

Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation

WINDSOR E. AGUIRRE*, KAITLYN E. ELLIS†, MARY KUSENDA‡ and MICHAEL A. BELL

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA

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Ancestral properties can influence patterns of evolutionary diversification, but ancestors can rarely be observed directly. We examined variation and sexual dimorphism of morphological traits in an anadromous threespine stickleback population representing the ancestral form for resident postglacial stickleback populations in the area. A combination of traditional and geometric morphometric methods were used to study variation over multiple years in an anadromous population that breeds in Rabbit Slough, Cook Inlet, Alaska. Major armor anomalies were extremely rare but their occurrence at measurable frequencies suggests that significant standing variation for armor phenotypes exists in anadromous populations. Sexual dimorphism was a major source of variation, and most traits differed significantly between sexes, particularly head length, length of the pelvic girdle, and body shape. Consequently, some degree of sexual dimorphism appears to be the ancestral condition for many traits in derived resident freshwater stickleback radiations. Morphological variation among years, especially in body shape, was significant in both sexes, but the magnitude of annual variation was always less than variation due to other factors. Phenotypic means were relatively stable over short time scales. Postglacial stickleback radiations are among the most enlightening cases of adaptive radiation, and our detailed study of variation in an anadromous stickleback population provides crucial insight into a key component of adaptive radiation, the variation on which directional selection acts at the onset of the radiation. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 465–478.

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INTRODUCTION

The role that ancestral properties play during adaptive radiation is a key issue in evolutionary biology. Some ancestral properties, such as high levels of variation, facilitate evolutionary diversification (Wayne & Miyamoto, 2006), whereas others, such as

ancestral covariance structure, may constrain it (Schluter, 1996). Most adaptive radiations lack direct knowledge of ancestral properties impeding examination of this issue, but this is not the case for threespine stickleback adaptive radiations.

Threespine stickleback fish, *Gasterosteus aculeatus*, are primitively oceanic, spending most or all of their lives in the ocean and entering fresh water only to reproduce. Sea-run (anadromous) threespine stickleback have repeatedly established resident populations in postglacial lakes and streams throughout much of the northern hemisphere. The resulting postglacial radiations are among the most enlightening cases of adaptive radiation known (Bell & Foster, 1994; Schluter, 2000; McKinnon & Rundle, 2002;

*Corresponding author. E-mail: waguirre@life.bio.sunysb.edu

†Current address: Tufts University School of Medicine and Department of Public Health and Family Medicine, Boston, MA 02111, USA.

‡Current address: Graduate Program in Genetics, Stony Brook University, Stony Brook, New York 11794, USA and Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.

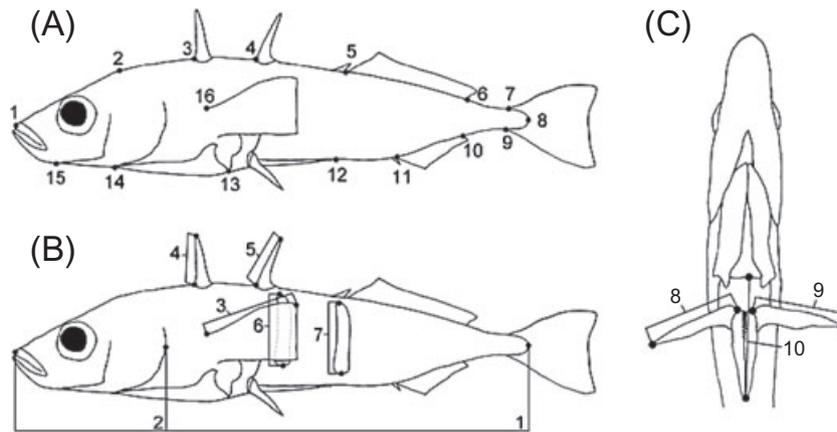


Figure 1. Landmarks (A) and linear measures (B, C) used in the present study.

Östlund-Nilsson, Mayer & Huntingford, 2007). Morphological differences between oceanic and resident freshwater stickleback include divergence of armor, trophic, and body shape traits (Hagen, 1967; Bell, Francis & Havens, 1985; McPhail, 1994; Reimchen, 2000; Walker & Bell, 2000; Bell, Aguirre & Buck, 2004). These changes have been particularly well studied along the Pacific Coast of North America, including Cook Inlet, Alaska, where a large postglacial radiation exists. Oceanic stickleback in this region are heavily armored, and have numerous bony lateral plates (modally 33) covering the entire flank, a well-developed pelvis with large pelvic spines, and three dorsal spines, the first two of which are also large (Fig. 1). Resident lake and stream stickleback generally have fewer lateral plates (< 10) restricted to the anterior part of the body, and the dorsal and pelvic spines are reduced in size. In extreme cases, resident freshwater populations may exhibit extreme pelvic reduction (loss of the elements of the pelvis) or have fewer than three dorsal spines (Bell *et al.*, 1985; Bell, 1987). The magnitude of this divergence is comparable to differences among species in other taxa, which is not uncommon for postglacial radiations (Bell & Andrews, 1997; Robinson & Schluter, 2000).

Despite long-standing interest in threespine stickleback radiation, relatively little is known about the ancestral anadromous form. Knowledge of ancestral variation could provide a greater understanding of patterns of radiation because levels of variation can influence rates of evolution and bias evolutionary trajectories (Sokal, 1978; Schluter, 1996; McGuigan, Chenoweth & Blows, 2005). The ancestral anadromous phenotype also provides a baseline to quantify the magnitude and rate of evolution of derived postglacial populations. Finally, genetic factors of large phenotypic effect account at least in part for the rapid

divergence between anadromous and postglacial resident freshwater stickleback (Peichel *et al.*, 2001; Colosimo *et al.*, 2004, 2005; Cresko *et al.*, 2004; Shapiro *et al.*, 2004; Shapiro, Bell & Kingsley, 2006; Kingsley & Peichel, 2007; Albert *et al.*, 2008). Careful screening of large samples of anadromous stickleback can provide minimal estimates of the ancestral frequency of alleles with large effects on armor phenotypes.

In the present study, we analyzed morphological variation and sexual dimorphism in an anadromous threespine stickleback population sampled multiple years between 1997 and 2005 from Rabbit Slough (RS), Alaska. The RS population runs into a drainage in an area harboring numerous derived lake resident stickleback populations (Bell & Ortí, 1994), including a recently established population in Loberg Lake that has been sampled annually between 1990 and 2008 and is evolving gradually in the direction of typical resident freshwater populations in the area (Aguirre, Doherty & Bell, 2004; Bell *et al.*, 2004; Aguirre, 2007). Given previous knowledge of postglacial radiation (McKinnon & Rundle, 2002) and other oceanic populations (Penczak, 1965; Gross, 1978), we expected to find some variation in armor in the RS population that could prime adaptive armor loss in freshwater environments. Previous studies have also documented sexual dimorphism (Kitano, Mori & Peichel, 2007) and relatively low levels of heterogeneity among oceanic populations (Walker & Bell, 2000). Consequently, we expected to find some sexual dimorphism and relatively little variation related to annual heterogeneity in the RS population. We contrast magnitudes of trait variation and discuss our findings in light of patterns of postglacial adaptive radiation.

Although we cannot be certain how much of the morphological variation measured reflects underlying genetic variation, many of the traits examined are highly heritable (Hagen, 1973; Aguirre *et al.*, 2004),

have been genetically mapped (Peichel *et al.*, 2001; Cresko *et al.*, 2004; Schluter *et al.*, 2004; Colosimo *et al.*, 2004, 2005; Albert *et al.*, 2008), or have experimentally been shown to possess a strong genetic basis (Spoljaric & Reimchen, 2007). Thus, much of the variation we document may form the genetic basis for evolutionary change in postglacial radiations.

MATERIAL AND METHODS

Rabbit Slough (61°32.065'N, 149°16.061'W) is located near Palmer, in the Matanuska-Susitna Borough (Mat-Su), Cook Inlet, Alaska. Fish were collected in the spring (generally in June) for 7 years between 1997 and 2005 with ten to 20 unbaited 1/4 and/or 1/8 inch minnow traps set overnight. Captured specimens were anesthetized with MS-222, fixed in 10% formalin, transferred to 50% isopropyl for storage, and bone was stained red with Alizarin Red S to visualize bony landmarks.

Complete samples collected over the 7 years (a total of 3440 specimens ranging between 99 and 1267 specimens annually) were screened for major armor anomalies in lateral plate, pelvic, and dorsal spine phenotypes. Anomalies were defined as an absence, a major size reduction (> 50% typical size as observed by inspection), or an excess number of dorsal spines, pelvic spines, or lateral plates (LP). Specimens with spines that were obviously broken were excluded.

A subset of 50 male and 50 female specimens collected on 25 June 1997, 12 June 2000, and 9 June 2003 (300 total) were subjected to more detailed morphological study. Ten linear measurements and two meristic counts (Fig. 1) for traits considered to be adaptively important in freshwater populations were taken. The morphometric traits measured were: (1) standard length (SL), from the tip of the upper jaw to the end of the vertebral column; (2) head length (HL); from the tip of the upper jaw to the posterior edge of the operculum; (3) pectoral fin length (PCTL), from the origin of the pectoral fin to the tip of the second pectoral fin ray; lengths of the (4) first (D1) and (5) second dorsal spines (D2) measured along their anterior edges; heights of the (6) eighth lateral plate (LP8) and (7) of the lateral plate ventral to third dorsal spine (LPD) measured between their dorsal and ventral tips; (8) lengths of the right (PLVR) and left pelvic spines (PLVL) measured along their anterior edges; and (10) length of the base of the pelvic girdle (PLVB) measured from the anterior-most point (to the left of medial suture) to the posterior-most tip. The two meristic traits counted were the number of gill rakers on the first left gill arch (GR) and the number of lateral plates on the left side of the body (LP). All linear traits were measured twice and the average of the two measures was used in the analysis. Both

measures were retaken if the difference between them in the same individual was greater than 5% of the mean. Linear measures were ln transformed for statistical analyses. Five males from 2000 and one from 2003 had broken spines and there were not enough intact males in these samples to find suitable replacements; these specimens were removed from the analyses. Repeatabilities of linear measurements were calculated following Baumgartner, Bell & Weinberg (1988). Five randomly chosen male and female specimens (ten in total) were measured three times each. A one-way analysis of variance was used to separate total phenotypic variance into among and within individual components, and the repeatability for each trait was computed as the ratio of the among-individual component to the total, $s^2_A/(s^2_A + s^2)$, where s^2_A is the among individual variance component and s^2 is the within groups variance. The repeatabilities were generally high, in the range 0.966–0.999 and averaging 0.985 (Table 1). Coefficients of variation [(SD/mean) × 100] were calculated for each trait.

The same specimens were held straight with insect pins inserted into the musculature for photography and subsequent body shape analysis. Landmarks on the midline not visible from the lateral aspect were also marked with insect pins. These specimens were photographed with a 3.3 megapixel Olympus Camedia C-3000 digital camera, and 16 landmarks were digitized on each (Fig. 1) to study body shape variation using geometric morphometric methods (Rohlf & Marcus, 1993; Adams, Rohlf & Slice, 2004). The landmarks are based on those previously used by Walker (1997) with the addition of a sixteenth landmark at the dorsal origin of the pectoral fin. Data were collected and analyzed using TPS software. Briefly, the x and y coordinates for the 16 landmarks were digitized from lateral images of specimens using tpsDig, version 1.40 (Rohlf, 2004a).

The specimens were aligned with Procrustes superimposition methods as implemented in tpsRelw, version 1.44 (Rohlf, 2006) to eliminate nonshape variation. The mean of all specimens was used as the reference shape to generate the shape variables (partial warps with uniform component). Deformation grids were created using tpsSpln, version 1.20 (Rohlf, 2004b).

To assess the relative magnitude of variation in mean body shape from year to year in RS, the level of divergence in yearly mean body shape was contrasted to the average difference in body shape between sexes in RS, and between male fish from RS and three derived resident freshwater populations from different habitat types located in the Mat-Su Borough. Only males were included to eliminate sexual dimorphism from the interpopulation comparisons. Samples of 20 specimens collected in 1990 and 2004

Table 1. Repeatabilities, size-adjusted means (mm), and coefficients of variation (CV) of linear traits

Variable	Repeatability	Adjusted ♂	Adjusted ♀	Difference %	CV ♂	CV ♀
SL	0.992	–	–	–	3.760	3.888
HL	0.979	20.662	19.137	7.666	3.718	3.693
PCTL	0.966	13.651	13.931	2.030	4.395	4.478
D1 ^{NS}	0.982	6.779	6.663	1.730 ^{NS}	6.898	7.184
D2	0.990	7.016	7.181	2.320	6.489	6.757
LP8	0.975	9.721	9.935	2.180	5.495	5.208
LPD	0.974	9.473	9.766	3.050	6.426	5.842
PLVL	0.999	9.708	9.986	2.820	5.380	5.683
PLVR	0.995	9.675	9.918	2.490	5.177	6.118
PLVB	0.995	13.754	14.701	6.658	5.134	5.517

SL, standard length, from the tip of the upper jaw to the end of the vertebral column; HL, head length, from the tip of the upper jaw to the posterior edge of the operculum; PCTL, pectoral fin length, from the origin of the pectoral fin to the tip of the second pectoral fin ray; D1, length of the first dorsal spine, measured along the anterior edge; D2, length of the second dorsal spine, measured along the anterior edge; LP8, height of the eighth lateral plate, measured between the dorsal and ventral tip; LPD, height of the lateral plate ventral to third dorsal spine, measured between the dorsal and ventral tip; PLVR, length of the right pelvic spine, measured along the anterior edge; PLVL, length of the left pelvic spine, measured along the anterior edge; PLVB, length of the base of the pelvic girdle, measured from the anterior-most point (to the left of medial suture) to the posterior-most tip.

Measurements were adjusted to grand mean SL (67.08 mm) of the specimens included. Difference % is the difference between male and female size-adjusted values expressed as a percentage of the average. All traits differed significantly (analysis of covariance: $P < 0.05$ after sequential Bonferroni correction) between males and females except D1. Females had higher size-adjusted mean values than males (higher values are shown in bold) for all traits except HL (which was larger in males) and D1 (which did not differ significantly). Specimens identified as one year olds were excluded. NS, not significant.

(40 per population) were included to account for potential temporal variation. The resident freshwater populations came from a deep lake, Long Lake (61.578N, 149.764W), a shallow lake, Mud Lake (61.563N, 148.949W), and a stream, Little Meadow Creek, which was sampled in 1990 at the intersection of Parks Highway and Big Lake Rd. (61°34'34.9N, 149°43'41.0W), and, in 2004, 2.3 km downstream where it crosses Kenlar Road, approximately 0.3 km from Big Lake Road (61°33'46.4N, 149°49'32.8W). Lakes were defined as deep or shallow based on the relative amount of littoral (habitat supporting macrophyte growth) area (RLA) that they possessed (Walker, 1997). The RLA was 30% and 100% for the deep and shallow lakes, respectively.

The magnitude of divergence was evaluated by comparing Euclidean distances in shape space formed by the first few principal components from a principle components analysis carried out with *tpsRelw*, version 1.44 (Rohlf, 2006), Procrustes distances between sample means in complete shape space, and by partitioning of partial variances (Tabachnick & Fidell, 1996; Langerhans & DeWitt, 2004). The Procrustes distance is defined as the square root of the sum of squared differences between the positions of two optimally superimposed configurations at unit

centroid size (Slice *et al.*, 1996). Procrustes distances were calculated between sample means with *tpsSpln*, version 1.20 (Rohlf, 2004b). Wilks' partial η^2 provides a measure of the strength of the association between factors being tested and variation in the dependent variables (Tabachnick & Fidell, 1996); in this case, the body shape variables. It was calculated following Tabachnick & Fidell (1996); Langerhans & DeWitt (2004). We evaluated the importance of sexual dimorphism relative to yearly variation, by testing the effects of Sex, Year, and the Sex \times Year interaction against the error SS matrix. The effects of variation among populations relative to yearly variation were estimated by testing Population independently (ignoring the nested term) and Year (nested within Population) over the error SS matrix. Wilks' lambda was calculated, and tests for significance were carried out, with *tpsRegr*, version 1.31 (Rohlf, 2005).

RESULTS

The SL distribution for both males and females had a long left tail (Fig. 2). Anadromous stickleback often have a 2-year life cycle (Baker, 1994), so these small individuals (two males, one female in 1997; three males, two females in 2003) are probably 1-year old

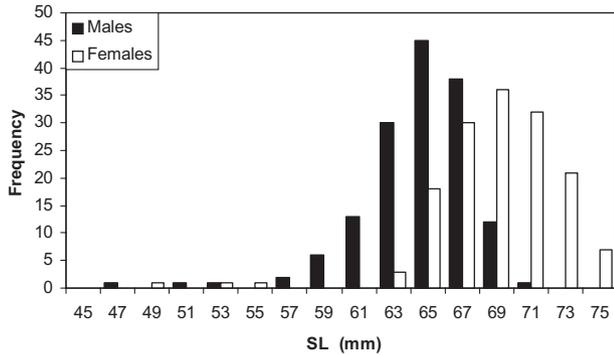


Figure 2. Size distribution of male and female stickleback from Rabbit Slough based on fifty males and females from samples made in 1997, 2000, and 2003 (300 fish in total). SL, standard length.

fish that came in to reproduce early. The frequency of these small individuals was in the range 0–4% (mean = 2.33%) of the spawning run across years. Because they probably represent a different year-class, these individuals were excluded from all further analyses.

MAJOR ARMOR ANOMALIES

Major armor anomalies (absences, reductions or excesses) in the RS population never exceeded 0.5%.

Only one of the 3440 specimens exhibited a LP anomaly. An individual collected in 2000 was missing a LP halfway along the flank of the body, approximately where the dorsal fin begins. The LP was missing on both sides of the body at the same location, suggesting a developmental origin for the missing plate and not the accidental loss of a plate due to injury. The observed anomaly does not represent a major phenotypic difference from the common phenotype and no partial or low morphs (*sensu* Hagen & Gilbertson, 1972; i.e. fish lacking numerous plates, see Bell & Foster, 1994), characteristic of resident freshwater populations in the area, were observed.

Loss and addition of spines was very rare; major size reduction (elements half or less of the typical size) was more common. The loss of pelvic spines was extremely rare, with a single individual collected in 2000 missing a left pelvic spine. Loss of dorsal spines (Fig. 3A) occurred more often but was also rare, with seven individuals collected over multiple years missing a dorsal spine (three missing D1; two missing D2, two missing D3). Major reductions of pelvic spines were the most common anomaly observed, with 16 individuals (0.47%) exhibiting this anomaly. Of these 16 cases, 11 had reduced right pelvic spines, five had reduced left pelvic spines, and one had both

pelvic spines reduced. This reduction did not differ statistically between sides from a random (*G*-test, $G_w = 2.236$, $P = 0.1348$). Reductions in the size of the first dorsal spine were less common than for pelvic spines but more common than for second dorsal spines (0.26% and 0.12% respectively; Fig. 3B, C). Extra dorsal spines were also extremely rare, with only five individuals exhibiting them (0.15%). A fourth dorsal spine was added in three ways: addition of a large dorsal spine, resembling the first or second spine, posterior to the second spine ($N = 1$; Fig. 3D), addition of an extra spine at the posterior base of the usual first spine ($N = 1$; Fig. 3E), and addition of a small dorsal spine anterior to the typical third dorsal spine ($N = 3$; Fig. 3F).

SEXUAL DIMORPHISM

Females were significantly larger than males, and the difference held across all years, even though SL differed significantly among years within sex [nested analysis of variance (ANOVA), fixed factor: sex, $F = 210.43$, $P = 0.005$; random factor: year, $F = 21.02$, $P = 0.045$; Fig. 2, Table 2]. Female SL exceeded that of males by 3.81–4.79 mm within years, with an unweighted average difference across all years of 4.46 mm, corresponding to 6.92% of the average length of males.

Females and males also differ for eight of the nine linear traits measured with size (SL) taken as the covariate (Table 1). PCTL, D2, LP8, LPD, PLVL, PLVR, and PLVB were all significantly larger in females than males, whereas HL is larger in males. D1 did not differ significantly between males and females. Head length was particularly divergent (Table 1), with very little overlap between males and females per unit size (Fig. 4). Interestingly, all of the sexually dimorphic armor traits were larger in females. For the meristic traits, GR do not differ between males and females (Mann–Whitney *U*-test: $Z = -1.196$, $P = 0.232$) but LP number does (Mann–Whitney *U*-test: $Z = -3.443$, $P = 0.001$). Males have more LP every year surveyed (Table 2), although the average difference is small (0.3 LP).

Body shape differed considerably between males and females. Males and females separated almost completely in the space formed by the first three principal components, which accounted for approximately 65% of the variation (Fig. 5). Differences in body shape between males and females were distributed throughout the body. As already indicated by the univariate measure of HL, the head and anterior region of the body were larger in males than in females. Thus, the trunk region, particularly the abdomen, was larger in females than in males, the distance between the dorsal and anal fins was

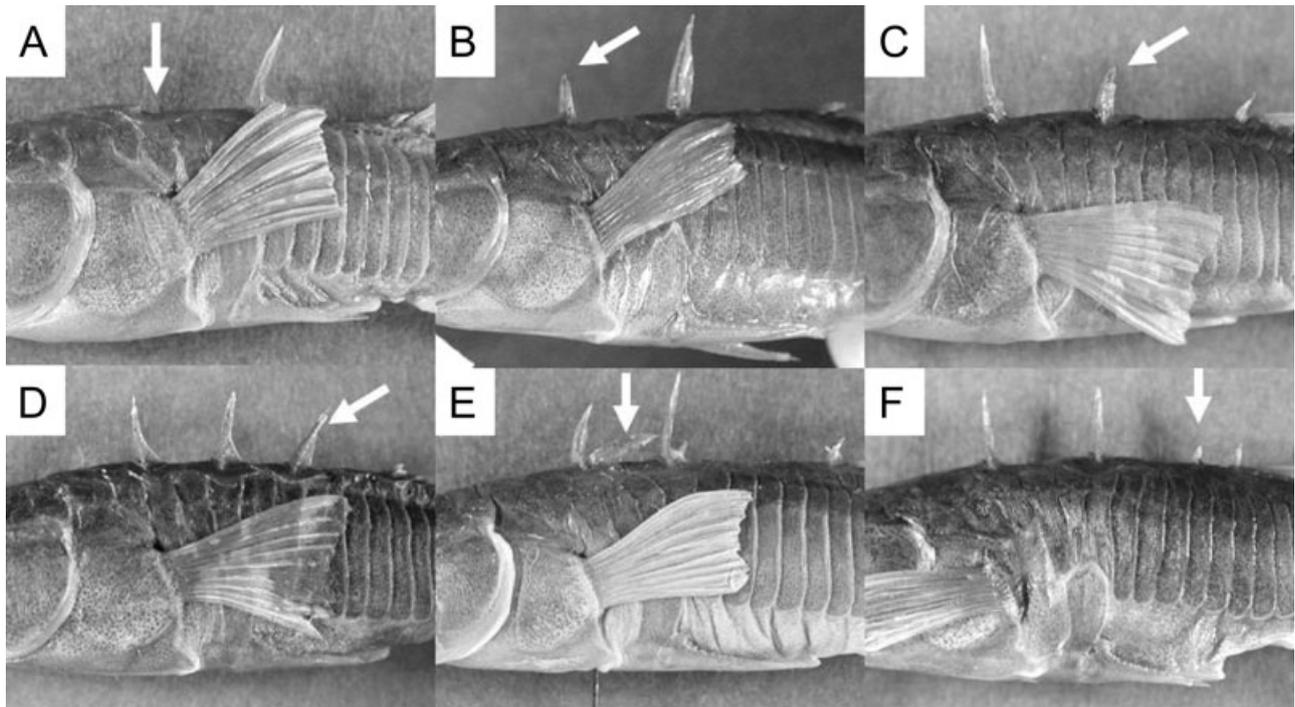


Figure 3. Examples of dorsal spine anomalies observed. A, first dorsal spine missing. B, first dorsal spine greatly reduced in length. C, second dorsal spine greatly reduced in length. D, large fourth dorsal spine located between typical second and third dorsal spines. E, fourth dorsal spine originating from base of typical first dorsal spine. F, fourth dorsal spine located anterior to typical third dorsal spine.

Table 2. Mean \pm SE, standard deviation (SL^{sd}) and range of standard length (SL^{rg}), gill raker number (GR) and lateral plate number (LP) based on samples of 50 specimens per sex per year

Year	SL	SL^{sd}	SL^{rg}	GR	GR^{sd}	GR^{rg}	LP	LP^{sd}	LP^{rg}
1997 ♂	64.07 \pm 0.50	3.54	47.58–68.71	22.50 \pm 0.24	1.72	19–26	33.34 \pm 0.08	0.59	32–35
1997 ♀	68.86 \pm 0.56	3.98	48.05–74.62	22.18 \pm 0.16	1.14	20–25	33.14 \pm 0.11	0.81	30–34
2000 ♂	64.07 \pm 0.33	2.33	59.06–68.37	22.06 \pm 0.14	1.02	19–25	33.56 \pm 0.10	0.71	32–35
2000 ♀	67.88 \pm 0.34	2.43	63.43–74.41	21.86 \pm 0.18	1.26	19–25	33.22 \pm 0.11	0.76	32–35
2003 ♂	65.30 \pm 0.52	3.69	50.45–70.78	22.38 \pm 0.18	1.28	19–25	33.40 \pm 0.08	0.57	32–35
2003 ♀	70.07 \pm 0.57	4.02	53.27–74.89	22.32 \pm 0.15	1.04	20–24	33.02 \pm 0.09	0.65	31–34
Total ♂	64.48 \pm 0.27	3.28	47.58–70.78	22.31 \pm 0.11	1.37	19–26	33.43 \pm 0.05	0.63	32–35
Total ♀	68.94 \pm 0.30	3.65	48.05–74.89	22.12 \pm 0.10	1.16	19–25	33.13 \pm 0.06	0.75	30–35

compressed, and the caudal peduncle was expanded in females relative to males.

ANNUAL VARIATION

Linear measurements for each sex were screened for significant differences among years. Although the difference in SL among years was not large for either sex (mean annual male and female SL differed by a maximum of 1.23 and 2.19 mm, respectively), it was significant in both males (ANOVA: d.f. = 2, 136; $F = 10.648$, $P < 0.001$) and females (ANOVA: d.f. = 2, 144; $F = 16.989$, $P < 0.001$), so analysis of covariance

(ANCOVA) using SL as the covariate, was used to screen the remaining variables. Seven of the nine variables measured did not differ significantly among years in either sex (ANCOVA: $P > 0.05$). Head length differed significantly among years in males (ANCOVA: $F = 5.767$, $P = 0.004$), and PCTL differed significantly in females (ANCOVA: $F = 9.461$, $P < 0.001$) after correcting for multiple tests using the sequential Dunn-Šidák method (Sokal & Rohlf, 1995). The two meristic traits, LP and GR number, did not differ significantly among years in either sex (Kruskal–Wallis test, $P > 0.05$).

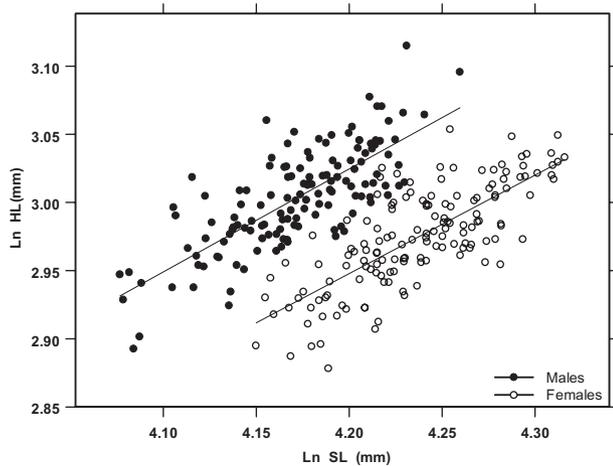


Figure 4. Sexual dimorphism of head length (HL). SL, standard length.

Body shape differed significantly among years in both males and females (tpsRegr permutation test: males, Wilks' lambda = 0.305; females, Wilks' lambda = 0.195; $P < 0.001$ in both tests). Although annual variation was significant, the magnitude of the difference was less than that related to sexual dimorphism in body shape and divergence between anadromous and resident freshwater stickleback (see below).

ANNUAL BODY SHAPE VARIATION COMPARED TO DIFFERENCES BETWEEN SEXES

The magnitude of the difference in body shape between males and females was larger than that among years within sex. The Procrustes distance between the consensus male and female for all years was 0.0355, and Procrustes distances between consensus males and females individually within each year yielded values ranging from 0.0341 to 0.0412 and averaging 0.0370. The Procrustes distance for the consensus configurations of females and males obtained from different years averaged 0.0161, or less than half the value obtained for sex. Variation in average body shape from year to year, however, was generally greater among females than males. The Procrustes distance between consensus configurations for the former was in the range 0.0127–0.0311 (mean = 0.0222), whereas for males it was in the range 0.0087–0.0108 (mean = 0.0099). A generally similar estimate of the relative influence of sex and year on body shape variation was obtained with Wilks' partial η^2 . For sex, Wilks' partial $\eta^2 = 0.922$; for year, Wilks' partial $\eta^2 = .438$; and, for the interaction of sex by year, Wilks' partial $\eta^2 = 0.220$. All factors had a significant influence on body shape variation

(Wilks' lambda = 0.078, 0.0316, and 0.609 for Sex, Year, and Sex \times Year respectively; permutation tests, $P < 0.001$).

ANNUAL BODY SHAPE VARIATION COMPARED TO DIFFERENCES AMONG POPULATIONS

Annual variation in body shape among male RS stickleback was also less than that between males from anadromous and resident freshwater populations. Rabbit Slough stickleback were completely separated from all resident freshwater stickleback along the first two principal components (which accounted for approximately 63.4% of the variation in the data), and the RS annual means were very similar to each other (Fig. 6). The resident freshwater populations segregated from RS along PC I, indicating the major axis of variation in body shape was associated with shape variation differentiating anadromous and resident freshwater populations. The deep lake sample was more divergent from RS along PC I whereas the shallow lake and stream samples largely overlapped along both PC axes. Variation in body shape among the resident freshwater populations was consistent with previous knowledge of the influence of habitat type on body shape (Lavin & McPhail, 1985, 1986, 1987; Reimchen, Stinson & Nelson, 1985; Walker, 1997). The deep lake population was much more elongate than any of the other populations, whereas the shallow lake and stream populations were deeper bodied (Fig. 6). The abdominal region tended to be larger in samples from resident freshwater populations, and the posterior tip of the pelvis and pectoral fin are shifted anteriorly relative to RS. The caudal peduncle also tended to be much more elongate in resident freshwater populations. The average Euclidean distance among the three annual means for RS in the space formed by PCs I and II was 0.0051, whereas the mean Euclidean distance between the RS grand mean shape and the means for the three freshwater populations was ten times greater (i.e. 0.0529). In the complete shape space, the average Procrustes distances among pairs of RS annual means was 0.0099, whereas the mean distance between RS and freshwater populations was approximately six-fold greater (0.0563).

Multivariate ANOVA (including only male RS fish from 1997 and 2003 to balance the design because only two annual samples of the resident freshwater populations were available) indicated that both Population and Year had significant effects on body shape variation (Wilks' lambda = 0.002 and 0.155 respectively; permutation tests, $P < 0.001$ for both tests). Estimates of Wilks' partial η^2 also indicate that the magnitude of the effect of Population on body shape variation (0.878) was 2.3-fold greater than Year (0.375).

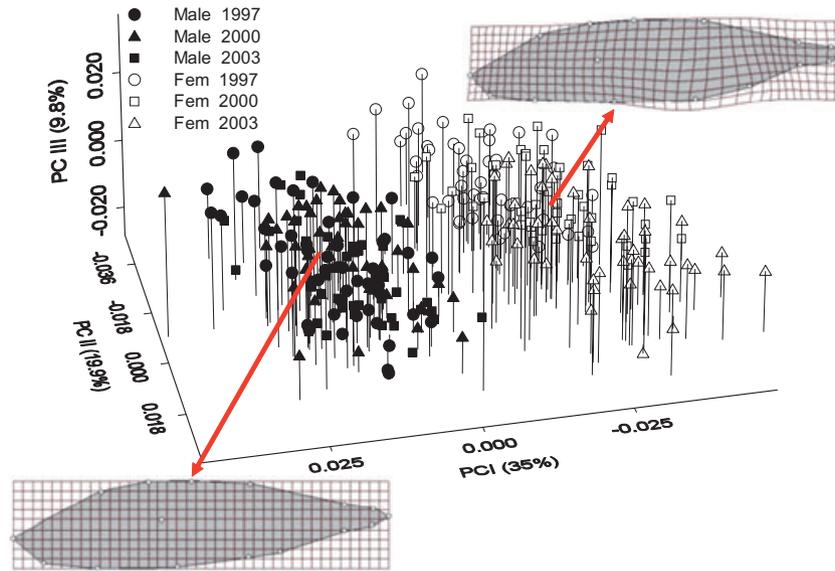


Figure 5. Principal component analysis plot of body shape variation between male and female stickleback (generally 50 specimens per year and sex). The female grid (top right) is depicted as a deformation of the male grid (exaggerated by a factor of two). PC I, II, and III account for 35, 19.9, and 9.8% of the variation, respectively.

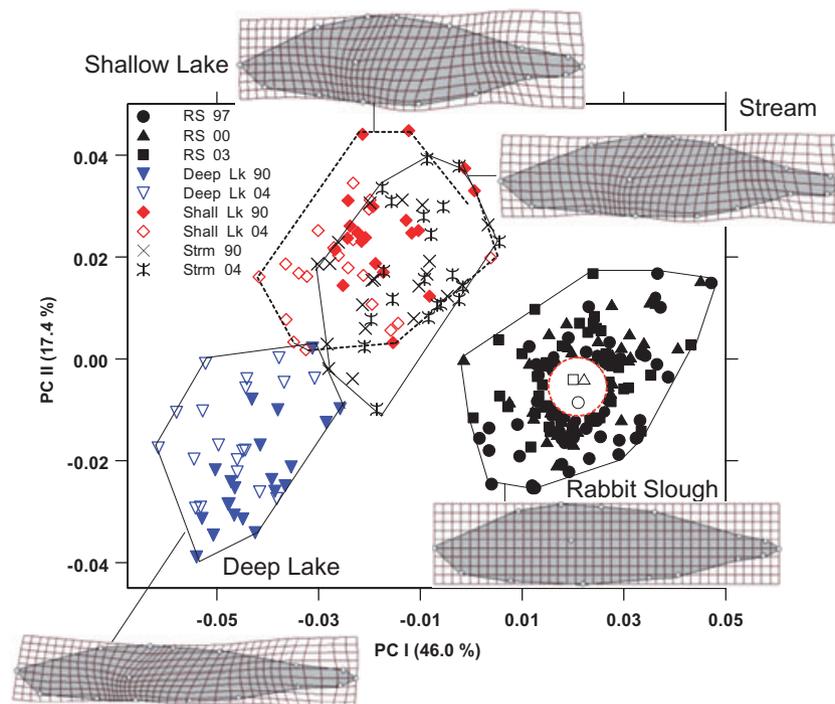


Figure 6. First two principal components of a principal components analysis depicting shape variation of male Rabbit Slough (RS) stickleback sampled in 1997, 2000, and 2003 (generally 50 specimens per year), compared to male stickleback from a deep lake, shallow lake, and stream sampled in 1990 and 2004 (20 specimens per year and population, 120 total). Clear symbols within the RS cluster indicate the approximate positions of annual means. Individual RS specimens falling within dashed circle circumscribing annual means were eliminated to facilitate visualization. The deformation grids are depicted as deformations of the consensus configuration for RS (exaggerated by a factor of two).

DISCUSSION

MAJOR ARMOR ANOMALIES AND ARMOR REDUCTION
IN RESIDENT FRESHWATER STICKLEBACK

Complete loss of spines and the addition of extra spines occurred at measurable frequencies, indicating that anadromous populations harbor the potential to produce stickleback varying in spine number. Variation in spine phenotypes in resident freshwater populations is observed in some derived freshwater populations in the region (Bell *et al.*, 1985; Bell & Ortí, 1994) and elsewhere (Penczak, 1965; Gross, 1978; Campbell, 1979, 1985; Reimchen, 1980, 1984; Bell & Baumgartner, 1984). Although care was taken to exclude specimens with broken spines, wounds occurring early in development may be difficult to recognize. Consequently, the frequency of specimens with armor loss/reduction may be overestimated.

Reductions in the size of armor structures were more common than total loss, and this pattern is consistent with variation in resident freshwater populations (Reimchen, 1980; Francis *et al.*, 1986). The frequencies of major reductions in the size of the left versus right pelvic spines did not differ from random in our study, but the result was in the same direction as directional asymmetry in pelvic reduction among derived resident lake populations in the area. Lake populations with pelvic reduction in Cook Inlet are more likely to have smaller pelvic vestiges on the right side than the left (Bell *et al.*, 1985; Bell, Khalef & Travis, 2006) as a consequence of variation at the *Pitx1* locus (Cole *et al.*, 2003; Shapiro *et al.*, 2004, 2006). Reduction of the size of right pelvic spines in RS specimens was more than twice as common as that of left spines (11 versus 5), raising the possibility that spine reduction in this population is due to variation in *Pitx1*. The lack of statistical significance of the test we performed could be due to a lack of power resulting from small sample size. Similarly, the frequency of individuals with major reductions in the size of the first dorsal spine was more than double that of the second dorsal spine. This is consistent with observations from derived freshwater populations in Paxton Lake, on Texada Island and fossil *Gasterosteus doryssus* from a Nevada deposit (Bell, 1974). However, the second dorsal spine is usually missing in the Boulton Lake population on the Queen Charlotte Islands (Reimchen, 1980). More work is needed to determine whether there is a typical pattern of dorsal spine reduction in freshwater populations.

The low frequency of individuals with four dorsal spines in RS is consistent with the sporadic occurrence of four-spined stickleback in freshwater environments (Penczak, 1965; Gross, 1978; Bell & Baumgartner, 1984), indicating that variation for production of four-spined stickleback is present in

anadromous populations. Moreover, how the fourth dorsal spine was most frequently added in the RS population (as a small dorsal spine between the typical second and third dorsal spines) is similar to observations by Penczak (1965) on Polish threespine stickleback. Bell & Baumgartner (1984) also reported the addition of a fourth dorsal spine between the typical second and third dorsal spines in a freshwater population from Boston (USA), but make no mention of spine size.

SEXUAL DIMORPHISM: THE MAJOR SOURCE OF
PHENOTYPIC VARIATION

Sexual dimorphism was substantial in this population, which is consistent with previous research on threespine stickleback, suggesting that many sexually dimorphic traits in resident freshwater populations in Cook Inlet and perhaps elsewhere are primitively dimorphic. However, this must be evaluated on a trait by trait basis because the magnitude of sexual dimorphism varies considerably among traits.

The most sexually dimorphic traits that we documented, including body size, head length, length of the base of the pelvis, and body shape, have been documented in previous studies (Kitano *et al.*, 2007), indicating that these dimorphisms are widespread. The larger size of females is common but not universal in threespine stickleback and other gasterosteids (for a list of cases, see Kitano *et al.*, 2007), and several hypotheses exist to explain this, including natural selection for greater egg production (Wootton, 1973; Andersson, 1994; Baker, 1994) and sexual selection based on male preference for larger females (Rowland, 1994). The differences in head length (Fig. 4) and body shape (Fig. 5) between males and females documented in the present study are particularly large, with little overlap between sexes. Sexual dimorphism of other head traits has been reported as well (Caldecutt & Adams, 1998; Caldecutt, Bell & Buckland-Nicks, 2001; Kristjánsson, Skúlason & Noakes, 2002), indicating that the head is particularly prone to sexual dimorphism in threespine stickleback. Understanding the adaptive significance of sexual dimorphism in these traits, if any exists, is a major avenue of future research. Significant sexual dimorphism that we document in other linear traits was not as great as for those listed above (generally 2–3% of the trait average) and varied among studies (Kitano *et al.*, 2007), indicating population-specific patterns of sexual dimorphism.

LP number was greater in RS males. Although Kitano *et al.* (2007) did not report significant differences in LP number between the sexes in the populations they surveyed, the left LP counts they

reported for populations with high mean plate counts (Kitano *et al.*, 2007: table 2) are greater in males than in females in six of seven samples. Their sample sizes were smaller than ours, suggesting that difference between studies may be due to differences in statistical power. Moodie & Reimchen (1976) also reported greater LP number in males in 20 of 22 mostly monomorphic low morph populations that they surveyed in the Queen Charlotte Islands. Reimchen & Nelson (1987) found a very similar pattern to ours for vertebral number, another meristic trait extending along the long axis of the body that should be correlated with LP number in complete morph fish because there is one vertebra (Moyle & Cech, 1996) and generally one LP per body segment in complete morph stickleback; but see also Penczak (1965). The reasons for LP dimorphism are unclear, but the similarity among studies suggests that it is common. Interestingly, Sargent *et al.* (1984) documented the opposite pattern in *Gasterosteus wheatlandi*, in which females have higher LP and vertebral counts.

ANNUAL MORPHOLOGICAL VARIATION: VARIATION AROUND STABLE PHENOTYPIC MEANS

Two of the linear traits (HL in males and PCTL in females) and body shape exhibited significant heterogeneity among years within sex. These slight differences may be due to phenotypic plasticity resulting from slight differences in the environmental conditions in different years. They may also be due to temporal changes in selection regimes, which often differ from year to year (Reimchen, 1995; Grant & Grant, 2002). Annual variation in sex-specific selection has been documented in threespine stickleback (Reimchen & Nosil, 2004). In addition, the annual samples we analyzed were collected during a single day. Thus, annual variation in the present study could include temporal variation within years, inflating our estimates of annual heterogeneity.

The magnitude of the annual variability was less than that related to sexual dimorphism within RS and among males from RS and freshwater populations. Gravity levels are a major source of body shape variation in female stickleback because of abdominal swelling in ripe females and may account for the greater annual variation in body shape observed in females. Differences in body shape among the freshwater and RS populations were consistent with previous results for the relationship between habitat and body shape (Reimchen *et al.*, 1985; Walker, 1997). The difference between RS and the three freshwater populations was the major sources of body shape variation among populations and was greater than the difference between limnetic and more benthic-feeding freshwater populations. Although there was no overlap in

body shape between anadromous and freshwater stickleback, there was substantial overlap between that of samples from shallow lakes and streams, which may be related to living in structurally complex habitats and feeding on benthic prey. The large differences in body shape between the RS and freshwater populations have evolved subsequent to the retreat of glaciers in the area, indicating great potential for body shape evolution in response to divergent selection based on habitat type. Mean body shape exhibited relatively little variation in RS stickleback among years, indicating stability in RS over the time scale examined compared to the evolutionary potential harbored by anadromous populations.

SOURCES OF VARIATION FOR THE ADAPTIVE RADIATION OF POSTGLACIAL STICKLEBACK POPULATIONS

The lack of phenotypic variation for armor structure exhibited by the RS population is consistent with observations from other Alaskan anadromous populations (von Hippel & Weigner, 2004; Karve, von Hippel & Bell, 2008; M. A. Bell, unpublished data). Oceanic populations in other regions can exhibit greater armor variation, especially in lateral plate and dorsal spine number (Münzing, 1963; Penczak, 1965; Gross, 1978; Klepaker, 1996; Kristjánsson *et al.*, 2002). Neutral genetic diversity in the RS population based on microsatellite markers is high (Cresko, 2000; Aguirre, 2007) and similar to those of oceanic populations in other regions (Taylor & McPhail, 2000; Reusch *et al.*, 2001; Mäkinen, Cano & Merilä, 2006), indicating that the low levels of phenotypic variation exhibited in RS are not due to genetic bottlenecks. In addition, the Cook Inlet region of Alaska harbors a large postglacial adaptive radiation exhibiting major armor reduction (Bell *et al.*, 1985; Bell & Ortí, 1994), and there is evidence of armor reduction evolving on contemporary time scales in a population located within 3 km of our RS sample, suggesting that there is abundant genetic variation for adaptive evolution in the region (Bell *et al.*, 2004; Aguirre, 2007). Cases of armor reduction evolving within decades have been reported from other regions as well (Klepaker, 1993; Kristjánsson *et al.*, 2002). Such rapid evolution of armor structures is unlikely to result from new mutations and strongly suggests selection on standing genetic variation. However, given the low levels of phenotypic variation observed in RS, what is the source of the variation for adaptive armor evolution in freshwater habitats?

The major armor anomalies that we documented are one potential source of variation. Although rare in our samples, individuals with major armor anomalies must exist in considerable numbers in oceanic

populations given their enormous population sizes. In addition, patterns of major armor variation in the RS population are often consistent with patterns of armor evolution in postglacial freshwater populations in the region. To contribute significant variation, the founding population sizes of resident freshwater populations must be large (on the order of several hundred at least), and the armor anomalies must be heritable. Unfortunately, we cannot infer the genetic basis of the observed anomalies, but, at the very least, their occurrence indicates that developmental pathways in anadromous stickleback are flexible enough to produce fish with highly divergent armor phenotypes that survive to reproduce.

Rare recessive alleles for major armor reduction in oceanic populations may be a major source of genetic variation for armor evolution. A recent survey of alleles at the *Eda* locus, a major gene regulating lateral plate expression, found that low morph *Eda* alleles fixed in freshwater populations throughout most of the world were derived from a common ancestral allele present at low frequencies (0.2–3.8%) but not expressed in oceanic populations (Colosimo *et al.*, 2005). Because they are recessive, major genes for armor reduction could persist in oceanic populations for long periods of time without resulting in major fitness costs, especially at low frequencies. Once again, relatively large founding population sizes are required for rare recessive alleles to contribute to adaptive evolution of resident freshwater populations.

Abundant quantitative variation is present in the RS population and must provide raw material for adaptive evolution of resident freshwater populations. For example, body shape differs considerably between anadromous and resident freshwater stickleback populations, as well as among freshwater populations (McPhail, 1984; Reimchen *et al.*, 1985; Walker, 1997; Walker & Bell, 2000; Spoljaric & Reimchen, 2007). Although mean body shape varied little in RS among years, there was substantial variation among individuals (Fig. 6). This broad intra-annual variation in body shape among individuals is probably an important source of variation for adaptation to freshwater habitats.

Another interesting potential source of adaptive genetic variation is that related to sexual dimorphism. Most of the traits measured in this study, including traits differing between anadromous and resident freshwater populations, exhibited significant sexual dimorphism. Sexual dimorphism of adaptively important traits indicates the presence of variation available for rapid evolution given the appropriate selective pressures and the modification of pathways involved in limiting expression to a particular sex. In a recent QTL study of body shape variation, Albert *et al.* (2008) proposed this mechanism to explain the

similarity in divergence between sexes within species and divergence between marine and lake stickleback species, as well as a decline in sexual dimorphism in the derived lake species. Others have also emphasized that sexual dimorphism can contribute substantially to ecomorphological diversity during adaptive radiation (Butler, Sawyer & Losos, 2007), as well as to evolutionary diversification in general (West-Eberhard, 2003). However, ecological sexual dimorphisms can also inhibit adaptive speciation (Bolnick & Doebeli, 2003), suggesting that the interaction between sexual dimorphism and adaptive radiation is complex.

In summary, even in southcentral Alaska, where phenotypic variation for armor traits in anadromous populations is severely limited, genetic variation for armor does not generally appear to be a limiting factor for adaptive evolution in freshwater environments. Anadromous and resident freshwater populations are highly divergent morphologically, and the high rate at which this divergence has evolved in the region and low levels of phenotypic variation in RS suggest that strong directional natural selection has caused evolutionary radiation of resident freshwater populations.

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