
Microgeographical diversification of threespine stickleback: body shape–habitat correlations in a small, ecologically diverse Alaskan drainage

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Adaptive radiations are a major source of evolutionary diversity in nature, and understanding how they originate and how organisms diversify during the early stages of adaptive radiation is a major problem in evolutionary biology. The relationship between habitat type and body shape variation was investigated in a postglacial radiation of threespine stickleback in the upper Fish Creek drainage of Cook Inlet, Alaska. Although small, the upper Fish Creek drainage includes ecologically diverse lakes and streams in close proximity to one another that harbour abundant stickleback. Specimens from ancestral anadromous and derived resident freshwater populations differed substantially and could be distinguished by body shape alone, suggesting that the initial stages of adaptation contribute disproportionately to evolutionary divergence. Body shape divergence among resident freshwater populations was also considerable, and phenotypic distances among samples from freshwater populations were associated with habitat type but not geographical distance. As expected, stream stickleback from slow-moving, structurally complex environments tended to have the deepest bodies, stickleback from lakes with a mostly benthic habitat were similar but less extreme, and stickleback from lakes with a mostly limnetic habitat were the most shallow-bodied, elongate fish. Beyond adapting rapidly to conditions in freshwater environments, stickleback can diversify rapidly over small geographical scales in freshwater systems despite opportunities for gene flow. This study highlights the importance of ecological heterogeneity over small geographical scales for evolutionary diversification during the early stages of adaptive radiation, and lays the foundation for future research on this ecologically diverse, postglacial system. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 98, 139–151.

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INTRODUCTION

Adaptive radiation is a central topic in evolutionary biology. Divergent natural selection plays a key role during adaptive radiation (reviewed in Endler, 1986; Schluter, 2000). Invasion of a new type of habitat or ‘adaptive zone’ (Simpson, 1953) can result in substantial divergence between ancestral and derived taxa over short time periods (Hendry & Kinnison, 1999; Kinnison & Hendry, 2001; Reznick & Ghalambor, 2001). Diversification within the adaptive zone can also proceed rapidly, as evidenced by phylogenetic

studies indicating relatively rapid bursts of speciation during the initial stages of adaptive radiation (for example, Hodges, 1997; Danley & Kocher, 2001) and the existence of young adaptive radiations in isolated habitats (for example, Echelle & Kornfield, 1984; Skúlason, Snorrason & Jonsson, 1999). Empirical studies documenting divergence and subsequent diversification in response to the invasion of a new adaptive zone can provide important insight into the process of evolutionary diversification. Unfortunately, studying the early phases of adaptive radiation is difficult. The lack of a known ancestor to establish a baseline and the lack of information on the conditions present during the early stages of the radiation, when much of the diversity originates, are typical limitations.

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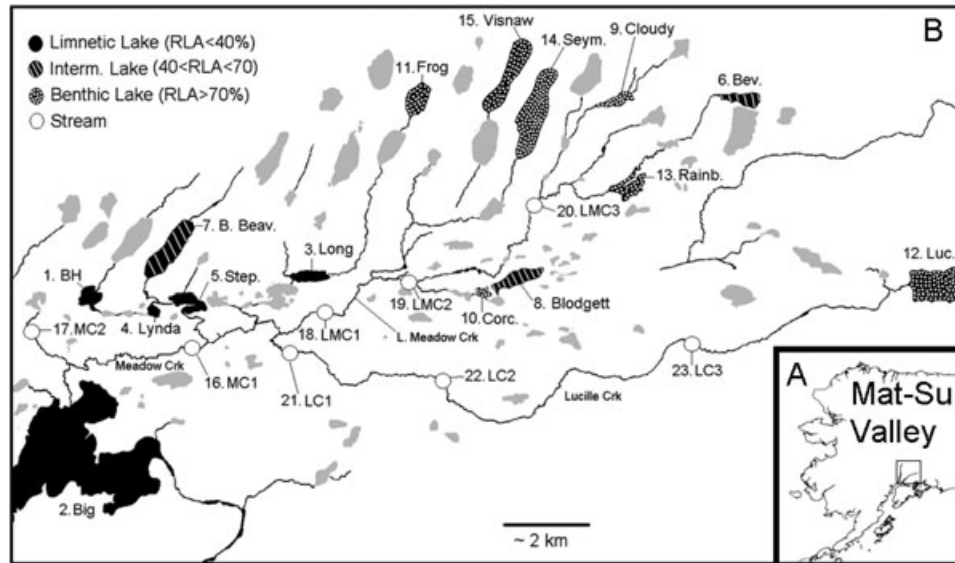


Figure 1. Map of collection sites. Lakes in plain grey were not sampled. A, approximate position of Matanuska-Susitna Valley. B, Lake and stream sites sampled. See Table 1 for detailed site information.

The threespine stickleback fish *Gasterosteus aculeatus* (referred to as stickleback hereafter) is a proven system to study the early stages of adaptive radiation (for example, Bell & Foster, 1994; Schluter, 2000). Stickleback are primitively oceanic, breeding in shallow ocean waters (marine) or in freshwater streams (anadromous). Thousands of new lakes and streams formed when glaciers retreated in coastal areas of the Northern Hemisphere beginning approximately 20 000 years ago. Anadromous stickleback established resident populations in this new adaptive zone, and repeatedly adapted to conditions in freshwater (Bell & Foster, 1994; Schluter, 2000). Post-glacial freshwater stickleback populations share a common suite of adaptations, such as armour reduction, which evolved in parallel throughout the Northern Hemisphere (for example, Bell, 1987; Bell & Foster, 1994; Colosimo *et al.*, 2005). However, resident freshwater populations also vary considerably in morphology based on the divergent selective pressures that they experience in contrasting freshwater habitats (for example, Bell, 1982; Reimchen, Stinson & Nelson, 1985; Baumgartner, 1992; Spoljaric & Reimchen, 2007).

Divergent natural selection for the exploitation of benthic (littoral, shallow and near-shore) vs. limnetic (deep, open-water) habitats and associated food items is one of the most common diversifying mechanisms affecting resident freshwater stickleback (for example, Walker, 1997; Spoljaric & Reimchen, 2007) and boreal fish in general (Robinson & Wilson, 1994; Schluter, 1996; Bell & Andrews, 1997). Resident freshwater stickleback populations evolve along a phenotypic con-

tinuum based on the relative importance of larger benthic food items associated with vegetation in shallow habitats or smaller planktonic prey found in open waters (Walker, 1997; Foster *et al.*, 2008). This divergence is most dramatic in several lakes in British Columbia where sympatric 'benthic' and 'limnetic' species pairs exist (for example, Bentzen & McPhail, 1984; McKinnon & Rundle, 2002). Benthics have deeper bodies better suited for manoeuvring in structurally complex habitats, whereas limnetics have narrower bodies that are better suited for sustained swimming in open waters (Walker, 1997). Sympatric species pairs exist only in a few lakes in British Columbia (McPhail, 1994); in other areas, variation along this continuum is more subtle, with extreme forms being allopatric (but see Cresko & Baker, 1996).

In this article, the phenotypic variation of stickleback is examined in a small, ecologically diverse Alaskan drainage. The upper portion of the Fish Creek drainage is unusual because it comprises adjacent, interconnected lakes that differ substantially in the amount of benthic vs. limnetic habitat types (Fig. 1). Stickleback in lakes and streams in the Fish Creek drainage have been used in numerous studies, suggesting substantial variability in the system (for example, Bell & Ortí, 1994; Walker, 1997; Baker & Foster, 2002; Cresko *et al.*, 2004; Purnell *et al.*, 2006), but no previous study has systematically screened phenotypic variation within the drainage. The primary objective of this study was to investigate whether body shape variation of stickleback in this drainage was associated with habitat type (broadly defined, see below) despite small geographical dis-

tances among sites and the potential for gene flow to homogenize phenotypes in the system. The magnitude of differentiation among populations in the system was also examined, together with how phenotypic divergence among freshwater populations compares with divergence between freshwater and anadromous populations.

Body shape was used as the measure of phenotypic differentiation because it is a complex trait that is strongly associated with ecological variables having important fitness consequences (for example, Walker, 1997; Spoljaric & Reimchen, 2007). It was expected that body shape would differ significantly among lake and stream sites (Hendry, Taylor & McPhail, 2002; Moore & Hendry, 2005), and that the pattern of variation would concord with previous ecomorphological models for the association between body shape and habitat depth (Walker, 1997; Spoljaric & Reimchen, 2007). Although phenotypic plasticity is common in fish and is probably important during the initial stages of adaptation to new environments (West-Eberhard, 2003; Hendry, Farrugia & Kinnison, 2008; Wund *et al.*, 2008), genetic factors account for more of the body shape variation among ecologically diverse postglacial stickleback populations (for example, Spoljaric & Reimchen, 2007), including in Alaskan stickleback (W. E. Aguirre and W. J. Caldecutt, unpublished data). In addition, genetic studies have found a strong genetic component to body shape variation (Lavin & McPhail, 1987; Schluter *et al.*, 2004; Albert *et al.*, 2008). Consequently, a genetic basis was assumed for much of the body shape variation observed.

This study contributes to the current knowledge of the microgeographical distribution of stickleback phenotypic variation. Few studies have explored the variation of body shape or trophic traits at the scale of the drainage (Reimchen *et al.*, 1985; Baumgartner, 1992); most have done so at the regional level (for example, Penczack, 1965; Hagen & Gilbertson, 1972; Moodie & Reimchen, 1976; Walker, 1997; Berner *et al.*, 2008; Spoljaric & Reimchen, 2008), between adjacent stream and lake sites (for example, Hendry *et al.*, 2002; Moore & Hendry, 2005) or in sympatric forms (for example, Baumgartner, Bell & Weinberg, 1988; Cresko & Baker, 1996; Karve, von Hippel & Bell, 2008).

MATERIALS AND METHODS

STUDY AREA

The Fish Creek drainage is a relatively young, small, postglacial drainage near Wasilla, located on the north shore of Cook Inlet in the Matanuska-Susitna Borough, Alaska. Glaciers covered the area occupied by the drainage during the late Wisconsin glaciation

and retreated within the last 20 000 years (Reger & Pinney, 1996). The drainage includes Meadow, Little Meadow and Lucille creeks and associated lakes in its upper (that is, above Big Lake) portion (Fig. 1). The creeks generally flow south-west into Big Lake, which, in turn, drains south into Fish Creek proper. Fish Creek runs south approximately 20.1 km from Big Lake before discharging into the Knick Arm of Cook Inlet, and there are few lakes associated with it south of Big Lake. A few ponds and lakes north and west of Big Lake also drain into Big Lake. The stream sites sampled above Big Lake are largely low gradient, slow flowing, shallow and rich in aquatic vegetation. Sites sampled in the upper Fish Creek drainage range from approximately 40 to 115 m in altitude. The lakes vary considerably in the amount of benthic vs. limnetic habitat. Some are very shallow (< 3m), and most of the stickleback habitat is benthic. Others can be relatively deep, for example Big Lake reaches a maximum depth of 27 m. In deep lakes, much of the available stickleback habitat is limnetic. Water quality also plays a critical role in the amount of benthic vs. limnetic habitat available in lakes. Solar radiation will penetrate to greater depths in lakes with clearer water, promoting macrophyte growth, whereas the inverse occurs in lakes with stained or turbid waters. A simple measure used to quantify the amount of benthic vs. limnetic habitat available, which takes both depth profiles and water quality into account, is the relative littoral area (RLA). The RLA is the proportion of littoral foraging habitat in a lake, and was estimated following Walker (1997) as the percentage of the lake surface area in which the depth of the bottom is above the euphotic zone depth (EZD), that is, is capable of supporting macrophyte growth. The EZD was estimated using the linear equation, $\log \text{EZD} = 1.9875 - 0.8787 \log \text{Pt}$, where Pt represents platinum cobalt units, a measure of water colour determined by comparing filtered lake water absorbance at 400 nm with a standard containing a known amount of platinum cobalt (Koenings *et al.*, 1987). Lakes with an RLA value of 100 are capable of supporting macrophyte growth throughout their entire bottom. Lakes with lower RLA values have larger amounts of limnetic habitat.

SAMPLING PROCEDURES

Twenty-three sites, including 15 lakes and eight stream locations in the upper Fish Creek drainage, were sampled (Table 1). Anadromous fish were collected from two additional sites in neighbouring drainages for comparison (Rabbit Slough and Mud Lake). The Rabbit Slough population was sampled over three different years (1997, 2000 and 2003). Anadromous stickleback typically do not occur in the

Table 1. Sampling sites

Num.	Site	Type	Lat. (N), Long. (W)	<i>N</i>	Year	SA	MaxDp	RLA
1	BH	Lake (L)	61.574, 149.869	20	2004	13.4	4.6	15.6
2	Big	Lake (L)	61.535, 149.826	30	2005	1009.7	27.4	1.0
3	Long	Lake (L)	61.576, 149.774	20	2004	18.0	5.2	30.2
4	Lynda	Lake (L)	61.570, 149.841	25	2004	4.5	7.6	24.2
5	Stepan	Lake (L)	61.570, 149.816	19	2005	24.2	5.2	34.0
6	Beverley	Lake (I)	61.613, 149.569	20	2005	17.0	6.4	53.3
7	BBeav	Lake (I)	61.578, 149.842	20	2005	65.2	5.2	41.7
8	Blodgett	Lake (I)	61.579, 149.671	20	2004	23.2	6.7	66.1
9	Cloudy	Lake (B)	61.612, 149.639	30	2005	12.1	1.8	100.0
10	Corcoran	Lake (B)	61.573, 149.693	20	2004	8.1	2.1	100.0
11	Frog	Lake (B)	61.614, 149.723	20	2005	25.9	8.2	100.0
12	Lucille	Lake (B)	61.579, 149.450	20	2004	146.5	6.1	87.8
13	Rainbow	Lake (B)	61.594, 149.632	20	2005	20.2	4.6	100.0
14	Seymour	Lake (B)	61.614, 149.670	20	2004	92.7	5.2	84.1
15	Visnaw	Lake (B)	61.614, 149.680	20	2004	52.9	5.2	100.0
16	MC1	Creek	61.563, 149.826	30	2005	–	–	–
17	MC2	Creek	61.566, 149.893	11	2005	–	–	–
18	LMC1	Creek	61.569, 149.760	30	2005	–	–	–
19	LMC2	Creek	61.576, 149.728	30	2005	–	–	–
20	LMC3	Creek	61.592, 149.666	30	2005	–	–	–
21	LC1	Creek	61.561, 149.779	17	2004	–	–	–
22	LC2	Creek	61.553, 149.708	18	2004	–	–	–
23	LC3	Creek	61.562, 149.602	26	2004	–	–	–
24	Mud Lake	Anad.	61.565, 148.947	18	2003	–	–	–
25	RS	Anad.	61.534, 149.268	50	1997	–	–	–
26	RS	Anad.	61.534, 149.268	50	2000	–	–	–
27	RS	Anad.	61.534, 149.268	46	2003	–	–	–

Num., site number (Fig. 1); Lat./Long., latitude/longitude in decimal degrees north and west, respectively; *N*, number of specimens measured; Year, year of collection; SA, surface area (ha); MaxDp, maximum depth (m); RLA, relative littoral area. For the lake sites: (B), lakes with mostly benthic habitat (RLA > 70%; abundant macrophyte growth); (I), intermediate lakes (40% < RLA < 70%); (L), lakes with mostly limnetic habitat (RLA < 40%). For the lake sites: BBeav, Big Beaver; BH, Beaverhouse. For the creek sites: LC, Lucille Creek; LMC, Little Meadow Creek; MC, Meadow Creek. For the anadromous sites: RS, Rabbit Slough; samples were collected in three different years ($N_{\text{total}} = 146$).

upper Fish Creek drainage, but anadromous populations in the region tend to be relatively homogenous phenotypically (Walker & Bell, 2000) and genetically (Cresko, 2000). Consequently, the anadromous samples are assumed to be representative of the anadromous fish that colonized the Fish Creek drainage. This study is conservative in terms of morphological diversity, because lakes lacking a stream connection and known to harbour stickleback populations with extreme armour reduction (Bell, Francis & Havens, 1985; Bell & Ortí, 1994) were excluded. Sampling was carried out to include lakes at the extremes for RLA and stream sites interspersed among them. Fish were collected in late May to June in 2004 and 2005, except for the anadromous samples, which were collected between 1997 and 2003. Six to 20 unbaited 0.64 mm and/or 0.32 mm mesh minnow traps were set overnight. Specimens were anaesthetized with

MS-222, fixed in 10% buffered formalin, transferred to 50% isopropyl for storage and stained with Alizarin Red S following Bell & Ortí (1994) to facilitate the visualization of landmarks (LMs) associated with bones.

COLLECTION OF BODY SHAPE DATA

Geometric morphometrics (Adams, Rohlf & Slice, 2004; Zelditch *et al.*, 2004) was used to study body shape. Only adult male specimens were included to minimize the variation related to allometry and sexual dimorphism. Males were identified by inspection of their gonads. Specimens infected with *Schistocephalus solidus* were also excluded because the worms may distort the body shape. In total, 680 specimens were included in the morphometric analysis. Specimens were photographed with a 3.3 mega-

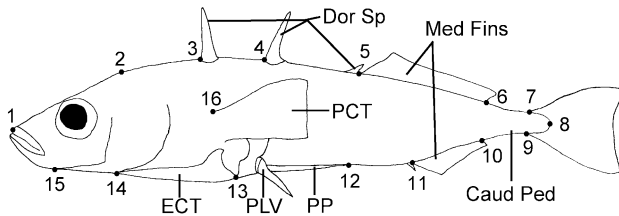


Figure 2. Landmarks used in this study (numbers 1–16). Anatomical structures mentioned in the text are labelled. ECT, ectocoracoid; PLV, pelvis; PP, posterior process of the pelvis; Caud Ped, caudal peduncle; Med Fins, median fins; Dor Sp, dorsal spines; PCT, pectoral fin.

pixel Olympus Camedia C-3000 digital camera, and two-dimensional coordinates were collected for 16 LMs digitized on each specimen (Fig. 2) using tpsDig version 1.40 (Rohlf, 2004). The LMs are based on Walker (1997), with the addition of a 16th LM at the dorsal origin of the pectoral fin. The LM data were aligned using Procrustes superimposition implemented in the program tpsRelw version 1.44 (Rohlf, 2006) to eliminate variation related to rotation, translation and size. All specimens were included in a single alignment to generate the shape variables.

MULTIVARIATE ANALYSES

Body shape is a complex multivariate trait, and so a combination of methods was used to examine variation among populations and its association with habitat type. Slight redundancy in the methods employed allowed the assessment of the robustness of the results.

First, variation in the body shape was explored using principal components analysis (PCA). PCA was used to evaluate patterns of differentiation among population means based on habitat type in a simple, two-dimensional space accounting for a large percentage of variation in the original dataset. PCA was performed in tpsRelw version 1.44 (Rohlf, 2006) using all individuals, and sample means were calculated afterwards. Procrustes distances calculated using tpsSmall 1.20 (Rohlf, 2003) between sample means were also used to examine differentiation among populations. The Procrustes distance is approximately the square root of the sum of squared differences between homologous LMs when the configurations are in Procrustes superimposition (Zelditch *et al.*, 2004). The significance of body shape divergence in relation to habitat type was tested using multivariate analysis of covariance (MANCOVA), as implemented in tpsRegr ver. 1.31 (Rohlf, 2005), and centroid size was included as a covariate to account for body size differences among populations. Significance was evaluated through a permutation test (1000 permutations).

Discriminant function analysis, as implemented in SPSS vers. 11.0.0 (SPSS Inc., 2001), was used to obtain another measure of the magnitude of differentiation among populations and to assess the predictability with which individuals could be correctly assigned to habitat type and population based on body shape. Two analyses were performed. In the first, sites were pooled by habitat type and the analysis determined rates of assignment to the correct habitat type. In the second, the analysis determined rates of correct classification based on the collection site. Shape variables (partial warps and uniform component) obtained from a single alignment of all specimens performed in tpsRelw vers. 1.44 (Rohlf, 2006) were used in the discriminant function analysis. Prior probabilities were obtained from group sizes and a leave-one-out classification was implemented. The leave-one-out classification classifies each specimen with functions derived from all individuals except that being classified.

Finally, Mantel tests, as implemented in PASSAGE version 1.1 (Rosenberg, 2004), were used to examine the association between body shape differentiation among population means and habitat type, and to take geographical distances among sites into account. Anadromous samples were not included in this analysis. The Mantel test is a test of the association between corresponding elements of two distance matrices, and has been widely used in biology (Mantel, 1967; Sokal, 1979). Three distance matrices were created: a geographical distance matrix, a habitat dissimilarity matrix and a phenotypic distance matrix. Significance was evaluated using permutation tests with 9999 permutations performed.

The geographical distance matrix consisted of natural logarithmically transformed distances in kilometres measured along the stream course between all sampling sites. One was added to the logarithmically transformed values for geographical distance to avoid having negative distances resulting from sites less than 1 km apart. An alternative geographical distance matrix was also evaluated because different distance schemes can have a significant impact on the results (for example, Sokal *et al.*, 1997). In the alternative geographical distance matrix, sites that were directly connected (between which you could travel without passing through any other sites) were given distance values of zero and sites connected indirectly were given distance values of unity. Both geographical distance matrices yielded similar results, and so only the analyses employing natural logarithmically transformed geographical distances along streams between sites are presented.

The habitat distance matrix used four categories: stream (eight samples), lakes with mostly benthic habitats (benthic lakes, seven samples), intermediate lakes (lakes with both significant benthic and lim-

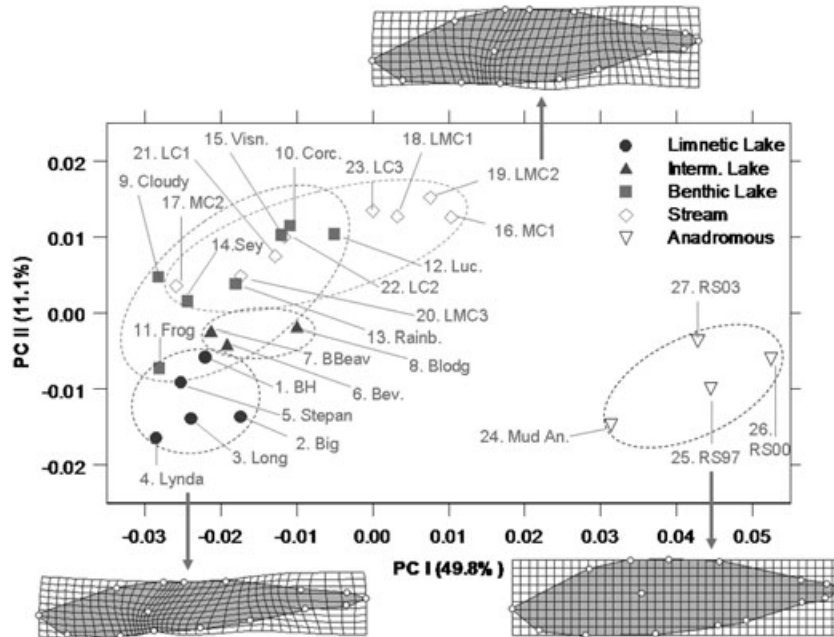


Figure 3. Principal components plot of sample means. The variation explained by each PC is in parentheses. Grids are included to depict major patterns of variation among the populations and represent consensus configurations of Rabbit Slough (for all three years), Little Meadow Creek site 2 and Lynda Lake, as deformations of the Rabbit Slough consensus (exaggerated by 2x to facilitate visualization).

netic habitats, three samples) and limnetic lakes (lakes with mostly limnetic habitats, five samples). The stream sites are largely slow flowing, shallow and structurally complex, and it was expected that the stickleback habitat would resemble that found in benthic lakes or ponds. Lake categories were based on RLA, as described above. Lakes with RLA < 40% were classified as limnetic, lakes with RLA of 40–70% as intermediate, and lakes with RLA > 70% as benthic. Scores of zero and unity were assigned to cells for pairwise comparisons of samples from the same and different habitat types, respectively, except that pairs comprising an intermediate lake plus a limnetic or benthic lake were given a value of 0.5. Using distance values of unity instead of 0.5 between intermediate and benthic/limnetic lakes, or changing the cut-offs for defining intermediate lakes (for example, defining Blodgett as a benthic lake, Big Beaver as a limnetic lake or Stepan as an intermediate lake), did not alter the results significantly. In addition, testing the relationship between consensus body shape and RLA directly for the lake populations (instead of dividing them into categories) yielded qualitatively similar results to the Mantel tests (consensus lake body shape was significantly associated with RLA; tpsRegr generalized Goodall F -test, $F = 5.5975$, 1000 permutations, $P = 0.001$). Procrustes distances (defined above) between sample means were used for the phenotypic distance matrix. A partial Mantel test

(Smouse, Long & Sokal, 1986) was used to test the association between phenotypic and habitat distance matrices, holding the geographical distances constant.

RESULTS

BODY SHAPE VARIATION AMONG POPULATIONS

The first two principal components (PCs) accounted for 60.9% of the variation in body shape (Fig. 3). The anadromous populations differed substantially from resident lake and stream populations, and segregation in the PCA occurred primarily along PC I. Resident freshwater populations also diverged substantially, with stream and limnetic lake samples being at the extremes, as expected.

The differences in body shape between anadromous and freshwater populations exceeded the differences among freshwater populations based on the configuration of sample means in the PC space (Fig. 3) and Procrustes distances between the consensus configurations of populations pooled by habitat type (Table 2). Procrustes distances between anadromous and resident freshwater forms were generally more than twice as large as between different freshwater forms. Anadromous stickleback tended to have a larger pelvis and ectocoracoid, as indicated by displacements of LMs 12, 13 and 14, a shorter caudal

Table 2. Procrustes distances between consensus configurations for populations pooled by habitat type

	Limnetic	Interm.	Benthic	Stream	An.
Limnetic	0	22.4	32.8	46.3	100.0
Interm.	0.015	0	20.9	31.3	91.0
Benthic	0.022	0.014	0	31.3	94.0
Stream	0.031	0.021	0.021	0	74.6
An.	0.067	0.061	0.063	0.050	0

Values below the diagonal are the actual Procrustes distances. Values above the diagonal are expressed as a percentage of the maximum Procrustes distance observed: 0.067 between anadromous and limnetic habitat types. Benthic are lakes with mostly benthic habitat, Interm. are intermediate lake types, Limnetic are lakes with mostly limnetic habitat and An. is anadromous.

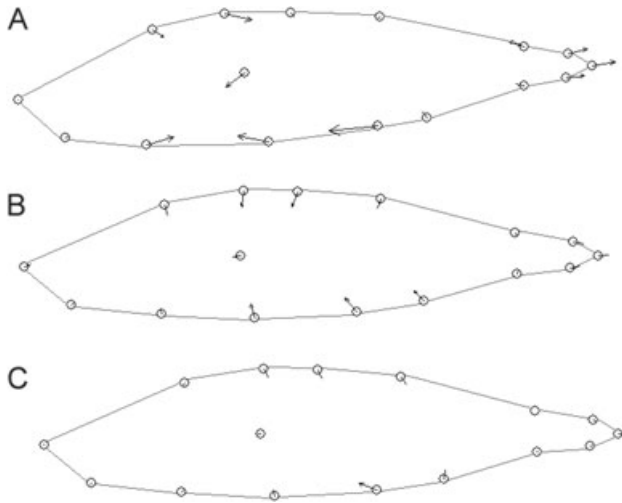


Figure 4. Body shape variation among consensus configurations for populations pooled by habitat type. A, change in position of landmarks of consensus freshwater configuration from consensus anadromous configuration. B, consensus limnetic lake from consensus stream configuration. C, consensus benthic lake from consensus stream configuration. Landmark displacements were exaggerated by 2x for visualization.

peduncle (LMs 6, 7, 9 and 10), an anterior first dorsal spine (LM 3), posterodorsally displaced pectoral fins (LM 16) and slightly longer median fins (LM 6 and 10; Fig. 4A). There was no overlap among anadromous and resident freshwater individuals in the space formed by PCs I and II (data not shown).

Resident freshwater population means tended to form a gradient from stream samples at the top centre through benthic and intermediate to limnetic lake samples at the bottom left corner of the two-dimensional PC space (Fig. 3). There was relatively broad overlap among the means for some habitat types, especially between stream and benthic lake and benthic lake and intermediate lake sample means. Divergence in body shape along this gradient

was largely associated with body depth. Stream and limnetic lake samples were the most divergent phenotypically among the freshwater samples. This held both in the two-dimensional PC space (Fig. 3) and for Procrustes distances (Table 2). Fish from stream samples tended to have much deeper bodies, shorter caudal peduncles (LMs 7, 9) and a posterior pectoral fin (LM 16) relative to fish from limnetic lake samples (Fig. 4B). Although there was some overlap, individuals from stream and limnetic lake sites largely occurred in different portions of the space formed by PCs I and II (data not shown), highlighting the magnitude of the differentiation among extreme resident freshwater forms. Intermediate-depth lakes tended to have stickleback that were on average intermediate in body shape between benthic lake and limnetic lake stickleback. Procrustes distances between the consensus body shape for intermediate lake stickleback and benthic and limnetic lake consensus configurations were small and similar (Table 2). Benthic lake and stream samples were relatively similar in body shape. Benthic lake fish were slightly less deep bodied, with the largest LM displacement occurring at the posterior tip of the pelvis (Fig. 4C). Stream stickleback tended to have a slightly longer pelvic process (LM 12).

MANCOVA (with centroid size included as a covariate) confirmed that the body shape of resident lake and stream populations differed significantly among habitat types (Wilks $\lambda = 0.180$, $F_s = 11.54$, $df_1 = 84$, $df_2 = 1254.3$, $P < 0.001$).

PREDICTABILITY OF CLASSIFICATION BY HABITAT TYPE

Discriminant function analysis yielded results consistent with the significant phenotypic divergence among populations by habitat type observed in the PCA. Based on body shape, 81.9% of individuals could be assigned correctly to the habitat type from which they came (Table 3). The assignment rate of anadro-

Table 3. Classification results (%) from discriminant function analysis of body shape data by habitat type

Source	<i>s</i>	<i>N</i>	Percentage classified as				
			Limnetic	Interm.	Benthic	Stream	Anadromous
Limnetic	5	114	80.7	4.4	8.8	6.1	0
Interm.	3	60	26.7	50.0	13.3	10.0	0
Benthic	7	150	4.0	3.3	78.7	14.0	0
Stream	8	192	3.6	1.0	15.6	79.7	0
Anadromous	2	164	0	0	0	0	100

Source, type of habitat from which the specimens originated; *s*, number of sites sampled per habitat type; *N*, number of specimens included per habitat type; Percentage classified as, percentage of fish assigned to each of the habitat types; Benthic, lakes with mostly benthic habitat; Interm., intermediate lake types; Limnetic, lakes with mostly limnetic habitat.

mous vs. resident freshwater stickleback was 100%. Among the resident freshwater populations, correct assignment percentages were similar for stream, benthic and limnetic lake samples (around 80%). Most misclassified stream fish were assigned to benthic lakes, and vice versa, confirming the similarity of body shape of stickleback from these habitat types. Stickleback from relatively limnetic lakes exhibited the highest frequency of correct classifications, with misclassified individuals being assigned to all other freshwater habitat types in similar proportions (4.4–8.8%). Correct assignment percentages were lowest for intermediate-depth lake populations, with only 50% of individuals correctly assigned. The other 50% of individuals were assigned in similar proportions to limnetic lake (26.7%) and stream or benthic lake (23.3% combined, see Table 3) habitat types.

Assignment rates differed substantially among resident freshwater populations, however. Misclassification to habitat types different from that of origin tended to be high in samples from a few populations within each category (Table 4), with most having relatively high correct assignment rates (> 70%). Three of the five limnetic lake populations (Big, Long and Lynda) had assignment rates above 95%. Beaverhouse Lake had an assignment rate of 75%, whereas Stepan Lake had a correct assignment rate of only 42.1%. Individuals from Stepan were assigned to all other habitat types, with the highest percentage corresponding to the benthic lake type (26.3%). Two of the intermediate-depth lakes had moderately high correct assignment percentages (> 75%), whereas the third, Blodgett Lake, had a correct assignment rate of only 35%. The highest percentage of misclassified individuals from Blodgett corresponded to the limnetic lake type (30%), although individuals were incorrectly assigned to benthic and stream types as well. Five of the seven benthic lake samples had

relatively high correct assignment rates ($\geq 85\%$). Rainbow had a correct classification rate of 65%, with 20% of individuals assigned to the stream type, whereas Lucille Lake had a correct classification rate of only 40%, with the majority of individuals (55%) assigned to the stream type. Finally, six of the eight stream sites had moderately high correct classification rates (> 70%) with most incorrectly assigned individuals being classified as benthic lake types. Lucille Creek site 2 had a correct classification rate of 66.7% and Meadow Creek site 2 had a correct classification rate of only 54.5%, with a relatively large number of individuals from this site surprisingly being classified as limnetic lake-type stickleback (27.3%). Assignment rates to the correct site of collection (rather than habitat type) were generally lower, as expected, varying between 30% (Rainbow) and 100% (Seymour Lake), with an unweighted average of 60.6% (Appendix S1, see Supporting Information).

ASSOCIATION BETWEEN BODY SHAPE AND HABITAT TYPE IS NOT A RESULT OF SPATIAL AUTOCORRELATION

Similar types of habitat were often located in close geographical proximity in the upper Fish Creek drainage (Fig. 1), raising the possibility that the association between body shape and habitat type was a result of the spatial autocorrelation of habitats. Spatial autocorrelation of habitat type was marginally nonsignificant (Mantel test, $r = 0.108$, $Z = 307$, $P = 0.064$), and variation in body shape among lake and stream samples was not associated with the geographical distances among them (Mantel test, $r = -0.013$, $Z = 44.31$, $P = 0.450$). Geographical structure was thus not responsible for patterning phenotypic divergence. Consistent with the results from PCA and discriminant function analysis, phenotypic distances among lake and stream samples were

Table 4. Classification results (%) from discriminant function analysis of body shape data by habitat type for individual populations

Num.	Site	Limnetic	Interm.	Benthic	Stream
1	Beaverhouse	75.0	0	15.0	10.0
2	Big	96.7	0	0	3.3
3	Long	95.0	0	5.0	0
4	Lynda	100.0	0	0	0
5	Stepan	42.1	15.8	26.3	15.8
6	Beverley	5.0	80.0	10.0	5.0
7	Big Beaver	15.0	75.0	10.0	0
8	Blodgett	30.0	35.0	15.0	20.0
9	Cloudy	6.7	3.3	86.7	3.3
10	Corcoran	0	0	85.0	15.0
11	Frog	10.0	5.0	85.0	0
12	Lucille	0	5.0	40.0	55.0
13	Rainbow	5.0	10.0	65.0	20.0
14	Seymour	0	0	100.0	0
15	Visnaw	0	0	95.0	5.0
16	MC1	0	0	0	100.0
17	MC2	27.3	9.1	9.1	54.5
18	LMC1	6.7	0	6.7	86.7
19	LMC2	0	0	6.7	93.3
20	LMC3	0	0	23.3	76.7
21	LC1	0	0	29.4	70.6
22	LC2	0	0	33.3	66.7
23	LC3	0	3.8	15.4	80.8

Num., site number; Benthic, lakes with mostly benthic habitat; Interm., intermediate lake types; Limnetic, lakes with mostly limnetic habitat; LC, Lucille Creek; LMC, Little Meadow Creek; MC, Meadow Creek. Percentages from habitat type of origin are given in bold.

significantly associated with the habitat type from which they were collected, regardless of whether (partial Mantel test between phenotypic distances and habitat type holding geographical distances constant, $r = 0.362$, $Z = 0.657$, $P = 0.0003$) or not (Mantel test, $r = 0.366$, $Z = 10.062$, $P = 0.0001$) geographical distances were taken into account.

DISCUSSION

The major axis of phenotypic variation in this study was between ancestral anadromous and derived resident freshwater populations. In conjunction with previous studies (for example, Walker & Bell, 2000; Leinonen *et al.*, 2006; Aguirre *et al.*, 2008), this result indicates that great phenotypic divergence often occurs early in the process of adaptive radiation. Indeed, empirical studies indicate that high evolutionary rates are associated with invasion of new habitats or adaptation to novel conditions (Hendry & Kinnison, 1999; Kinnison & Hendry, 2001), suggesting that the earliest stages of adaptive radiation contribute disproportionate amounts of biological diversity.

The magnitude of phenotypic divergence within the new adaptive zone (that is, among freshwater resident populations) was also substantial. Within the upper Fish Creek drainage, the body shape of resident freshwater stickleback correlated with habitat type, even when geographical distances among sites were taken into account, in accordance with previous knowledge of evolutionary divergence associated with adaptation to benthic vs. limnetic environments (McPhail, 1984, 1994; Reimchen *et al.*, 1985; Walker, 1997; Spoljaric & Reimchen, 2007). Streams and benthic lakes in the upper Fish Creek drainage tend to be ecologically similar. Both are relatively shallow and have stagnant or relatively slow-moving water rich in vegetation and woody debris. As expected, stickleback in these habitats were generally similar in body shape and tended to have deep bodies that favoured manoeuvrability in structurally complex habitats (Walker, 1997). Although there was variation among sites, on average, stream fish tended to have deeper bodies than benthic lake fish. This is not surprising as the benthic lakes sampled probably have more limnetic habitats available for stickleback to exploit than do streams. For example, based on

their RLA, Lucille and Seymour Lakes have 12.2 and 15.9% limnetic habitat by area, respectively. Even lakes with 100% RLA can have significant open-water habitat (W. E. Aguirre, pers. observ.). At the other extreme, limnetic lakes were inhabited by elongate, shallow-bodied stickleback, which is expected to favour reduced drag for prolonged swimming in open waters (Walker, 1997). There was marked differentiation between extreme stream and limnetic lake stickleback samples, with relatively little overlap on PCs I and II.

There was significant variation among populations within habitat types, as well as among individuals within samples. The spread along PCs I and II among sample means for benthic lake and stream sites was considerable (Fig. 3), and classification rates by habitat type were quite low (< 50%) for a few populations (that is, Stepan, Blodgett and Lucille lakes). Although habitat misclassifications often involved similar habitat types (for example, stream and benthic lake sites, as was the case for the Lucille lake population), many individuals were classified to habitat types containing fish with highly divergent consensus configurations. For example, 27.3% of the individuals at Meadow Creek site 2 were misclassified as limnetic lake fish, and 15.8% of the individuals from Stepan Lake, a limnetic lake, were misclassified as stream fish. The habitat classification used is quite broad and unmeasured factors may be influencing both mean and individual variation in body shape. In addition, both of these sites are close to ecologically divergent sites (Meadow Creek site 2 is just downstream of Orchid Lake, and Stepan Lake is just upstream of Meadow Creek site 1, see Fig. 1), suggesting that gene flow may be influencing phenotypic variation at some sites.

The influence of gene flow on adaptive divergence is a particularly important topic in evolutionary biology (for example, Endler, 1977; Slatkin, 1985; Lenormand, 2002). Previous studies have documented both constraints (Stearns & Sage, 1980) and adaptive divergence despite gene flow (for example, Hendry *et al.*, 2002; Moore *et al.*, 2007; Rosenblum, Hickerson & Moritz 2007). The lack of a significant correlation between phenotypic variation and geographical distances among sites suggests that gene flow among geographically adjacent sites is probably not a major factor constraining adaptive divergence at the system-wide level in the upper Fish Creek drainage. However, direct measures of gene flow are necessary to confirm this. In addition, the analyses performed have limited power to detect factors acting locally at a few sites, and gene flow among neighbouring sites may well account for some of the variation in body shape within habitat types (for example, Meadow Creek site 2 located immediately downstream of a

lake). The extent of gene flow in the system is unknown. Local adaptation may be occurring despite significant gene flow, or there may be very little gene flow with extreme morphotypes (for example, stream and limnetic lake populations) being reproductively isolated. Research on genetic variation in the upper Fish Creek drainage is presently underway.

The occurrence of morphologically divergent individuals at the same site (for example, individuals classified as stream and deep lake fish) raises questions about the extent of individual specialization within populations in this system. The importance of individual specialization in biology is another area of active research, and recent studies have suggested that populations are often collections of individuals specialized for distinct ecological roles (for example, Bolnick *et al.*, 2003, 2007). It is unclear whether stickleback in lakes with substantial habitat heterogeneity in the upper Fish Creek drainage are generalists or a mix of specialized limnetic and benthic individuals, but individual specialization has been documented in other stickleback populations (Araujo *et al.*, 2008). More detailed studies may provide insight into the extent of individual specialization in the Fish Creek system.

In summary, this study indicates that stickleback populations in the upper Fish Creek drainage have diverged substantially from their anadromous ancestor and from one another in response to local conditions, despite the relative youth of the radiation and the opportunities for gene flow among ecologically divergent sites. Rates of adaptation to local conditions appear to be sufficiently high to produce population differentiation, even if lake populations occasionally go extinct and are recolonized by stream fish; selection seems to be rapid compared with the rate of extinction in this system. It is unclear whether any of the phenotypically divergent forms in the drainage are or will evolve into reproductively isolated biological species. Some of the most extreme phenotypes in the upper Fish Creek drainage, such as fish in Big and Lynda lakes (limnetic-type fish) and stream fish at adjacent stream sites, occur in geographical proximity. Documenting whether significant assortative mating exists among these ecologically divergent forms is a major direction for future research. Efforts to measure genetic differentiation and gene flow throughout the drainage are presently under way and will provide much greater insight into the biological dynamics of the system.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Classification results of discriminant function analysis of body shape data by sample. Asterisks (*) indicate zero values.

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