

Intraspecific vicariant history and the evolution of adaptive morphological diversity in a fish species (*Osmerus mordax*)

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Vicariant geographic isolation and resource partitioning have long been independently identified as processes contributing to the morphological divergence of closely-related species. However, little is known about the extent to which vicariant history influences the adaptive ecological divergence associated with resource partitioning and trophic specialization within species. The present study thus quantified the contribution of vicariant historical genetic divergence to the adaptive contemporary morphological divergence of intraspecific feeding specialists in the Rainbow smelt (*Pisces: Osmerus mordax*). This species is characterized by the polyphyletic origin of two lacustrine feeding specialists originating in two intraspecific lineages associated with independent glacial refuges. The historical genetic segregation was initiated approximately 350 000 years ago, whereas the lacustrine trophic segregation arose within the past 10 000 years. Wild caught lacustrine smelt populations were grouped a priori based on known historical genetic identities (Acadian and Atlantic mitochondrial DNA clades) and contemporary feeding specializations (microphageous and macrophageous morphotypes). The present study demonstrated that independent suites of correlated morphological traits are associated with either vicariant history or contemporary feeding specializations. Second, functionally-similar feeding specialists exhibit distinct morphologies resulting largely from vicariant historical processes. Although, the evolutionary processes producing historical phenotypes remains unknown, the results obtained demonstrate how adaptive radiation associated with ecological resource partitioning and feeding specializations can be strongly influenced by intraspecific phenotypic diversification resulting from relatively recent vicariant histories. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 97, 140–151.

ADDITIONAL KEYWORDS: feeding specializations – historical genetic divergence – intraspecific diversity – traits association.

INTRODUCTION

Intraspecific morphological diversity is often explained by a process of adaptive divergence hypothesized to involve the partitioning of restricted resources among specialists adapted to different ecological niches (Schluter, 2001). Natural selection may favour morphological divergence through ecological character displacement, in the presence of intraspecific competition, or character release, in the absence

of intraspecific competition (Brown & Wilson, 1956; Slatkin, 1980; Schluter & McPhail, 1992; Robinson & Wilson, 1994; Losos, 2000; Pfennig & Murphy, 2003). For example, distinct morphological and behavioural adaptations in fishes are associated with distinct feeding specializations (Robinson & Wilson, 1994). In freshwater fishes, limnetic morphotypes are specialized for actively filtering zooplankton in the water column, whereas benthic morphotypes are specialized in preying upon macrozoobenthos (Coregonidae: Rogers, Gagnon & Bernatchez, 2002; Gasterosteidae: Bentzen & McPhail, 1984; Salmonidae: Bourke,

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Magnan & Rodriguez, 1999; Osmeridae: Jardine & Curry, 2006). Evolution of these feeding specializations independently in so many fish species clearly identifies them as adaptive responses and not the product of genetic drift. Such ecological divergence has resulted in reproductive isolation in many disparate taxa (Funk, Nosil & Etges, 2006) and, thus, may ultimately lead to speciation (McPhail, 1994; Doebeli & Dieckmann, 2000; Via, 2001; Coyne & Orr, 2004).

Numerous intraspecific phylogeographic studies have shown that many vertebrate species are composed of morphologically distinct lineages (fish: Bernatchez & Wilson, 1998; Hickerson & Ross, 2001; amphibians: Smith & Green, 2004; reptiles: Lenk *et al.*, 1999; Janzen *et al.*, 2002; birds: Liukkonen *et al.*, 2002; Stevens & Hogg, 2003; mammals: Avise, Walker & Johns, 1998; Galbreath & Cook, 2004; Michaux *et al.*, 2004). These lineages result from the vicariant isolation of various intraspecific groups in isolated refuges, such as islands, lakes, isolated valleys or mountain tops, or refuges created during Pleistocene continental glaciation events. Historical vicariant differentiation may have arisen through genetic drift and/or adaptation to distinct environmental conditions prevailing in distinct refuges (Avise, 2004). In such cases, we are faced with the problem of disentangling to what extent variation in morphological traits among contemporary populations is attributable to adaptation to the current environment or to vicariant history (Edwards & Kot, 1995).

The general objective of the present study was to quantify the relative importance of vicariant historical morphological diversity in determining contemporary ecological adaptations observed at the intraspecific level. The model species we studied is the Rainbow smelt (*Osmerus mordax*), a highly abundant, carnivorous, schooling fish found in the lakes, rivers and estuaries of eastern Canada and north-eastern USA (Scott & Crossman, 1974). The Acadian and Atlantic intraspecific lineages [respectively, mitochondrial (mt)DNA clade A and clade B] diverged approximately 350 000 years ago in isolated Pleistocene refuges (Baby, Bernatchez & Dodson, 1991; Taylor & Bentzen, 1993a; Bernatchez, 1997). Introgression of the two lineages has been observed in contemporary anadromous and lacustrine populations (Lecomte & Dodson, 2004), M.-F. Barrette & J. J. Dodson, unpubl. data), indicating that complete post-zygotic reproductive isolation has not developed between them. The two lineages have independently evolved several cases of feeding specializations subsequent to postglacial lake colonizations, approximately 10 000 years ago (Bernatchez, 1997). The morphological and ecological specializations of Rainbow smelt reflect the common adaptive

dichotomy observed among many species pairs and intraspecific morphotypes (Taylor, 1999). In lakes, the exploitation of trophic resources of different sizes has produced microphageous and macrophageous feeding specialists (Bentzen *et al.*, 2006; Jardine & Curry, 2006). Microphageous smelt have developed a lean body shape with high gill raker density and proportionately large eyes to feed on small zooplanktonic prey, whereas macrophageous smelt have developed a deep body with a large mouth to feed on larger benthic and pelagic prey, including fishes (McAllister, 1963; Nellbring, 1989). The two feeding specialists may co-occur in the same lake (in sympatry) or be isolated from each other in different lakes (in allopatry). Several studies have demonstrated that, in sympatry, the two morphs exhibit a certain degree of pre-zygotic reproductive isolation (Taylor & Bentzen, 1993b; Saint-Laurent, Legault & Bernatchez, 2003). If vicariant divergence between the two historical lineages, regardless of its source, contributes significantly to contemporary intraspecific morphological diversity, the role attributed to relatively recent historical processes in the phenotypic divergence of feeding specialists may be underestimated.

The occurrence of two historical lineages of Rainbow smelt in a diversity of trophic habitats allows us to evaluate the relative importance of two processes influencing intraspecific morphological diversity. On the one hand, the ecological specialization hypothesis proposes that contemporary ecological processes are the main source of morphological divergence which forms the basis of adaptive radiation and speciation (Schluter, 2001). On the other hand, vicariant history may influence relatively recent ecological diversification, as demonstrated in several taxa (Darwin's finches: Grant, 1986; Caribbean Anolis lizards: Losos *et al.*, 1998; Langerhans, Knouft & Losos, 2006; surfperch: Bernardi, 2005; montane grasshopper: Knowles & Richards, 2005). We thus first established if the two historical smelt lineages possess characteristic morphologies. Second, we assessed how any such historically-based differences in morphology are involved in the evolution of derived character states. Accordingly, we evaluated the extent to which intraspecific vicariant history influences morphological divergence within each historical lineage when exposed to divergent selective pressures (feeding specializations in each lineage: microphagy versus macrophagy).

MATERIAL AND METHODS

SAMPLE COLLECTIONS

Thirteen populations were sampled in 11 lakes aiming to include all possible combinations of histori-

Table 1. Feeding specializations, sample size, mitochondrial (mt)DNA genotypes and location (state or province) of 13 native lacustrine Rainbow smelt populations sampled in the present study

Feeding specializations	Sample size	mtDNA frequency		Geographic coordinate
		Acadian	Atlantic	N/W
Microphageous populations				
1. Lake Winnepesaukee (NH)	11	1.00	0.00	43°35'/71°18'
2. Lake George (ME)	40	1.00	0.00	45°06'/69°40'
3. Lake Muskrat (ON)	44	0.00	1.00	45°40'/76°55'
4. Lake Perreault (QC)	29	0.00	1.00	46°09'/76°04'
5. Aux Rats River (QC)	50	0.00	1.00	49°15'/72°17'
6. Lake Utopia (NB)*	25	1.00	0.00	45°09'/66°46'
7. Lake Heney (QC)*	39	0.00	1.00	46°02'/75°55'
Macrophageous populations				
8. Long Lake (ME)	40	1.00	0.00	47°11'/68°14'
9. Grand Lake (NS)	39	1.00	0.00	44°54'/63°35'
10. Lake Champlain (VT)	45	0.00	1.00	44°54'/73°23'
11. Lake Quatre-Lieux (QC)	41	0.00	1.00	50°30'/67°20'
12. Lake Utopia (NB)*	28	1.00	0.00	45°09'/66°46'
13. Lake Heney (QC)*	41	0.00	1.00	46°02'/75°55'

*Populations coexisting with the alternate morph in a same lake.

cal lineages and feeding specialists (Table 1). Because the morphological divergence between feeding specialists is expected to be greater between sympatric morphotypes than between allopatric ones (Taylor & Bentzen, 1993b; Schluter, 2001), we also included two sympatric pairs to extend the possible range of ecological divergence observed in lacustrine Rainbow smelt, one pair for each historical lineage (Fig. 1). From the Acadian lineage, we analysed three microphageous populations ($N = 76$ fish) and three macrophageous populations ($N = 107$ fish). From the Atlantic lineage, we analysed four microphageous populations ($N = 162$ fish) and three macrophageous populations ($N = 127$ fish). Adult fish were collected, either by dip-net, gill net, or hand-line ice fishing, from February to September 2002. Fish were frozen in water and kept at -20 °C prior to morphological and genetic analyses. Samples included mature fish of both sexes (age range 1–5 years; size range 64–310 mm).

LINEAGE ASSIGNMENT

To assign each fish to its historical lineage and thus establish its historical identity, we identified mtDNA haplotypes *sensu* Pigeon, Dodson & Bernatchez (1998). The diagnostic marker that we used discriminates mtDNA lineages, but mtDNA alone cannot detect introgression. Consequently, we cannot rule out the possibility that, at one time, two lineages may

have coexisted and that, after a period of introgression, one maternal lineage has been fixed through stochastic lineage extinction. This bias reduces the resolution of the analysis and reduces the chance of observing morphological differences between the two lineages. As such, interpretations of the analysis are conservative.

MORPHOLOGICAL ANALYSIS

Each fish was identified a priori as either a putative microphageous or macrophageous specialist using the bimodality of frequency distributions of fork length (Lanteigne & McAllister, 1983) and total gill raker counts (Taylor & Bentzen, 1993b) within each age class. For cases of sympatry, only sites where no fish of intermediate fork length and gill raker counts occurred were retained to restrain our analysis to clearly differentiated morphotypes. To quantify phenotypic diversity, we measured 37 continuous morphometric traits and nine discrete meristic traits from 472 fish encompassing all freshwater Rainbow smelt types (Fig. 2). SIGMA-SCAN software was used to capture images with a video camera combined with an Olympus binocular scope for morphometric measures and gill raker counts. Fin structures were measured with a digital calliper. Total gill raker counts were excluded and size-effects (see below) removed from further analyses such that the analysis of phenotypic diversity did not include variables that were used to initially define trophic morphotypes.

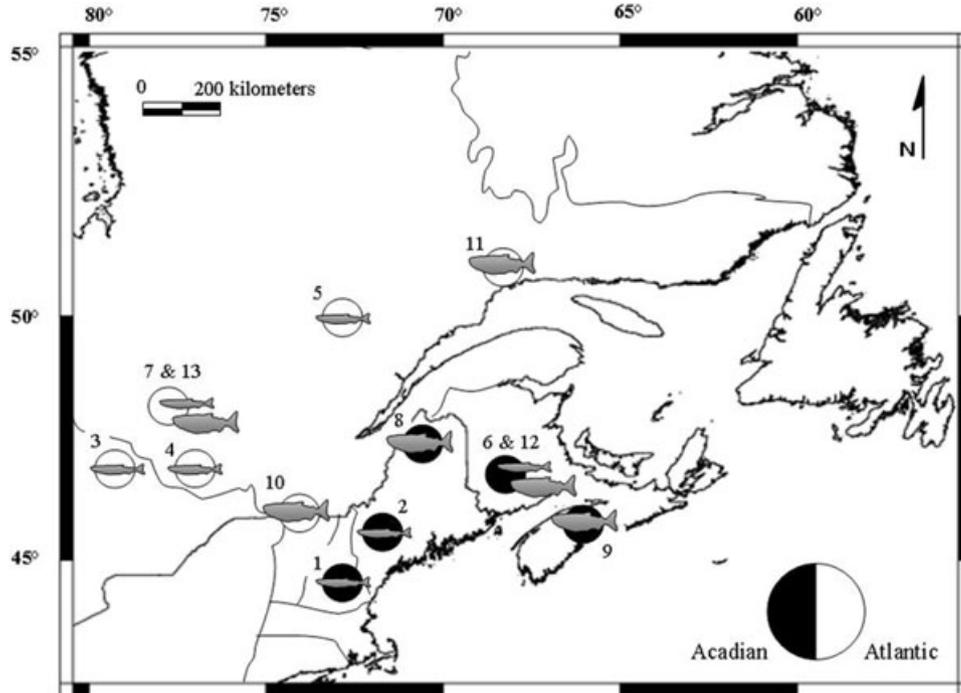


Figure 1. Geographic distribution of 13 native lacustrine Rainbow smelt populations and frequency distribution of Acadian and Atlantic historical lineages (mtDNA clades) found throughout north-east North America. Numbers refer to sites listed in Table 1. Slender fish symbols represent microphageous populations, deeper bodied fish symbols represent macrophageous populations, and both symbols present at a same location designate the co-existence in sympatry of both feeding specialists.

The ecological basis of the morphological dichotomy, considered in the present study and elsewhere as indicative of feeding specializations, has been confirmed in many species of freshwater fishes (see above), including smelt (Bentzen *et al.*, 2006; Jardine & Curry, 2006). However, because we did not conduct a detailed feeding study of the 13 smelt populations included in our analyses, we do not know the extent of the association between the two morphotypes and their specialization on distinct diets. The morphological differences that we defined as micro- and macrophageous may very well involve other aspects of the ecology of smelt not directly related to diet.

STATISTICAL ANALYSIS

We first standardized morphometric measures for fish of different sizes in a principal component analyses (Gardner, Walker & Greer, 1988; Douglas & Matthews, 1992). Statistical methods are detailed in the Supporting information (Doc. S1). Although we removed the size effect, residual values are not allometry free and may still be correlated with body size measurements in subsequent analyses (Lecomte & Dodson, 2004). Because morphotypes differed substantially in size, we examined allometries of traits.

Accordingly, we used the regression of each morphometric trait on fork length, and compared regression slopes among the four groups of populations formed according to their historical identity (Atlantic or Acadian smelt) and contemporary feeding specializations (microphageous or macrophageous smelt). The relationships between the 36 morphological variables and fork length were compared among groups using the following allometric model:

$$Y_{ij} = (\alpha + \alpha_i) x^{(\beta + \beta_i)} \quad i = \text{group } 1, 2, 3, 4$$

α_i and β_i are parameters specific to each group and are expressed as deviations to parameters α and β in common with all groups. The relationships are considered the same if the following null hypothesis is accepted: $H_0: (\alpha_i = 0, \beta_i = 0), \forall i \in \{1, 2, 3, 4\}$. However, to maintain the type I error rate at the specified level, a Bonferroni correction was used and each of the 36 relationships were compared at the level of $0.05/36 \approx 0.001$.

Second, to investigate the relative importance of alternative sources of intraspecific morphological divergence, we combined multivariate analysis of variance (MANOVA), discriminant function analyses (DFA) and analyses of variance (ANOVA) (see Sup-

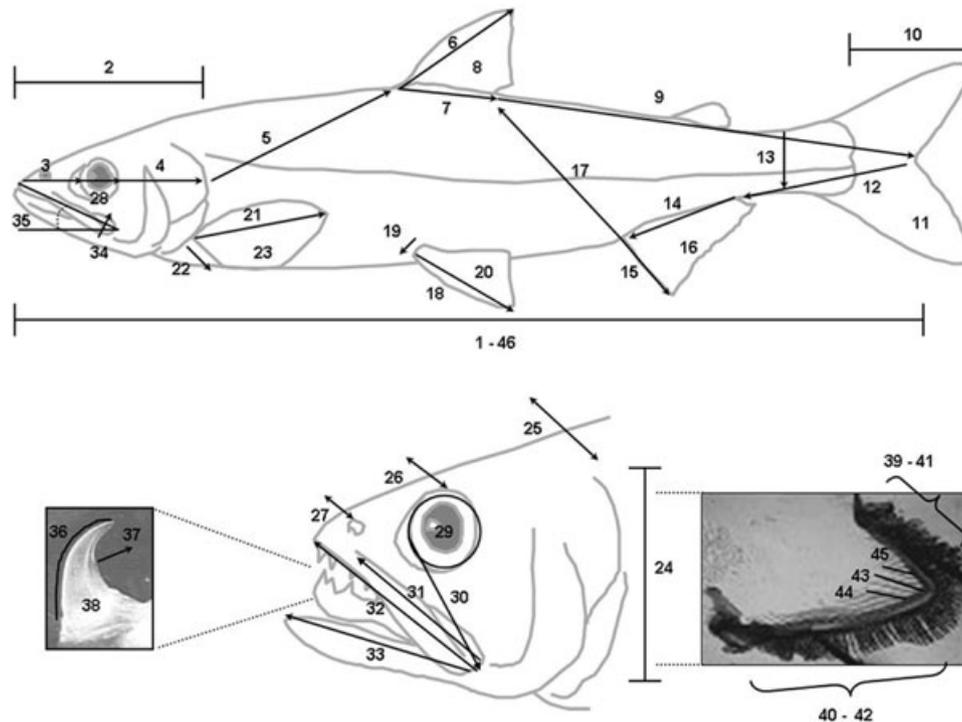


Figure 2. Morphometric and meristic traits measured on 472 Rainbow smelt: 1, fork length; 2, head length; 3, snout length; 4, post-orbital length; 5, trunk length; 6, dorsal fin length; 7, dorsal fin base; 8, dorsal fin ray count; 9, dorsal fin to fork length; 10, caudal fin length; 11, caudal fin ray count; 12, fork to anal fin length; 13, caudal peduncle depth; 14, anal fin base; 15, anal fin length; 16, anal fin ray count; 17, anal fin to dorsal fin length; 18, pelvic fin length; 19, pelvic fin base; 20, pelvic fin ray count; 21, pectoral fin length; 22, pectoral fin base; 23, pectoral fin ray count; 24, head depth; 25, head width; 26, inter orbital width; 27, inter nasal width; 28, eye diameter; 29, eye area; 30, eye to maxillary length; 31, maxillary length; 32, upper jaw length; 33, lower jaw length; 34, maxillary depth; 35, maxillary angle; 36, lingual tooth length; 37, lingual tooth curve; 38, large lingual tooth count; 39, dorsal gill arch length; 40, ventral gill arch length; 41, dorsal gill raker count; 42, ventral gill raker count; 43, 44, 45, gill raker length [first and third ventral (1st v. and 3rd v.) and third dorsal [3rd d.]]; 46, vertebrae count.

porting information). MANOVAs were first used to test the hypothesis of significant morphological distinction between historical lineages and between predetermined feeding specialists.

In the case of significant differences, DFAs were conducted to identify suites of correlated morphological traits distinguishing among four groups of populations formed according to their historical identity and contemporary feeding specializations. The DFA also served to evaluate the percentage of reclassification to assess the robustness of observed differences. In a first DFA, we grouped all populations according to one criterion: the historical lineages. We thereby identified traits characteristic of each lineage, hence morphological patterns reflecting vicariant history. In a second DFA, we grouped all populations according to two criteria: historical lineages and predetermined feeding specializations. We thereby documented to what extent suites of traits associated with vicariant history, as identified

in the first DFA, were involved in contemporary feeding specializations. We advocate the interpretation of these suites of correlated traits, rather than a trait by trait analysis, because individual traits are not free to evolve independently.

We partitioned the observed variation of suites of correlated traits into two components: 'historical traits association', based on shared vicariant history associated with mtDNA genetic divergence, and 'contemporary traits association' based on shared contemporary function associated with micro- and macrophagy. This amounted to quantifying the morphological variance observed among and within smelt populations for each 'traits association' previously identified as discriminant functions, according to historical (intraspecific mitochondrial lineages) and contemporary (morphotypes) sources of intraspecific morphological variation. The historical and contemporary sources are included as independent cross factors in an ANOVA model. The ANOVA model addi-

tionally quantified the within population variation which is not provided by DFAs alone.

RESULTS

THE IMPRINT OF HISTORICAL LEGACY

Historical lineages clearly differed morphologically (MANOVA: $F_{\text{Wilk's } \lambda} = 18.35$; d.f. = 43, 428;

Table 2. Highly-correlated morphological traits, composing the single axis of variation of the discriminant function analysis (DFA) differentiating the two historical lineages

Variables	Loadings
Anal fin base	-0.3150
Pectoral fin base	-0.3118
Pectoral fin length	-0.2214
Dorsal gill raker count	-0.2182
Maxillary depth	-0.1468
Maxillary angle	0.1734
Eye diameter	0.1788
Lower jaw length	0.1809
Upper jaw length	0.1862
Maxillary length	0.1912
Ventral gill arch length	0.1950
Head depth	0.1971
Anal fin length	0.2202
Eye to maxillary length	0.2206
Eye area	0.2712

Significant loadings were greater than +0.14 or lower than -0.14; Fisher's inverse hyperbolic tangent transformation (see Supporting information Doc. S1). Average position of both lineages on the axis are -1.70 (Acadian) and +1.08 (Atlantic) ($N = 472$; $P_{\text{Acadian versus Atlantic}} < 0.0001$). Traits with a negative loading on the DFA axis had higher trait values for Acadian smelt (located on the negative end of the axis) and lower trait values for Atlantic smelt (located on the positive end of the axis). Conversely, a positive loading on this axis had a higher trait value for Atlantic smelt and a lower trait value for Acadian smelt.

$P < 0.0001$). Fifteen morphological traits discriminated lacustrine smelt populations (Table 2) when individuals were classified in a DFA on the sole basis of their historical origin. The discrimination was robust as 87.4% of Acadian smelt and 89% of Atlantic smelt were successfully reclassified to their historical lineage of origin. Additionally, of the total variance in this traits association, 65.1% was attributed to the genetic divergence between lineages and 33.1% was attributed to within-population variation (Table 3). When grouping all smelt according to their historical lineage, regardless of their contemporary feeding specializations, 30 morphological traits (Fig. 2) were not involved in the morphological divergence between Atlantic and Acadian populations and are thus not considered to be associated with vicariant history.

Vicariant history remains the primary source of morphological divergence among populations when individuals were classified in a DFA according to the two historical lineages and the two feeding specializations (Fig. 3). The morphology of these four morphotype groups differed significantly (MANOVA: $F_{\text{Wilk's } \lambda} = 10.88$; d.f. = 129, 1277.4; $P < 0.0001$). The traits association accounting for the greatest degree of intraspecific morphological divergence in Rainbow smelt discriminated between the two historical lineages (Axis 1; Table 4). This suite of traits included those previously identified as characteristic of vicariant history and hence is identified as a 'historical traits association' (Table 2).

The second-most important traits association discriminated morphotypes, with a far lesser degree of morphological discrimination associated with vicariant history, hence identified as a 'contemporary traits association'. This secondary traits association involved traits previously identified as not being characteristic of vicariant history (Table 4), as well as some traits considered as characteristic of vicariant history.

Table 3. Variance explained by vicariant history when all lacustrine populations are subdivided in two groups in a discriminant function analysis (DFA): all Acadian smelt (six populations) and all Atlantic smelt (seven populations)

Axis 1	d.f.	Variance		Wald chisquare	P-value	Part of the total variance
		Estimate	Bootstrap standard error			
H	1	1.8826	0.1281	215.98	< 0.0001	65.09%
Pop _(H)	11	0.0530	0.0353	2.25	0.1333	1.83%
Error	459	0.9569	0.0580	272.19	< 0.0001	33.08%
Total		2.8925				

The random analysis of variance model (with nested factors) partitioned variance, on the single canonical axis of the DFA, among (1) historical lineage 'H'; (2) the remaining among populations variance nested within each lineage 'Pop_(H)'; and (3) the within population variability 'Error'. d.f., degrees of freedom.

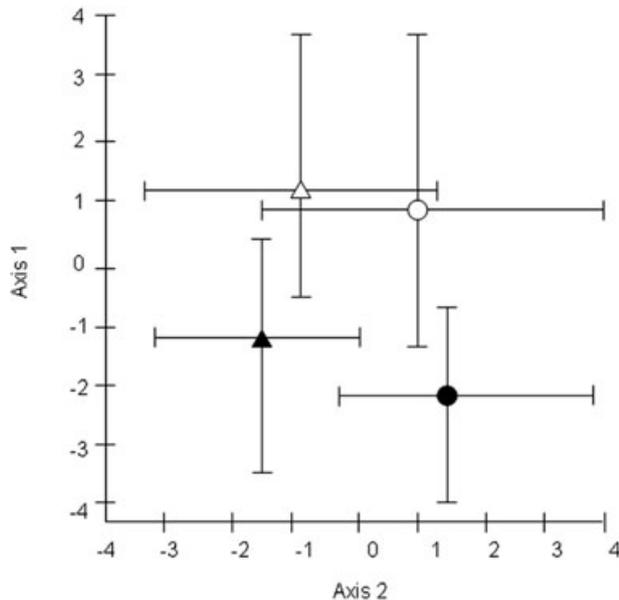


Figure 3. Discriminant function analysis (DFA) significantly differentiating microphageous and macrophageous feeding specialists derived independently from the two historical lineages ($P < 0.0001$). Canonical axes have a relative discriminative power of 53.6% (Axis 1) and 34.2% (Axis 2); $N = 472$. For morphological variables that contribute significantly to forming each canonical axis, see Table 4. Each point represents the mean group value. Horizontal and vertical bars show the range of observations (i.e. the distribution of all fishes) around the mean value. Circles denote groups of microphageous smelt and triangles denote groups of macrophageous smelt. Open symbols denote groups from the Atlantic historical lineage and filled symbols denote groups from the Acadian historical lineage.

Acadian smelt showed a greater morphological divergence than Atlantic smelt. Based on the two morphological traits associations identified, smelt of Acadian origin were more successfully reclassified to their respective morphotypes (80.3% microphageous, 82.2% macrophageous) than smelt of Atlantic origin (75.3% microphageous, 72.4% macrophageous). Additionally, the DFA performed on lacustrine morphotypes within and among the two mtDNA lineages did not discriminate between microphageous and macrophageous feeding specialists of an Atlantic origin (Axis 1 for Atlantic micro- and macrophageous smelt, $P = 0.4204$) but otherwise significantly discriminated all four groups on Axis 1 and 2 ($P < 0.0001$). These results suggest that apparent feeding specializations were much less distinct among smelt of Atlantic origin.

Contemporary functional convergence in apparent feeding specialization was not accompanied by complete morphological convergence of the two intraspecific lineages. Morphological variation characterizing

the primary traits association (DFA Axis 1) was mainly explained by historical divergence (61.6%; Table 5) and, to a lesser degree, by population-specific effects [$\text{Pop}_{(\text{H} \times \text{FS})} = 3.5\%$; Table 5]. Morphological variation characterizing the second-most important traits association (DFA Axis 2) was mainly explained by contemporary functional divergence (50.8%; Table 5) and, to a lesser degree, by population-specific effects [$\text{Pop}_{(\text{H} \times \text{FS})} = 7.8\%$; Table 5]. Rainbow smelt thus diverged in morphology under different contemporary selective pressures, associated with microphagy and macrophagy, but they diverged to a greater extent under similar selective pressures (i.e. for a specific feeding specialization) for those traits associated with vicariant history.

THE INFLUENCE OF ALLOMETRY

Allometry had a minor but significant influence on lacustrine Rainbow smelt morphological diversity. The four groups of populations formed according to their historical origin and contemporary feeding specializations varied in body size: mean \pm SD fork lengths microphageous Atlantic smelt = 97 ± 18 mm; micro-Acadian = 104 ± 25 mm; macro-Atlantic = 175 ± 54 mm; and macro-Acadian = 178 ± 42 mm. However, only six of the 36 morphometric variables examined as a function of fork length exhibited significant allometric differences. Of these, only jaw measurements (upper jaw length, lower jaw length and maxillary length) were involved in the discrimination of historical lineages and feeding specialists (Tables 2, 3). In all cases, the Atlantic microphageous feeding specialists exhibited significantly greater allometric slopes than the other three groups (results not shown). This difference in allometry most likely contributes to the similarity of microphageous and macrophageous feeding specialists within the Atlantic lineage.

DISCUSSION

The present study aimed to evaluate the contribution of relatively recent historical vicariant events in shaping contemporary intraspecific adaptations. Accordingly, we statistically associated distinct traits associations with different sources of variation, either historical or contemporary. We considered morphological traits associated with the divergence of two historical lineages as indicative of vicariant history. Traits associated with two feeding specializations observed in lakes that have been in existence for no more than 10 000 years were considered as indicative of more contemporary influences. We clearly demonstrated that vicariant historical processes are the main source of intraspecific morphological variation observed today in lacustrine smelt populations. Each

Table 4. Highly-correlated morphological traits, composing the two main axes of variation of a discriminant function analysis (DFA) differentiating Rainbow smelt when grouped as four morphotypes: two feeding specialists each descending independently from the two historical lineages

Axis 1		Axis 2	
Variables	Loadings	Variables	Loadings
Anal fin base	-0.3632	Dorsal gill arch l.	-0.2458
Pectoral fin base	-0.3394	Head width	-0.2145
Pectoral fin l.	-0.2649	Ventral gill arch l.	-0.2110
Dorsal gill raker ct	-0.2469	Dorsal fin ray ct	-0.2029
Maxillary d.	-0.1401	Eye to maxillary l.	-0.1809
Head width	0.1542	Upper jaw l.	-0.1610
Head d.	0.1707	Lower jaw l.	-0.1610
Eye diameter	0.1818	Pectoral fin base	0.1764
Lower jaw l.	0.2100	Anal fin base	0.1766
Upper jaw l.	0.2190	Head d.	0.1846
Maxillary l.	0.2257	Pelvic fin base	0.1946
Anal fin l.	0.2293	Caudal fin l.	0.1960
Ventral gill arch l.	0.2340	Pectoral fin l.	0.2279
Eye to maxillary l.	0.2650	Ventral gill raker ct	0.4017
Eye area	0.2657	Maxillary angle	0.4107

Traits identified in the first DFA (Table 2) as being associated with vicariant history are shown in bold. Measures of lengths (l.), depths (d.), and counts (ct.). For measurement details, see Fig. 2.

Table 5. Variance explained by vicariant history and feeding specialization when lacustrine populations are subdivided in four morphotype groups in a discriminant function analysis (DFA): Acadian and Atlantic microphageous smelt and Acadian and Atlantic macrophageous smelt

	d.f.	Variance Estimate	Bootstrap standard error	Wald chisquare	P-value	Part of the total variance
Axis 1						
H	1	1.8919	0.1349	196.76	< 0.0001	61.58%
FS	1	0.0553	0.0436	1.61	0.2046	1.80%
H × FS	1	0.0880	0.0672	1.72	0.1899	2.87%
Pop _(H × FS)	9	0.1085	0.0496	4.79	0.0286	3.53%
Error	459	0.9285	0.0596	243.10	< 0.0001	30.22%
Total		3.0722				
Axis 2						
H	1	0.0000	0.0018	0.00	1.0000	0.00%
FS	1	1.1275	0.0948	141.52	< 0.0001	50.83%
H × FS	1	0.0376	0.0361	1.08	0.2978	1.69%
Pop _(H × FS)	9	0.1730	0.0504	11.78	0.0006	7.80%
Error	459	0.8802	0.0641	188.80	< 0.0001	39.68%
Total		2.2183				

The random analysis of variance models (with crossed and nested factors) partitioned variance, on the two main canonical axes of the DFA, among (1) historical lineage 'H'; (2) feeding specialization 'FS'; (3) the interaction of historical lineage and feeding specialization 'H × FS'; (4) the remaining among populations variability nested within lineage and feeding specialization 'Pop_(H × FS)'; and (5) the within population variability 'Error'. d.f., degrees of freedom.

lineage has produced more or less distinct feeding specialists but not all microphageous or macrophageous smelt are morphologically similar. Hence, contemporary ecological diversification has not led to a complete morphological convergence in lacustrine Rainbow smelt as each feeding specialist has been derived from two morphologically distinct historical lineages.

INTRASPECIFIC VICARIANCE AND CONTEMPORARY ADAPTATIONS

Adaptations for morphological specializations such as microphagy and macrophagy have been explained in a wide range of fish species by a process of competition between co-existing populations, which leads to resource partitioning (Robinson & Wilson, 1994). In Rainbow smelt, contemporary resource partitioning can be accountable for functional specializations only since the postglacial formation of contemporary lakes, which occurred approximately 10 000 years ago. However, smelt morphotypes were morphologically more similar within their historical lineage than between lineages. The largest part of the morphological variation described in the present study consisted of traits characteristic of the two intraspecific historical lineages. By contrast, only a few additional traits, not associated with historical lineages, were identified as playing a secondary, but significant, role in the morphological diversification of Rainbow smelt. For example, the increase in gill raker count on the ventral gill arch for all microphageous smelt, regardless of historical origin, involved the modification of a trait that was not associated with vicariant history. Historical morphologies arose relatively recently in pre-landlocked anadromous smelt, approximately 350 000 years ago, which is well short of the time required for the development of post-zygotic reproductive isolation in allopatry. Divergent selective pressures, as exemplified by feeding specializations, have shaped the different traits characteristic of Rainbow smelt intraspecific historical lineages in producing different morphs specialized for a similar feeding mode. Thus intraspecific vicariant history has the potential to contribute to ecologically significant morphological variation that predates potential speciation events.

Historical divergence, predating contemporary selective pressures, appears to have influenced morphological divergence between microphageous and macrophageous smelt morphotypes. Previous studies on feeding specializations in fishes propose that intraspecific morphotypes may develop a greater gill raker density when faced with the need to sieve small prey, and may develop a bigger jaw to specialize their feeding on large mobile prey. This is indeed what we

observed in Rainbow smelt. When smelt from both ancestral lineages are confounded, microphageous smelt have shorter gill arches and a greater number of gill rakers on the ventral arch hence a greater gill raker density compared to macrophageous smelt. By contrast, macrophageous smelt exhibit a bigger jaw compared to microphageous smelt. Furthermore, when both morphotypes are confounded, we observed that smelt of an Acadian origin also have an overall greater gill raker density (a shorter ventral gill arch and a greater number of gill rakers on the dorsal arch) compared to smelt of an Atlantic origin. Atlantic smelt in turn exhibited a bigger jaw compared to Acadian smelt. Thus smelt from an Acadian origin appear predisposed for microphagy and smelt from an Atlantic origin appear predisposed for macrophagy. Nevertheless, both lineages have experienced parallel morphological changes in producing the alternative morphotype, and hence succeeded in exploiting similar ecological conditions making use of different available attributes.

ALTERNATIVE HYPOTHESES

The absence of complete contemporary morphological convergence for functionally-similar morphotypes may also be the result of differential phenotypic plastic responses of the two historical lineages, or different selective regimes that each historical lineage experienced during and after the colonization of post-glacial lakes. Although plastic responses within each historical lineage could be an important factor in generating morphological differences between contemporary morphotypes (Meyer, 1987), such a difference is in itself indicative of an historical difference distinguishing the two lineages.

Given the broad geographic range of the studied lakes, it could be argued that the two historical lineages have been exposed to somewhat different selection regimes subsequent to the founding of the contemporary populations, approximately 10 000 years ago. The two historical lineages are also hypothesized to have followed different post-glacial colonization routes (Bernatchez, 1997). We cannot directly assess the existence of large-scale selection regimes because we did not document the trophic ecology of the 13 populations investigated in the present study. We can only speculate about the ecological pressures that prevailed during population founding. An analysis of longitudinal and latitudinal gradients in individual morphological traits revealed no significant trends (results not presented), arguing against large-scale geographic selection regimes. Population-specific effects revealed by the ANOVA may provide a more robust test of different selection regimes acting across the distributional range of the

species. In particular, the significant but rather minor contribution of population-specific effects on the divergence of traits characterizing morphotypes, but not historical lineages (Table 5), provides only weak evidence for large-scale geographic selection regimes. If the distributional areas of the two historical lineages encompassed two selection regimes, we would expect a far more significant population effect within feeding specializations. Vicariant historical differences arising prior to population founding thus remains the more parsimonious hypothesis in explaining the intraspecific morphological diversity observed in Rainbow smelt.

THE PAST AS PROLOGUE

At this point in the evolution of Rainbow smelt, vicariant history can be considered as a diversifying force with the potential to contribute to species formation when associated with ecological polymorphism. The contemporary adaptations resulting from divergent selective pressures associated with feeding specializations have shaped morphological traits, some of which can be associated with (and others of which cannot be associated with) vicariant history. The results obtained in the present study suggest that each lineage, although apparently predisposed for a specific evolutionary path towards either microphagy or macrophagy, succeeded in developing the alternate functional morphology. We also assume that, if changes in a series of traits cannot occur under specific selective pressures to reach a contemporary adaptive peak, other suites of traits, largely unconstrained by vicariant history, may be involved. In this context, we may ask when and to what extent is divergent vicariant history a creator of diversity or an impediment to adaptation? The time scale between the moment of historical divergence and contemporary ecological specialization might play a key role (Westoby, Leishman & Lord, 1995; McPeck, 2000). Differences generated by recent vicariant history may act as a source of novelty, whereas the accumulation of differences between taxa over longer periods of time may be more resistant to current adaptive forces. To conclude, our attempt to disentangle the role of historical and more recent evolutionary events in generating intraspecific morphological diversity has revealed a predominant role for relatively recent vicariant events in the adaptive ecological divergence associated with intraspecific resource partitioning. The generality of this observation across taxa remains to be tested.

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REFERENCES

- Awise JC. 2004.** *Molecular markers, natural history and evolution*, 2nd edn. Sunderland, MA: Sinauer.
- Awise JC, Walker D, Johns GC. 1998.** Speciation durations and pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London Series B, Biological Sciences* **265**: 1707–1712.
- Baby M-C, Bernatchez L, Dodson JJ. 1991.** Genetic structure and relationships among anadromous and landlocked populations of rainbow smelt, *Osmerus mordax*, as revealed by mtDNA restriction analysis. *Journal of Fish Biology* **39** (Suppl. A): 61–68.
- Bentzen P, Hasselman D, Anstey L, Bradford R. 2006.** Adaptive morphological divergence vs. neutral genetic divergence in sympatric morphs of rainbow smelt *Osmerus mordax*. *Journal of Fish Biology* **69** (Suppl. C): 231–231.
- Bentzen P, McPhail JD. 1984.** Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialisation for alternative trophic niches in Enos Lake species pair. *Canadian Journal of Zoology* **62**: 2280–2286.
- Bernardi G. 2005.** Phylogeography and demography of sympatric sister surfperch species, *Embiotoca jacksoni* and *E. lateralis* along the California coast: historical versus ecological factors. *Evolution* **59**: 386–394.

- Bernatchez L. 1997.** Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt (*Osmerus mordax*) and their reproductive isolation in the St-Lawrence R. estuary (Québec, Canada). *Molecular Ecology* **6**: 73–83.
- Bernatchez L, Wilson CC. 1998.** Comparative phylogeography of nearctic and palearctic freshwater fishes. *Molecular Ecology* **7**: 431–452.
- Bourke P, Magnan P, Rodriguez MA. 1999.** Phenotypic responses of lacustrine brook charr in relation to the intensity of interspecific competition. *Evolutionary Ecology* **13**: 19–31.
- Brown WL, Wilson EO. 1956.** Character displacement. *Systematic Zoology* **5**: 49–65.
- Coyne JA, Orr HA. 2004.** *Speciation*. Sunderland, MA: Sinauer Associates.
- Doebeli M, Dieckmann U. 2000.** Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *American Naturalist* **156**: S77–S101.
- Douglas ME, Matthews WJ. 1992.** Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. *Oikos* **65**: 213–224.
- Edwards SV, Kot M. 1995.** Comparative methods at the species level: geographic variation in morphology and group size in grey-crowned babblers (*Pomatostomus temporalis*). *Evolution* **49**: 1134–1146.
- Funk DJ, Nosil P, Etges WJ. 2006.** Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 3209–3213.
- Galbreath K, Cook J. 2004.** Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. *Molecular Ecology* **13**: 135–148.
- Gardner AS, Walker AF, Greer RB. 1988.** Morphometric analysis of two ecologically distinct forms of Arctic charr, *Salvelinus alpinus* (L.), in Loch Rannoch, Scotland. *Journal of Fish Biology* **32**: 901–910.
- Grant PR. 1986.** *Ecology and evolution of Darwin's finches*. Princeton, NJ: Princeton University Press.
- Hickerson MJ, Ross JRP. 2001.** Post-glacial population history and genetic structure of the northern clingfish (*Gobiosox maeandricus*), revealed from mtDNA analysis. *Marine Biology* **138**: 407–419.
- Janzen FJ, Krenz JG, Haselkorn TS, Brodie ED. 2002.** Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in western North America: implications for regional historical forces. *Molecular Ecology* **11**: 1739–1751.
- Jardine TD, Curry RA. 2006.** Unique perspectives on the influence of size and age on consumer $\delta^{15}N$ from a rainbow smelt complex. *Journal of Fish Biology* **69**: 215–223.
- Knowles LL, Richards CL. 2005.** Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Biology* **14**: 4023–4032.
- Langerhans RB, Knouft JH, Losos JB. 2006.** Shared and unique features of diversification in greater Antillean Anolis ecomorphs. *Evolution* **60**: 362–369.
- Lanteigne J, McAllister DE. 1983.** *The pygmy smelt, Osmerus mordax Cope, 1870, a forgotten sibling species of eastern North America fish*. Ichthyology Section, National Museum of Natural Sciences, National Museum of Canada.
- Lecomte F, Dodson JJ. 2004.** Role of early life-history and resource polymorphism in the segregation of sympatric populations of an estuarine fish. *Evolutionary Ecology Research* **6**: 631–658.
- Lenk P, Fritz U, Joger U, Wink M. 1999.** Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Molecular Ecology* **8**: 1911–1922.
- Liukkonen AT, Uimaniemi L, Orrel M, Lumme J. 2002.** Mitochondrial DNA variation and the phylogeography of the grey partridge (*Perdix perdix*) in Europe: from Pleistocene history to present day populations. *Journal of Evolutionary Biology* **15**: 971–982.
- Losos JB. 2000.** Ecological character displacement and the study of adaptation. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 5693–5695.
- Losos JB, Jackman TR, Larson A, de Queiroz K, Rodriguez-Schettino L. 1998.** Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**: 2115–2118.
- McAllister DE. 1963.** A revision of the smelt family, Osmeridae. *National Museum of Canada Bulletin* **191**: 1–53.
- McPeck MA. 2000.** Predisposed to adapt? Clade-level differences in characters affecting swimming performance in damselfishes. *Evolution* **54**: 2072–2080.
- McPhail JD. 1994.** Speciation and the evolution of reproduction isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In: Bell MA, Foster SA, eds. *The evolutionary biology of the three-spined stickleback*. Oxford: Oxford University Press, 399–437.
- Meyer A. 1987.** Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* **41**: 1357–1369.
- Michaux JR, Libois R, Paradis E, Filippucci MG. 2004.** Phylogeographic history of the yellow-necked fieldmouse (*Apodemus flavicollis*) in Europe and in the Near and Middle East. *Molecular Phylogenetics and Evolution* **32**: 788–798.
- Nellbring S. 1989.** The ecology of smelts (Genus *Osmerus*): a literature review. *Nordic Journal of Freshwater Research* **65**: 116–145.
- Pfennig DW, Murphy PJ. 2003.** A test of alternative hypotheses for character divergence between coexisting species. *Ecology* **84**: 1288–1297.
- Pigeon D, Dodson JJ, Bernatchez L. 1998.** A mtDNA analysis of spatio-temporal distribution of two sympatric larval populations of rainbow smelt (*Osmerus mordax*) in the St. Lawrence river estuary, Québec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 1739–1747.
- Robinson BW, Wilson DW. 1994.** Character release and displacement in fishes: a neglected literature. *American Naturalist* **144**: 596–627.
- Rogers SM, Gagnon V, Bernatchez L. 2002.** Genetically based phenotype-environment association for swimming

- behavior in lake whitefish ecotypes (*Coregonus clupeaformis* Mitchill). *Evolution* **56**: 2322–2329.
- Saint-Laurent R, Legault M, Bernatchez L. 2003.** Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). *Molecular Ecology* **12**: 315–330.
- Schluter D. 2001.** Ecology and the origin of species. *Trends in Ecology and Evolution* **16**: 372–380.
- Schluter D, McPhail JD. 1992.** Ecological character displacement and speciation in sticklebacks. *American Naturalist* **140**: 85–108.
- Scott WB, Crossman EJ. 1974.** Freshwater fishes of Canada. *Bulletin of the Fisheries Research Board of Canada* **184**: 335–342.
- Slatkin M. 1980.** Ecological character displacement. *Ecology* **61**: 163–177.
- Smith MA, Green DM. 2004.** Phylogeography of *Bufo fowleri* at its northern range limit. *Molecular Ecology* **13**: 3723–3733.
- Stevens MI, Hogg ID. 2003.** Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology* **12**: 2357–2369.
- Taylor EB. 1999.** Species pairs of north temperate freshwater fishes: Evolution, taxonomy, and conservation. *Reviews in Fish Biology and Fisheries* **9**: 299–324.
- Taylor EB, Bentzen P. 1993a.** Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. *Evolution* **47**: 813–832.
- Taylor EB, Bentzen P. 1993b.** Molecular genetic evidence for reproductive isolation between sympatric population of smelt *Osmerus* in Lake Utopia, south-western New Brunswick, Canada. *Molecular Ecology* **2**: 345–357.
- Via S. 2001.** Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology and Evolution* **16**: 381–390.
- Westoby M, Leishman MR, Lord JM. 1995.** On misinterpreting the ‘phylogenetic correction’. *Journal of Ecology* **83**: 531–534.

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