Variable and Asymmetric Introgression in a Hybrid Zone in the Toads, *Bufo americanus* and *Bufo fowleri*

DAVID M. GREEN AND CHRISTINE PARENT

The structure and dynamic history of a mosaic hybrid zone are investigated using an analysis of morphometric variation to determine the extent of interbreeding between the species involved in different regions. The hybrid zone between the toads *Bufo americanus* and *Bufo fowleri* is geographically widespread. Natural hybrids occur in Ontario at Long Point but not along the nearby Niagara peninsula although the species are sympatric in both places. Discriminant analysis of variation in 27 morphological characters from 286 preserved museum specimens of male toads and putative hybrids was compared with previous allozyme genetic characterization of the same specimens. Reference samples from allopatric populations were used for comparison. *Bufo americanus* from Long Point were significantly different from other samples of the same species, demonstrating introgression in the direction of sympatric *B. fowleri*. Long-term hybridization in this region has been confined to Long Point despite sympatry elsewhere. Thus, the hybrid zone between these toads does not conform to gradient or "tension zone" models. Rather, it is a mosaic in that hybrids appear at some times and in some places but not in others and it conforms to the concept of a "localized sympatric" hybrid zone.

THE biogeographic expression of interspecific hybridization is a hybrid zone, definable as an instance where two distinguishable species that overlap both spatially and temporally interbreed to produce viable and at least partially fertile offspring (Arnold, 1997). Thus, a hybrid zone is that place and time where and when you find hybrids. Hybrid zones among animals were once presumed to be temporary phenomena in evolutionary time (Mayr, 1942; Remington, 1968). Under the view that species are reproductively isolated, by definition, and characterized by tightly coadapted complexes of genes, natural hybridization must be considered maladaptive in that it necessarily disrupts the gene complexes. It cannot be seen as having much evolutionary importance, either, other than that disruptive selection against inferior hybrids should lead to greater isolation of gene pools, character displacement and reinforcing speciation (Mayr, 1970). It was thus logically concluded that natural hybridization should only lead to either speciation (i.e., complete reproductive isolation) through reinforcement or to the fusion of the two species involved through introgression (Remington, 1968).

More recent models of interspecific hybrid zones, though, have been derived based upon the hybridizing interactions themselves and not upon expectations from a strict biological species concept. Rather than dictate instability, more realistic theories have tried instead to account for the evident persistence of many, if not most, hybrid zones (Harrison, 1993, Arnold, 1997). Natural hybridization is a dynamic phenomenon, and hybrids may be continuously generated by mismatings between the species concerned even though there is selection against those hybrids (Barton and Hewitt, 1985). Prevailing models of hybridizing interactions differ largely in their expectations of the fitness of the hybrid offspring. Spatially, hybrid zones may be manifested as "tension zones," that is, gradients or clines located between the ranges of species or else may take the form of mosaics consisting of dispersed regions or patches where hybridization occurs (Harrison, 1986; Arnold, 1997; Klingenberg et al., 2000).

Amphibians are particularly useful in the study of concepts of hybridization because of the ease with which interspecific crosses occur in nature. The *Bufo americanus* group toads are notorious hybridizers (Blair, 1963, 1972; Green, 1996). The American toad, *B. americanus*, enters into hybrid zones in the form of clines both at the northwest of its range with the Canadian toad, *Bufo hemiophrys* (Cook, 1983; Green, 1983; Green and Pustowka, 1997), and at the southeast of its range where it contacts the Southern toad, *Bufo terrestris* (Neill, 1949; Weatherby, 1982). Hybridization between *B. americanus* and Fowler's toad, *Bufo fowleri*, however, is geographically widespread (Jones, 1973; Green, 1984, 1996) and does not conform to a gradient or tension zone model for a hybrid zone (Barton and Hewitt, 1985; Arnold, 1997).

Bufo americanus and *B. fowleri* have extensively overlapping geographical ranges. *Bufo american-*

us occurs from southern Labrador and northern Ontario south to the edge of the Gulf Coastal Plain, whereas *B. fowleri* occurs from the Great Lakes to the Gulf coast (Conant and Collins, 1991). Natural hybrids have been documented from various regions of their overlapping range since early this century, including records from North Georgia (Allard, 1908), Illinois (Brown, 1964; Hubbs, 1918), Indiana (Blair, 1941; Cory and Manion, 1955; Volpe, 1952), New Jersey (Myers, 1927; Zweifel, 1968), and southern Ontario (Green, 1984). Artificial crosses between the species produce viable F_1 progeny (Blair, 1941), and both artificial and natural F_1 hybrids are fertile in backcrosses (Green, 1981).

In southern Ontario, *B. americanus* and *B. fowleri* are known to hybridize at Long Point, a sand spit extending 35 km into Lake Erie and the only site along the Lake Erie shoreline where hybrid individuals have been found in appreciable numbers (Green, 1984). Although both species also live along the Lake Erie shoreline of the Niagara Peninsula, no hybrids have been recorded there. This suggests either that there is differential isolation of breeding activities of the two species in the two regions or that hybrids have simply not been detected. *Bufo americanus* typically prefers forest and forest edge habitats, whereas *B. fowleri* usually occupies open, sandy areas such as lakeshores and dunes (Blair 1941; Wright and Wright, 1949; Breden, 1988); yet both species breed in similar shallow water habitats. Ordinarily, *B. americanus* tends to breed earlier and at colder temperatures than *B. fowleri* (Barbour, 1971; Green, 1982). Nevertheless overlaps in breeding season do occur and the species may emerge from winter dormancy almost synchronously under the right spring weather conditions (Green, 1984).

Understanding the structure and dynamics of a mosaic hybrid zone requires determining the extent and history of interbreeding and backcrossing between the species involved. This is possible with *B. americanus* and *B. fowleri* at Long Point and along the Niagara Peninsula using an analysis of morphological variation. Because morphologies are generated by large numbers of individual genes, we reason that we can detect the legacy of past hybridization, or its absence, among preserved specimens without recourse to genetic analysis of a more limited suite of loci. The results of this morphological approach can be compared to the genetic analysis of allozymes previously done by Green (1984). In this manner, we tested whether hybridization has happened along the Niagara Peninsula and the extent and direction of intro-
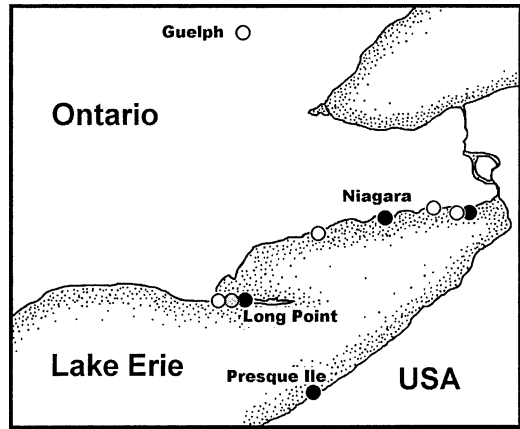

Fig. 1. Eastern Lake Erie and adjacent southern Ontario showing sample localities of toads. The northern limit of *Bufo fowleri* is along the north shore of Lake Erie. Open circles indicate samples of *Bufo americanus*, closed circles indicate samples of *B. fowleri*, the grey circle at Long Point indicates the collection of hybrid individuals.

gression between the two species in those places where hybrization has occurred.

MATERIALS AND METHODS

We examined 286 preserved museum specimens of *B. americanus*, *B. fowleri*, and their putative hybrids, which had been collected from sites along the Lake Erie shoreline in the Niagara Peninsula and at Long Point, in southern Ontario, Canada (Fig. 1; Appendix 1). The majority of these specimens had been collected between 1978 and 1981 and are deposited at the Canadian Museum of Nature, Ottawa. These were the same specimens previously studied with allozyme electrophoresis of allozyme variation by Green (1984). Other specimens had been collected between 1990 and 1995 and are deposited in the Redpath Museum, McGill University, Montreal. For the calculation of discriminant function scores, we used reference samples of *B. americanus* from Guelph, Ontario (Canadian Museum of Nature), north of the range of *B. fowleri*, and of *B. fowleri* from Presque Ile, Pennsylvania (Carnegie Museum, Philadelphia) on the south shore of Lake Erie (Appendix 1). Putative hybrids among the specimens examined had all been identified upon collection according to their calls (Zweifel, 1968; Green, 1982) and the extent of ventral spotting, which is a reliable physical characteristic to discriminate between the two species and their hybrids (Wright and Wright, 1949; Green, 1984). Thus, although ventral spotting has been used previ-

TABLE 1. MORPHOLOGICAL CHARACTERS AND THEIR WEIGHTS USED IN CALCULATING DISCRIMINANT FUNCTION SCORES OF HYBRIDIZING TOADS.

		Character	Discriminant weight
1	SVL	snout–vent length	0.075
2	NS	nostril separation	0.773
3	CLR	cranial crest length (right)	−3.079
4	CLL	cranial crest length (left)	1.809
5	NPR	nostril to parotoid (right)	−1.519
6	NPL	nostril to parotoid (left)	1.179
7	CA	cranial crest width (anterior)	−1.114
8	CP	cranial crest width (posterior)	−0.251
9	HW	head width	0.913
10	TD	tympanum diameter	−0.269
11	PLR	parotoid length (right)	−0.045
12	PLL	parotoid length (left)	−0.370
13	PWR	parotoid width (right)	−0.810
14	PWL	parotoid width (left)	0.072
15	PS	parotoid separation	0.466
16	SLR	length of largest spot (right)	−0.258
17	SLL	length of largest spot (left)	0.409
18	SWR	width of largest spot (right)	−0.540
19	SWL	width of largest spot (left)	1.095
20	WWR	wart width (right)	0.058
21	WWL	wart width (left)	−1.032
22	WSR	number of warts per spot (right)	0.385
23	WSL	number of warts per spot (left)	−0.004
24	T	tibia length	0.996
25	WTR	diameter of largest wart on tibia (right)	−1.556
26	WTL	diameter of largest wart on tibia (left)	−1.026
27	TAL	tarsus length	−0.891

ously in several studies of *B. americanus* and *B. fowleri* (Cory and Manion, 1955; Zweifel, 1968; Jones, 1973), it was omitted in the morphometric analysis. Thus, seven samples of toads were examined, all males, all of snout–vent length > 45 mm so as to exclude juveniles: *B. americanus* from Long Point ($n = 43$), from the Niagara Peninsula ($n = 44$), and from Guelph (reference, $n = 25$), *B. fowleri* from Long Point ($n = 64$), from the Niagara Peninsula ($n = 62$), and Presque Ile (reference, $n = 24$), and putative hybrids of *B. americanus* and *B. fowleri* ($n = 24$) all of which came from Long Point. All specimens used for morphometric analysis were fixed in 10% formalin and preserved in 70% ethanol for at least a year to control for shrinkage artifacts in preservation (Lee, 1982).

We used similar measurements and method for discriminant function analysis as described by Cook (1983) and Green and Pustowka (1997) in their studies of hybridization involving *B. americanus* and related species. On all specimens, 27 morphological characters (Table 1) were measured with dial calipers to the nearest 0.1 mm. Morphological features peculiar to toads that were available for measure included

aspects of the bony cranial crests present on the top of the head and the paired, poison-bearing parotoid glands at the back of the head behind each eye. These have been used as diagnostic features distinguishing *B. americanus* from *B. fowleri* in other studies (Blair, 1941; Jones, 1973). Measurements were taken on both sides of the body to detect and control for morphological asymmetry.

Sympatric samples of the two species, as well as the reference samples, were analyzed by means of canonical discriminant analysis, calculated using Systat version 9 software, to determine the characters contributing most to their divergence, judged by means of *F*-statistics. The two reference samples of relatively "pure" species were further employed to establish a univariate discriminant function (see Cook, 1983) that would maximally separate individuals of either species. Using the canonical discriminant function coefficients obtained for each morphological character, a discriminant function (df) score < 0 for increasingly *B. americanus*-like toads and > 0 for increasingly *B. fowleri*-like toads could be calculated for every individual. Significant differences between the df scores of

the samples could be examined using *t*-tests at $\alpha = 0.05$. Because it has been shown in at least one instance (Dowling et al., 1989) that evidence of hybridization from morphology and from allozymes are not necessarily correlated, individual df scores were also compared with individual genetic hybrid index scores (**B**) previously calculated for most of the same individuals by Green (1984) based on five diagnostic allozyme loci. The correlation between morphological and genetic variation was tested with an *F*-statistic. Comparison of sample means was done via multivariate discriminant anaylsis employing all seven samples. Morphological asymmetry was measured for nine pairs of characters by computing the variance of the scaled differences between measures from the left and right sides of the body.

RESULTS

The discriminant analysis was able to distinguish unambiguously between putatively pure individuals of either species in sympatry, as well as among the reference samples. For the reference samples, the most significant characters were tibia length ($F = 9.73$), right cranial crest length ($F = 4.76$), right parotoid gland width ($F = 3.82$), left spot width ($F = 3.14$), and tarsus length ($F = 3.14$). For the Niagara samples, the most significant characters were tibia length ($F = 18.05$), parotoid gland separation ($F = 15.19$), right largest wart length ($F = 14.50$), left largest wart length ($F = 6.63$), left nostril to parotoid gland distance ($F = 6.61$), and right parotoid gland length ($F = 5.98$). For the Long Point samples, the most significant characters were parotoid gland separation ($F = 21.03$), right largest wart length ($F = 3.70$), left largest wart length ($F = 3.19$), nostril separation ($F = 3.65$), and snout–vent length ($F = 3.36$). Thus, the same sets of characters were not necessary the most important differentiating features at each site (Table 2). In general, *B. fowleri* were smaller, with shorter legs, shorter head, and smaller, more widely separated parotoid glands than *B. americanus*. But *B. fowleri* at Long Point were shorter, with shorter tibiae and tarsi, and with not so widely separated parotoid glands compared to *B. fowleri* from the other sites. *Bufo fowleri* from both Long Point and Niagara had smaller spots than *B. americanus* but not so the *B. fowleri* from Presque Isle. At Long Point, both species had smaller warts than conspecific individuals from elsewhere. There were no detectably different levels of morphological asymmetry between samples.

The reference samples were, necessarily, well differentiated from each other morphologically (Fig. 2) using the discrimant function. The mean df score for the reference sample of *B. americanus* from Guelph was -4.00 ± 1.08 (range: -6.07 to -1.97) and 4.79 ± 0.91 (range: 3.15 to 6.22) for the reference sample of *B. fowleri* from Presque Ile, PA. Overall, individual df scores ranged from -7.47 for a *B. americanus* (NMC 21783–2, from Pt. Abino in the Niagara sample) to 7.04 for a *B. fowleri* (NMC 29404–7, likewise from Pt. Abino in the Niagara sample).

Samples of *B. americanus* and *B. fowleri* from both Niagara and Long Point were significantly different from each other in terms of df score ($P < 0.01$). The means of the samples of the two species from Long Point were, however, considerably closer to each other and, unlike the Niagara samples, there was substantial overlap in df score distribution (Fig. 2). For Niagara samples, the mean df score for *B. americanus* was -3.47 ± 1.61 (range $= -7.47$ to 0.03) and 3.88 ± 1.11 (range = 1.37 to 7.04) for *B. fowleri*. For Long Point samples, though, the mean df score for *B. americanus* was -1.38 ± 1.86 (range $= -5.22$ to 2.32) and 2.97 ± 0.95 (range $= -0.36$ to 4.94) for *B. fowleri*. The sample of hybrids had a mean df score of 0.30 ± 1.41 (range $= -3.65$ to 2.69) and differed significantly from all pure samples of either species from Long Point at $P < 0.01$. The genetic hybrid index, **B**, devised by Green (1984) based on five isozyme loci, correlated significantly with the df scores per individual ($r = 0.778$, $P < 0.001$; Fig. 3).

Among the samples of *B. americanus* in the multivariate discriminant analysis (Fig. 4), toads from Long Point were significantly different from the toads from both Niagara ($P < 0.01$) and the reference ($P < 0.01$), but the Niagara sample and the reference sample from Guelph were not significantly different ($P = 0.11$). All three samples of *B. fowleri* were significantly different from each other at $P < 0.01$, with the sample from Presque Ile the most divergent (Fig. 5). The characters most contributing to the separation of these seven samples (Table 2) were tibia length ($F = 12.14$), posterior cranial crest width ($F = 9.12$), parotoid gland separation ($F = 7.76$), anterior cranial crest width ($F = 7.62$), tarsus length ($F = 6.76$), left largest wart length ($F = 5.06$), and snout–vent length ($F = 4.08$). The hybrids, overall, were more similar to the sympatric *B. americanus* than they were to the sympatric *B. fowleri* (Fig. 5), with considerable overlap in their distributions in the first two dimensions (Fig. 4).

TABLE 2. Dimensions (in mm) of the 12 Most Significant Morphological Characters (Means ± Standard Errors) Distinguishing Samples of *Bufo americanus* from *Bufo fowleri*, and Each from Their Hybrids.

Character	*Bufo americanus*			Hybrids	*Bufo fowleri*		
	Guelph	Niagara	Long Point		Long Point	Niagara	Presque Ile
Snout–vent length	62.12 ± 0.75	65.2 ± 0.91	62.33 ± 1.19	55.61 ± 0.70	49.79 ± 0.33	58.59 ± 0.47	58.49 ± 0.79
Parotoid separation	7.96 ± 0.24	8.65 ± 0.17	8.98 ± 0.19	8.67 ± 0.20	8.91 ± 0.08	10.57 ± 0.10	10.68 ± 0.20
Tibia	24.89 ± 0.37	25.78 ± 0.35	24.29 ± 0.53	21.84 ± 0.32	20.89 ± 0.18	24.98 ± 0.18	24.99 ± 0.45
Right largest wart length	3.42 ± 0.10	3.40 ± 0.07	2.88 ± 0.11	2.00 ± 0.09	1.12 ± 0.03	1.39 ± 0.04	1.42 ± 0.05
Left largest wart length	3.50 ± 0.10	3.49 ± 0.09	2.88 ± 0.12	2.09 ± 0.08	1.08 ± 0.03	1.36 ± 0.03	1.28 ± 0.04
Left spot width	3.91 ± 0.14	3.57 ± 0.09	3.62 ± 0.15	3.14 ± 0.07	2.22 ± 0.04	2.88 ± 0.07	3.76 ± 0.10
Nostril separation	3.72 ± 0.04	3.98 ± 0.06	3.68 ± 0.07	3.50 ± 0.04	3.52 ± 0.03	3.70 ± 0.03	3.61 ± 0.04
Tarsus length	16.30 ± 0.27	16.38 ± 0.27	15.02 ± 0.38	12.68 ± 0.18	12.12 ± 0.14	13.47 ± 0.11	14.8 ± 0.23
Right cranial crest length	13.27 ± 0.16	13.69 ± 0.15	13.15 ± 0.20	12.15 ± 0.13	11.25 ± 0.07	12.21 ± 0.08	12.37 ± 0.15
Right parotoid width	7.96 ± 0.17	7.22 ± 0.15	6.72 ± 0.14	6.26 ± 0.11	5.73 ± 0.06	6.33 ± 0.09	5.95 ± 0.15
Right parotoid length	12.76 ± 0.20	12.85 ± 0.22	11.85 ± 0.27	11.45 ± 0.19	10.48 ± 0.09	12.08 ± 0.12	11.98 ± 0.22
Left nostril–parotoid distance	13.82 ± 0.19	14.43 ± 0.17	14.11 ± 0.25	12.55 ± 0.13	11.86 ± 0.08	12.84 ± 0.09	12.97 ± 0.16

References

Niagara

Long Point

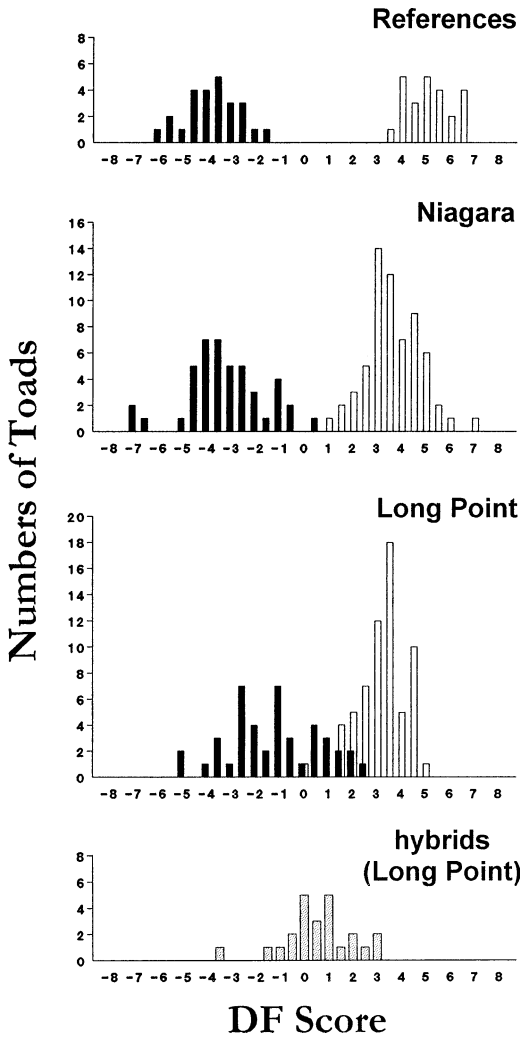**hybrids (Long Point)**

Numbers of Toads

DF Score

Fig. 2. Distributions of univariate discriminant function (df) scores for toads from the Niagara Peninsula, Long Point, and reference samples. Hybridization and introgression at Long Point has evidently shifted the morphologies of *Bufo americanus* (black bars) and *Bufo fowleri* (white bars) closer to each other compared to toads from the Niagara Peninsula such that the distributions df scores overlap. In contrast, no hybrids have been detected along the Niagara shoreline and there is no overlap in distributions of df scores of sampled toads.

DISCUSSION

Green (1984) concluded that although back-crossing between *B. americanus* and *B. fowleri* in southern Ontario was a possibility, its effect was likely negligible. However, the present morphological evidence from the same specimens tends to indicate much more extensive and long-standing introgression between the species at
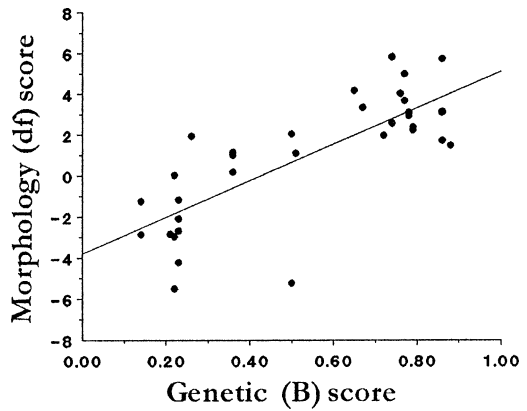

Fig. 3. Correlation of morphological univariate (df) scores versus genetic hybrid index (B) scores (Green, 1984) for all suspected hybrids ($r = 0.778$).

Long Point. The sample of *B. americanus* from Long Point was significantly different from both the reference sample of *B. americanus* and *B. americanus* from the Niagara Peninsula, and demonstrably more similar to coexisting *B. fowleri*. The introgression of genes is largely from *B. fowleri* into the gene pool of *B. americanus*.

This is demonstrably not the case along the Niagara Peninsula. Although the two species are sympatric and will breed in the same sites, there is no evidence here that any hybridization has occurred. The hybrid zone, therefore, is a will-
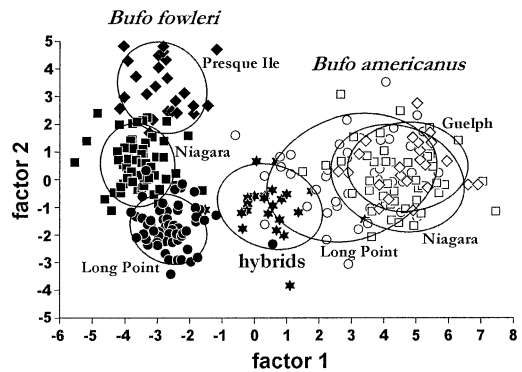

Fig. 4. Discriminant function plots (Factor 1 and Factor 2) of multiple discriminant function analyses for populations of *Bufo americanus* and *Bufo fowleri* and their hybrids. Ninety-five percent centroids are plotted. The samples of *B. americanus* are not significantly different from each other nor is there significant difference among them in their morphological distance from *B. fowleri*. However, the Long Point sample is closest to the reference sample of *B. fowleri*. The distribution of the hybrids overlaps more extensively with *B. americanus* than with *B. fowleri*, but there is no significant difference between the average distance between the hybrids and either species.
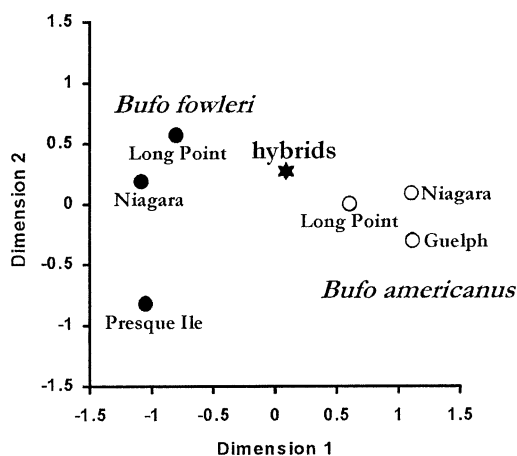
Fig. 5. Multidimensional scaling plot (in two dimensions) of Euclidian distances between sample means, derived from the discriminant analysis (Fig. 4).

o′-the-wisp, present here and absent there, sometimes intense and sometimes weak. The toads themselves are demonstrably not the same everywhere, either, as different characters contribute most to their diagnosis in different places. Their variation may either have something to do with their propensity for hybridization or else be the legacy of it. *Bufo fowleri* on the northern shore of Lake Erie tend to be small and grey with small spots compared to the large, brown and blotchy conspecifics south of the lake. At Long Point, especially, even ostensibly pure individuals of either species tend to be smaller and more like each other than are members of the same species from other sites (Table 2).

The relative fitness of hybrids may dictate the overall dynamics of a hybrid interaction in nature but the asymmetric introgression between the two species of toads at Long Point is a puzzle. One possible reason may lie with the timing of breeding of the two species. *Bufo americanus* will commence breeding at body temperatures of 9 C, whereas *B. fowleri* will not sing until they are nearly 14 C. The lowest body temperature recorded at Long Point for a calling male *B. fowleri*, among some 500 recorded, was 13.8 C (DMG, unpubl.). Therefore, male *B. americanus* emerge first in spring, followed by the female *B. americanus*, then male *B. fowleri*, followed at the last by female *B. fowleri*. The hybrids emerge at intermediate temperatures and times (Green, 1981), and therefore a calling male hybrid would more likely encounter a female *B. americanus* than any other female. However, any female hybrids would most likely encounter male *B. fowleri*. Malmos et al. (2001) found similarly

asymmetrical hybridization between *B. microscaphus* and *B. woodhousii* in Arizona. All hybrid *B. microscaphus* × *woodhousii* possessed mtDNA from *B. woodhousii*, which Malmos et al. attributed to the relative abundances of the two species. *Bufo woodhousii* males were greatly outnumbered and outcompeted for females of either species by male *B. microscaphus*.

A different possible explanation for the asymmetry of introgression between the two species of toads at Long Point may be differential survival or viability of the two sexes among the hybrids according to Haldane's rule (Haldane, 1922; Craft, 1938; Laurie, 1997). In a hybrid combination where there is differential survival between the two sexes among the offspring, the rule is that it is the heterogametic sex that is at the disadvantage. Thus mules (horse × donkey hybrids), in accordance with the rule, are female because mammalian females are the homogametic sex. The absence of male-mediated gene flow in a hybrid zone has been detected between hybridizing races of shrews, presumably caused by male, but not female, sterility among the hybrids (Balloux et al., 2000). If Haldane's rule were to apply to the hybrids between *B. americanus* and *B. fowleri*, male hybrids should be the favored sex in that *Bufo* have ZW female/ZZ male sex determination. In over 25 years of surveys of toads at Long Point beginning in 1978 (Green, 1984, 1997, and unpubl.), a female hybrid has never been identified.

Much attention has been paid to understanding those hybrid zones that take the form of steep clines between two erstwhile allopatric hybridizing taxa, sometimes called ''tension zones'' or ''gradient'' hybrid zones (Remington, 1968; Harrison, 1990; Arntzen, 1997). Barton and Hewitt (1985) described the shape of these hybrid zones in terms of diffusion models, and their formulations have successfully been applied to numerous hybrid interactions of like kind (Symura and Barton, 1986, 1991; Barton and Gale, 1993). The tension zone model, first voiced by Remington (1968) and elaborated upon by Barton and Hewitt (1985), is a description of clines and assumes the selective inferiority of the hybrids compared to the parental species. It relies upon the continual generation of hybrids in the region between the respective ranges of the parental species, the tension zone. The tension zone is at the periphery of both species and is populated by immigration caused by dispersal. Thus, the geographic location of the tension zone may shift and may or may not be constrained by an environmental gradient.

The bounded hybrid superiority model (Moore, 1977; Good et al., 2000) does not as-

sume lack of fitness on the part of the hybrids. Instead of a tension zone between two colliding species ranges, there lies a zone, which neither species finds optimal. Its intermediate environmental characteristics lend themselves to the success of individuals that are intermediates between the parental species: hybrids. These competitively superior intergrades, capable of backcrossing, could exclude genetically pure individuals of the parental species and exert dispersal pressure to countervail against immigration of pures from either side of the hybrid zone.

Arnold (1997) proposed a more general model of hybrid zone formation as an attempt to reconcile the causative aspects of previous and partially conflicting theories of hybrid zone origination and maintenance. Arguing that the tension zone model does not account sufficiently for the influence of the environment, Arnold's (1997) evolutionary novelty model invokes rarity of hybrid formation, endogenous selection against certain hybrid combinations, and exogenous selection acting either for or against certain hybrid combinations to predict the formation of long-standing hybridizing lineages. The hybridization of *B. americanus* and *B. fowleri*, however, is not a cline nor does it feature competitively superior hybrids nor is it consistent from place to place.

Describing hybrid zones, rather than attempting to account for them, Woodruff (1973) distinguished "allopatric" hybrid zones, characterized by narrow clines, from other hybridizing situations, including those where there is broad overlap of the ranges of the hybridizing taxa. The incidence of hybridization is spatially complex and patchily distributed (Klingenberg et al., 2000) such that they have been modeled as mosaics of dispersed gradient hybrid zones between disjunct patches of ranges or, conversely, as patches of hybridization within overlapping, continuous ranges (Harrison, 1986; Arnold, 1997). The fire-bellied toads, *Bombina bombina* and *Bombina variegata* of eastern Europe, for example, individually have fragmented ranges but the range patches of the two species alternate with each other. Where the ranges meet, the species hybridize (Szymura and Barton, 1986, 1991; Szymura et al., 2000), and so hybridization is itself patchily distributed.

The distinction between cline and mosaic models may not be absolute (Arntzen, 1997) but may be considered a function of tile size in the mosaic, each tile being a patch of the range of the species. Where but two tiles meet, the hybrid zone is a classic cline as modeled by Barton and Hewitt (1985) or Arnold (1997). Smaller tiles lead to a geographically distributed interaction as seen in *Bombina*. Exceedingly small tiles would give the appearance of instances of hybridization in broad sympatry. This is how the hybridizing *B. americanus* and *B. fowleri* scattered across eastern North America may best be perceived.

Bufo americanus and *B. fowleri* conform to Woodruff's (1973) concept of a "localized sympatric" hybrid zone. It is a mosaic in that hybrids appear at some times and in some places and not in others. At Long Point, there is ample evidence of past hybridization in the introgression of alleles of *B. fowleri* into *B. americanus* even if hybrids themselves may not be present at all times. On the contrary, despite their close proximity along the Niagara shoreline, there is no hint of introgression now or in the past.

Some 60 years ago, Blair (1941) found and characterized abundant hybrids between *B. americanus* and *B. fowleri* near Bloomington, Indiana. Those same sites were revisited 30 years later by Jones (1973), who failed to find hybrids and concluded that the hybrid zone had disappeared. Jones (1973) concluded that hybridization had vanished because better isolating mechanisms between the species had evolved. This interpretation was criticized by Loftus-Hills (1975) and, on the face of the present evidence, appears ever less likely. Hybridization at Long Point comes and goes, but it has left a legacy of morphological introgression that cannot be detected on the Niagara Peninsula. There is no guarantee that the two species will not hybridize again at Long Point, or at numerous other sites, with the same consequences of limited introgression.

Literature Cited

ALLARD, H. A. 1908. *Bufo fowleri* (Putnam) in northern Georgia. Science 26:655–656.

ARNOLD, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, Oxford.

ARNTZEN, J. W. 1997. Patterns of ecology and scale integrate the gradient and mosaic models of hybrid zone structure in *Bombina* toads and *Triturus* newts. Israel J. Zool. 42:111–119.

BALLOUX, F. H. BRUNNER, N. LUGON-MOULIN, J. HAUSSER, AND J. GOUDET. 2000. Microsatellites can be misleading: an empirical and simulation study. Evolution 54:1414–1422.

BARBOUR, R. W. 1971. Amphibians and reptiles of Kentucky. Univ. Press of Kentucky, Lexington.

BARTON, N. H., AND K. S. GALE. 1993. Genetic analysis of hybrid zones, p. 13–45. *In:* Hybrid zones and the evolutionary process. R. Harrison (ed.). Oxford Univ. Press, New York.

———, AND G. M. HEWITT. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

BLAIR, A. P. 1941. Variation, isolating mechanisms, and hybridization in certain toads. Genetics 26: 398–417.

BLAIR, W. F. 1963. Evolutionary relationships of North American toads of the genus *Bufo*: a progress report. Evolution 17:1–16.

———. 1972. Evidence from hybridization, p. 93–101. *In:* Evolution in the genus *Bufo*. W. F. Blair (ed.). Univ. of Texas Press, Austin.

BREDEN, F. 1988. The natural history and ecology of Fowler's toad, *Bufo woodhousei fowleri* (Amphibia: Bufonidae) in the Indiana Dunes National Lakeshore. Fieldiana Zool. 49:1–16.

BROWN, L. E. 1964. An electrophoretic study of variation in the blood proteins of the toads *Bufo americanus* and *B. woodhousei*. Syst. Zool. 13:92–95.

CONANT, R. AND J. T. COLLINS. 1991. A field guide to reptiles and amphibians of eastern and central North America. 3d ed. Houghton Mifflin, Boston, MA.

COOK, F. R. 1983. An analysis of toads of the *Bufo americanus* group in a contact zone in central northern North America. Nat. Mus. Can, Publ. Nat. Sci. 3:1–89.

CORY, L., AND J. J. MANION. 1955. Ecology and hybridization in the genus *Bufo* in the Michigan–Indiana region. Evolution 9:42–51.

CRAFT, W. A. 1938. The sex ratio in mules and other hybrid mammals. Q. Rev. Biol. 13:19–39.

DOWLING, T. E., G. R. SMITH, AND W. M. BROWN. 1989. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (family Cyprinidae): comparison of morphology, allozymes, and mitochondrial DNA. Evolution 43:620–634.

GOOD, T. P., J. C. ELLIS, C. A. ANNETT, AND R. PIEROTTI. 2000. Bounded hybrid superiority in an avian hybrid zone: effects of mate, diet, and habitat choice. Evolution 54:1744–1783.

GREEN, D. M. 1981. Theoretical analysis of hybrid zones derived from an examination of two dissimilar zones of hybridization in toads (genus *Bufo*). Unpubl. Ph.D. diss., Univ. of Guelph, Guelph, ON, Canada.

———. 1982. Mating call characteristics of hybrid toads (*Bufo americanus* × *B. fowleri*) at Long Point, Ontario. Can. J. Zool. 60:3293–3297.

———. 1983. Allozyme variation through a clinal hybrid zone between the toads *Bufo americanus* and *B. hemiophrys* in southeastern Manitoba. Herpetologica. 39:28–40.

———. 1984. Sympatric hybridization and allozyme variation in the toads *Bufo americanus* and *B. fowleri* in southern Ontario. Copeia 1984:18–26.

———. 1996. The bounds of species: hybridization in the *Bufo americanus* group of North American toads. Israel J. Zool. 42:95–109.

———. 2003a. *Bufo fowleri* (Fowler's toad). *In:* Status and conservation of U.S. amphibians. M. J. Lannoo (ed.). Smithsonian Institution Press, Washington, DC (In Press).

———. 2003b. *Bufo americanus* (American toad). *In:* Status and conservation of U.S. amphibians. M. J. Lannoo (ed.). Smithsonian Institution Press, Washington, DC (In Press).

———, AND C. PUSTOWKA. 1997. Correlated morphological and allozyme variation in the hybridizing toads, *Bufo americanus* and *Bufo hemiophrys*. Herpetologica 53:218–228.

HALDANE, J. B. S. 1922. Sex-ratio and unisexual sterility in hybrid animals. J. Genet. 12:101–109.

HARRISON, R. G. 1986. Pattern and process in a narrow hybrid zones. Heredity 56:337–349.

———. 1990. Hybrid zones: windows on the evolutionary process. Oxf. Surv. Evol. Biol. 7:69–128.

———. 1993. Hybrids and hybrid zones: historical perspective, p. 3–12. *In:* Hybrid zones and the evolutionary process. R. G. Harrison (ed.). Oxford Univ. Press, New York.

HUBBS, C. L. 1918. *Bufo fowleri* in Michigan, Indiana and Illinois. Copeia 55:40–43.

JONES, J. M. 1973. Effects of thirty years hybridization on the toads *Bufo americanus* and *Bufo woodhousei fowleri* at Bloomington, Indiana. Evolution 27:435–448.

KLINGENBERG, C. P., J. R. SPENCE, AND C. K. MIRTH. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. J. Evol. Biol. 13:756–765.

LAURIE, C. L. 1997. The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics 147:937–951.

LEE, J. C. 1982. Accuracy and precision in anuran morphometrics: artifacts of preservation. Syst. Zool. 31:266–281.

LOFTUS-HILLS, J. J. 1975. The evidence for reproductive character displacement between the toads *Bufo americanus* and *Bufo woodhousei fowleri*. Evolution 29: 368–369.

MALMOS, K. B., B. K. SULLIVAN, AND T. LAMB. 2001 Calling behavior and directional hybridization between toads (*Bufo microscaphus* × *B. woodhousii*) in Arizona. Evolution 55:626–630.

MAYR, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.

———. 1970. Populations, species and evolution. Belknap Press. Cambridge, MA.

MOORE, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. Q. Rev. Biol. 52:263–277.

MYERS, G. S. 1927. The differential characters of *Bufo americanus* and *Bufo fowleri*. Copeia 163:50–53.

NEILL, W. T. 1949. Hybrid toads from Georgia. Herpetologica 5:30–32.

REMINGTON, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evol. Biol. 2: 321–428.

SZYMURA, J. M., AND N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40:1141–1159.

———, AND ———. 1991. The genetic structure of

the hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. *Ibid.* 45:237–161.

———, T. UZZELL, AND C. SPOLSKY. 2000. Mitochondrial DNA variation in the hybridizing fire-bellied toads, *Bombina bombina* and *B. variegata*. Mol. Ecol. 9:891–899.

VOLPE, E. P. 1952. Physiological evidence for natural hybridization of *Bufo americanus* and *B. fowleri*. Evolution 6:393–406.

WEATHERBY, C. A. 1982. Introgression between the American toad *Bufo americanus* and the Southern toad *B. terrestris* in Alabama. Unpubl. Ph.D. diss., Auburn Univ., Auburn, AL.

WOODRUFF, D.S. 1973. Natural hybridization and hybrid zones. Syst. Zool. 22:213–218.

WRIGHT, A. H., AND A. A. WRIGHT. 1949. Handbook of frogs and toads of the United States and Canada. Comstock Publishing Co., Ithaca, NY.

ZWEIFEL, R. G. 1968. Effects of temperature, body size and hybridization on mating calls of toads *Bufo a. americanus* and *Bufo woodhousei fowleri*. Copeia 1968: 269–285.

REDPATH MUSEUM, MCGILL UNIVERSITY, 859 SHERBROOKE STREET WEST, MONTREAL, QUEBEC, CANADA. PRESENT ADDRESS: (CP) DEPARTMENT OF BIOLOGICAL SCIENCES, SIMON FRASER UNIVERSITY, BURNABY, BRITISH COLUMBIA, CANADA. E-mail: (DMG) david.m.green@ mcgill.ca. Send reprint requests to DMG. Submitted: 26 Nov. 2001. Accepted: 22 July 2002. Section editor: R. M. Wood.

APPENDIX 1

Specimens examined. Museum abbreviations: NMC—Canadian Museum of Nature, Ottawa; RM— Redpath Museum, McGill University, Montreal; CM— Carnegie Museum, Yale University, Philadelphia.

Bufo americanus.—Reference: Guelph, Ontario (NMC 28344[1C10], 23710 [2C3, 5C17]). Long Point: Long Point, Ontario (RM 2396, 2397, 2400, 2401, 2403, 2404, 2406, 2407; NMC 21739, 21741, 21761, 21799[1C2], 21855[1], 21888, 21900, 21902, 21907[1C3], 21912 [1C3], 21914, 21918[1C3], 21919[1C6], 21920[1C6], 21928, 21964[1C3]). Niagara Peninsula: Point Abino, Ontario (NMC 21929, 21944[1C4], 21947[1C4], 21783[1C2], 21940[1C2]), Port Colborne, Ontario (NMC 21777, 21791, 21793[1C3], 21866). Sugarloaf Pond, Port Colborne, Ontario (NMC 21797[1C6], 21921[1C4]). Featherstone Point (NMC 21915[1C4], 21945[1C6]). "Lake Erie," Ontario (NMC 21923[1C6])

Bufo fowleri.—Reference: Presque Ile, Pennsylvania (CM 4190 [ACC], 2104, 2114, 2115, 2116, 7658 [1C17]). Long Point: Long Point, Ontario (RM 2498C2501, 2510, 2539; NMC 21735, 21736, 21750, 21756, 21775[1,3C6], 21779, 21790[1C4], 21792[1C5,6,7], 21802[1C5], 21813 [1C2], 21860[1C3], 21890[1C3], 21908, 21910[1C6], 21939, 21941[1C6], 21943[1C7], 21957[1C2], 21965 [1C4]). Niagara: Point Abino, Ontario (NMC 21776 [1], 21782[1C2], 21784[1C11], 21796[1C2], 21842 [1C6], 21930, 21948[1C12], 21950[1C2], 21959, 29404 [1C5,7]). Port Maitland, Ontario (NMC 21807[2], 21883, 21949[1C8], 21952[1C6]). Rock Point, Ontario (NMC 21794).

Bufo americanus × *fowleri.*—Hybrids: Long Point Ontario (RM 2383, NMC 21778, 21795[1C3], 21899, 21905, 21906[1C2], 21911[1C4], 21958, 21963[1C12], 29447).