INTRODUCTION

Understanding how organisms adapt to new environmental conditions is an important challenge given the rapidly changing environmental conditions that many ecosystems are experiencing. Studies of contemporary microevolution (i.e. evolution within species that can be measured when it is under way) can be particularly enlightening because they provide integrated information on the rate, pattern, and causes of evolution. Until recently, there have been few such studies, although compelling evidence now exists that populations can evolve substantially within a few generations (Reznick et al., 1990; Hendry & Kinnison, 1999, 2001; Kinnison, Hendry & Stockwell, 2007; Ellner, Geber & Hairston, 2011) and that the magnitude of evolutionary divergence can be comparable to that observed among species (Liem & Kaufman, 1984; Bell, 1995; West-Eberhard, 2003). Studies of contemporary evolution also allow us to test hypotheses about the factors that facilitate and constrain adaptation. For example, the role of ancestral covariance structure in evolutionary diversification has attracted great interest recently (Schluter, 1996; McGuigan, Chenoweth & Blows, 2005; Hunt, 2007; Revell et al., 2007; Berner, Stutz & Bolnick, 2010). Traits do not evolve independently; they evolve with other traits with which they are related through a complex covariance structure. This covariance structure can influence how organisms respond to natural selection by ‘constraining’ evolution to dimensions with heritable variation (Fisher, 1930) or biasing evolution in the direction of maximum variation in multivariate space or ‘line of least resistance’ (Schluter, 1996). Populations evolving on contemporary time scales from a known ancestral form can provide powerful tests of the role of ancestral covariance structure in constraining or biasing evolutionary trajectories.

Studies of postglacial evolution of threespine stickleback fish, *Gasterosteus aculeatus*, have greatly increased our understanding of how organisms diversify in nature (Bell & Foster, 1994a; Östlund-Nilsson, Mayer & Huntingford, 2006; Hendry et al., 2009).
Three-spine stickleback are ancestrally oceanic and many anadromous populations enter fresh water to reproduce. Anadromous stickleback established resident freshwater populations that adapted to novel surroundings throughout coastal areas of the northern hemisphere. Some adaptations, such as the evolution of lateral plate phenotypes, are dramatic and relatively easy to quantify (Bell, Aguirre & Buck, 2004; Colosimo et al., 2005). However, many other aspects of the stickleback phenotype diverge after colonization of fresh water, and most are quantitative traits exhibiting subtle variation (Bell, 1984; Bell & Foster, 1994b).

Complex quantitative traits are more difficult to study, although documenting their evolution is crucial for understanding evolutionary diversification. Body shape is one such trait. Fishes vary greatly in body shape, and the differences often reflect divergent ecological roles (Webb, 1982). Body shape differences among stickleback populations often reflect differences in habitat use, such as those between anadromous and resident freshwater (Walker & Bell, 2000; Aguirre et al., 2008), between stream and lake (Hendry, Taylor & McPhail, 2002; Berner et al., 2008; Bolnick et al., 2009) or between benthic (deep-bodied bottom-feeders in shallow waters) and limnetic (shallow-bodied planktivores in deep waters) populations (McPhail, 1994; Walker, 1997; Aguirre, 2009; Willacker et al., 2010). Body shape evolves in response to other ecological variables as well, such as the presence of predatory fishes (Walker, 1997). Divergence in body shape among populations can thus reflect adaptation in response to diverse ecological factors.

In the present study, the evolutionary trajectory of body shape is described in the newly-established Loberg Lake population as it adapts to conditions in the lake. The native Loberg Lake stickleback population was exterminated by rotenone poisoning in 1982 to increase the suitability of the lake for recreational fishing. The stickleback population inhabiting the lake was typical of resident lake populations in the area, exhibiting significant armour reduction relative to its anadromous ancestor. Anadromous stickleback recolonized the lake sometime between 1983 and 1989. Annual sampling beginning in 1990 revealed rapid lateral plate evolution in the direction of local resident lake populations (Bell et al., 2004), indicating that the population has experienced strong directional selection. Contemporary evolution in Loberg Lake stickleback thus simulates the natural process of freshwater colonization and divergence of anadromous populations that produced postglacial adaptive radiation of stickleback, and can provide a rare glimpse into the early stages of adaptive radiation.

Body shape was chosen for the present study because it is a complex quantitative trait that captures morphological variation associated with multiple ecological factors and has a demonstrated genetic basis in stickleback (Baumgartner, 1995; Albert et al., 2008). The evolutionary trajectory of the Loberg Lake population is plotted in a multivariate space formed by body shape variation of neighbouring threespine stickleback populations in the Cook Inlet Basin, Alaska. Differences in body shape between anadromous and resident lake stickleback have been relatively well documented (Walker & Bell, 2000; Spoljaric & Reimchen, 2007; Aguirre, 2009), allowing predictions to be made regarding how stickleback body shape will change over time in Loberg Lake. If the newly-established stickleback population is adapting to conditions in Loberg Lake from a population with an anadromous phenotype, its mean body shape should gradually diverge from an anadromous type body shape to that typical for neighbouring resident lake populations. In addition, resident freshwater stickleback typically have smaller ectocoracoids, a shorter posterior process of the pelvis, longer caudal peduncles, more posteriorly positioned dorsal spines, and shorter median fins than anadromous stickleback (Aguirre, 2009). Parallel changes in body shape (i.e. in the direction of resident freshwater stickleback) are expected in the extant Loberg Lake population.

In the present study, the pattern of body shape change is examined in multivariate space as the Loberg Lake population adapts to conditions in the lake, spatial body shape variation is tested within the lake, and associations between body shape and armour phenotypes are also investigated. An estimate of how quickly complex quantitative traits such body shape can evolve upon invasion of a new type of habitat is also obtained, and whether phenotypic change is uniform through time as the population adapts is examined. Finally, the present study tests whether ancestral phenotypic covariance structure, which is inferred from a neighbouring anadromous stickleback population (Aguirre et al., 2008), constrained its evolutionary trajectory.

**MATERIAL AND METHODS**

**SITES, SAMPLING, AND SPECIMEN PREPARATION**

Loberg Lake is a small lake (approximately 4.45 ha) in the Matanuska-Susitna Borough of Southcentral Alaska (61°33′35″N, 149°15′30″W). Its location, a map of the lake with collection sites (see also Supporting information, Appendices S1 and S2), the physical and biological properties of the lake, and the sampling and preservation methods employed are reported elsewhere Bell et al. (2004) and at the
Gasterosteus aculeatus were sampled from five sites around the perimeter of the lake, except between 1990 and 1993, when only site A was sampled. Most Loberg Lake samples were collected with minnow traps in late May or early June. The 1990 sample was collected with a seine in July, and the 1991 sample was trapped through the ice in November. Specimens were preserved and stained and lateral plate morphs were scored \textit{sensu} Bell et al. (2004). Lateral plate morphs in Loberg Lake include the low, partial, and complete morphs. The samples collected have been deposited in the fish collection of the Division of Vertebrate Zoology at the Yale Peabody Museum. Samples from 20 neighbouring lakes, five streams, and two anadromous populations were included in the analysis (generally 20 specimens per sample; see Supporting information, Appendix S3) to put body shape evolution of the Loberg Lake population into the context of local stickleback diversity. Thirteen of the 20 lake populations included were sampled during 2 years (usually 1990 and 2004). An anadromous population from Rabbit Slough (RS; Aguirre et al., 2008) and minimizing the effects of allometry (Walker, 1993) on shape variation. Males were identified by inspection of gonads. Specimens infected with \textit{Schistocephalus solidus} (Cestoda) were also excluded. At least fifty male fish were included each year, except for samples collected before 1994 (see Supporting information, Appendix S2). A sample of ten male fish from the extinct Loberg Lake population in 1982 (frozen before fixation) was also included. Specimens from this sample, as well as those from 1990 and 1991 (because of their small sample sizes), were photographed three times to reduce measurement errors associated with straightening fish and digitizing landmarks. Consensus configurations for each individual were used in the morphometric analyses to reduce measurement error. With small sample sizes, measurement error of individual specimens can have disproportionately large effects.

**COLLECTION OF BODY SHAPE DATA**

Geometric morphometric methods (Zelditch et al., 2004) were used to study body shape variation. Specimens were photographed with a 3.3 megapixel Olympus Camedia C-3000 digital camera, and two-dimensional coordinates were collected for 16 landmarks digitized on each specimen (Fig. 1) using TPSDIG, version 1.40 (Rohlf, 2004a). The landmarks are based on previous studies by Aguirre et al. (2008) and Aguirre (2009). The landmark data were aligned using the Procrustes superimposition method in TPSRELW, version 1.44 (Rohlf, 2006). All specimens in the study were included in a single alignment from which the shape variables were generated.

Only males exceeding 32 mm standard length (i.e. tip of upper jaw to end of last vertebra) were included to eliminate the effects of sexual dimorphism (Kitano, Mori & Peichel, 2007; Aguirre et al., 2008) and minimize the effects of allometry (Walker, 1993) on shape variation. Males were identified by inspection of gonads. Specimens infected with \textit{Schistocephalus solidus} (Cestoda) were also excluded. At least fifty male fish were included each year, except for samples collected before 1994 (see Supporting information, Appendix S2). A sample of ten male fish from the extinct Loberg Lake population in 1982 (frozen before fixation) was also included. Specimens from this sample, as well as those from 1990 and 1991 (because of their small sample sizes), were photographed three times to reduce measurement errors associated with straightening fish and digitizing landmarks. Consensus configurations for each individual were used in the morphometric analyses to reduce measurement error. With small sample sizes, measurement error of individual specimens can have disproportionately large effects.

**MULTIVARIATE ANALYSIS**

Principal component analysis (PCA) was used to capture the evolutionary trajectory of the Loberg Lake population in a two-dimensional morphospace representing the first two major axes of body shape variation of stickleback populations in the region. The PCA was carried out with TPSRELW, version 1.44 (Rohlf, 2006) on the annual Loberg Lake samples plus all populations sampled, and mean sample PC scores were calculated subsequently. Confidence intervals (CIs) were calculated for bivariate means of selected
samples in the morphospace sensu Sokal & Rohlf (1995) with BIOMSTAT, 3.30 (Applied Biostatistics, Inc.).

Using annual samples from the Loberg Lake population from 1994 (the first year for which five sites were sampled) and 2006 (a year towards the end of the time series), we tested whether year (1994 versus 2006), lateral plate morph (complete versus low), collection site, and the interactions between these factors, were associated with significant body shape variation in the Loberg Lake population and contrasted the relative contributions of these factors. Centroid size (Zelditch et al., 2004) was included as a covariate. All factors were tested over the residual Sum of Squares error matrix with TPSREGR, version 1.31 (Rohlf, 2005). The magnitude of the effects of these variables was evaluated using procrustes distances (Zelditch et al., 2004) among sample means calculated with TPSSPLIN, version 1.20 (Rohlf, 2004b) and Wilks’ partial η² (Langerhans & DeWitt, 2004).

Body shape was regressed on year of collection (using TPSREGR, version 1.31), with centroid size included as a covariate (to control for allometry), to confirm that there is a temporal trend in the evolution of body shape in the Loberg Lake population. The regression was run with all specimens in the Loberg Lake time series (1990–2009), all specimens collected during a different time of year. If ancestral phenotypic covariance structure influences the evolutionary trajectory of the Loberg Lake population, we expect to be small and possibly increase over time (Schluter, 1996). Confidence intervals for the calculated angles were generated by bootstrapping (sampling with replacement) the rows (specimens) of the shape variable matrices, carrying out the procedure described above 1000 times, and using the upper and lower 2.5% values of the distribution to define the 95% CI.

Because p_max can bias z even if the angle θ between these vectors is greater than 0, we examined whether the angles between p_max-RS and z_RS-L90 through z_RS-L09 were below the null expectation for two random vectors of the same dimensions in accordance with the general approach outlined by Hunt (2007). We used the algorithm of Knuth (1981) and created a null distribution of angles between 10 000 pairs of random vectors of the same dimensionality as p_max and z (vectors with 28 scalars, the number of shape variables corresponding to 16 landmarks).

The mean of the 10 000 angles generated was 81.3° and 95% of the angles fell between 65.8 and 89.6°, forming the null expectation for the angle between two random vectors with 28 scalars. We considered z_i to be biased in the direction of p_max if the empirically derived 95% CIs for θ did not overlap with the null expectation of 81.3°.

We also examined whether the divergence vector, z_i, between Loberg Lake samples collected in consecutive generations was biased in the direction of p_max within the Loberg Lake time series by examining the angle between the vectors p_max-L0t and z_L0t-L0t+2, where p_max-L0t is p_max for the Loberg Lake population in year t (the ‘ancestral’ sample within the pair of samples) and z_L0t-L0t+2 is the divergence vector between the Loberg Lake annual samples collected in years t and t + 2, the latter representing the ‘derived’ sample within the pair. We compared years t and t + 2 because stickleback in Cook Inlet generally, and specifically in Loberg Lake, appear to have a mean generation time of 2 years (Havens et al., 1984; Bell et al., 2004). However, assuming a generation time of 1 year yielded similar results. z_i was considered to be
biased in the direction of $p_{\text{max}}$ if the 95% CIs did not overlap with the null expectation for $\theta$ of 81.3°. Angles, bootstrap values, and random vectors were calculated using MATLAB, version 6.5.0 (The MathWorks, Inc.).

**Stable isotope analysis**

The first sample from the extant population was made in 1990, and specimens in this sample resembled anadromous stickleback for several traits, including armour phenotype (Bell et al., 2004). Thus, they may have been anadromous stickleback that had migrated from the ocean to breed. Several lines of morphological evidence discussed below suggest that this is not the case and that specimens in the 1990 sample developed entirely within Loberg Lake. Nevertheless, we compared the δ13C and δ15N stable isotope ratios (Post, 2002) from specimens in the 1990 sample with those of known resident Loberg Lake and anadromous stickleback. Although there can be variation among lakes, δ13C tends to be enriched (values are less negative) in the tissues of marine relative to freshwater consumers (Fry, 2006). Stable C and N isotope ratios were measured in all six specimens from the Loberg Lake 1990 sample, five anadromous specimens from two populations (three from Rabbit Slough collected in 2000 and two from Bishop Creek collected in 1992), and eight resident Loberg Lake stickleback collected in 1992 and 1993 (four per year). A small piece of muscle was cut from the caudal peduncle of each specimen and allowed to dry at 50–70 °C for approximately 1 week. Between 3 and 7 mg of tissue (dry weight) per specimen was ground, dried again at 50–70 °C overnight, and then packed in a tin capsule and sent to the Institute of Ecology at the University of Georgia’s Stable Isotope Laboratory, where δ13C and δ15N were measured using mass spectrometry.

**Results**

**Body shape variation among Cook Inlet threespine stickleback**

PCs I to V accounted for 34.3%, 15.5%, 10.1%, 8.9%, and 7.3% of the variation, respectively. Biologically interpretable variation was present in the space formed by PCs I and II so we focus on these. Deformation grids for body shape variation at the extremes of PCs III, IV, and V are available in the Supporting information (Appendix S4). In the space formed by PCs I and II, anadromous samples grouped together and were segregated from the freshwater samples (Fig. 2). The stream samples also grouped in a relatively small part of the PC space and overlapped with samples from shallow lakes and Loberg Lake. Lake samples were widely dispersed and contributed a large portion of the body shape variation among populations. In most cases in which populations were sampled in 2 years, means from the same population were generally close together. Exceptions include Zero Lake (pelvic reduction of some individuals in this population may be influencing the results) and Lower Ohmer. Biologically interpretable variation among samples tended to be diagonally oriented to PCs I and II. Variation distributed from the top right (where the anadromous samples occur) to the bottom left of the space [Nowack (21) and Zero Lake (15) samples] tended to be largely associated with a shift in position of landmark 12, which is at the tip of the posterior process of the pelvis. The posterior process of the pelvis is longer in anadromous fish than in lake resident fish, whereas Zero and Nowack both exhibit significant pelvic reduction, resulting in displacement of the posterior end of their pelvis forward. There were other smaller differences, including the length of the ectocoracoid, median fins, and caudal peduncle, and the position of the pectoral fin. Variation along the other diagonal, from bottom right to the top left of the space, tended to be associated with relative body depth. Stream and shallow lake samples [e.g. Mud (10) and Tern (12) lakes] were deeper bodied and located towards the bottom right of the space, whereas samples from deep lakes [e.g. Stormy (11), Big (16), Nancy (20), and Long (8) lakes] had much more elongated bodies and were located towards the top left of the space.

**Body shape variation in the Loberg Lake population**

Centroid size, year of collection (1994 versus 2006), lateral plate morph, collection site, and the interaction between lateral plate morph and collection site significantly influenced body shape variation of Loberg Lake stickleback (Table 1). However, year of collection had the largest effect on body shape variation, as indicated by the Wilks’ partial $\eta^2$ values. Temporal variation of body shape for the entire time series is described in greater detail below. Lateral plate morph had the second largest influence on body shape. Differences in body shape between completes and lows from the same year were subtle, although variation was greater in 1994 than in 2006 based on Procrustes distances between consensus configurations (0.0105 and 0.0063, respectively). Lows had a slightly smaller ectocoracoid (LM 14) and posterior process of the pelvis (LM 12), and the first dorsal spine (LM 3) was displaced backward (see Supporting information, Appendix S5). The effect of the other variables on body shape was smaller and not discussed.
The mean for the extinct 1982 Loberg sample was located far from the anadromous samples in the morphospace (Fig. 2). Major differences in body shape from anadromous RS stickleback (Fig. 3A) include a smaller ectocoracoid (LM 13 and 14), a much smaller pelvic posterior process (LM 12), shorter median fins and a longer caudal peduncle (LM 6, 7, 9, and 10),

**Table 1.** Multivariate analysis of variance and partial $\eta^2$ estimates of body shape variation in the Loberg Lake 1994 and 2006 samples related to centroid size, year of collection, lateral plate morph (LPM), collection site, and interactions among the main factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Wilks’ $\lambda$</th>
<th>$F$</th>
<th>d.f. 1</th>
<th>d.f. 2</th>
<th>$P$</th>
<th>Wilks’ partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centroid size</td>
<td>0.7704</td>
<td>3.012</td>
<td>28</td>
<td>283</td>
<td>&lt; 0.001</td>
<td>0.230</td>
</tr>
<tr>
<td>Year of collection</td>
<td>0.3760</td>
<td>16.780</td>
<td>28</td>
<td>283</td>
<td>&lt; 0.001</td>
<td>0.624</td>
</tr>
<tr>
<td>LPM</td>
<td>0.7563</td>
<td>3.257</td>
<td>28</td>
<td>283</td>
<td>&lt; 0.001</td>
<td>0.244</td>
</tr>
<tr>
<td>Collection site</td>
<td>0.5479</td>
<td>1.645</td>
<td>112</td>
<td>1126.6</td>
<td>&lt; 0.001</td>
<td>0.141</td>
</tr>
<tr>
<td>Year × LPM</td>
<td>0.9255</td>
<td>0.813</td>
<td>28</td>
<td>283</td>
<td>0.738</td>
<td>NS</td>
</tr>
<tr>
<td>Year × Site</td>
<td>0.6474</td>
<td>1.164</td>
<td>112</td>
<td>1126.6</td>
<td>0.126</td>
<td>NS</td>
</tr>
<tr>
<td>LPM × Site</td>
<td>0.6220</td>
<td>1.277</td>
<td>112</td>
<td>1126.6</td>
<td>0.033</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Lower Wilks’ $\lambda$ values imply higher significance. Wilks’ partial $\eta^2$ provides a measure of the strength of the association between factors being tested and variation in the dependent variables. Higher Wilks’ partial $\eta^2$ values imply greater effect strengths for the particular factor (Langerhans & DeWitt, 2004); NS, not significant.

**Evolutionary Trajectory of the Loberg Lake Population**

The mean for the extinct 1982 Loberg sample was located far from the anadromous samples in the morphospace (Fig. 2). Major differences in body shape from anadromous RS stickleback (Fig. 3A) include a smaller ectocoracoid (LM 13 and 14), a much smaller pelvic posterior process (LM 12), shorter median fins and a longer caudal peduncle (LM 6, 7, 9, and 10),

posteriorly displaced first and second dorsal spines (LM 3 and 4), and a ventrally displaced pectoral fin (LM16). By contrast, the mean shape of the 1990 Loberg Lake sample was similar to that of anadromous samples (Fig. 3B). The similarity in body shape between the 1990 Loberg sample and anadromous populations is consistent with other traits measured, including armour phenotypes and gill-raker number (Bell et al., 2004).

Several lines of evidence suggest that the 1990 Loberg Lake stickleback were lake residents, not anadromous fish that had migrated into the lake. They were smaller than typical anadromous fish; all 1990 stickleback were smaller than all but one of 146 Rabbit Slough fish included in the present study. Most stickleback in the 1990 Loberg Lake sample were also heavily parasitized by Schistocephalus solidus (Cestoda) tapeworms, which are rare in adult anadromous stickleback (M. A. Bell and W. E. Aguirre, unpubl. data) but common in freshwater populations (Heins & Baker, 2003). A few fish in the 1990 sample were intermediate partial morphs (i.e. with multiple interruptions in an otherwise complete plate row; Bell et al., 2004), whereas anadromous stickleback in Cook Inlet are phenotypically monomorphic for the complete lateral plate morph (Aguirre et al., 2008; M. A. Bell and W. E. Aguirre, unpubl. data ). Finally, δ¹³C values of 1990 specimens were indistinguishable from those of

**Figure 3.** Body shape variation of the Loberg Lake population. Landmark displacements of consensus configurations of (A) the 1982 sample of the extinct Loberg Lake population from the anadromous Rabbit Slough (RS) sample, (B) the 1990 Loberg Lake sample from the RS sample, (C) the 1992 Loberg Lake sample from the 1990 Loberg Lake sample, (D) the 2009 Loberg Lake sample from the RS sample, and (E) the 2009 Loberg Lake sample from the 1982 sample of the extinct Loberg Lake population. Vectors are exaggerated two-fold for visualization.
resident Loberg Lake samples from 1992 and 1993 but contrasted strikingly with values from the anadromous samples (Fig. 4), indicating that fish in the 1990 sample had assimilated carbon from the same source as the 1992 and 1993 samples during development. There can be little doubt that specimens in the 1990 Loberg Lake sample were lake residents.

The 1991 Loberg Lake sample mean is located far from the anadromous and all other Loberg Lake annual samples in the morphospace. We include the 1991 sample for completeness, although differences in collection date and mean body size relative to the other samples make this comparison questionable, and it is not discussed further. There is a large gap between mean body shapes for the 1990 and 1992 Loberg Lake samples, suggesting substantial change in body shape early in the Loberg Lake time series. The change in body shape between the 1990 and 1992 sample means includes a decrease in the lengths of the posterior process of the pelvis (LM 12) and the ectocoracoid (LM 13), a slight decrease in body depth, and a slight increase in length of the caudal peduncle (Fig. 3C). The rate of change in body shape in the rest of the time series was relatively uniform as demonstrated by plotting the Euclidean distance between mean PC scores for consecutive annual samples against time (data not shown). The 1992–2009 annual means for the Loberg Lake population clustered in a relatively small part of the morphospace among the stream and shallow lake samples, indicating gradual but somewhat irregular change in body shape after 1992 (Fig. 2). However, Loberg Lake is not a shallow lake. Its relative littoral area (Walker, 1997), which is an index of the amount of habitat above euphotic zone depth, is 27.1, indicating that most of the lake bottom (72.9%) receives too little light to support macrophytes.

Upon close inspection, the 1992–2009 Loberg sample means displayed a striking temporal pattern, with annual means generally progressing towards the centre of the morphospace of typical lake populations (Fig. 5). Although the change in position between consecutive years is erratic, the overall trend is clear. The direction of displacement of landmarks of the consensus configuration for the 2009 Loberg Lake sample from the Rabbit Slough sample (Fig. 3D) is similar to that between the extinct 1982 sample and Rabbit Slough (Fig. 3A), suggesting substantial parallelism in the evolution of body shape between the exterminated and extant populations. Displacement vectors between the extinct 1982 and the 2009 Loberg samples are relatively small reflecting similar body shapes (Fig. 3E). By 2009, the Procrustes distance between the means for the Loberg Lake and Rabbit Slough samples is 68% of the Procrustes distance between the means for the extinct Loberg Lake (1982) and Rabbit Slough samples.

We explored the temporal pattern further by regressing body shape of Loberg Lake stickleback on year of collection (with centroid size included as a covariate to control for allometry). The results were generally similar, regardless of whether we regressed

all specimens from 1990 to 2009, only specimens collected between 1992 to 2009, or only low morphs from site A collected between 1992 and 2009 to eliminate variation related to lateral plate morph and spatial heterogeneity. Both year of collection and centroid size accounted for significant components of the variation in body shape ($P < 0.001$ in all cases), with estimates of Wilks' partial $\eta^2$ in the range 0.623–0.637 for year and 0.335–0.358 for centroid size. Body shape change over time in the extant Loberg Lake population based on the regression analysis largely involved decreases in length of the posterior process of the pelvis (LM 12) and ectocoracoid (LM 13 and 14), a decrease in body depth, the posterior movement of the landmarks associated with the supraoccipital notch and the dorsal spines (LM 2, 3, and 4), and an elongation of the caudal peduncle (Fig. 6). Allometric body shape change (not shown) resulted in a more elongate body form, especially in the trunk region, a reduction in the length of the caudal peduncle, and an increase in length of the median fins with size.

THE INFLUENCE OF ANCESTRAL COVARIANCE STRUCTURE

The major axis of phenotypic variation in the Rabbit Slough population, $p_{\text{max-RS}}$, explained only 22.5% of the variation in body shape. The angle between $p_{\text{max-RS}}$ and $z_{\text{RS-LS0}}$ was 83.8° (95% CI = 56.9–89.3), which is in line with that expected between two random vectors. The divergence between the Loberg Lake 1990 and Rabbit Slough sample means is thus not biased in the direction of $p_{\text{max-RS}}$. The angle between $p_{\text{max-RS}}$ and $z_{\text{RS-LS2}}$ (the first year in the main cluster of Loberg annual samples; Figs 2, 5) was 77.7° (95% CI = 62.1–89.1), which was also not significantly less than the null expectation of 81.3°. Angles between Rabbit Slough and all other annual samples of the Loberg Lake population were similarly large, in the range 70.4–87.2° and none was significantly less than the null expectation. Consequently, there appears to be no detectable relationship between the evolutionary trajectory of the Loberg Lake population and ancestral phenotypic covariation inferred from the Rabbit Slough sample.

The major axis of variation within individual annual samples for the Loberg Lake population also typically accounted for a relatively small proportion of the body shape phenotypic variance (see Supporting information, Appendix S6). The mean variance explained by $p_{\text{max}}$ was 22.7% and ranged from 18.7% in the 1994 sample to 31.7% in the 2009 sample (excluding the small 1990 and 1991 samples). The angles between $p_{\text{max-Lobt}}$ and $z_{\text{Lobt-Lobt+2}}$ were generally lower than those calculated between the anadromous ancestral Rabbit Slough sample and the Loberg Lake annual samples; however, in most cases, the empirically bootstrapped 95% CIs overlapped with the null expectation for $\theta$ of 81.3° (see Supporting information, Appendix S7). The mean value for $\theta$ within the Loberg Lake time series was 65.5° and individual estimates ranged from 33.0° between the 2000 and 2002 samples to 82.9° between the 2007 and 2009 samples. The assumption of a 1-year generation time yielded similar results. There was no temporal trend to the angles, indicating that the population is not evolving in a direction more closely or distantly influenced by $p_{\text{max}}$ over time (correlation analysis,

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Predicted body shape change over time in the Loberg Lake population between 1990 and 2009 (from TPSREGR, version 1.31 with centroid size included as a covariate in the analysis). A, body shape early in the time series. B, body shape late in the time series. Deformation grids indicate deformation from the mean shape across all Loberg Lake samples and are exaggerated by three-fold.
Influences of spatial heterogeneity, lateral plate morph, and allometry on body shape variation in the Loberg lake population

Significant variation in body shape among sites in Loberg Lake is not unexpected. Although it is small, there is probably substantial spatial heterogeneity in vegetation, substratum, food resources, and predators throughout the lake. When examined closely, subtle spatial differences in morphology, often associated with ecological factors, are typical for resident lake and stream stickleback (Reimchen, 1980; Bell, 1982; Baumgartner, 1992; Hendry et al., 2002). In addition, spatial heterogeneity of lateral plate morph frequencies was reported previously from Loberg Lake stickleback (Bell et al., 2004). The influence of spatial heterogeneity on body shape variation appears to be relatively small compared to other factors. Based on Wilks’ partial η² values, year of collection (1994 versus 2006) was four-fold more important than spatial heterogeneity (Table 1).

Parallel evolution is one of the most remarkable outcomes of natural selection. The numerous instances of parallel evolution documented in the threespine stickleback system illustrate the power of natural selection acting on closely-related organisms subjected to similar environmental conditions. The present study differs from most previous examples of parallel evolution in that we document the evolution of a complex quantitative trait over a 20-year time series, beginning from a sample that is phenotypically similar to the ancestral form, and ending with samples that are phenotypically almost indistinguishable from typical derived resident lake forms. The trajectory of the population was illustrated by plotting the change in mean body shape of the Loberg Lake population in a multivariate shape space that includes body shape variation among 27 neighboring oceanic, resident lake, and stream populations, many of which were sampled over multiple years. With previous knowledge of stickleback variation, we were able to correctly predict that the mean body shape of the Loberg Lake population would move towards the body shape typical of resident freshwater populations in the region over time. We were also able to predict many of the actual changes in body shape that occurred. Although the results obtained must be interpreted with caution because this is a single case study, the Loberg Lake population provides a rare glimpse into how body shape changes as stickleback populations adapt to lakes.

The role of ancestral covariance

We found little evidence that patterns of ancestral phenotypic covariance meaningfully constrained the evolutionary trajectory of the extant Loberg Lake population. Derived samples in the Loberg Lake time series was reported previously from Loberg Lake stickleback (Bell et al., 2004). The association between lateral plate morph and body shape may be a result of genetic linkage or pleiotropy. Quantitative trait loci (QTL) linked to a major gene, Ectodysplasin (Eda), which strongly influences lateral plate morph in stickleback (Colosimo et al., 2005), also affected body shape in a cross between marine and benthic stickleback (Albert et al., 2008). Albert et al. (2008) found displacements of landmarks associated with the first dorsal spine and the ectocoracoid (among others) in response to variation at loci linked to a QTL within Eda. This was similar to the results that were obtained for variation between lows and completes in the present study. Armor plate variation in the Loberg Lake population also depends strongly on Eda genotype (W. E. Aguirre and M. A. Bell, unpubl. data). The results of the present study are consistent with the hypothesis that Eda or other loci linked to Eda have a geographically widespread influence on body shape and facilitate parallel evolution of armour and body shape upon colonization of freshwater environments (Albert et al., 2008).

Allometry accounted for a small but significant fraction of body shape variation in the Loberg Lake population and is similar to that documented among Cook Inlet resident lake populations by Walker (1997). Specimens included were generally > 32 mm standard length and allometry is likely to be a minor source of variation after sexual maturity (Walker, 1993).
series did not evolve along the major axis of variation of the sample representative of the presumed ancestral phenotype, the anadromous Rabbit Slough sample. Nor was there a strong relation between $p_{max}$ for a particular annual sample within the Loberg Lake time series and the divergence vector between it and the next generation in the lake.

Are patterns of ancestral covariance generally unimportant for the evolutionary trajectory of derived populations? The results of empirical studies addressing this issue have been mixed (Schluter, 1996; Hunt, 2007; Revell et al., 2007; Berner et al., 2008, 2010), suggesting that the importance of genetic constraints may be trait- and taxon-specific. Several factors may have contributed to the results obtained in the present study. The appropriate test for the influence of ancestral variation on evolutionary divergence employs genetic covariances (Schluter, 1996; McGuigan et al., 2005), which we did not employ. However, several studies have found that phenotypic covariances may be suitable substitutes for genetic covariances (Cheverud, 1988; Schluter, 1996), and the use of phenotypic data to examine evolutionary phenomena is not uncommon (Steppan, 1997; Leinonen et al., 2006; Hunt, 2007; Revell et al., 2007; Berner et al., 2008). Instead, we suspect that lack of strong major axes of body shape variation in the samples analyzed is an important factor. It is unlikely that evolution could be constrained when the covariance structure is as weak as it was for ancestral body shape in the present study. A study by Schluter (1996) found a strong relationship between genetic covariance and variation among populations, and $g_{max}$ accounted for 71% of the additive genetic variance for the traits that were analyzed. The low level of phenotypic variance in body shape accounted for by the dominant eigenvectors in the present study may be a consequence of the nature of body shape variation and the methods that we employed. Body shape is a complex trait that is probably influenced by many genes of moderate effect (Schluter et al., 2004; Albert et al., 2008). Geometric morphometric methods eliminate size variation which is a major source of variability, and we also only used males, which eliminated variation related to sexual dimorphism. In addition, the scatter of individuals along PCs I and II is extremely broad compared to differences among sample means (W. E. Aguirre, unpubl. data). The results of the present study suggest that there is substantial flexibility in the response of body shape to natural selection in threespine stickleback.

LOBERG LAKE AS A CASE STUDY OF ADAPTATION TO NOVEL ENVIRONMENTS

As is the case for lateral plates (Bell et al., 2004) and operculum shape (Arif, Aguirre & Bell, 2009), body shape has evolved substantially in the newly-established Loberg Lake population in the general direction of typical resident lake populations. The divergence in body shape was similar to that documented in previous comparisons of lake and anadromous stickleback (Walker & Bell, 2000; Aguirre et al., 2008). The similarity of body shape between the Loberg 2009 and the extinct Loberg 1982 samples and of their pattern of divergence from the oceanic Rabbit Slough sample is particularly striking (Fig. 3A, D, E). The direction of the displacement vectors is remarkably similar, although the lengths of the vectors were greater for the extinct 1982 Loberg Lake sample than for the 2009 sample, presumably because the latter is still adapting to conditions in the lake. This result is consistent with widespread parallelism in the evolution of freshwater stickleback populations (Bell, 1974; Bell & Foster, 1994b; Cresko et al., 2004; Schluter et al., 2004; Colosimo et al., 2005).

Change in body shape was not uniform throughout the time series. The rate of body shape change of the Loberg Lake population was initially high, and, by 1992, the population had diverged substantially from the ancestral phenotype. Body shape evolved more slowly after 1992 and proceeded toward the centre of the distribution of lake populations in the region. The 95% CI for the 1990 Loberg Lake sample mean is large and approaches the cluster of sample means between 1992–2009 (Fig. 5). Thus, we cannot rule out the possibility that the large shift in body shape occurred at least as far back as 1990 and reflects phenotypic plasticity associated with developing in a freshwater environment versus the ocean. Phenotypic plasticity is common in fish and can play an important role during the early stages of adaptation (Baker & Foster, 2002; West-Eberhard, 2003; Hendry, Farrugia & Kinnison, 2008; Wund et al., 2008), and this would hardly be unexpected.

Our best estimate for the Loberg 1990 sample indicates that the population was likely similar in body shape to anadromous populations. If this is correct, the major shift in body shape occurred between 1990 and 1992, in which case we could rule out the simple phenotypic plasticity scenario outlined above because the major impact of plasticity should be observed in the first generation of stickleback that developed in the lake. Phenotypic plasticity could still account for the shift in body shape between 1990 and 1992 if there was a rapid shift in environmental conditions in the lake between these years causing a plastic response of body shape. However, it is unclear what purely environmental factor could cause such a dramatic phenotypic shift in body shape in the observed direction. Alternatively, body shape evolution caused by a change in selection regime for body shape between 1990 and 1992 may have resulted in the shift.
in body shape observed. Some of the landmarks that exhibited the greatest change are associated with armour structures, including landmarks at the posterior tip of the pelvis (12) and the ectocoracoid (13 and 14). Consequently, selection for armour reduction, which is known to have been strong in Loberg Lake (Bell et al., 2004), could indirectly have produced an evolutionary response for body shape. Finally, if the 1990 sample is the first generation that developed in the lake, maternal effects could be involved.

Interestingly, most Loberg Lake samples (1992–2006) group with stream and benthic lake populations in the shape space, even though Loberg Lake is relatively deep. The population has been evolving a more streamlined body shape since 1992 (Fig. 6), consistent with the physical characteristics of the lake (i.e. deep lakes should select for elongate body shapes; Walker, [1997]) and is moving towards the centre of the morphospace (Fig. 2). The similarity of Loberg Lake stickleback with deep-bodied populations from shallow lakes and streams suggests that postglacial populations may go through intermediate phases resembling other freshwater ecomorphs in body shape as they adapt. Future studies of recently established lake populations or anadromous populations introduced into lakes could help determine how general this result is.

Postglacial habitats for fishes in Alaska have existed for thousands of years. Although this represents a single case study, contemporary evolution in the Loberg Lake population suggests that much of the phenotypic diversity exhibited by divergent freshwater threespine stickleback in postglacial lakes evolved very soon after invasion of fresh water (Bell et al., 2004). In the present study, we demonstrate that complex quantitative traits like body shape are capable of evolving substantially within decades of establishment of lake populations. This is consistent with the findings reported by Gelmond, von Hippel & Christy’s (2009) for stickleback isolated from their oceanic ancestors in lakes during the Great Alaskan Earthquake of 1964. Combined with the relative temporal stability observed among presumably older, native populations sampled over multiple years in the present study, and the association between morphology and the environment typically observed in postglacial stickleback populations (Walker, 1997; Spolaric & Reimchen, 2007), our results suggest that resident lake and stream stickleback populations are generally located near adaptive peaks. Thus, the scenario emerging from study of the Loberg Lake population is consistent with observations in the fossil record of stasis punctuated by bouts of rapid evolution (Gould & Eldredge, 1993; Bell, Travis & Blouw, 2006; Hunt, Bell & Travis, 2008), and with the contention of Kinnison & Hendry (2001) that macroevolution results from microevolution ‘writ in fits and starts’. Studies of other recently established lake populations, including artificial introductions of oceanic stickleback to lakes, would be extremely useful for evaluating the generality of the results documented in Loberg Lake.

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Appendix S2. List of Loberg Lake specimens used in this study.

Appendix S3. Populations used in morphometric analysis. The latitudes and longitudes are in decimal degrees.

Appendix S4. Deformation grids depicting predicted body shapes at the extremes of principal components III (a, b), IV (c, d), and V (e, f) for the principal component analysis of all specimens included in the study.

Appendix S5. Body shape variation between consensus configurations for low and complete morph stickleback in 1994. Arrows indicate the direction and magnitude of landmark displacements of the low morph consensus from that of the complete morph. Although statistically significant, there was relatively little difference between morph means within years so the vectors are exaggerated by five-fold to facilitate visualization. The difference between morph means was less in 2006 and is not shown.
Appendix S6. Variance explained by principal components 1 – 3 for the anadromous Rabbit Slough and Lober Lake annual samples.

Appendix S7. Angles (θ) within the Loberg Lake time series between $p_{max}$, the major axis of variation within annual samples, and $z$, the divergence vector between annual samples. $z_{Lobt-Lobt+2}$ is the divergence vector between Loberg Lake annual samples in years $t$ and $t+2$ (e.g. 1990 and 1992). Years $t$ and $t+2$ are used because stickleback in Cook Inlet lakes and in Loberg Lake generally have two year generation times (Havens et al., 1984; Bell et al., 2004). Assuming a one year generation time did not significantly alter the results. 95% Confidence intervals (95% CI) were calculated by bootstrapping (sampling with replacement) the shape variable matrices, calculating $θ$ at each iteration, and using the upper and lower 2.5% values of the distribution as 95% CIs.

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