleys*	8.14	2	**0.017**
Period 2	Pelee ($n = 156$), North Bass ($n = 108$), Middle Bass ($n = 61$)	13.84	4	**0.008**
	North Bass, Middle Bass*	2.83	2	0.243
Period 3	Kelleys ($n = 146$), North Bass ($n = 36$), Middle Bass ($n = 117$), South Bass ($n = 97$)	4.54	6	0.604
Period 4	Pelee ($n = 56$), Kelleys ($n = 980$), North Bass ($n = 153$), Middle Bass ($n = 423$), South Bass ($n = 499$)	35.91	8	**<0.001**
	North Bass, Middle Bass, South Bass*	8.26	4	0.083
	North Bass, Middle Bass, South Bass, Kelleys*	34.13	6	**<0.001**
	North Bass, Middle Bass, South Bass, Pelee*	18.84	6	0.093
	Kelleys, Pelee*	1.73	2	0.422
C. Between small islands and nearest large island				
Period 1	Pelee ($n = 195$), Middle ($n = 27$)	6.67	2	**0.036**
Period 2	Pelee ($n = 156$), Middle ($n = 19$)	3.39	2	0.183
Period 2	Middle Bass ($n = 61$), Rattlesnake ($n = 15$)	0.44	2	0.803
Period 3	Middle Bass ($n = 117$), Sugar ($n = 34$)	0.73	2	0.694
Period 4	Middle Bass ($n = 423$), Sugar ($n = 23$)	1.26	2	0.533
Period 4	South Bass ($n = 499$), Gibraltar ($n = 43$)	1.17	2	0.556

Note: Period 1 = 1980–1985, Period 2 = 1988–1992, Period 3 = 1996–1998, Period 4 = 2000–2003. Number of sites within islands (A) and sample sizes (A, B, C) are shown in parentheses. Significant *P*-values are shown in **bold**. In (B), all large islands for which morph frequency data were available were compared, followed by comparisons among subsets of large islands (follow-up comparisons are marked with asterisks).

($G = 102.42$, d.f. $= 36$, $P < 0.001$). Thus, morph frequency did vary among time periods. When the islands were analysed separately, all islands except Middle Island showed significant temporal variation ($P < 0.05$) (sample sizes for Middle Island were small; Table 2). However, examination of morph frequencies across periods revealed no strong pattern of change that was consistent among islands (Table 2, Fig. 1). Ordinal regression confirmed that there was no significant temporal trend from 1980 to 2003 (effect of period: Wald statistic $= 0.026$, $P = 0.872$), whereas there were significant differences in morph frequency

Table 2. Temporal variation in Lake Erie watersnake morph frequency

Island	Date	Source	n	Frequency		
				Unbanded	Intermediate	Banded
Pelee	1933–1937	Museum	21	0.43	0.43	0.14
	Before 1937	Conant and Clay (1937)	36	0.61	0.39	0.00
	1947–1950	Museum	33	0.42	0.42	0.15
	1959–1961	Museum	7	0.14	0.439	0.43
	1980–1985	This study	195	0.11	0.47	0.42
	1989–1991	This study	156	0.21	0.31	0.48
	2003	This study	56	0.20	0.39	0.41
Middle	1949	Camin *et al.* (1954)	131	0.68	0.25	0.07
	1945–1958	Musuem	16	0.50	0.44	0.06
	1980–1985	This study	27	0.22	0.22	0.56
	1988–1992	This study	19	0.05	0.47	0.47
Kelleys	Before 1937	Conant and Clay (1937)	16	0.13	0.75	0.13
	1980–1984	This study	108	0.22	0.48	0.30
	1996–1998	This study	146	0.48	0.27	0.25
	2000–2003	This study	980	0.21	0.46	0.33
North Bass	1980–1985	This study	230	0.24	0.53	0.24
	1988–1992	This study	108	0.22	0.51	0.27
	1996–1998	This study	36	0.39	0.36	0.25
	2001–2003	This study	153	0.37	0.35	0.28
Middle Bass	Before 1937	Conant and Clay (1937)	51	0.28	0.63	0.10
	1981–1984	This study	102	0.19	0.49	0.32
	1989–1992	This study	61	0.18	0.43	0.39
	1996–1998	This study	117	0.38	0.29	0.33
	2001–2003	This study	423	0.28	0.34	0.38
South Bass	1893–1901	Museum	9	0.22	0.44	0.33
	1930–1940	Museum	21	0.48	0.48	0.05
	Before 1937	Conant and Clay (1937)	157	0.39	0.56	0.04
	1948–1953	Museum	21	0.29	0.57	0.14
	1996–1998	This study	97	0.41	0.31	0.28
	2001–2003	This study	499	0.28	0.38	0.34

Note: Data from museum specimens are from King (1987).

among islands, as anticipated from analysis of spatial patterns of variation (for individual islands, Wald statistic = 0.789–11.79, $P = 0.001$–0.374). When ordinal regression analysis was repeated to include historical data, there was a significant temporal trend with banded morphs occurring consistently less frequently among samples collected before 1961 than in samples from 1980 to the present (Wald statistic = 47.498, $P < 0.001$) (Table 2, Fig. 1).

DISCUSSION

Spatial variation in colour pattern

Previous analyses of colour pattern variation in Lake Erie watersnakes have focused on differences between island and mainland populations. These analyses have demonstrated that differences in selective regimes are responsible for marked differences in morph frequency over a distance of about 15 km (Camin *et al.*, 1954; Camin and Ehrlich, 1958; Ehrlich and Camin, 1960; King, 1993b). Analyses presented here indicate that morph frequencies also differ significantly, although to a lesser extent, among islands. Differences in morph frequency were most apparent between Pelee and the Bass Islands, locations separated by a minimum of 9.4 km. Other locations separated by comparable distances also sometimes differed significantly in morph frequency (e.g. Pelee and Middle Island in Period 1, separated by 5.1 km; Kelleys Island and the Bass Islands in Period 4, separated by 7.7 km; Kelleys and Pelee Island in Period 1, separated by 9.5 km). However, neither sites within islands (separated by 0.3–10.5 km) nor islands separated by only short distances (the Bass Islands, 0.8–4.5 km; Rattlesnake and Middle Bass Island, 1.6 km; Sugar and Middle Bass Island, 0.4 km; Gibraltar and South Bass Island, 0.2 km) differed significantly. These observations are consistent with the pattern of isolation by distance seen in allozyme-based estimates of gene flow among island and mainland sites (King and Lawson, 1995). Apparently, gene flow within islands and between close islands occurs frequently enough that detectable differences in morph frequency are lacking, whereas islands separated by greater distances (e.g. Pelee and the Bass Islands) exchange migrants less frequently and are less homogeneous in morph frequency.

Temporal variation in colour pattern

Analyses presented here demonstrate relative stability in morph frequencies since 1980. Although log-linear analysis revealed significant differences in morph frequency among periods, ordinal regression suggests that there was no temporal trend to these differences. Examination of morph frequencies (Table 2) suggests that unbanded and intermediate morphs have varied more over time than have banded morphs. This may reflect differences in morph classification among investigators. In particular, more unbanded and fewer intermediate morphs were recorded during Period 3 (1996–1998), whereas the frequency of banded morphs varied little (Table 2). In contrast to relative constancy of morph frequencies since 1980, a marked increase in the frequency of banded morphs occurred some time before 1980 (from 7–13% banded in samples from before 1961 to >25% banded in 1980–2003 samples; Table 1, Fig. 2).

Synthesis

Differences in morph frequency between island and mainland watersnake populations are readily explained by differences in selective regimes (Camin *et al.*, 1954; Camin and Ehrlich, 1958; Ehrlich and Camin, 1960; King, 1992, 1993b). Reasons for spatial and temporal variation in morph frequency within the island region are less clear. In particular, two patterns warrant explanation: (1) spatial and temporal variation in morph frequency since 1980, and (2) increases in banded morph frequency from historic (1893–1961) to recent (1980–2003) times.
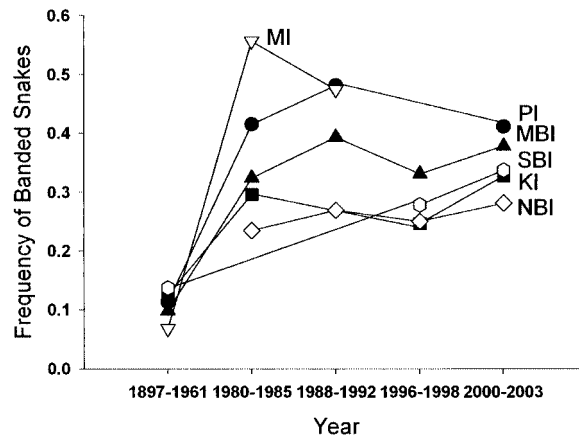
Fig. 2. Temporal change in morph frequency (frequency of banded morph) in *Nerodia sipedon insularum* on six islands. MI = Middle Island, PI = Pelee Island, MBI = Middle Bass Island, SBI = South Bass Island, KI = Kelleys Island, NBI = North Bass Island. Not all islands were sampled in all periods. Data for 1897–1961 are from museum specimens and published frequencies.

Patterns of allozyme variation within and among island watersnake populations (King and Lawson, 1995, 2001) provide a context for interpreting spatial variation in morph frequency since 1980. In particular, allozyme-based estimates of F_{ST} provide a measure of population divergence owing to neutral processes [see Bittner and King (2003) for evidence that allozyme variation is functionally neutral in syntopic gartersnake populations]. Diversifying and stabilizing selection on other traits (e.g. colour pattern) should result in higher and lower F_{ST} values, respectively. Because colour pattern is apparently influenced by a major locus with a regular pattern of dorsal and lateral blotches dominant to a reduced pattern (King, 1993a), colour pattern allele frequencies can be estimated as the square root of the frequency of reduced pattern morphs (see, for example, Table 5 in King and Lawson, 1995), making it possible to compute F_{ST} for this trait. We note that allele frequency estimates generated in this way may be biased because selection favouring reduced pattern morphs in island populations will result in genotype frequencies that deviate from Hardy-Weinberg equilibrium. However, the magnitude of this bias is small. Under directional selection favouring a recessive allele, the ratio of estimated to true allele frequency is ≤1.06 for a wide range of allele frequencies and selection coefficients (data not shown). Based on seven allozyme loci, genetic differentiation among five island watersnake populations is low with $F_{ST} = 0.016$ (see Table 1 in King and Lawson, 2001). F_{ST} for the putative colour pattern locus is in close agreement with this value, equalling 0.018 for the nine islands included here and 0.019 for the five islands for which allozyme data are available (in computing F_{ST} for the putative colour pattern locus, colour pattern allele frequencies were estimated separately for each island and time period and then averaged across time periods). In contrast, F_{ST} for the putative colour pattern locus is 0.493 (compared with 0.0737 for allozymes) when Ohio and Ontario mainland populations, where regular pattern alleles appear to be fixed (Conant and Clay, 1937; King and Lawson, 1995), are included.

Observed allele frequencies at the putative colour pattern locus also provide a means for understanding temporal variation in morph frequency since 1980. Temporal changes in allele frequency can be used to estimate effective population size (N_e) and yield $N_e = \infty$ when

changes can be explained by sampling error alone (Nei and Tajima, 1981; Waples, 1989). Using the formulation in Waples (1989) for N_e and its 95% confidence interval, none of 13 estimates of N_e generated from differences in putative colour pattern allele frequencies between sampling periods on seven islands differed significantly from ∞ (note that numerical estimates of N_e depend on the estimated generation time but tests of whether N_e differs from ∞ do not). This result suggests that although morph frequencies sometimes differed significantly, underlying allele frequencies showed no greater variation than expected from sampling error alone. In contrast, four of five estimates of N_e generated from differences in putative colour pattern allele frequencies between historic and recent samples do differ significantly from ∞ (Middle Bass, Middle, Pelee, and South Bass but not Kelleys Island), suggesting that changes in colour pattern allele frequency over this longer time frame are greater than expected from sampling error. The observation that frequencies of the regular pattern allele (and banded morphs; Fig. 2) have increased on all five islands suggests that deterministic processes (e.g. an island-wide change in strength of selection or rate of gene flow) are responsible.

Temporal variation in strength of selection may have occurred as a result of changes in predator assemblages and visual environments. Nesting colonies of gulls and herons are present on some islands but not others, and the density and location of these colonies have changed over time (Weseloh et al., 1988). Feral pigs, released in the 1800s to reduce snake populations, are no longer present (Hatcher, 1945; Conant, 1997). Human persecution of watersnakes has sometimes been severe (Ehrlich and Camin, 1960; Conant, 1997) but has been reduced through legal prohibitions and public outreach efforts (US Fish and Wildlife Service, 2003). Potential fish predators on watersnakes have changed (Reutter and Hartman, 1988), as has the visual environment in which these predators encounter watersnakes. Water clarity and rooted aquatic vegetation within the island region both decreased during a period of extreme eutrophication in the 1960s and 1970s (Stuckey, 1971, 1989; Bolsenga and Herdendorf, 1993). More recently, water clarity and rooted aquatic vegetation have increased as a result of reductions in nutrient loading and the inadvertent introduction of filter-feeding zebra and quagga mussels (Holland, 1993; Stuckey and Moore, 1995; McGuken, 2000; Ludsin et al., 2001). Although the timing of eutrophication of the lake coincides with increases in the frequency of banded watersnakes, a mechanistic connection remains elusive. Further information on important predators and the visual environment under which predation occurs would be of value.

Temporal variation in rate of gene flow may have occurred as a result of changes in watersnake population size. Island watersnake populations decreased dramatically during the 1900s but have recently begun to increase, possibly as a result of reduced human impacts and a new and abundant food source, the round goby, an invasive fish introduced into the Great Lakes in the 1990s (Ray, 2004; King et al., 2006). Decreases in island watersnake population size may have resulted in an increased impact of mainland immigrants, consistent with increases in the frequency of regularly banded morphs seen from historic to recent times (King, 1987). However, morph frequencies have remained relatively stable since 1980 despite increases in island watersnake population size – increases that should decrease the impacts of mainland immigrants. Finally, genetic models of watersnake evolution using empirical estimates of selection and gene flow suggest that island and mainland populations should show even greater differences in morph frequency (King and Lawson, 1995; Hendry et al., 2001). The higher-than-predicted frequency of banded morphs in island populations led King and Lawson (1995) to suggest that island populations were still approaching selection-gene flow equilibrium. However, increases (rather than decreases) in the frequency of banded morphs

in island populations between historic and recent samples, and stability of morph frequencies since 1980, contradicts this interpretation. In short, explanations for changes in morph frequency between historic and recent samples remain speculative.

Before the present analysis, colour pattern differences between Lake Erie island and mainland watersnake populations were understood to represent a case of rapid evolution on a fine spatial scale. Colour pattern differences arose in about 4000 years and over a distance of approximately 15 km (King and Lawson, 1995, 1997). Our results demonstrate that colour pattern variation has also been influenced by microevolutionary processes operating over a much shorter time frame, a result consistent with other long-term studies (Milner et al., 1999; Grant and Grant, 2002). Recognizing the temporal and spatial scale of microevolutionary processes also has conservation implications (e.g. Stockwell et al., 2003; Stockwell and Ashley, 2004). Our analysis suggests that selection acts uniformly within the island region and that observed differences in morph frequency among islands are small and no greater than would be expected by chance given current patterns of gene flow. This observation, coupled with high rates of gene flow among islands (King and Lawson, 1995), suggests that the island region as a whole represents a single evolutionary unit. Thus, for conservation purposes, the entire island region might be treated as a single management unit. Furthermore, concerns that gene flow might swamp the effects of selection following decreases in island population size (King, 1987) appear not to have materialized (cf. Rhymer and Simberloff, 1996). However, islands or sites within islands may represent independent or semi-independent demographic units or a metapopulation requiring a more complex management strategy. This possibility is addressed by the *Population Persistence* criterion of the Lake Erie Watersnake Recovery Plan (US Fish and Wildlife Service, 2003), which specifies that both overall and island-specific population size requirements be met on the US islands before delisting of the Lake Erie watersnake be considered. Continued monitoring of population size and morph frequency, together with demographic analyses, will aid in refining management strategies and may provide further insight into the evolutionary causes of fine-scale spatial and temporal variation.

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