Stony Brook University

The Pattern and Process of Evolutionary Diversification:

Lessons from a Threespine Stickleback Adaptive Radiation

A Dissertation Presented

by

Windsor Efren Aguirre

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor in Philosophy

in

Ecology and Evolution

Stony Brook Univeristy

August 2007

Stony Brook University

The Graduate School

Windsor Efren Aguirre

We, the dissertation committee for the above candidate for the Doctor of

Philosophy degree, hereby recommend acceptance of this dissertation.

Michael A. Bell, Dissertation Advisor
Professor, Department of Ecology and Evolution, Stony Brook University

F. James Rohlf, Chairperson of Defense
Distinguished Professor, Department of Ecology and Evolution, Stony Brook University

John R. True
Assistant Professor, Department of Ecology and Evolution, Stony Brook University

R. Geeta
Associate Professor, Department of Ecology and Evolution, Stony Brook University

Andrew P. Hendry
Associate Professor, Redpath Museum and Department of Biology,
McGill University

This dissertation is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Dissertation

The Pattern and Process of Evolutionary Diversification:

Lessons from a Threespine Stickleback Adaptive Radiation

by

Windsor Efren Aguirre

Doctor of Philosophy

in

Ecology and Evolution

Stony Brook University

2007

How do organisms diversify or "radiate" in nature? I studied an Alaskan threespine stickleback adaptive radiation to examine the rate at which organisms adapt to novel environmental conditions and the importance of different factors that facilitate or constrain adaptive radiation in nature. My dissertation research consisted of two main components.

First, I exploited a recently established population in Loberg Lake to examine the rate and pattern of stickleback adaptation in nature. I established baseline phenotypic variation and covariation using its most likely ancestor, a sea-run population from the same drainage. Within 25 years of establishment, the Loberg Lake population evolved from the ancestral phenotype to become almost indistinguishable from typical resident lake populations in the area, suggesting that adaptation to freshwater environments occurs within decades after

freshwater populations form. Evolutionary rates were often highest early in the time series, levels of phenotypic variation remained high during adaptation to lake conditions, and ancestral phenotypic variation was abundant and did not appear to substantially constrain the evolution of the Loberg Lake population. Genetic variation in the Loberg Lake population is high compared to neighboring lake and stream populations, indicating that high levels of genetic variation were also conserved during founding.

Second, I examined the relative importance of gene flow and natural selection on phenotypic divergence within a phenotypically diverse stickleback lake-stream radiation, in a small Alaskan drainage. Genetic distances among populations were associated with geographic distances, indicating that they were generally more important than the nature of the environment for structuring of genetic diversity. Morphological distances, however, were strongly associated with environmental conditions. Consequently, even within small drainages, local environmental conditions can select for adaptively important genes, despite genetic exchange with phenotypically contrasting, neighboring populations.

This study combined powerful morphometric, molecular and geographical methods to examine microevolutionary processes in nature, and provides a novel perspective on evolutionary processes during adaptive radiation of natural populations.

Dedication

This dissertation is dedicated to my parents, Efren and Carmen Aguirre, in honor of their support, sacrifice, and love.

Table of Contents

List of Tables

List of Figures

Acknowledgments

My dissertation research has been aided by an enormous number of people. Although I can not list them all, I am very grateful for their contributions. All my chapters include other authors; please see Appendix 1 for a listing of their contributions to this dissertation. My research was supported by Turner Summer Research Grants in 2003-2005, Sokal Award for Statistical Research (Dept. Ecology and Evolution, Stony Brook University), NSF Doctoral Dissertation Improvement Grant DEB0509070, NSF EAR9870337 and DEB0211391 to Michael A. Bell, and DEB0322818 to Michael A. Bell and F. J. Rohlf. REU supplements to the latter grant supported participation by undergraduates in the research.

I must thank my advisor, Michael A. Bell, for his guidance, support, and friendship. His contributions to this dissertation were invaluable and it is difficult to describe how much he has influenced my thinking. I was also very fortunate to possess a wonderful dissertation committee and am grateful to R. Geeta, F. James Rohlf, John True, and Andrew Hendry for their guidance, support, and patience over the years. They have all been truly inspiring and I will carry their lessons with me for the rest of my career.

There is a long list of other people that made important contributions to this dissertation. A.C. Havens provided the 1982 Loberg Lake sample and information on the fisheries management in the lake. The Alaska Department of Fish and Game granted annual permits for sampling the stickleback. F. A. von Hippel and his students in the Department of Biological Sciences at the University of Alaska Anchorage provided facilities and greatly assisted with field work in Alaska. R. Geeta provided facilities for the molecular procedures

and was always extremely generous with lab space. Contributions to my research by undergraduate students were numerous and I highlight only the most significant. Rashad Rahim, Tom Torres, Joanne Soong, Erika Kalabacas, and Kaitlyn Ellis assisted with genotyping specimens, Sumera Akram, Vanessa Gopez and Michael Tiongson helped collect lateral plate morph frequencies and gill raker counts from the Loberg Lake population, and Kaitlyn Ellis and Mary Kusenda helped collect morphometric data from the Rabbit Slough population. My lab mates, especially Matt Travis and Peter Park, but also Summer Ostrowski, Bill Caldecutt, and Joe Ward, assisted my research enormously and helped keep me sane (well, they tried). The W. Burghardt Turner Fellowship Program and the Alliance for Graduate Education and the Professoriate were extraordinary sources of support and warmth. I am eternally indebted to them.

It would have been impossible to complete this dissertation without the support of my family. Carmen, Michael, Liz, my nephews and niece, my uncles and aunts, my cousins, and above all my parents. My father is my hero and my mother is my saint. I will never understand what I did to deserve such wonderfully loving and supportive parents. They were always there when I needed them. I am also grateful for having met Julia Johanson towards the end of my Ph.D. studies. She has been so loving, patient, and inspiring.

Finally, I must thank my extended family, the E&E community at Stony Brook. They have been an endless source of guidance, support, inspiration, and friendship. Thank you all.

Chapter 1

Introduction

How do organisms diversify? Typically, this question brings to mind processes related to the formation of species. Speciation, however, begins with differences among individuals in demes, and the divergence of demes within species. Understanding the processes leading to evolutionary divergence of populations within species can thus provide insight into the origin and evolution of biological diversity. My dissertation research focuses on examining the factors that facilitate and constrain the evolutionary diversification of populations, and I use threespine stickleback fish, *Gasterosteus aculeatus*, as a model system to do so. My research addresses issues at all stages of diversification within species. The first part of my dissertation concerns the properties of forms ancestral to extant adaptive radiations, the second part evaluates rates, patterns and evolutionary trajectories of a population adapting to new environmental conditions, and the final part focuses on factors influencing morphological and genetic divergence of young populations inhabiting an ecologically diverse system.

A key question in evolutionary biology is the role of ancestral properties in the adaptive radiation of derived taxa. Some ancestral properties, such as high levels of variation, facilitate evolutionary diversification (e.g., Bowler, 2005; Wayne and Miyamoto, 2006) whereas others, such as ancestral covariance structure, may constrain it (Schluter, 1996; Phillips and McGuigan, 2006). The threespine stickleback system is ideal to examine how ancestral properties influence adaptive radiation. Stickleback are primitively oceanic,

spending most or all of their lives in the ocean and entering fresh water only to reproduce. Oceanic 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 and Foster, 1994; Schluter, 2000; McKinnon and Rundle, 2002; Östlund-Nilsson et al., 2006). Most research on stickleback has focused on resident freshwater populations, with little attention typically paid to the ancestral oceanic populations. My second chapter seeks to fill this void by studying phenotypic variation, covariation, and sexual dimorphism in an oceanic (sea-run) population in Rabbit Slough, Cook Inlet, Alaska sampled over multiple years. Although differences among oceanic stickleback populations are generally small (e.g., Walker and Bell, 2000), I found abundant phenotypic variation for several traits within this population, consistent with the high evolutionary rates observed among postglacial stickleback populations. Sexual dimorphism was particularly common. Temporal variation was relatively small, as was variation of covariance structure of body shape data, suggesting relative phenotypic stability of oceanic populations at least over the time scale of decades. Finally, correlations among structurally and functionally related traits indicate significant morphological integration. This detailed study of variation in an oceanic stickleback population provides crucial insight into a key component of adaptive radiation, the variation present at the onset of the radiation.

Until recently, there have been few studies of contemporary microevolution (evolution within species occurring within hundreds of years). Now, it is apparent that populations can evolve substantially on contemporary time scales (e.g., Endler, 1980, 1986; Reznick et al., 1997, Gibbs and Grant, 1987; Losos et al., 1997; Hendry and Kinnison, 1999,

2001a), and that the magnitude of evolutionary divergence among populations can be comparable to that observed among species (Liem and Kaufman, 1984; West-Eberhard, 2003). Contemporary evolutionary studies can thus serve as a valuable tool to increase our understanding of evolutionary diversification in nature (Hendry and Kinnison, 2001b). Recent studies of contemporary evolution in stickleback suggest that major morphological changes can evolve in a matter of decades (Klepaker, 1993; Bell, 2001; Kristjánsson et al., 2002; Bell et al, 2004; von Hippel and Weigner, 2004). In chapters 3 and 4, I describe rates and patterns of evolution in a recently established population in Loberg Lake, Alaska (Bell, 2001; Aguirre et al., 2004; Bell et al., 2004) that is providing insight into how postglacial populations originate in nature. The native Loberg Lake population was exterminated in 1982 and was morphologically typical of lake populations in the area (Bell et al., 2004). Oceanic stickleback must have recolonized the lake sometime between 1983, after the native population was exterminated, and 1989, the year before a sample resembling oceanic stickleback was collected (Bell et al., 2004).

In Chapter 3, I examine the evolutionary trajectory of the Loberg Lake population using body shape, a complex trait that captures phenotypic variation throughout the body and is associated with ecologically important variation (e.g., Reimchen et al. 1985; Baumgartner et al., 1988; Walker, 1997; Spoljaric and Reimchen, 2007). I also examine change in phenotypic variance within the Loberg Lake population over time and test whether ancestral covariance structure influenced its evolutionary trajectory. I found that the Loberg Lake population is evolving towards the phenotype typical of lake populations in the area, that the greatest change occurred early in the time series, that variance in body shape within populations was high compared to differences among populations, and that ancestral

covariance structure did not substantially constrain body shape evolution in this population. In Chapter 4, I document rates of evolution of armor and feeding traits, and confirm that the recently established Loberg Lake population is rapidly evolving in the direction of the original population inhabiting the lake for multiple adaptively important traits. Using five microsatellite markers, I also found that the armor morphs inhabiting the lake form a single deme, as expected, and that levels of genetic diversity were comparable to neighboring resident lake populations indicating that the Loberg Lake population was not bottlenecked more severely than is typical for lake populations in the area, and can thus be used to generalize about adaptation to postglacial environments. Overall, my research on the Loberg Lake population indicates that phenotypes characteristic of resident lake populations may evolve within decades of establishment, suggesting that evolution proceeds rapidly during the early stages of adaptation to new environmental conditions followed by relative stasis once populations are near adaptive peaks.

Understanding the factors that influence the early stages of adaptive radiation is an important problem in evolutionary biology. Divergent natural selection plays a key role during adaptive radiation, and its influence has been documented in numerous empirical studies (reviewed in Endler, 1986; Schluter, 2000). The impact of other factors, like gene flow between ecologically contrasting sites, is less clear. Gene flow can inhibit adaptive divergence by reducing the fitness of individuals adapted to ecologically contrasting habitats (Endler, 1977; Lenormand, 2002; Hendry et al., 2002; Hendry and Taylor, 2004; Moore and Hendry, 2005), or it can enhance it by providing adaptive genetic variation. However, the relative importance of gene flow may vary among traits and taxa and depend on the geographic details of the system (e.g., Hendry and Taylor, 2004; Moore et al., 2007). In

Chapter 5, I use an ecologically diverse postglacial stickleback radiation in the upper Fish Creek drainage of Cook Inlet, Alaska to investigate the influence of habitat type and geographic distance among sites on phenotypic and neutral genetic variation. Divergent natural selection, inferred from habitat phenotype correlations, is the major factor influencing phenotypic variation in the system in accordance with expectations, while neutral (i.e., microsatellite) genetic variation segregates primarily based on geographic distances among sites. Thus, phenotypic and neutral genetic variation are disconnected in this system. On a system-wide basis, divergent natural selection was sufficient to drive phenotypic differentiation, despite the potential for "swamping" due to gene flow from ecologically contrasting sites, although gene flow may constrain phenotypic divergence at particular sites. I also describe patterns of genetic diversity and genetic and phenotypic divergence throughout the drainage. This research has important implications for our understanding of the relative importance of gene flow and natural selection and on the dynamics of phenotypic and neutral genetic variation during the early stages of adaptive radiation.

In summary, my research provides a rare glimpse into the factors influencing the earliest stages of evolutionary diversification and spans all stages of diversification within species. I combine powerful morphometric, molecular and geographical methods to examine microevolutionary processes in nature, and provide insight into the rates, patterns and processes affecting adaptive radiation.

LITERATURE CITED

Aguirre, W.E., P. Doherty, and M.A. Bell. 2004. Genetics of lateral plate and gill raker phenotypes in a rapidly evolving population of threespine stickleback. Behaviour 141:1465-1483.

Baumgartner, J.V., M.A. Bell, and P. H. Weinberg. 1988. Body form differences between the Enos Lake species pair of threespine sticklebacks (*Gasterosteus aculeatus* complex). Canadian Journal of Zoology 66:467-474.

Bell, M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. Genetica 112-113:445-461.

Bell, M.A., and Foster, S.A. 1994. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell. M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Bowler, P.J. 2005. Variation from Darwin to the modern synthesis. Pages 9-27 in B. Hallgrímsson and B.K. Hall (eds.). Variation, a central concept in biology. Elsevier Academic Press, London.

Endler, J.A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76-91.

Endler, J.A. 1986. Natural selection in the wild. Monographs in Population Biology 21. Princeton University Press, Princeton.

Gibbs, H.L., and P.R. Grant. 1987. Oscillating selection on Darwin's finches. Nature 327:511-513.

Hendry, A.P., and M.T. Kinnison. 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution 53:1637-1653.

Hendry, A.P., and M.T. Kinnison. 2001a. Microevolution. Rate, pattern, process. Genetica (special issue) 112-113:1-535.

Hendry, A.P., and M.T. Kinnison. 2001b. An introduction to microevolution: rate, pattern and process. Genetica 112-113:1-8.

Hendry, A.P., and E.B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319-2331.

Hendry, A.P., E.B. Taylor, and J.D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:1199-1216.

Klepaker, T. 1993. Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1231-1258.

Kristjánsson, B.K., S. Skúlason, and D.L.G. Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659-672.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology and Evolution 17:183-189.

Liem, K.F., and L.S. Kaufman. 1984. Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. Pages 203-215 in A.A. Echelle and I. Kornfield (eds.), Evolution of fish species flocks. University of Maine at Orono Press, Orono.

Losos, J.B., K.I. Warheit, and T.W. Schoener. 1997. Adaptive differentiation following experimental island colonization in Anolis lizards. Nature 387:70-73.

McKinnon, J.S., and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model system. Trends in Ecology and Evolution 17:480-488.

Moore, J.S., and A.P. Hendry. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. Evolutionary Ecology Research 7:871-886.

Moore, J.S., J.L. Gow, E.B. Taylor, and A.P. Hendry. 2007. Quantifying the constraining influence of gene flow on adaptive divergence in the lake-stream threespine stickleback system. Evolution, in press.

Östlund-Nilsson, S., I. Mayer, and F. A. Huntingford (eds.). 2006. Biology of the three-spined stickleback. CRC Press, Boca Raton.

Phillips, P.C., and K.L. McGuigan. 2006. Evolution of genetic variance-covariance structure. Pages 310-325 in C.W. Fox and J.B. Wolf (eds.). Evolutionary genetics, concepts and case studies. Oxford University Press, Oxford.

Reimchen, T.E., E.M. Stinton, and J.S. Nelson. 1985. Multivariate differentiation of parapatric and allopatric threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Canadian Journal of Zoology 63:2944-2951.

Reznick, D.N., F.H. Shaw, F.H. Rodd, and R.G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934-1937.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution

50:1766-1774.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Spoljaric, M.A., and T.E. Reimchen. 2007. 10,000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. Journal of Fish Biology 70:1484-1503.

von Hippel, F.A., and H. Weigner. 2004. Sympatric and anadromous-resident pairs of threespine stickleback species in young lakes and streams at Bering Glacier, Alaska. Behaviour 141:1441-1464.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Walker, J.A., and M.A. Bell. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). Journal of Zoology 252: 293-302.

Wayne, M.L., and M.M. Miyamoto. 2006. Genetic variation. Pages 14-31 in C.W. Fox and J.B. Wolf (eds.). Evolutionary genetics, concepts and case studies. Oxford University Press, Oxford.

West-Eberhard, M.J. 2003. Developmental plasticity and evolution. Oxford University Press. Oxford.

Chapter 2

Phenotypic Variation, Covariation, and Sexual Dimorphism in an Alaskan Anadromous Threespine Stickleback Population

ABSTRACT

Ancestral properties can greatly influence patterns of evolutionary diversification but most systems in which evolution is studied lack direct knowledge of ancestors. In this study, we examine variation and covariation of morphological traits in an anadromous threespine stickleback population that represents the ancestral form from which resident postglacial stickleback populations in the area have evolved. 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 present at the onset of the radiation. 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 supports the notion that significant standing variation for armor evolution exists in anadromous populations. Sexual dimorphism was a major source of variation, and most traits measured differed significantly between sexes, with body size, head length, length of the pelvic girdle, and body shape differing most. Morphological variation among years, especially in body shape, was significant in both sexes, but the magnitude of yearly variation was small relative to other forms of variation, suggesting relative phenotypic stability over short time scales. Covariance structure of body shape data

also was similar, though not identical, from year to year, and the major axes of body shape variation in the study did not account for much of this variation. Finally, structurally and functionally related traits, especially armor traits, exhibited substantial phenotypic correlations, indicating significant morphological integration.

INTRODUCTION

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 and Foster, 1994; Schluter, 2000; McKinnon and Rundle, 2002). Morphological differences between oceanic and resident freshwater stickleback include divergence of armor (e.g., Hagen and Gilbertson 1972; Bell et al., 1985, 2004) trophic (McPhail, 1994) and body shape (Walker and Bell, 2000) traits. 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 pelvic reduction or have fewer than three dorsal

spines (Bell, 1974, 1987; Bell et al., 1985). The magnitude of this divergence is comparable to differences among species in other taxa, which is remarkable given the youth of the postglacial radiation (within 22,000 years; Reger and Pinney 1996).

Despite interest in threespine stickleback radiation, little is known about the ancestral anadromous form because anadromous stickleback exhibit limited morphological (Walker and Bell, 2000) and behavioral (Foster, 1994) variation, and it is easier to sample fish in streams and lakes than in the ocean. Knowledge of ancestral variation is crucial for understanding patterns of radiation, however, because levels of variation can influence rates of evolution and bias evolutionary trajectories (Sokal, 1978; Schluter, 1996; McGuigan et al., 2005). The ancestral anadromous phenotype also provides the baseline to quantify the magnitude and rate of evolution of derived postglacial populations. Postglacial adaptive radiations of threespine stickleback are exceptional because the ancestral form is known and readily available for study, making it possible to incorporate this key element lacking from most other radiations and thus obtain a better understanding of how adaptive radiations occur. Finally, enormous progress has been made recently on identifying the genetic basis of adaptive armor loss in threespine stickleback, and it is becoming clear that genetic elements of large phenotypic effect account for the rapid divergence between anadromous and postglacial resident freshwater stickleback (Peichel et al., 2001; Colosimo et al., 2004, 2005; Cresko et al., 2004; Kingsley et al., 2004; Shapiro et al., 2004, 2006; reviewed by Kingsley and Peichel 2007). Careful screening of phenotypic variation in large samples of anadromous stickleback can provide minimal estimates of the frequency of expression of genes of large effect on armor phenotypes in anadromous populations.

In this study, we analyze morphological variation, covariation and sexual dimorphism in an anadromous threespine stickleback population sampled multiple years between 1997 and 2005 from Rabbit Slough (RS), Alaska. This population runs into a drainage in an area harboring numerous derived lake resident stickleback populations (Bell and Ortí, 1994), which includes Loberg Lake (61º 33' 35" N, 149º 15' 30" W). The Loberg Lake population was established within the last 25 years from anadromous stickleback and is evolving rapidly for armor structure (Bell et al., 2004; Aguirre et al., 2004), body shape and trophic morphology (Aguirre et al., unpublished data) in the direction of typical resident freshwater populations in the area. Careful examination of phenotypic variation of the RS population can provide insight into the variability present at the onset of stickleback adaptive radiations and baseline information on the most likely ancestor of the rapidly evolving Loberg Lake population. We discuss our findings in light of patterns of adaptive radiation observed among postglacial populations.

MATERIALS AND METHODS

Rabbit Slough (61º32.065', 149º16.061') is located near Palmer, in the Matanuska-Susitna Borough, Cook Inlet, Alaska. Fish were collected in the spring (generally in June) for seven years between 1997 and 2005 with 10 to 20 unbaited 1/4 and/or 1/8 inch minnow traps set overnight. Specimens were then 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 seven years (a total of 3,440 specimens) were screened for major armor anomalies in lateral plate, pelvic and dorsal spine phenotypes.

Anomalies were defined as absence, 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 ignored.

A subset of 50 male and 50 female specimens collected on June 25, 1997, June 12, 2000, and June 9, 2003 were subjected to more detailed morphological study. Ten linear measurements and two meristic counts (Fig.1) for traits thought to be adaptively important in freshwater populations were taken. The morphometric traits measured were (1) standard length (SL), distance 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 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 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 et al. (1988). Five randomly chosen male and female specimens (10 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, ranging from 0.966 to 0.999 and averaging 0.985 (Table 1). Coefficients of variation ([standard deviation/mean]*100) were calculated for each trait and variances were tested for heterogeneity between males and females following Lewontin (1966), with probability values adjusted using the sequential Dunn-Šidák method (Sokal and Rohlf 1995).

The same 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 and Marcus, 1993; Adams et al., 2004). The landmarks are based on those used by Walker (1997) with the addition of a sixteenth at the origin of the pectoral fin. Data were collected and analyzed using TPS software. Briefly, lateral images of specimens were captured with a digital camera and X and Y coordinates for the 16 landmarks were digitized using tpsDig vers. 1.40 software (Rohlf, 2004a). The specimens were aligned with Procrustes superimposition methods as implemented in tpsRelw vers. 1.44 (Rohlf, 2006) to eliminate non-shape variation. The mean of all specimens was used as the reference shape to generate the shape variables (partial warps with uniform component). With p = 16 landmarks, 2p – 4 = 28 shape variables were generated. Deformation grids were created using tpsSplin vers. 1.20 (Rohlf, 2004b) and, unless otherwise indicated, they are deviations from the grand mean shape.

Body shape is a particularly interesting aspect of the phenotype because it captures variation related to multiple functions that vary among populations (e.g., Walker, 1997), and

application of geometric morphometric methods allows this variation to be evaluated as a composite trait. To assess the relative magnitude of variation in mean body shape from year to year in RS, the level of divergence in annual mean body shape was contrasted to the average difference in body shape between sexes in RS, and between male fish from RS and those from 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 inter-population comparisons, and because female shape may be affected by reproductive state (i.e., gravidity), and males have typically been used in these types of studies (Walker, 1997; Walker and Bell, 2000). 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. (61º 34’ 34.9N, 149º 43’ 41.0W), and in 2004 approximately 2.3 km downstream where it crosses Kenlar Rd., about 0.3 km from Big Lake Rd. (61º 33' 46.4N, 149º 49' 32.8W). Lakes were defined as deep or shallow based on the relative amount of littoral (habitat supporting macrophyte growth) area (RLA) they possessed (Walker, 1997). The deep lake had a RLA of 30 % whereas the shallow lake had a RLA of 100 %. To account for potential temporal variability of body shape in these populations, samples of 20 specimens collected in 1990 and 2004 (40 per population) were included.

The magnitude of divergence was evaluated by comparing Euclidean distances in shape space formed by the first few principal components from a PCA carried out with tpsRelw vers. 1.44 (Rohlf, 2006), Procrustes distances between sample means in complete shape space, and by partitioning of partial variances (Tabachnick and Fidell, 1996; Langerhans and DeWitt, 2004). The Procrustes distance is a distance measure used in

geometric morphometric studies and 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 tpsSplin vers. 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 and Fidell, 1996), in this case the body shape variables. Higher Wilks' partial η^2 values indicate a stronger association. Wilks' partial η^2 was calculated as:

$$\text{Partial } \eta^2 = 1 - \lambda^{1/s}$$

where λ is

$$\text{Wilks' } \lambda\text{: } = |SS_{error}|/|\ SS_{effect} + SS_{error}|$$

the ratio of the determinant of the error cross-products matrix to the determinant of the sum of the error and effect cross-product matrices, and

$$s = \text{SQRT}\ [(p^2(df_{effect})^2 - 4)/(\ p^2 + (df_{effect})^2 - 5)]$$

where p is equal to the number of dependent variables (28 for the body shape data) and df_{effect} is the degrees of freedom of the effect variable. Although not ideal, this combination of approaches provides a rough estimate of the relative importance of factors. We evaluated the importance of sexual dimorphism relative to annual variation, by testing the effects of Sex, Year, and the Sex x Year interaction against the error SS matrix. For testing the effects of variation among populations relative to annual variation, we tested Population independently (ignoring the nested term) and Year (nested within Population) over the error SS matrix. Wilks' λ was calculated, and tests for significance were carried out, with tpsRegr vers. 1.31 (Rohlf, 2005).

Pair-wise phenotypic correlations were calculated among size-corrected $\log_{10}$-transformed linear measures (with SL used as the measure of size) separately for males and females. The data were size-corrected because differences in size among individuals will result in trivial positive correlations among all traits (e.g., a larger individual will have a larger head, a larger pectoral fin, a larger first dorsal spine, etc.). Size-correction was carried out using the allometric method described by Reist (1986). Briefly, all traits were individually assessed for heterogeneity of slopes among years within sex with Biomstat vers. 3.30o (Applied Biostatistics, Inc.), and only right pelvic spine length in females was statistically significant ($P = 0.0077$; P for all other traits in both sexes > 0.05). The common with-group slope for this trait ($b = 0.645$) was comparable to that of left pelvic spine in females ($b = 0.582$), and the heterogeneity of slopes among years appeared to be largely driven by a few large females with large right pelvic spines in 2003, so we assumed a common slope for this trait. Size-corrected values for all traits were calculated with the equation $\log Y_{ij} - \beta_j (\log SL_i - \log SL_{GM})$ where Y_{ij} is the value for variable j in specimen i, β_j is the common within-groups slope for variable j, SL_i is the standard length for individual i, and SL_{GM} is the grand mean SL across all years within each sex. Phenotypic correlations were calculated from $\log_{10}$ transformed variables for each sex both for individual years and across all years. Significance of the pairwise correlations ($r \neq 0$) was evaluated using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995) following Verhoeven et al. (2005). This method is much less conservative than traditional sequential Bonferroni methods and relatively robust to lack of independence caused by positive correlations among tests (Benjamini and Yekutieli, 2001). Although the FDR method substantially reduces the frequency of type II errors, a small number of type I errors are expected (Verhoeven et al.,

2005). Consequently, we focus on larger correlations that are consistent across years within each sex to avoid spurious correlations.

The body shape variables generated using geometric morphometric methods capture body shape variation when taken as a whole set but have no biological meaning individually (Rohlf, 1998). Assessing correlations among body shape variables is thus not very informative. Instead, the structure and stability of the covariance structure was evaluated across years for each sex by calculating matrix correlation coefficients between correlation matrices of body shape variables. We also used Flurry's hierarchical common principal components (CPC) approach to examine heterogeneity of covariance matrices across years. The matrix correlation coefficients provide a general measure of association among corresponding elements of the body shape correlation matrices, and we used correlations instead of covariances to account for potential differences in variance among variables. The CPC method allows comparison of the structure of two or more covariance matrices in a hierarchical fashion to examine the level at which they share a common structure. Covariance matrices can be equal, proportional (equal eigenvectors, eigenvalues differing by a proportional value), share a common principal components structure (equal eigenvectors, different eigenvalues), share some principal components, or be unrelated (Steppan 1997). The CPC analyses were carried out using the program CPC, distributed by Patrick Phillips (2007). The number of common principal components shared was limited to the five principal components explaining the highest percentage of variance (which accounted for at least 68 % of the total variance) for computational simplicity. The step-up model building approach described by Phillips (2007) was used to evaluate the level at which the covariance matrices share a common structure, and the Akaike Information Criterion (AIC) was used to

choose the best fitting model, as recommended by Flurry (1988). The AIC balances the fit of the model against the number of parameters required and models minimizing the AIC value are preferred (Steppan, 1997).

RESULTS

The standard length (SL) distribution for both males and females from specimens measured in 1997, 2000 and 2003 had a long left tail (Fig. 2). Anadromous stickleback often have a two-year life cycle (Baker, 1994), so these small individuals (2 males, 1 female in 1997; 3 males and 2 females in 2003) are probably one-year old fish that came in to reproduce early. The frequency of these small individuals ranged from 0 to 4% of the spawning run across years and averaged 2.33%. Since 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% (Table 2).

Only one of the 3,440 specimens exhibited an LP anomaly (Fig. 3). 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 fortuitous loss of a plate due to injury. Furthermore, we did not observe any lesions in the area from which the plate was missing and there is evidence that removed LP are regenerated (Penczak, 1961). The observed anomaly does not represent a major phenotypic difference from the common

phenotype and no partial or low morphs (fish lacking numerous or most plates), characteristic of resident freshwater populations in the area, were observed. Lateral plate polymorphism thus was undetectable in this anadromous population.

Loss of dorsal and pelvic spines was very rare, but major size reduction (elements half or less of the typical size) was more common (Figs. 4 & 5). 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. 5a) occurred more often but was also very rare, with seven individuals collected over multiple years missing a dorsal spine (3 missing D1; 2 missing D2, 2 missing D3). Major reductions of pelvic spines were the most common anomaly observed, with 16 individuals (0.47%) exhibiting this anomaly (Fig. 4). Of these 16 cases, 11 had reduced right pelvic spines, five had reduced left pelvic spines, and one had both pelvic spines reduced. This did not differ statistically from a random reduction (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. 5b & c).

The addition of dorsal spines was also extremely rare, with only five individuals exhibiting an extra dorsal spine (0.15%). There were three different ways to add a fourth dorsal spine. One specimen added a large dorsal spine, resembling the first or second spine, posterior to the second spine (Fig 5d). Another had an extra spine at the posterior base of the usual first spine and bending backwards (Fig. 5e). The most common way (i.e., 3 specimens) in which a fourth dorsal spine was added, however, was through placement of an extra small dorsal spine anterior to the typical third dorsal spine (Fig 5f).

Sexual Dimorphism:

As documented for other anadromous stickleback populations (e.g., Heuts, 1947; Kitano et al., 2007), females were significantly larger than males, and the difference held across all years, even though SL differed significantly among years within sex (Nested anova, Fixed factor: sex, F= 210.43, p=0.005; random factor: year, F=21.02, p=0.045; Fig. 2, Table 3). Female SL exceeded that of males by 3.81 to 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 as a 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. It is worth noting that all of the sexually dimorphic armor traits were disproportionately larger in females. Of the sexually dimorphic traits, HL and PLVB were particularly divergent (Fig. 6a and b), with the difference between size-adjusted means corresponding to 7.38 and 6.89% of the male trait values, respectively. 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 3), although the magnitude of the difference (0.3 LP) is small, it was significant when the data were pooled across years.

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 in the original data set (Fig. 7). Differences in body shape between males and females were not localized in a particular

region, but appeared to be 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 and the distance between the dorsal and anal fins was compressed and the caudal peduncle expanded in females relative to males.

Annual Variation:

Linear measures for each sex were screened for significant differences among years. Although the magnitude of 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) the difference was significant in both males (anova; df= 2, 136; F=10.648; P<0.001) and females (ANOVA; df= 2,144; F=16.989; P<0.001), so analysis of covariance (ANCOVA), with 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 males or females (ANCOVA, P>0.05). Head length differed significantly among years in males (ANCOVA, F=5.767, P=0.004), and PCTL differed significantly among years in females (ANCOVA, F=9.461, P<0.001), after correcting for multiple tests with a sequential Bonferroni test using the Dunn-Šidák method (Sokal and Rohlf, 1995). The two meristic traits, LP and GR number, did not differ significantly among years in males or females (Kruskal-Wallis test, P>0.05).

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 minor

compared to sexual dimorphism in body shape and divergence between anadromous and resident freshwater stickleback (see below).

Annual Body Shape Variation Relative to Differences Between Sexes:

The magnitude of the difference in body shape between males and females was much larger than that among years within sex. The Procrustes distance between the consensus male and female across all years was 0.0355, and assessing Procrustes distances between consensus males and females individually for each year yielded similar 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 was generally greater among females than males, however. The Procrustes distance between consensus configurations for the former ranged from 0.0127 to 0.0311 and averaged 0.0222, whereas for males it ranged from 0.0087 to 0.0108 and averaged 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 (Table 4). For sex, Wilks' partial $\eta^2 = 0.922$, for year it was 0.438, and for the interaction of sex by year it was 0.220. All factors had a significant influence on body shape variation ($P < 0.001$, permutation test).

Annual Body Shape Variation Relative to Differences Among Populations:

Annual variation in body shape among male RS stickleback was also small compared to body shape differences between male anadromous and resident freshwater populations. Rabbit Slough stickleback separated completely 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 (Fig. 8). 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 population was more divergent from RS along PC I than the shallow lake and stream populations, and it segregated almost completely along PC I from the shallow lake and stream populations, which overlapped along both PC axes. The overlap between the shallow lake and stream populations may be related to the ecological similarities of the habitats in which these populations occur. The stream population was collected in a shallow, slow-moving stream with abundant vegetation and complex three-dimensional structure. Shallow lakes also have dense vegetation and complex structure, and both habitats should have relatively abundant benthic prey. Variation in body shape among the resident freshwater populations was consistent with previous knowledge of the influence of habitat type on body shape (Reimchen et al., 1985; Lavin and McPhail, 1985, 1986, 1987). 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. 8). The abdominal region tended to be larger in resident freshwater populations, and the posterior tip of the pelvis and pectoral fin are shifted anterially 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 PC's 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). The average divergence among RS annual means in the complete shape space,

as calculated from Procrustes distances, was 0.0099, whereas the mean distance between RS and freshwater populations in the complete shape was approximately 6 times greater (i.e., 0.0563).

MANOVA was carried out to test the significance of differences in body shape among populations and also year nested within populations (Table 4). For this analysis, only male RS fish from 1997 and 2003 were included to balance the design since resident freshwater population samples were only available for two years (1990 and 2004), and years were nested within population. Both population of origin and year nested within population had significant effects on body shape variation (permutation test, $P < 0.001$). Estimates of Wilks' partial η^2 also indicate that the magnitude of the effect of population of origin on body shape variation was higher than year, with the estimate of Wilks' partial η^2 for population being over 2.3 times greater than for year (Table 4).

Correlations Among Linear Traits:

Size-corrected measures were used to assess correlations among linear traits. There were large correlations among traits, particularly among structurally or functionally related traits. The larger correlations tended to be similar in males and females and held across the three years evaluated (Fig. 9). Matrix correlation coefficients among yearly correlation matrices were smaller for males (ranging between 0.628 and 0.721) than for females (ranging between 0.748 and 0.845). To simplify evaluation, correlations were pooled among years for each sex (Table 5). There were 36 possible pairwise correlations among the nine linear measures evaluated for each sex, of which 28 were significantly different from zero in males and 29 in females after implementing the FDR method to correct for multiple tests

(Benjamini and Hochberg, 1995; Verhoeven et al., 2005). Results differed little if the FDR method was not implemented (i.e., uncorrected P value of 0.05 used for all pairwise correlations); 28 and 30 of the correlations for males and females differed significantly from zero, respectively. All correlations were positive.

The largest correlation among traits, both in males and females (pooled across years) was the correlation between PLVL and PLVR (Table 5, Fig. 9a). These are elements of the pelvis that occur bilaterally, thus a high correlation between them is expected. The next highest correlations were between D1 and D2 and the length of the two LP measured. These are serially homologous traits so the large correlations are not surprising either. These first three pairs of traits were not only highly correlated, but the magnitudes of the correlations were similar between males and females and also similar across years (Fig 9a). Correlations between PLVB and the pelvic spines (PLVS, includes both PLVL and PLVR), which are part of the same structural complex, were substantially lower (especially in males). The magnitude of the correlations was also markedly heterogeneous between males and females relative to the first three pairs of traits discussed above (Fig. 9a). Correlations among the lengths of the dorsal and pelvic spines were significant in both males and females and tended to be relatively high (Table 5, Fig. 9b). Dorsal and pelvic spines are functionally related; together they serve as a defense against predators (Reimchen, 1983). There are three sets of other traits that tended to have consistently high correlations but are not structurally of functionally related in obvious ways. The length of the head was significantly correlated with the lengths of the two LP measured in both males and females, and HL was also significantly correlated with PCTL in females but not males. The difference in the magnitude of the correlations between males and females for this last pair of traits was surprising, correlations

were relatively similar from year to year in each sex, but tended to be twice as large in females.

Stability and Strength of Covariance Among Body Shape Variables:

Matrix correlation coefficients calculated between annual correlation matrices of the body shape variables were significantly different from zero for both sexes across all annual comparisons (Mantel test, $P<0.001$ for all comparisons), and tended to be larger in females than in males, ranging between 0.670 to 0.756 in females and 0.465 to 0.508 in males. Thus, the correlation structure of the body shape variables tended to be more stable in females.

The principal component structure was generally similar across years in both sexes, and there was not a particularly strong PC I in any sample (Table 6). PC I ranged between 23.24 and 24.62% of the variance across years in males (average = 23.74%), and between 24.3 and 31.37% of the variance in females (average = 28.15%). Pooling specimens across all years, the strength of the major PC's decreased very slightly in males (PC I = 22.30%) and increased in females (PC I = 38.51%). CPC indicated that the body shape covariance matrices shared a similar principal component structure across years. The level minimizing the AIK value (Steppan, 1997) was common principal component structure (tested over sharing five principal components in common) in both sexes, indicating that the eigenvectors were shared across years (the directions of variation in multivariate space were the same), but eigenvalues differed (the amount of variance accounted for by each eigenvector differed from year to year). However, a model of proportional covariance matrices (indicating that eigenvalues differed by a proportional amount from year to year) fit significantly better that the common principal component structure model that minimized the AIK value. In any case,

the results indicate similarity in eigenvector structure and heterogeneity of variances from year to year.

DISCUSSION

Variation of armor phenotypes in oceanic populations of threespine stickleback, including LP polymorphism, has been documented in only a few populations from other regions (i.e., Münzing, 1963; Klepaker, 1996). Variation of other traits that characteristically differ between anadromous stickleback and their freshwater descendants have received even less attention (e.g., Gross and Anderson, 1984; McPhail, 1994; Kristjánsson et al., 2002; Karve et al., 2007). Our study characterizes a wide range of traits in an anadromous population from Cook Inlet, Alaska, where several studies have analyzed adaptive radiation of freshwater populations. Furthermore, the wide range of traits included in this study provides a phenotypically broader basis for interpretation of divergence in freshwater threespine stickleback radiations elsewhere. Although we cannot be certain of the extent to which the phenotypic results documented have an underlying genetic basis, many of the traits evaluated are highly heritable and have a strong genetic basis in other populations (e.g., Hagen, 1973; Peichel et al., 2001; Cresko et al., 2004; Aguirre et al., 2004; Schluter et al., 2004; Spoljaric and Reimchen, 2007), and phenotypic and genetic correlations of morphological characters are often similar (Chevarud, 1988). This suggests that much of the variation observed here primes evolutionary change in postglacial radiations.

Major Armor Anomalies and Adaptive Radiation of Resident Freshwater Stickleback:

We found that major armor anomalies occurred at low but measurable frequencies (Table 2). This is significant, because although they are rare, major armor anomalies must exist in considerable numbers given the enormous sizes of oceanic stickleback populations. Coupled with emerging data on the presence of alleles for major armor reduction in oceanic populations hidden as recessive variation (e.g., Colosimo et al., 2005), our results suggest that significant standing variation for the evolution of armor reduction in freshwater habitats is available in ancestral oceanic populations.

Lateral plate variation was the rarest anomaly documented. While loss of the corresponding plate on both sides does not appear to represent an injury, it also does not appear to be related to reduction of plates from the complete morph of most anadromous stickleback (and all Cook Inlet anadromous stickleback) to the abbreviated anterior row (i.e., low morph) that characterizes most freshwater populations. The absence of pelvic and dorsal spines was also extremely rare, and the values in Table 2 may be overestimates. Although care was taken to exclude specimens in which a missing spine had been lost, it is impossible to verify that this was not the case. Wounds resulting from the loss of a spine early in development may heal making detection unlikely.

Reduction in the sizes of spines is much more common than complete loss in the RS population, and this is consistent with variation in derived resident freshwater populations (e.g., Reimchen, 1980; Francis et al., 1986). Although the frequencies of major reductions in the size of the left vs. right pelvic spines did not differ from random in our study, the test was not very powerful due to the small sample size and the result was in a direction consistent with biases in pelvic reduction among resident lake populations in the area. Lake populations with pelvic reduction in Cook Inlet tend to have smaller pelvic vestiges on the right side than

the left (Bell et al., 1985, 2006). Pelvic reduction is strongly influenced by *Pitx1*, and the left bias in pelvic vestiges appears to be due to partial functional compensation by another gene *Pitx2,* which is preferentially expressed on the left side (Shapiro et al., 2004, 2006). Over twice as many individuals had major reductions in the size of the right pelvic spine than did the left (11 to 5). 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 in RS. This is consistent with observations from Paxton Lake, on Texada Island (Bell, 1974), fossil *G. doryssus* from a Nevada deposit (Bell, 1974) and some populations in Cook Inlet (Bell, unpubl. data). However, it is unclear how common this trend is; the second dorsal spine is usually the one missing in the Boulton Lake population on the Queen Charlotte Islands (Reimchen, 1980). The observed relative frequencies of pelvic and dorsal spine size reduction thus appear generally consistent with evolutionary trends in derived resident freshwater populations.

Additions of dorsal spines were rare but occurred at measurable frequencies. Ancestral anadromous populations thus harbor the potential to produce stickleback with more than three spines, which is occasionally observed in derived freshwater populations (e.g., Bell and Baumgartner, 1984; Bell et al., 1985). The most common way RS stickleback added a fourth dorsal spine was by adding a spine anterior to, and about the same size as the typical third dorsal spine. This is similar to observations by Penczak (1965) on Polish threespine stickleback.

Sexual Dimorphism - The Major Source of Phenotypic Variability:

Sexual dimorphism was substantial in this population, which is consistent with previous research on threespine stickleback. Size, eight of the nine linear traits, LP number, and body shape all differed significantly between the sexes. Only D1 and GR number did not differ. The widespread sexual dimorphism found in this study indicates that many stickleback traits are probably ancestrally sexually dimorphic in derived resident freshwater populations in Cook Inlet and elsewhere. Studying levels of sexual dimorphism in resident freshwater populations differing in ecological characteristics may provide much needed insight into how ecological factors influence sexual dimorphism.

Females were larger, which is widespread (but not universal) in threespine stickleback and other gasterosteids (See Kitano et al., 2007 for a list). The difference in size may be due to a number of different factors, including natural selection, because larger female stickleback produce more eggs (Wootton, 1973; Baker, 1994), and sexual selection based on male preference for larger females (Rowland 1994). Larger female size as a consequence of selection for greater egg production is common in groups with continual growth (Andersson, 1994).

Kitano et al. (2007) also reported larger male head size and list similar cases from populations in Japan, Canada, Iceland, and Europe, as well as in the gasterosteid genus *Pungitius*. Besides the length of the head, sexual dimorphism of other head traits has been reported (Caldecutt and Adams, 1998; Caldecutt et al., 2001; Kristjánsson et al., 2002). Our finding of a longer PLVB in females is also consistent with Kitano et al.'s (2007) finding. They proposed adaptive explanations for the sexual dimorphism of these traits, but these have not been properly tested. We also found substantial differences in body shape between males and females that were distributed throughout the body. The larger head in males was

associated with an overall expansion of the anterior portion of the body, extending back at least to the origin of the pectoral fin (Fig. 7). The posterior trunk region and especially the abdomen were larger in females than in males, as was the caudal peduncle. The expansion of the abdominal region in females may be an adaptation to accommodate large egg masses. Generally similar dimorphism in body shape was reported by Kitano et al. (2007), though they used a different set of landmarks prohibiting direct comparison.

Significant sexual dimorphism in the other linear traits that we document was not as large as for those listed above (generally 2 – 3 % of the trait average), and our results are not always consistent with previous studies of other populations. Besides a relatively long PLVB in females, we observed significantly longer pelvic spines, LP, and D2 in females after size correction; armor traits tend to be relatively large in females. Kitano et al. (2007) found that D1 differed significantly between sexes in some of the populations they studied, but it was larger in males in two populations and larger in females in one. Pelvic spine lengths did not differ significantly in their study, and the non-significant differences observed were heterogeneous among populations. The reason that females have significantly larger armor structures in our study is not clear. Greater armor expression in females has been documented previously, however. Reimchen (1980), for example, found that female stickleback in Boulton Lake are more likely to have a full set of dorsal and pelvic spines than males. This seemed to be an adaptation for sexually dimorphic habitat use in lakes and resulting differences in exposure to different types of predators. Our results raise the intriguing possibility that greater armor development in females may be an ancestral condition. The selective advantage for sexual dimorphism in armor size in anadromous stickleback is

unclear though; anadromous stickleback spend most of their lives in the ocean, and differences in selection regimes for males and females in the ocean are unknown.

We also found that PCTL was significantly greater in females and LP number was greater in males. Kitano et al. (2007) did not find a significant difference in LP number between sexes among the 10 populations they surveyed. The mean left lateral plate counts they reported for populations with high mean plate counts (Table 2 in Kitano et al., 2007), however, are greater in males than in females in six of seven samples. The sample sizes in that study were smaller than in ours, suggesting that the difference in the reported results may be due to differences in statistical power. Moodie and Reimchen (1976) also reported greater LP number in males in 20 of 22 populations that they surveyed in the Queen Charlotte Islands, although most populations they surveyed were monomorphic for the low plate morph. Similarly, although not dealing with LP number, Reimchen and Nelson (1987) found a very similar pattern to ours for vertebral number, which is probably correlated with LP number in complete morph fish. As is the case with vertebrae (Moyle and Cech, 1996), there is generally one LP per body segment in complete morph fish. Consequently, vertebral number and LP number should be correlated in completes (but see Penczak, 1965). In their study of resident freshwater fish from Drizzle Lake (a monomorphic low morph population in the Queen Charlotte Islands), Reimchen and Nelson (1987) found that although smaller than females, males had 0.18 more vertebrae. Expanding their study to 10 surrounding lake and stream populations they found an average of 0.3 more vertebrae in males than in females. These values are remarkably similar to those we found for LP number and SL in this study. The reasons for this difference in LP number between males and females in RS are unclear, but the similarity among multiple studies from different regions, different LP morphs, and

correlated meristic traits, suggests that this may be widespread and deeply-rooted phenomenon. Interestingly, Sargent et al. (1984) documented the opposite pattern in the sister species of *G. aculeatus, G. wheatlandi*, in which females had higher LP and vertebral counts.

Annual Morphological Variation - Variation Around a Common Phenotypic Theme:

Two of the linear traits, HL in males and PCTL in females, and body shape exhibited significant heterogeneity among years within sex. The reasons for this variability are unclear. The selection regimes experienced by oceanic populations may differ slightly from year to year in relation to any number of variables as has been well documented in other cases (e.g., Grant and Grant, 2002; Reimchen and Nosil, 2004). The differences we documented may thus reflect subtle shifts in selection regimes among years. The yearly differences may also be a consequence of our sampling design. The samples we analyzed for each year were collected on a single day, thus annual variation in our study is potentially confounded with intra-annual variation. Our sampling design will tend to overestimate yearly heterogeneity in RS, which is a conservative error for our contention that annual variation in RS is relatively small. Nonetheless, the magnitude of the yearly variability was small relative to sexual dimorphism within RS and among populations for males. Annual variation in RS thus seems to reflect subtle variation around a mean that is stable compared to the evolutionary potential for divergence that andromous populations harbor.

Trait Correlations and Structuring of Variance - Phenotypic Integration of Armor and Flexibility of Body Shape Evolution:

There were strong correlations among the size-corrected linear measures. It is surprising that so many traits were correlated even after size-correction. This suggests a fairly high degree of phenotypic integration (e.g., Olson and Miller, 1958; Pigliucci and Preston, 2004). Significant integration of armor traits has been reported before for resident freshwater stickleback (Francis et al., 1986; Baumgartner, 1995). Furthermore, correlations, especially high correlations, were relatively homogeneous from year to year and between the sexes. Curiously, phenotypic correlations tended to be greater among females than males both for linear and body shape variables. The difference between sexes suggests greater integration of the female phenotype and consequently greater independence of trait variation in males. Whether this is adaptive or simply due to lower canalization of developmental pathways in males is unknown. For the linear traits, we limit our discussion to the highest correlations, which occurred among structurally or functionally related traits.

The correlation between pelvic spines is trivial and will not be discussed further. Lengths of the two dorsal spines and lateral plates were also strongly correlated (D1 to D2 and LP8 to LPD) in both sexes and among all years (Fig. 9). Since these are serially homologous traits, it is not surprising that they are correlated. What is surprising is the magnitude of the correlations. For both sets of traits, the correlations were similar in magnitude to the correlation between the two pelvic spines. If there is an important genetic component to these correlations, the correlations documented here may help account for the speed with which armor reduction evolves in postglacial resident freshwater populations (e.g., Klepaker, 1993; Bell, 2001; Kristjánsson et al., 2002; Bell et al, 2004). Surprisingly, phenotypic correlations between the pelvic spine lengths and plevic base length, which all

form part of the pelvic girdle, were much lower for both males and females than for the traits listed above.

The next highest correlations were between the dorsal and pelvic spines. The correlations we found were generally similar between sexes and among years. Although not structurally related, pelvic and dorsal spines are functionally related. When erect, the pelvic and dorsal spines function together to increase the effective size of stickleback, which is important protection against gape-limited predators (Hoogland et al., 1957; Reimchen, 1983). The correlations we found between these traits suggest phenotypic integration for functionally linked traits. Some previous studies, including QTL mapping studies, indicated correlations between pelvic structure and dorsal spines. For example, Hagen and Gilbertson (1972) found that dorsal and pelvic spine lengths were correlated ($r > 0.4$) within most populations they surveyed from the Pacific coast of North America. Other studies have not found such correlations, however. There was no association between dorsal spine number and pelvic structure in fossil *G. doryssus* when time averaging was eliminated (Bell et al., 1989). Reimchen (1980) also found no correlation between the loss of pelvic and dorsal spines in the Boulton Lake population.

Research on the genetics of armor variation in threespine stickleback is ongoing, and some of our results are consistent with genetic correlations documented among armor traits, whereas others are not. Peichel et al. (2001) found that QTLs associated with variation for D2 and pelvic spine length mapped to the same QTL whereas the lengths of the dorsal spines, D1 and D2 mapped to different chromosomes in an analysis of highly derived resident lake populations. Thus they found evidence of a genetic correlation between the lengths of the second dorsal spine and pelvic spines, but they did not find evidence of a

genetic correlation between dorsal spine lengths. Shapiro et al. (2004) found that relatively large portions of the variation of pelvic spine and pelvic girdle lengths mapped to similar QTLs. However, more work on the genetic basis of armor variation is needed before our findings become more readily interpretable in this context.

Correlation matrices for the body shape variables were significantly correlated among years, suggesting stability of correlation structure over short time scales. Common principal component analyses were consistent with these results and indicated that the eigenvector structure was conserved within sexes from year to year, but eigenvalues differed. That is, the major axes of body shape variation persisted over the time-scale examined, but the magnitude of the variance they account for differs significantly among years. The major axis of body shape variation (PC I) was consistently small across years in both males and females (Table 6). PC I tended to be larger in females, particularly when specimens were pooled across years, but this appears to reflect variation resulting from differences in abdominal expansion, possibly reflecting gravidity.

Body shape variation within anadromous populations seems to be substantial; we did not notice lower levels of intrapopulation variation in anadromous stickleback relative to that of the other freshwater populations assessed (Fig. 8). The lack of strongly dominant principal components in the body shape data sets suggests that individuals tend to vary in idiosyncratic ways. At the phenotypic level, this suggests substantial flexibility in the directions in which anadromous populations can evolve in response to selection. Strong axes of variation, which may bias evolutionary responses to selection (e.g., Schluter, 1996), appear to be lacking. Our findings do not preclude the existence of strong genetic axes of variation, however, and studies aimed at addressing this issue are presently under way (Bell et al., unpublished data).

LITERATURE CITED

Adams, D.C., F.J. Rohlf, and D.E. Slice. 2004. Geometric morphometrics: ten years of progress following the "revolution". Italian Journal of Zoology 71:5-16.

Aguirre, W.E., P. Doherty, and M.A. Bell. 2004. Genetics of lateral plate and gill raker phenotypes in a rapidly evolving population of threespine stickleback. Behaviour 141:1465-1483.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Baker, J.A. 1994. Life history variation in female threespine stickleback. Pages 144-187 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Baumgartner, J.V. 1995. Phenotypic, genetic, and environmental integration of morphology in a stream population of the threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Fisheries and Aquatic Sciences 52:1307-1317.

Baumgartner, J.V., M.A. Bell, and P. H. Weinberg. 1988. Body form differences between the Enos Lake species pair of threespine sticklebacks (*Gasterosteus aculeatus* complex). Canadian Journal of Zoology 66:467-474.

Bell, M.A. 1974. Reduction and loss of the pelvic girdle in *Gasterosteus* (Pisces): a case of parallel evolution. Natural History Museum of Los Angeles County 257:1-36.

Bell, M.A. 1987. Interacting evolutionary constraints in pelvic reduction of threespine sticklebacks, *Gasterosteus aculeatus* (Pisces: Gasterosteidae). *Biol. J. Linn. Soc.* 31:347-382.

Bell, M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica* 112/113:45-461.

Bell, M.A., and J.V. Baumgartner. 1984. An unusual population of *Gasterosteus aculeatus* from Boston, Masachusetts. Copeia 1984:258-262.

Bell, M.A. and S.A. Foster, eds. 1994. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M.A. and G. Ortí. 1994. Pelvic reduction in threespine stickleback from Cook Inlet lakes: geographic distribution and intrapopulation variation. *Copeia* 1994:314-325.

Bell, M.A., R.C. Francis, and A.C. Havens. 1985. Pelvic reduction and its directional asymmetry in threespine sticklebacks from the Cook Inlet Region, Alaska. Copeia 1985:437-444.

Bell, M.A., C.E. Wells, and J.A. Marshall. 1989. Mass-mortality layers of fossil stickleback fish: catastrophic kills of polymorphic schools. Evolution 43:607-619.

Bell, M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Bell, M. A., V. Khalef, and M. P. Travis. 2006. Directional asymmetry of pelvic vestiges in threespine stickleback. Journal of Experimental Zoology (Mol. Dev. Evol.) 306B:189-199.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B 57:289-300.

Benjamini, Y., and D. Yekutieli. 2001. The control of the false discovery rate in multiple testing under dependency. The Annals of Statistics 29:1165-1188.

Caldecutt, W.J., and D.C. Adams. 1998. Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. Copeia 1998:827-838.

Caldecutt, W.J., M.A. Bell, and J.A. Buckland-Nicks. 2001. Sexual dimorphism and geographic variation in dentition of threespine stickleback, *Gasterosteus aculeatus*. Copeia 2001:936-944.

Chevarud, J.M. 1988. A comparison of genetic and phenotypic correlations. Evolution 42:958-968.

Colosimo, P.F., C.L. Peichel, K. Nereng, B.K. Blackman, M.D. Shapiro, D. Schluter, & D.M. Kingsley. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PloS Biology 2:635-641.

Colosimo, P.F., K.E. Hoseman, S. Balabhadra, G. Villareal Jr., M. Dickson, J. Grimwood, J. Schmtz, R.M. Myers, D. Schluter, and D.M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928-1933.

Cresko, B., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. Bell, C. Kimmel, & J. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. Proceedings of the National Academy of Sciences, USA 101:6050-6055.

Flurry, B. 1988. Common principal components and related multivariate models. Wiley,

New York.

Foster, S. A. 1994. Evolution of the reproductive behaviour of threespine stickleback. Pages 381-398 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Francis, R.C., J.V. Baumgartner, A.C. Havens, and M.A. Bell. 1986. Historical and ecological sources of variation among lake populations of threespine sticklebacks, *Gasterosteus aculeatus*, near Cook Inlet, Alaska. Canadian Journal of Zoology 64:2257-2265.

Grant, P.R., and B.R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296:707-711.

Gross, H.P., and J.M. Anderson. 1984. Geographic variation in gillrakers and diet of European threespine sticklebacks, *Gasterosteus aculeatus*. Copeia 1984:87-97.

Hagen, D.W. 1973. Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculeatus*. Heredity 30:303-312.

Hagen, D. W. and L. G. Gilbertson. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific northwest, America. Evolution 26:32-51.

Heuts, M.J. 1947. The phenotypical variability of Gasterosteus aculeatus (L.) populations in Belgium: its bearing on the general geographical variability of the species. *Verhandelingen van de Koninklijke Vlaamse Academie Voor Wetenschappen Letteren en Schone Kunsten van België, Klasse der Wetenschappen* 9:1-63.

Hoogland, R.D., D. Morris, and N. Tinbergen. 1957. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as a means of defence against predators (*Perca* and *Esox*). Behaviour 10:205-236.

Karve, A.D., F.A. von Hippel, and M.A. Bell. 2007. Isolation between sympatric anadromous and resident threespine stickleback species in Mud Lake, Alaska. Environmental Biology of Fishes, in press.

Kingsley, D. M. and C. L. Peichel. 2007. The molecular genetics of evolutionary change in sticklebacks. Pages 41-81in S. Östlund-Nilsson, I. Mayer, and F. A. Huntingford (eds.). Biology of the three-spined stickleback. CRC Press, Boca Raton.

Kingsley, D., B. Zhu, K. Osoegawa, P. J. de Jong, J. Schein, M. Marra, C. Peichel, C. Amemiya, D. Schluter, S. Baldhadra, B. Friedlander, Y. M. Cha, M. Dickson, J. Grimwood, J. Schmutz, W. S. Talbot, and R. Myers. 2004. New genomic tools for molecular studies of evolutionary change in sticklebacks. Behaviour 141:1331-1344.

Kitano, J., S. Mori, and C.L. Peichel. 2007. Sexual dimorphism in the external

morphology of the threespine stickleback (*Gasterosteus aculeatus*). Copeia 2007: 336-349.

Klepaker, T. 1993. Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1231-1258.

Klepaker, T. 1996. Lateral plate polymorphism in marine and estuarine populations of the threespine stickleback (*Gasterosteus aculeatus*) along the coast of Norway. Copeia 1996:832-838.

Kristjánsson, B.K., S. Skúlason, and D.L.G. Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659-672.

Langerhans, R.B., and T.J. DeWitt. 2004. Shared and unique features of evolutionary diversification. The American Naturalist 164:335-349.

Lavin, P. A. and J. D. McPhail. 1985. The evolution of freshwater diversity in threespine stickleback (*Gasterosteus aculeatus*): site-specific differentiation of trophic morphology. Canadian Journal of Zoology 63:2632-2638.

Lavin, P. A. and J. D. McPhail. 1986. Adaptive divergence of trophic phenotype among freshwater populations of threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 43:2455-2463.

Lavin, P. A. and J. D. McPhail. 1987. Morphological divergence and the organization of trophic characters among lacustrine populations of the threspine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 44:1820-1829.

Lewontin, R.C. 1966. On the measurement of relative variability. Systematic Zoology 15:141-142.

McGuigan, K., S.F. Chenoweth, and M.W. Blows. 2005. Phenotypic divergence along lines of genetic variance. The American Naturalist 165:32-43.

McKinnon, J. S. and H. D. Rundle. 2002. Speciation in nature: the threespine stickleback model systems. Trends in Ecology and Evolution 17:480-488.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. Pages 399-437 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Moodie, G.E.E., and T.E. Reimchen. 1976. Phenotypic variation and habitat differences

in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25: 49-61.

Moyle, P.B., and J.J. Cech, Jr. 1996. Fishes, an introduction to ichthyology. Prentice Hall, Upper Saddle River, NJ.

Münzing, J. 1963. The evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus*. Evolution 17: 320-332.

Olson, E.C., and R.L. Miller. 1958. Morphological integration. University of Chicago Press, Chicago.

Peichel, C.L., C.S. Nereng, K.A. Ohgi, B.L.E. Cole, P.F. Colosimo, C.A. Buerkle, D. Schluter, and D.M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901-905.

Penczak, T. 1961. Significance of the regeneration of lateral plates of the stickleback (*Gasterosteus aculeatus* L.). Nature 191:621.

Penczak, T. 1965. Morphological variation of the stickleback (*Gasterosteus aculeatus* L.) in Poland. Zoologica Poloniae 15:3-49.

Phillips, P. 2007. CPC- Common Principal Components Program. University of Oregon, http://www.uoregon.edu/~pphil/software.html.

Pigliucci, M., and K. Preston (eds.). 2004. Phenotypic integration, studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford.

Reger, R. D. and D. S. Pinney. 1996. Late Wisconsin glaciation of the Cook Inlet region with emphasis on Kenai lowland and implications for early peopling. Pages 15-35 in N.Y. Davis and W.E. Davis (eds.). Adventures through time: readings in the anthropology of Cook Inlet, Alaska. Cook Inlet Historical Society, Anchorage.

Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 68:1232-1244.

Reimchen, T.E. 1983. Structural relationship between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). Evolution 37:931-946.

Reimchen, T.E., and J.S. Nelson. 1987. Habitat and morphological correlates to vertebral number as shown in a teleost, *Gasterosteus aculeatus*. Copeia 1987:868-874.

Reimchen, T.E., and P. Nosil. 2004. Variable predation regimes predict the evolution of

sexual dimorphism in a population of threespine stickleback. Evolution 58:1274-1281.

Reimchen, T.E., E.M. Stinson, and J.S. Nelson. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Canadian Journal of Zoology 63:2944-2951.

Reist, J.D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Canadian Journal of Zoology 64:1363-1368.

Rohlf, F.J. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology 47:147-158.

Rohlf, F.J. 2004a. TpsDig version 1.40. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2004b. TpsSplin version 1.20. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2005. TpsRegr software version 1.31. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2006. TpsRelw version 1.44. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J., and L. Marcus. 1993. A revolution in morphometrics. Trends in Ecology and Evolution 8:129-132.

Rowland, W. J. 1994. Proximate determinants of stickleback behavour: an evolutionary perspective. Pages 297-344 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Sargent, R.C., M.A. Bell, W.H. Krueger, and J.V. Baumgartner. 1984. A lateral plate cline, and phenotypic variation in the black-spotted stickleback, *Gasterosteus wheatlandi*. Canadian Journal of Zoology 62:368-376.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766-1774.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Schluter, D. E.A. Clifford, M. Nemethy, and J.S. McKinnon. 2004. Parallel evolution and inheritance of quantitative traits. The American Naturalist 163:809-822.

Shapiro, M.D., M.E. Marks, C.L. Peichel, B.K. Blackman, K. S. Nereng, B. Jónsson, D. Schluter, and D.M. Kingsley. 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. Nature 428:717-723.

Shapiro, M. D., M. A. Bell, and D. M. Kingsley. 2006. Parallel genetic origins of pelvic reduction in vertebrates. Proceedings of the National Academy of Sciences, USA 103:13753-13758.

Slice, D.E., F.L. Bookstein, L.F. Marcus, and F. J. Rohlf. 1996. Appendix I. A glossary for geometric morphometrics. Pages 531-551 in L.F. Marcus et al. Advances in morphometrics. Plenum Press, New York.

Sokal, R.R. 1978. Population differentiation: something new or more of the same? Pages 215-239 in P.F. Brussard (ed.). Ecological genetics: the interface. Springer-Verlag, New York.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry. W.H. Freeman and Company, New York.

Spoljaric, M.A., and T.E. Reimchen. 2007. 10,000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. Journal of Fish Biology 70:1484-1503.

Steppan, S.J. 1997. Phylogenetic analysis of phenotypic covariance structure. I. Contrasting results from matrix correlation and common principal component analyses. Evolution 51:571-586.

Tabachnick, B.G., and L.S. Fidell. 1996. Using multivariate statistics. Harper Collins College Publishers, New York.

Verhoeven, K.J.F. K.L. Simonsen, and L.M. McIntyre. 2005. Implementing false discovery rate control: increasing your power. Oikos 108:643-647.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Walker, J.A., and M.A. Bell. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). Journal of Zoology 252: 293-302.

Wooton, R.J. 1973. Fecundity of the three-spined stickleback, *Gasterosteus aculeatus* (L.). Journal of Fish Biology 5:683-688.

Table 1. Repeatabilities, size-adjusted means (mm), and coefficients of variation (CV) of linear traits. Acronyms are defined in the methods. Measurements were adjusted to grand mean SL (67.08 mm) of the specimens included. Diff. % is the difference between male and female size-adjusted values expressed as a percentage of the average. All traits differed significantly (Ancova, $P < 0.05$ after sequential Bonferroni correction) between males and females except D1. Females had higher size-adjusted mean values than males (higher values 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.

Variable	Repeat.	Adj.♂	Adj.♀	Diff. %	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

Table 2. Major osteological anomalies in the Rabbit Slough population during seven years. Variables are sample size, n; number of specimens missing lateral plates (LP); number of specimens with a missing or greatly reduced (i.e., missing/reduced) pelvic structure (PLV) and first (D1), second (D2) or third dorsal spine (D3); or an extra dorsal spine (D4).

Year	n	LP	PLV	D1	D2	D3	D4
1992	99	0	0/0	1/0	0/0	0	0
1997	235	0	0/4	0/2	0/0	0	1
2000	599	1	1/1	0/1	0/1	1	1
2001	124	0	0/0	1/0	0/0	0	0
2003	154	0	0/1	1/0	1/0	0	0
2004	962	0	0/5	0/3	0/0	1	2
2005	1,267	0	0/5	0/3	1/3	0	1
Total	3,440	1	1/16	3/9	2/4	2	5
Percentage		0.03	0.03/0.47	0.09/0.26	0.06/0.12	0.06	0.15

Table 3. Mean (± standard error), 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/sex/year.

Year	SL	SL^{sd}	SL^{rg}	GR	GR^{sd}	GR^{rg}	LP	LP^{sd}	LP^{rg}
1997♂	64.07±0.50	3.54	47.58-68.71	22.50±0.24	1.72	19 – 26	33.34±0.08	0.59	32 – 35
1997♀	68.86± 0.56	3.98	48.05-74.62	22.18±0.16	1.14	20 – 25	33.14±0.11	0.81	30 – 34
2000♂	64.07± 0.33	2.33	59.06-68.37	22.06±0.14	1.02	19 – 25	33.56±0.10	0.71	32 – 35
2000♀	67.88± 0.34	2.43	63.43-74.41	21.86±0.18	1.26	19 – 25	33.22±0.11	0.76	32 – 35
2003♂	65.30± 0.52	3.69	50.45-70.78	22.38±0.18	1.28	19 – 25	33.40±0.08	0.57	32 – 35
2003♀	70.07± 0.57	4.02	53.27-74.89	22.32±0.15	1.04	20 – 24	33.02± 0.09	0.65	31 – 34
Total ♂	64.48±0.27	3.28	47.58-70.78	22.31± 0.11	1.37	19 – 26	33.43±0.05	0.63	32 – 35
Total ♀	68.94±0.30	3.65	48.05-74.89	22.12±0.10	1.16	19 – 25	33.13±0.06	0.745	30 – 35

Table 4. MANOVA of the effects of sex and year on body shape variation in RS, and population and year on RS vs. resident freshwater populations. Only males from 1997 and 2003 were used in the RS vs. freshwater populations analysis and year was treated as a nested factor in that analysis. Wilks' λ is the multivariate test criterion used in MANOVA, and lower values imply greater significance. Partial η^2 is a measure of the strength of association between each factor and body shape variation, with higher values indicating greater association. All factors were significant ($P < 0.001$, permutation tests).

Within RS MANOVA

Factor	Wilks' λ	partial η^2
Sex	0.0779	0.922
Year	0.3158	0.438
Sex X Year	0.609	0.220

RS vs. Resident Freshwater Populations MANOVA

Pop	0.0019	0.878
Year	0.1547	0.375

Table 5. Pairwise correlations among size-corrected linear measures pooled among years. Male trait correlations are below the diagonal, and female trait correlations are above the diagonal.

	HL	PCTL	D1	D2	LP8	LPd	PlvL	PlvR	PlvB
HL	1	0.414	0.272	0.234	0.480	0.464	0.222	0.216	0.350
PCTL	0.215	1	0.098	0.087	0.299	0.310	0.164	0.114	0.219
D1	0.079	0.113	1	0.718	0.239	0.143	0.704	0.607	0.298
D2	0.103	0.189	0.765	1	0.267	0.228	0.669	0.584	0.301
LP8	0.466	0.227	0.250	0.218	1	0.769	0.213	0.154	0.273
LPd	0.386	0.148	0.200	0.211	0.813	1	0.199	0.147	0.341
PlvL	0.112	0.187	0.574	0.599	0.280	0.236	1	0.865	0.495
PlvR	0.119	0.187	0.528	0.561	0.260	0.239	0.829	1	0.488
PlvB	0.158	0.044	0.209	0.226	0.230	0.262	0.281	0.247	1

Table 6. Variation in body shape (expressed as a percentage) accounted for by each of the first five principal components in male and female RS fish for the three years sampled. Pooled is the percentage of variation accounted for when specimens from all years were pooled for the PCA. Total % is the cumulative variation accounted for by the first five PC's.

	Males				**Females**			
	1997	**2000**	**2003**	**Pooled**	**1997**	**2000**	**2003**	**Pooled**
PC I	24.62	23.24	23.36	22.30	24.30	28.77	31.37	38.51
PCII	20.78	21.83	15.88	17.48	19.20	19.72	16.06	15.36
PCIII	12.63	9.21	12.67	10.34	11.27	11.19	10.72	9.53
PCIV	7.88	7.81	9.92	8.21	8.61	8.70	9.65	6.82
PCV	5.34	5.96	7.39	5.93	6.32	5.43	4.96	4.06
Total %	71.25	68.05	69.21	64.26	69.70	73.79	72.77	74.27

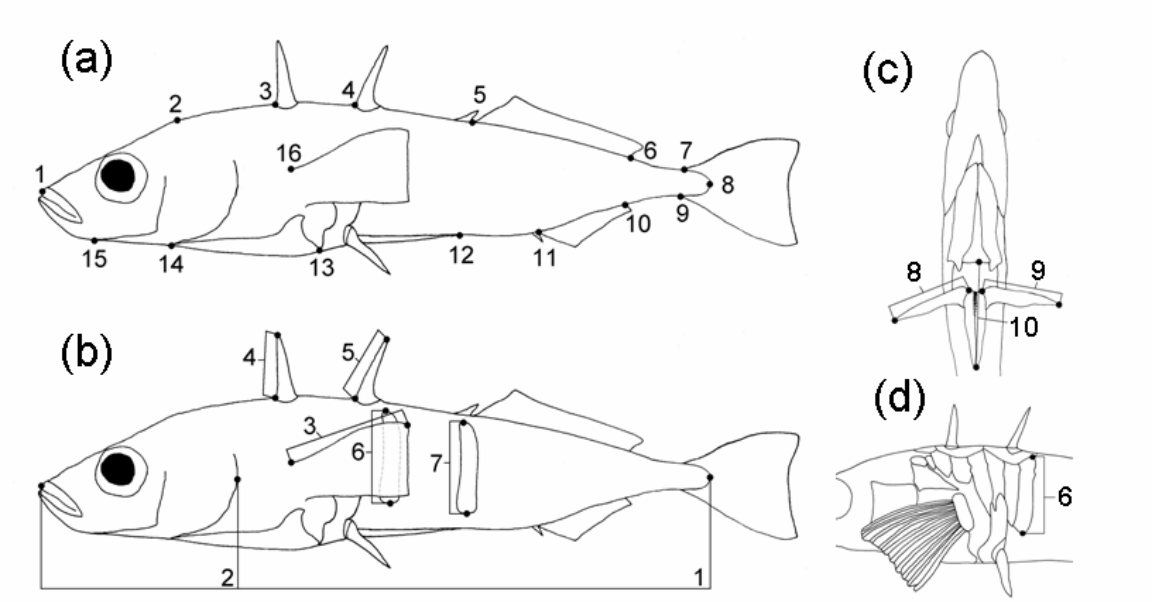


Fig. 1. (a) Landmarks and (b-d) linear measures used in this study. Landmarks are derived from Walker (1997) with the exception of landmark 16.

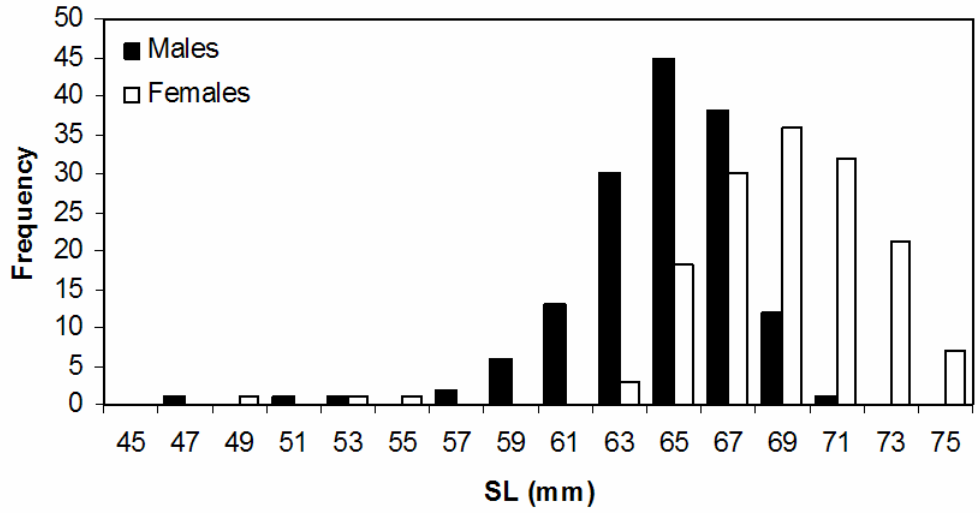


Fig. 2. Size distribution of male and female stickleback based on fifty males and fifty females from 1997, 2000, and 2003 (300 fish total).

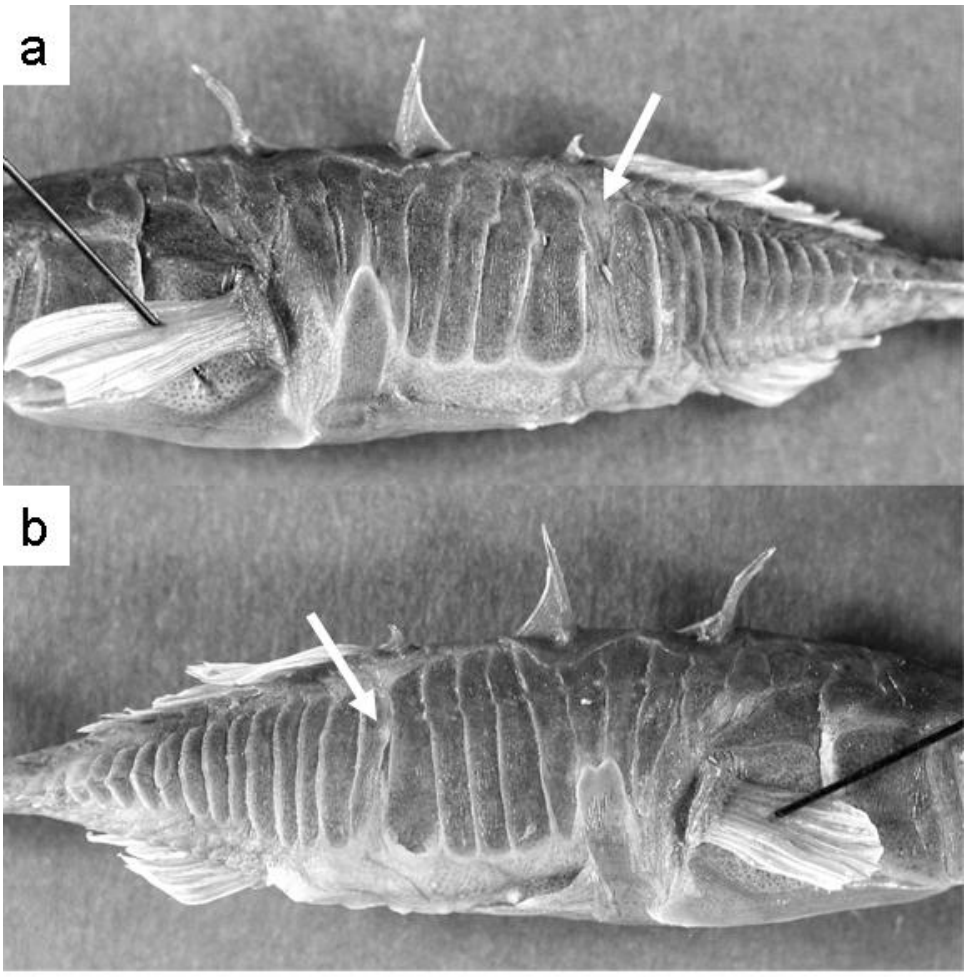


Fig. 3. Anomalous specimen collected in 2000 missing a lateral plate on both flanks. This was the only specimen of 3,440 collected with a lateral plate anomaly. (a) left side, (b) right side.

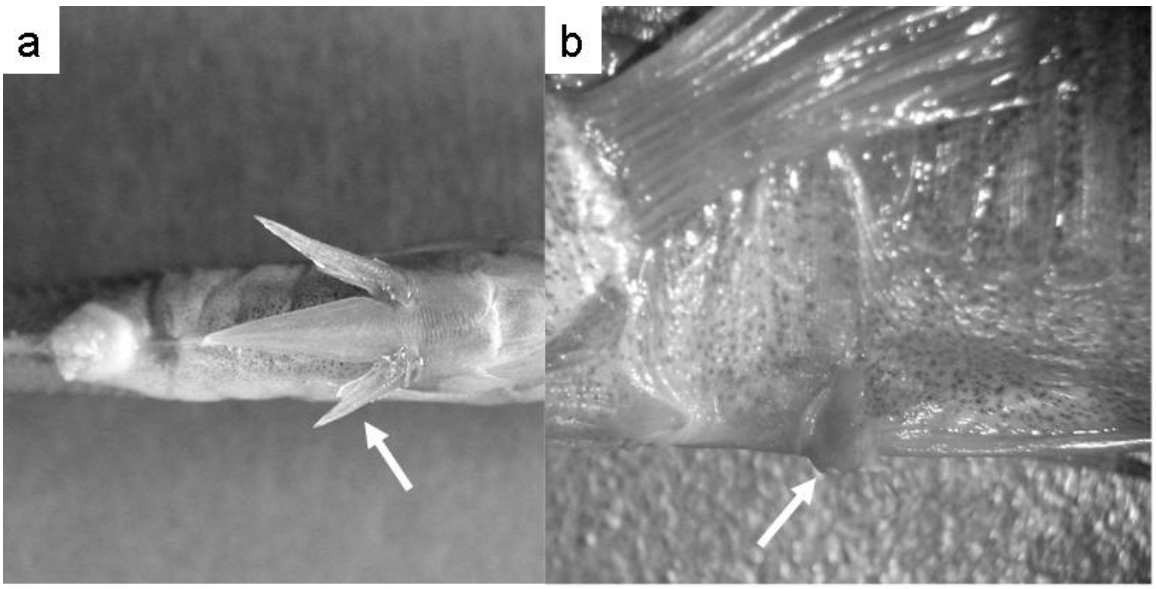


Fig. 4. Examples of pelvic structure anomalies (arrows); (a) reduced left pelvic spine, and (b) spine with base but missing the distal shaft.

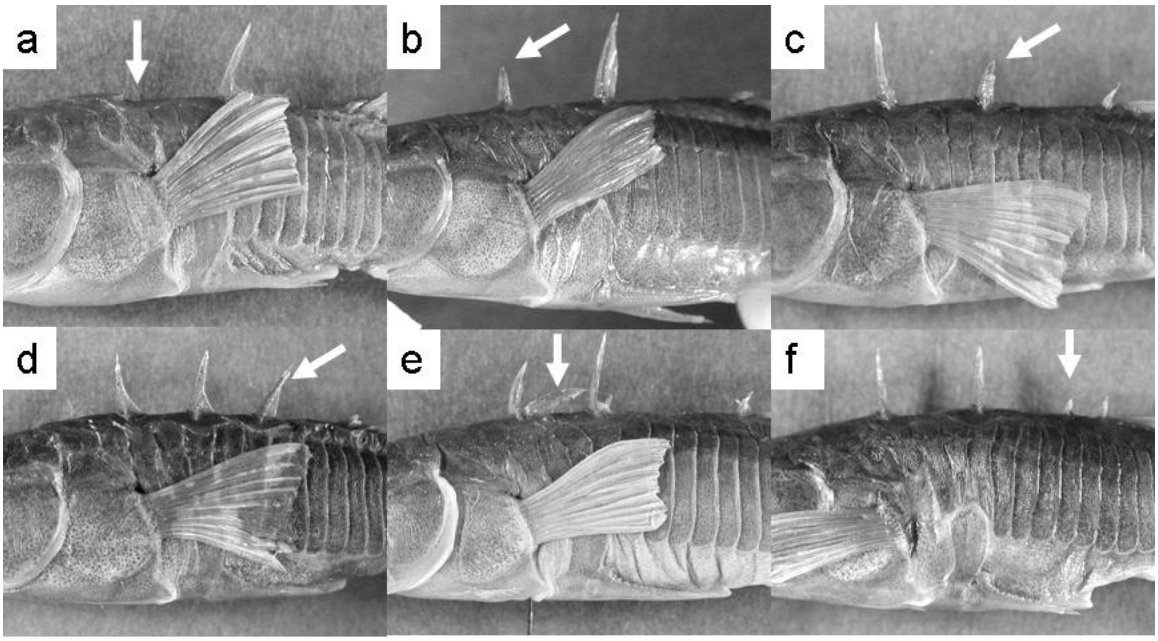
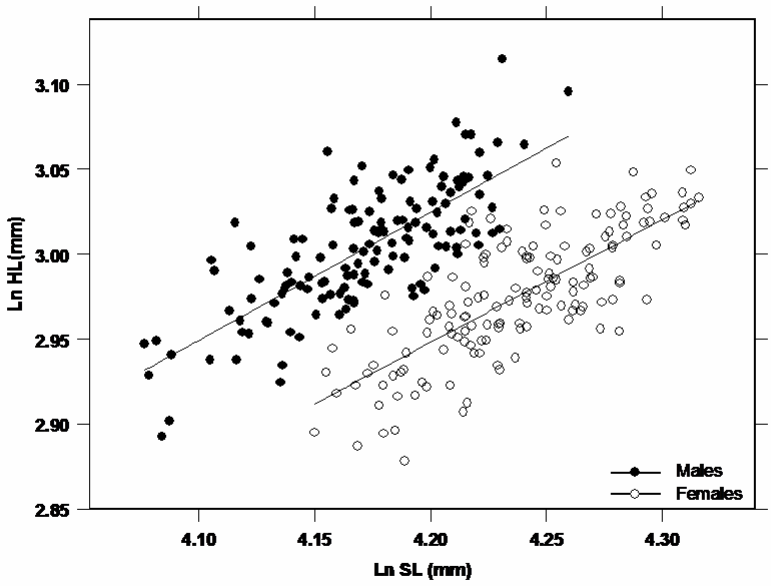


Fig. 5. Examples of dorsal spine anomalies observed: (a) first dorsal spine missing, (b) first dorsal spine greatly reduced in length, (c) second dorsal spine greatly reduced in length, (d) large fourth dorsal spine located between typical second and third dorsal spines, (e) fourth dorsal spine originating from base of typical first dorsal spine, and (f) fourth dorsal spine located anterior to typical third dorsal spine. The latter form (f) was the most common way for an extra dorsal spine to be added.

(a)



(b)

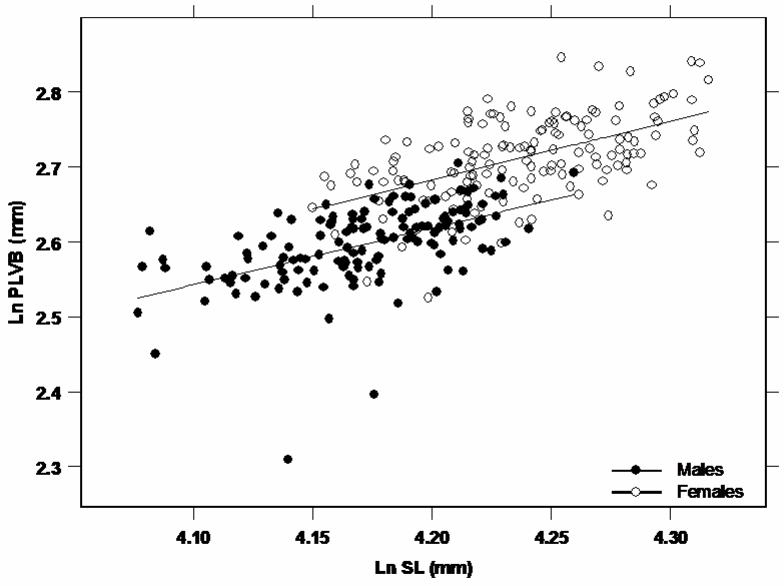


Fig. 6. The two linear traits (a. head length and b. pelvic girdle length) exhibiting the greatest sexual dimorphism.

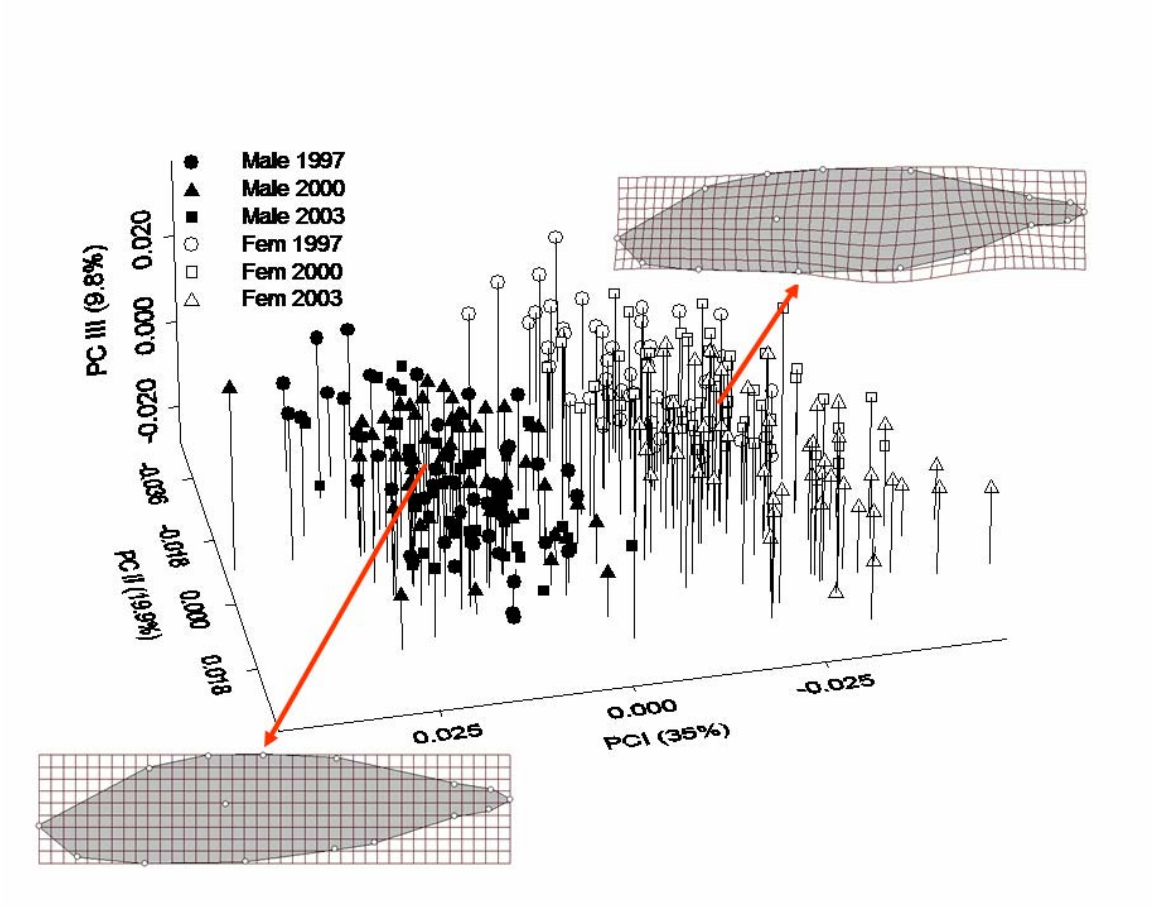


Fig. 7. Principal component analysis plot of body shape variation between male and female (Fem) stickleback for all years. Note that males and females segregate completely along principal component (PC) I. The female grid (top right) is depicted as a deformation of the male grid (exaggerated by a factor of two to facilitate visualization). PC I, II, and III accounted for 35, 19.9, and 9.8% of the variation respectively.

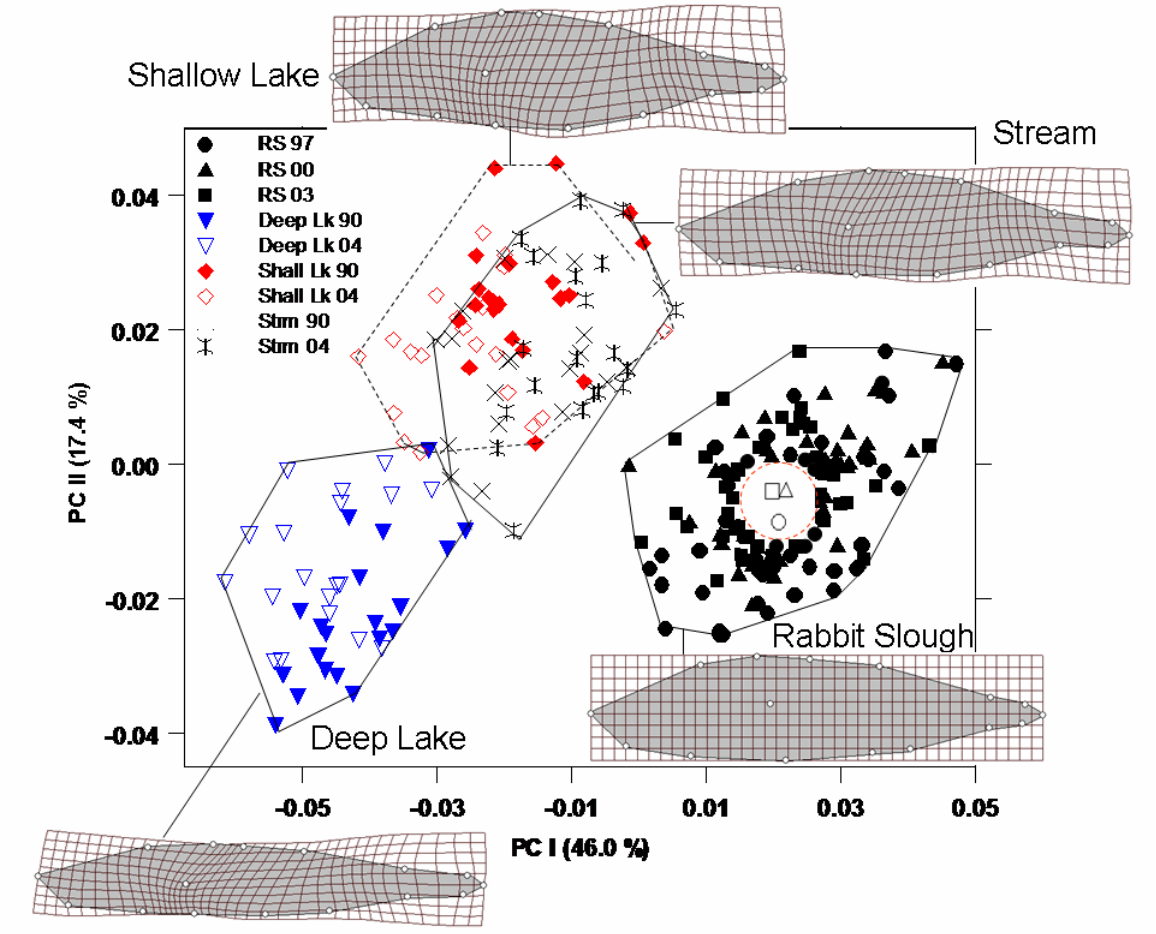


Fig. 8. First two principal components of PCA depicting shape variation of male Rabbit Slough (RS) stickleback sampled in 1997, 2000, and 2003, compared to male stickleback from a deep lake, shallow lake, and stream sampled in 1990 and 2004. Clear symbols (circle, triangle and square) within the RS cluster 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 to facilitate visualization).

(a) (b)

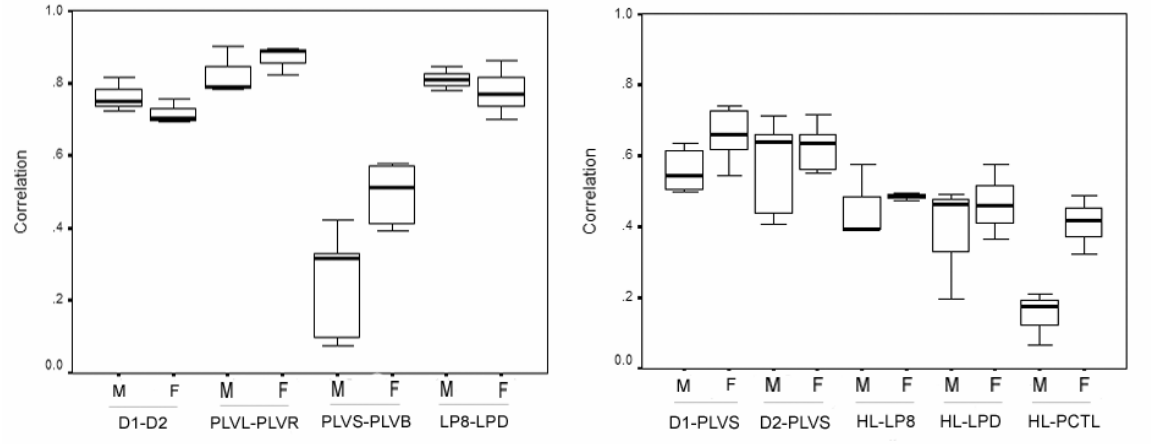


Fig. 9. Box plots of the highest pairwise correlation coefficients for the linear traits by year. Males (M) and female (F) correlations are depicted separately. D1 is the length of the first dorsal spine, D2 is the length of the second dorsal spine, PLVL is the length of the left pelvic spine, PLVR is the length of the right pelvic spine, PLVS is pelvic spines and includes both PLVL and PLVR, PLVB is the length of the base of the pelvis, LP8 is the length of the eighth lateral plate, LPD is the length of the lateral plate directly below the third dorsal spine, and HL is head length. (a) Correlations among structurally related traits. (b) Correlations among structurally unrelated traits.

Chapter 3

The Evolutionary Trajectory of a Recently Established Threespine Stickleback Lake Population

ABSTRACT

We describe body shape evolution in a recently established threespine stickleback population in Loberg Lake, Alaska using geometric morphometric methods. Loberg Lake was poisoned in 1982, and a new population was founded sometime between 1983 and 1989 by anadromous stickleback. We analyzed the evolutionary trajectory of the Loberg Lake population in the context of variation among neighboring stickleback populations. The Loberg Lake population was very similar to its anadromous ancestor in 1990, but diverged markedly in the direction of other lake populations by 1992. Between 1993 and 2006 the population evolved much more slowly in the direction of the original Loberg Lake population, and by 2006 it had diverged from its anadromous ancestor approximately 71% of the distance separating its ancestor and the original population. Variation associated with temporal evolution in Loberg Lake resembles the major sources of variation among neighboring lake populations, with the greatest change being associated with body depth and the size of armor structures. Temporal evolution is the main source of variation in the study, but spatial heterogeneity, lateral plate morph phenotype, and allometry all contribute small but significant variation. Variation in body shape does not exhibit a temporal trend, remaining high during adaptation to lake conditions, and there is little evidence that patterns of ancestral phenotypic variation

meaningfully constrain the evolutionary trajectory of this population. Lack of strong axes of variation and broad scatter of individuals within populations suggests substantial flexibility in the response of body shape to natural selection. Temporal variation in the Loberg Lake population provides a rare glimpse into the evolutionary response of a complex trait to natural selection after a major habitat shift.

INTRODUCTION

Until recently, there have been few studies of contemporary microevolution (evolution within species occurring within hundreds of years). It is now apparent that populations can evolve substantially on contemporary time scales (e.g., Endler, 1980, 1986; Reznick et al., 1990, Gibbs and Grant, 1987; Losos et al., 1997; Hendry and Kinnison, 1999, 2001a) and that the magnitude of evolutionary divergence among populations can be comparable to that observed among species (Kettlewell, 1973; Liem and Kaufman, 1984; West-Eberhard, 2003). Studies of contemporary evolution can thus serve as a valuable tool to increase our understanding of evolutionary diversification in nature because the ecological factors that influenced it can be measured with more confidence (Hendry and Kinnison, 2001b). Most documented cases of contemporary evolution lack major components of the process, however. Very few cases have documented divergence soon after establishment of isolates, incorporated a complete time series detailing the pattern of change as the population evolved, or traced the population's fate until it diverged substantially from its ancestor. Moreover, no previous case involved the evolution of a population that is known to represent the ancestor of a large, naturally occurring adaptive radiation.

Postglacial radiations of threespine stickleback represent one of the most promising systems for increasing our understanding of how organisms diversify in nature and the factors that facilitate and constrain diversification (e.g., Bell and Foster, 1994a; Schluter, 2000; McKinnon, and Rundle, 2002; Östlund-Nilsson et al., 2006). Threespine stickleback are primitively oceanic, but many populations are anadromous, entering freshwater only to reproduce. As glaciers retreated over much of the northern hemisphere beginning about 20,000 years ago, anadromous (sea-run) stickleback colonized newly formed lakes and streams and established resident populations that adapted to their novel surroundings. Reduction in armor is among the most dramatic adaptations to freshwater environments (Bell and Foster, 1994b). Anadromous stickleback tend to be heavily armored and to have numerous (>30) of bony lateral plates (LP, superficial bony armor structures) covering both body flanks, they are "complete" morphs. Resident lake and stream stickleback generally have a few LP (< 10) restricted to the anterior part of the body, they are "low" morphs (reviewed in Bell and Foster, 1994b). Many other aspects of the phenotype also diverge after colonization of fresh water, including body form and trophic morphology (e.g., reviewed by Bell and Foster, 1994b; Walker and Bell, 2000). Resident freshwater populations differ from one another as well, with relative depth of the environments inhabited, and the presence of different types of prey and predators having major influences on phenotypic variability (e.g., Hagen and Gilbertson, 1972; Moodie and Reimchen 1976; Gross and Anderson, 1984; Baumgartner, 1992; Bell et al., 1993; McPhail, 1994; Reimchen, 1994; Walker, 1997). The magnitude of the divergence among stickleback populations is comparable to differences among species in other taxa, which is remarkable given the recent origin of the postglacial radiation (within 20,000

years). Moreover, recent studies suggest that major morphological changes can evolve in threespine stickleback populations in a matter of decades (e.g., Klepaker, 1993; Bell 2001; Kristjánsson et al., 2002; Bell et al, 2004; von Hippel and Weigner, 2004).

One such rapidly evolving lake population occurs in Loberg Lake, Alaska (Bell, 2001; Aguirre et al., 2004; Bell et al., 2004). The native Loberg Lake stickleback population was exterminated in 1982 to improve the lake for recreational fishing. It was typical of resident lake populations in the area, exhibiting significant armor reduction relative to its anadromous ancestor. Anadromous threespine stickleback, similar to those that founded the freshwater stickleback radiation in Cook Inlet, colonized the lake sometime between 1983 and 1989. Annual sampling from 1990 has revealed rapid armor evolution in the direction of local resident lake populations (Bell et al. 2004). The evolution of reduced LP number is particularly striking. Between 1990 and 2001, the frequency of complete morphs declined from 95.9 to 11.2% (Bell et al., 2004) and has continued to drop thereafter (unpublished data). Bell et al. (2004) presented evidence that other aspects of the phenotype are evolving as well, and Aguirre et al (2004) found significant heritable variation for adaptively important traits. Loberg Lake may thus provide general insights into the tempo and mode of evolutionary change after a population moves from one adaptive zone to another (see Simpson, 1953) and a rare glimpse into how postglacial stickleback adaptive radiations originate in nature.

In this study, we build on our previous work (Aguirre et al., 2004; Bell et al., 2004) to describe the evolutionary trajectory of the newly established Loberg Lake population in a multivariate space formed by variation of neighboring resident freshwater populations in Cook Inlet, Alaska. We focus on body shape because it is a composite

measure capturing morphological variation throughout the body and is representative of the overall morphological phenotype. Additionally, variation in body shape is strongly associated with multiple ecological variables that have important fitness consequences (e.g., Moodie and Reimchen, 1976; Taylor and McPhail, 1986; Walker, 1997; Spoljaric and Reimchen, 2007). We also evaluate changes in variance as the population adapts to novel lake conditions, and the influence of ancestral phenotypic variation on the evolutionary trajectory of the population.

MATERIALS AND METHODS

Sampling Procedure and Processing of Specimens:

Loberg Lake is a small lake (~4.45 ha surface area) in the Matanuska-Susitna Borough, Cook Inlet, Alaska (61˚ 33’ 35”N, 149˚ 15’ 30”W). The location, a map of the lake with collection sites, physical and biological aspects of the lake, and the sampling and preservation methods employed, can be found in Bell et al. (2004) and at the Alaska Department of Fish and Game Loberg Lake website (http://www.sf.adfg.state.ak.us/statewide/LakeData/index.cfm/FA/main.lakeDetail/MgtAreaID/2/LakeID/351). Briefly, *G. aculeatus* were sampled from five sites around the perimeter of Loberg Lake, except between 1990 and 1993, when only one site (site A) was sampled. Most Loberg Lake samples were collected with minnow traps in late spring or early summer. The 1990 sample was collected with a seine in July, and the 1991 sample was trapped through the ice in November. Preserved stickleback were stained with an alkaline aqueous solution (< 1% KOH wt/wt) of Alazarin Red S to visualize LP, and lateral plate morph (LPM) was scored following Bell et al. (2004; see also Hagen and Gilbertson 1972).

To put body shape evolution of the Loberg lake population in the context of variability among neighboring stickleback populations, samples from 20 other lakes, 5 streams, and 2 anadromous populations were collected (Appendix 2). An effort was made to include lake populations at the extremes of body shape variability in the analysis carried out by Walker (1997). Of the 20 lakes included, 13 were sampled two years (typically 1990 and 2004, see Appendix 2 for details). The analysis included a total of 1805 specimens. An anadromous population collected in Rabbit Slough (RS), which is in the same drainage as Loberg Lake, was sampled three years, and is emphasized in most comparative analyses in this study because it is the most likely ancestor for the Loberg Lake population.

Collection of Body Shape Data:

Geometric morphometric methods (Rohlf and Marcus, 1993; Adams et al., 2004; 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 those used by Walker (1997) with the addition of a sixteenth landmark located at the origin of the pectoral fin (Fig. 1). The landmark data were aligned using the Procrustes superimposition method as implemented in the program tpsRelw version 1.44 (Rohlf, 2006) to eliminate variation related to rotation, translation, and size. All specimens in the study were included in a single alignment from which the shape variables (partial warps and uniform component) were generated.

Only adult male specimens were included in this study to minimize variation related to allometry and sexual dimorphism, and the largest males were generally selected. Specimens infected with *Schistocephalus solidus* worms were not included either because they may distort body shape. At least fifty male fish were included for each year for Loberg Lake, except in 1990, 1991, 1992, and 1993 when only 6, 10, 44, and 25 specimens were available respectively. Most years, specimens included in the analysis were primarily from site A; specimens from other sites were included as necessary to increase sample sizes. More specimens were included in the samples from 1994 (n=177) and 2006 (n=150) to test for body shape differences between low and complete LP morphs and to test for spatial heterogeneity in body shape around the lake, as well. A sample of 10 male fish from the extinct Loberg Lake population collected when the lake was poisoned was also included. These fish were frozen prior to fixation, which decreased their quality for morphometric analysis. Each specimen was photographed and landmarks digitized three times to reduce measurement errors. Triplicate landmark coordinates were aligned for each specimen and the consensus configuration was used for each individual. The same procedure was carried out with the Loberg Lake 1990 and 1991 samples because sample sizes were small. Samples from the other populations included for comparative purposes generally consisted of 20 males (see Appendix 2 for exact numbers).

Multivariate Analyses:

We tested for heterogeneity in size among years in the Loberg Lake population with a regression analysis of annual mean centroid size (ln transformed) against year of

collection, and included centroid size as a covariate in all multivariate tests carried out. Centroid size is calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration (Zelditch et al., 2004). The 1991 Loberg Lake sample was excluded from most analysis because it was smaller and collected at a different time of the year.

To examine whether variation in body shape is significantly associated with spatial heterogeneity in lake conditions or with LP phenotypes, we carried out a MANOVA with samples from 1994 (the first year that the lake was sampled at five sites) and 2006 (the last year). We tested for the significant influences of Year (1994 vs. 2006), LP morph (complete vs. low), collection site, the interactions between these factors, and included centroid size as a covariate. All factors were tested over the residual SS error matrix with tpsRegr 1.31 (Rohlf, 2005). The magnitude of the effects of these variables was evaluated using procrustes distances and Wilks' partial η^2. The procrustes distance is a distance measure used in geometric morphometric studies and 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 with tpsSplin 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 and Fidell, 1996), in this case the body shape variables, and higher Wilks' partial η^2 values indicate a stronger association. Wilks' partial η^2 was calculated as:

$$\text{Partial } \eta^2 = 1 - \lambda^{1/s}$$

where λ is:

$$\text{Wilks' } \lambda\text{: } = |SS_{error}|/|SS_{effect} + SS_{error}|$$

the ratio of the determinant of the error cross-products matrix to the determinant of the sum of the error and effect cross-product matrices, and

$$s = \sqrt{[(p^2(df_{effect})^2 - 4)/(p^2 + (df_{effect})^2 - 5)]}$$

where p is equal to the number of dependent variables (28 for the body shape data) and df_{effect} is the degrees of freedom of the effect variable. Wilks' λ was calculated, and tests of significance were carried out, with tpsRegr 1.31 (Rohlf, 2005).

Principal Components Analysis (PCA) was used to visualize the evolutionary trajectory of the Loberg Lake population in a low-dimensional morphospace representing the 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 full data set, and mean sample PC scores calculated afterwards.

Temporal patterns in variance of the Loberg Lake population were evaluated to examine how levels of intrapopulation variance changed as the population adapted to the novel lake environment. Variance for each annual sample was quantified by summing the variances of PC scores along the first two PC axes, the first five PC axes, and all PC axes. This allowed direct comparison of shifts in variance in the two dimensional shape space and the full shape space. The total variance accounted for by the body shape variables and PC scores across all PCs is the same.

Finally, the potential influence of phenotypic variation on the evolutionary trajectory of the Loberg Lake population was examined in both the two dimensional PC space and the complete shape space. Principal axes (Sokal and Rohlf, 1995) were used to visualize the relationship between the major axes of variation in relation to the evolutionary trajectory of the Loberg Lake population in the space formed by PCs I and

II. Principal axes represent the major trend lines through a bivariate scatter of points, and were calculated with BIOMstat 3.30o (Rohlf, 2002).

We followed the approach outlined by Schluter (1996) to examine the relationship between ancestral variation and the direction of phenotypic evolution in the complete shape space, with the important distinction that our analysis is based on patterns of ancestral phenotypic variation, not genetic variation of a derived population. Efforts to examine this question using genetic covariances are presently under way (Bell et al., In Progress). Briefly, the covariance matrix of the ancestral RS population was calculated from the shape variables (partial warps and uniform component). The first eigenvector of this covariance matrix is $\mathbf{p_{max}}$, the direction of maximum ancestral phenotypic variation. Vectors, $\mathbf{z_i}$, representing the direction of the line separating the ancestral RS and Loberg Lake population annual means were calculated as

$$z_i = [X_a - X_i] [(X_a - X_i)' (X_a - X_i)]^{-1/2}$$

where X_a is the vector of mean partial warp scores for the RS population, and X_i is the vector of mean partial warp scores for each of Loberg Lake population annual means (1990-2006) assessed individually. The angle, θ, between $\mathbf{p_{max}}$ and $\mathbf{z_i}$ was calculated as

$$\theta = \cos^{-1} [(\mathbf{p_{max}})'\mathbf{z_i}]$$

If ancestral phenotypic covariance structure influences the evolutionary trajectory of the Loberg Lake population, we expect θ to be small early in the time series and increase over time. Because $\mathbf{p_{max}}$ only accounted for 22.5% of the variation in the RS sample, we also carried out the analysis with $\mathbf{p_2}$, the second eigenvector of the RS covariance matrix, which accounted for 17.4% of the variation.

RESULTS

Size Evolution in the Loberg Lake Population:

Body size in the modern Loberg Lake population declined significantly over time (Fig. 2; $r^2 = 0.580$, $F = 19.316$, $P = 0.001$). There was a large decline between the anadromous RS sample and the first sample from the modern Loberg Lake sample collected in 1990 (mean centroid size declined by 23.32%), and another large decline between the 1990 Loberg Lake sample and the rest of the time series (decline of 21.54% between 1990 and 1992). Specimens in the 1991 sample are unusually small because it was collected at a different time of year. The decline in body size after 1992 was much more gradual, although the trend was still significant when the 1990 sample was excluded ($r^2 = 0.508$, $F = 13.442$, $P = 0.003$). Interestingly, body size in the sample of the extinct 1982 Loberg Lake population was larger than all of the annual samples of the modern Loberg Lake population except the 1990 sample (Multiple comparisons test, GT2 method (see Sokal and Rohlf, 1995), $P < 0.05$ between 1982 and each annual sample of the extant population).

Spatial and Armor Related Variation in Body Shape in the Loberg Lake Population:

Centroid size, year of collection (1994 vs. 2006), LPM, site of collection, and the interaction between LPM and site of collection 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 η^2 values, Procrustes distances among consensus configurations, and distribution of specimens along the first two principal components. Centroid size (implicating allometry), LPM,

and site of collection had much smaller, but significant, influences on body shape variation. The LPM X Site interaction was only marginally significant and seemed to have a relatively small effect on body shape variation.

We focus on body shape variation related to year of collection and LPM here, and allometry is addressed below. Deviations of landmarks between the consensus configurations within morphs between 1994 and 2006 samples were similar in direction and magnitude. The greatest displacement appeared to occur at landmark 12, located at the end of the posterior process of the pelvis (Fig. 3a). This landmark was displaced substantially forward in the 2006 specimens, suggesting that the pelvis became smaller between 1994 and 2006. Body depth also appeared to decline and the caudal peduncle was more elongate in 2006 fish than in 1994 fish. The differences in landmark consensus configurations for completes and lows from the same year were small and difficult to visualize even after exaggerating the vector lengths by a factor of five (Fig. 3b). Variation in body shape associated with LPM was greater in 1994 than in 2006 based on Procrustes distances between consensus configurations, which were 0.01053 and 0.00629, respectively.

A PCA carried out on the 1994 and 2006 Loberg Lake specimens yielded qualitatively similar results. The first two PCs accounted for 32.1 and 12.8 % of the variation, respectively. Specimens segregated largely along PC I, the major axis of body shape variation, by year of collection, but completes and lows overlapped within years on PCs I and II (Fig. 4). Variation of the predicted body shapes at the extremes of PC I was similar to the variation in body shape associated with divergence between 1994 and 2006 consensus configurations. Clearly, year of collection is associated with a large amount of

the variation in body shape in Loberg Lake, and we focus on this factor further below. We begin by describing variation in body shape of neighboring stickleback populations.

Phenotypic Variation Among Cook Inlet Threespine Stickleback:

PCs I to V accounted for 35.6, 15.2, 9.8, 8.9, and 7.3% of the variation, respectively. Although the first two PCs accounted for only 50.8% of the variation, the first two PCs in the PCA carried out on sample consensus configurations (resulting in a virtually identical distribution of sample means along the first two PCs) accounted for ~70% of the variation, indicating that the first two PCs account for a large portion of the variation among samples.

The anadromous samples grouped together in a relatively small area of PC space and were segregated from the freshwater populations, particularly along PC I (Fig. 5). The stream samples also grouped in a relatively small area of the PC space, but they overlapped with some shallow lake samples and the Loberg Lake annual samples. Most of the Loberg Lake annual samples also tended to group together in a relatively small area of the PC space. Other lake samples were widely dispersed, and they represent a large portion of the variation in body shape.

Biologically interpretable variation among samples tended to be oriented diagonally to PCs I and II. Variation distributed from the top right (where the anadromous samples occur) to the bottom left of the space (where Nowack [21] and Zero Lake [15] samples occurred) tended to be largely associated with a shift in position of landmark 12, which is at the posterior end of the pelvis. This structure was much longer in anadromous samples, which is probably related to the greater armor of these

populations. Zero and Nowack both exhibit significant pelvic reduction, resulting in a shorter pelvic posterior process. There were other smaller differences as well, including the length of the ectocoracoid, the length of the median fins and caudal peduncle, and the position of the pectoral fin. Variation distributed along the other diagonal from the 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 segregated towards the bottom right distribution of freshwater samples, and 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 and left of the freshwater populations.

Thirteen of the twenty lake populations (excluding Loberg) were sampled in two different years. In most cases, differences between sample means for fish collected in different years were relatively small, means from the same population were generally close and always in the same region of the shape space (Fig. 5). The same is true for the anadromous population, RS, sampled three different years. A MANOVA including population and year of collection as factors for the thirteen lake populations indicated that both factors were significant (Table 2), but estimates of Wilks' partial η^2 and the distribution of sample means in the two-dimensional PC space indicate that population had a stronger effect (Fig. 5).

Evolutionary Trajectory of the Loberg Lake Population:

The mean for the 1982 sample from the extinct Loberg Lake population was relatively isolated from other populations in the shape space formed by PC I and PC II

(Fig 5). Divergence in body shape relative to the anadromous form was generally comparable to that of other resident lake stickleback (Fig. 6). The posterior process of the pelvis was smaller, as was the ectocoracoid, the caudal peduncle was more elongate, and the median fins were shorter, and the dorsal spines (especially the first) were displaced backwards, among other more subtle changes.

Body shape differences between the 1990 and 1992 samples of the extant Loberg population were substantial. The first sample from the extant population collected in 1990 was very similar in body shape to that of anadromous samples (Fig. 7a). The Loberg Lake 1991 sample mean is isolated from the rest of the samples. It was collected at a different time of year than the rest (in the winter), and its position in shape space may reflect the smaller size of the fish and differences in conditioning due to harsh winter conditions that individuals were experiencing when collected. The reasons for the difference between the 1990 and 1992 samples are unclear. Several lines of evidence indicate that fish included in the 1990 Loberg Lake sample developed in freshwater like the later samples, precluding phenotypic plasticity. They were smaller than typical anadromous fish including the RS population (Fig. 2), being smaller than all but one of the 146 RS fish included in this study (data not shown). Most stickleback in the 1990 Loberg Lake sample were also heavily parasitized with *Schiztocephalus* worms, which are rare in anadromous stickleback but common in freshwater populations. Finally, a few fish in the 1990 sample, including one of the males used in the body shape analysis, were intermediate partial morphs (see Bell et al., 2004 for a definition). Anadromous stickleback in this area are virtually monomorphic for the complete lateral plate morph (Aguirre, In prep.), and thus the presence of several non-complete phenotypes in such a

small sample also indicates that the 1990 sample represents a lake resident population. Thus, the large shape change between 1990 and 1992 seems to have evolved after at least one generation had developed in Loberg Lake.

The change in mean body shape between 1990 and 1992 involves a decrease in size of the posterior process of the pelvis and ectocoracoid, a slight decrease in body depth, as indicated by ventral displacement of the landmarks at the supraoccipital and first and second dorsal spines, and a dorsal displacement of landmarks located at the angular and anterior tip of the ectocoracoid, and a slight increase in length of the caudal peduncle (Fig. 7b).

The 1992-2006 annual means for the Loberg Lake population clustered in a relatively small part of the two-dimensional shape space, relatively far from both the anadromous populations and the extinct Loberg Lake population, among the stream and shallow lake samples (Fig 5). Loberg Lake is not a shallow lake, however. Its relative littoral area (RLA- See Walker, 1997), an index of the amount shallow habitat, is 27.1, indicating that most of the lake bottom cannot support macrophyte growth. Upon close inspection, the 1992-2006 Loberg sample means displayed a striking temporal pattern, with annual means generally progressing towards the center of the shape space spanned by PCs I and II, and the mean for the extinct 1982 sample (Fig. 8). The change in position between any pair of adjoining years is erratic; samples can move in virtually any direction from year to year. The trend is clear across multiple years, however. The direction of displacement of landmarks of the consensus configuration for the Loberg Lake 2006 sample from the RS sample (Fig 6c) is strikingly similar to that between the

extinct 1982 sample and RS (Fig 5a), suggesting substantial parallelism in the evolution of body shape between the exterminated and extant populations.

We explored the temporal pattern further by regressing body shape variation of Loberg Lake stickleback on year of collection (using tpsRegr version 1.31), with centroid size included as a covariate (to control for allometry). The results were generally similar whether we regressed all specimens from 1990 to 2006, only specimens collected between 1992 to 2006, or only low morphs from site A collected between 1992 and 2006 to eliminate variation related to LPM 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 η^2 ranging between 0.5602 – 0.6066 for year, and between 0.3393 – 0.3450 for centroid size. Variation in body shape within samples was high compared to differences among samples, which was also the case for neighboring stickleback populations (see below). Consequently, the percentage of the variation in body shape accounted for by year of collection and centroid size combined was small, ranging between 5.98 and 10.05%.

The predicted pattern of body shape evolution over time (Fig. 9a) was generally similar to the comparison between consensus configurations for the 1994 and 2006 complete morph fish discussed previously (Fig. 3a). Over time, the extant Loberg Lake population is evolving a more elongate body and head, and a smaller ectocoracoid and posterior process of the pelvis. Allometric body shape change also resulted in a more elongate body form especially in the trunk region (Fig. 9b). The landmark at the supraoccipital notch was displaced dorsally, suggesting an increase in head depth, and the landmark at the posterior edge of the angular was displaced posteroventrally, suggesting

an increase in the relative length of the mouth with size. The largest body shape change related to allometry, however, was the reduction in the length of the caudal peduncle and increase in length of the median fins with size.

Levels of Variation in the Evolving Loberg Lake Population:

The correlation between variance among all samples accounted for by PCs I and II versus PCs I toV was 0.714, between PC's I and II versus all PCS was 0.671, and between PCs I to V and versus all PCS was 0.987, so we discuss only patterns related to PCs I and II and all PCs. There was no significant trend in variance over time in the Loberg Lake population either with PCs I to II or all PCs (correlation analysis; $r = 0.259$, $P = 0.317$, and $r = -0.448$, $P = 0.071$, respectively). With the exception of the Loberg 1990 sample, levels of variation were also not divergent from typical levels of variance in the other resident lake populations sampled (Table 3). The variance for the Loberg Lake 1990 sample was above the range of typical lake populations in the region when all PCs were taken into account, hinting at a possible increase in variance in the first sample, but the 1990 sample is very small, which hinders accurate estimation of its variance. If this increase was biologically meaningful, it disappeared very early in the time series. The Loberg Lake population clearly did not go through a significant phenotypic bottleneck; levels of variation were always within the typical range for resident lake populations in Cook Inlet, Alaska, or higher (Table 3). Cook Inlet stickleback populations tend to possess abundant phenotypic variation for body shape evolution, and the Loberg Lake population did not lose much, if any, of this variation during colonization.

The Influence of Ancestral Variation on the Evolutionary Trajectory of the Loberg Lake Population:

The principal axis of body shape variation in the anadromous RS population does not point in the direction of most of the Loberg Lake annual samples or of other resident freshwater populations in the two-dimensional PC space (Fig. 10). The mean for the Loberg Lake population 1990 sample falls very close to the RS principal axis, however, and is displaced in the opposite direction from other freshwater populations, which would be expected if it had been influenced by the major axis of phenotypic variation of its anadromous ancestor. Unfortunately, the 95% confidence ellipse of the 1990 bivariate mean is enormous (because of the small sample size), compromising the reliability of this result. If this result is biologically meaningful, however, the influence of ancestral covariance is extremely short lived. The main axis of divergence among Loberg Lake annual samples (1992-2006) is in a completely different direction, indicating that within as few as two years, the population began to evolve independently of the principal axis of phenotypic variation in the ancestral form.

In the complete shape space, there was no relationship between either $\mathbf{p}_{max}$ or $\mathbf{p}_2$ and $\mathbf{z}_i$. The angles (θ) between them were large, ranging from 76.2 – 89.2˚ (avg. = 84.0˚) and 47.0 – 82.8˚ (avg. = 72.1˚), between $\mathbf{p}_{max}$ and $\mathbf{p}_2$ and the $\mathbf{z}_i$ series, respectively (Fig. 11). That is, in the complete shape space, the two major axes of variation in the anadromous RS sample point in very different directions to the vector of divergence between most RS and the Loberg Lake population annual mean vectors. There was also little evidence of an increase in θ over time; θ between $\mathbf{p}_{max}$ (PCI) and $\mathbf{z}_i$ was large from the beginning of the time series and did not increase over time as expected. The angle

between $\mathbf{p_2}$ and $\mathbf{z_i}$ for 1990 was smaller than for the rest of the time series, but at 47.0 it was still quite large and provides little evidence of a meaningful constraint on the extant population.

The lack of a strong major axis of variation in the ancestral RS population and broad distribution of individuals in PC space may play a part in the pattern observed. PC I, II, and III only accounted for 22.5, 17.4, and 10.0 % of the variation respectively. The scatter of individuals along PCs I and II is extremely broad, both for the RS (Fig. 12a) and the derived Loberg Lake annual samples as illustrated by the 1992 and 2006 samples (Fig. 12b). Two individuals from the RS population and one from the Loberg Lake 1990 sample, appeared as outliers in the PC space (indicated by an arrow and circled by a small ellipse), falling very close to the means of early years of the Loberg Lake population. This indicates that rare individuals, similar in body shape to derived Loberg Lake stickleback, already existed as standing variation in the anadromous RS population.

DISCUSSION

Predictable Decline and Subsequent Overshoot of Body Size in the Loberg Lake Population:

The body-size (as captured by centroid size) decline in the Loberg lake population with time is consistent with size variation among stickleback populations in the region. Anadromous stickleback are larger (von Hippel and Weigner, 2004; Karve et al., 2007), and this size difference occurs throughout the Holarctic distribution of the species complex (Baker, 1994). The temporal pattern was similar to that of body shape divergence in that the largest declines occurred early in the time series, becoming much

more gradual after 1992. Body size in the extinct 1982 Loberg Lake population exceeded that of most annual samples of the extant Loberg Lake population (Fig. 2), indicating that it has evolved a smaller size than that of the original population inhabiting the lake. The reasons for this are unclear. The population is still evolving morphologically, and it is possible that body size had not reached equilibrium yet. On the other hand, conditions in the lake differ from those experienced by the original population. Sport fishes (especially rainbow trout and coho salmon) have been stocked annually in the lake since the original population was exterminated, and their presence may affect the ecology of the lake enough to result in a different optimum size for the stickleback. Founder effects may also play a role. Continued monitoring of body size is necessary to evaluate how it changes in the future.

Allometry accounted for a small but significant amount of the variation in body shape in the Loberg Lake population. The body shape change associated with allometry is similar to that documented among Cook Inlet resident lake populations by Walker (1997). Only adult fish were included in this study, so the small amount of variation accounted for by allometry is consistent with previous research indicating that it is a minor source of body shape variation after sexual maturity (Walker, 1993).

Influences of Spatial Heterogeneity and Lateral Plate Morph on Body Shape Variation in Loberg Lake:

Significant variation in body shape among sites in Loberg Lake is not surprising. Although Loberg is small, there is likely substantial spatial heterogeneity in vegetation, substratum, food resources, and predators throughout the lake. When examined closely,

subtle, but significant spatial differences in morphology, often associated with ecological factors, seem to be the norm for resident lake and stream stickleback (e.g., Bell and Richkind, 1981; Bell, 1982; Baumgartner 1992; Reimchen, 1994; Hendry et al., 2002; Reimchen and Nosil, 2004). Spatial heterogeneity in morphology (LPM frequency) had been reported previously from Loberg Lake (Bell et al., 2004). As in LPM frequency, the magnitude of spatial heterogeneity of body shape is small, however, relative to other factors, including evolution.

The significant variation in body shape we found associated with LPM, although also apparently small, was not anticipated. Differences in body shape were subtle, but lows seemed to have smaller ectocoracoids, a smaller posterior process of the pelvis and a first dorsal spine displaced slightly backwards (Fig. 3b). The first two of these differences suggest that reduction in LP number may be associated with a general reduction in the size of armor structures, which is a major selective factor in the lake (see below). The association between LPM and body shape may be ecological; it is possible that there are selective advantages for completes and lows to have distinct body shapes, for example when inhabiting limnetic versus the littoral environments in the lake. It also may be due to genetic linkage or pleiotropy. QTLs linked to a major gene, ectodysplasin (*Eda*), influencing LPM phenotypes in stickleback (Colosimo et al., 2005), have recently been found to affect body shape variation (Albert et al., in press), and a similar mechanism may be operating in the Loberg Lake population.

Body Shape Variation among Cook Inlet Threespine Stickleback:

Patterns of morphological variation among stickleback populations have been studied previously (see Walker, 1997 for a treatment especially relevant to this study, but also Moodie and Reimchen, 1976; Reimchen et al., 1985; Bell and Foster, 1994b; McPhail, 1994; Walker and Bell, 2000; Spoljaric and Reimchen, 2007), so we limit our discussion to the most important findings. We tried to include lake populations at the extremes of the PC space for body shape in Walker's (1997) study, and 13 of the 20 lakes we used were in his analysis. Consequently, the general patterns of body shape variation are similar. Body depth, the length of the posterior process of the pelvis, the lengths of the median fins and caudal peduncle, and the position of the dorsal spines all varied substantially among populations. Several of these features appear broadly variable among stickleback populations from other regions (e.g., Spoljaric and Reimchen, 2007), suggesting parallel selection on body shape similar to what is observed for armor traits (Colosimo et al., 2005). The grouping of the five stream populations is consistent with an association between habitat type and morphology (Reimchen et al., 1985; McPhail, 1994; Walker and Bell, 2000; Hendry et al, 2002; Spoljaric and Reimchen, 2007), and the stream populations tended to be similar to shallow lake populations, which is also in accordance with similarities in habitat type. The stream populations were collected in shallow, slow-moving streams with abundant vegetation and rich in benthic prey, like conditions that prevail in shallow lakes. Lakes were very broadly distributed in the PC space suggesting substantial evolutionary divergence among resident lake populations as previously documented (e.g., Moodie and Reimchen, 1976; Reimchen et al., 1985; Walker, 1997; Walker and Bell, 2000; Spoljaric and Reimchen, 2007), and the phenotypic stability over

multiple years suggests relative stability of the selective pressures driving evolutionary diversification of lake populations, at least over the time scale of decades.

Variation and the Evolution of the Loberg Lake Population:

Variation in body shape is abundant in ancestral anadromous and derived freshwater populations, and the Loberg Lake population indicates that levels of variation are maintained during adaptation to lake conditions, despite selection resulting in relatively rapid evolutionary change. This is generally consistent with observations on neutral genetic variation, which is higher in anadromous populations but still reasonably high in derived freshwater populations of *G. aculeatus* (e.g., Buth and Haglund, 1994; Taylor and McPhail, 2000; Reusch et al., 2001; Mäkinen et al., 2006). How variation is maintained in natural populations has long been a central question in evolutionary biology (Lewontin, 1974), but its abundance in Loberg Lake suggests that resident freshwater populations are founded by enough individuals to maintain large amounts of variation.

Evidence that patterns of ancestral phenotypic variation biased the evolutionary trajectory of the extant Loberg Lake population is scarce, and any influence seems extremely short-lived. The results of studies testing the influence of genetic variances on evolution have been mixed (see Revell et al., 2007), suggesting that the importance of genetic constraints may be trait- and taxon- specific. Several factors may have contributed to our negative result. The appropriate test for the influence of ancestral variation on evolutionary divergence employs genetic covariances (Schluter, 1996; McGuigan et al., 2005). It is possible that genetic covariances have biased the evolution

of the Loberg Lake population and our result reflects a lack of correspondence between phenotypic and genetic covariances. However, several studies have found that phenotypic covariances may be suitable substitutes for genetic covariances (e.g., Schluter, 1996; Chevarud, 1988; Waitt and Levin, 1998; but see Willis et al., 1991), especially for morphological traits, and the use of phenotypic data to examine evolutionary phenomena in lieu of genetic data is not uncommon (e.g., Steppan, 1997; Leinonen et al., 2006; Revell et al., 2007). In any case, an analysis that employs genetic covariance and the evolutionary trajectory in the Loberg Lake population is presently under way and should clarify this issue (Bell, in progress).

The lack of strong axes of body shape variation in the RS population ($\mathbf{p}_{max}$ explained only 22.5% of the variation) may also be important. Schluter's (1996) study found a strong relationship between genetic covariance and variation among populations, but $\mathbf{g}_{max}$ accounted for 71% of the additive genetic variance for the traits he analyzed. The low amounts of phenotypic variation accounted for by the dominant eigenvectors in our study may be a consequence of the nature of body shape variation and the methods that we employed. Geometric morphometric methods eliminate size variation which is a major source of variability in conventional morphometric studies, and is often the most common morphological feature to evolve in response to divergent selection. When not removed, size variation can result in concordance between genetic covariance structure and evolutionary divergence (e.g., Begin and Roff, 2004). The scatter of individuals in the PC space was also quite broad compared to the differences among population means. Given the lack of strong axes of body shape variation and the broad scatter of individuals in shape space, our phenotypic data suggest substantial flexibility in the direction of

response to natural selection in threespine stickleback. Other important differences between Schluter's (1996) analysis and ours that could account for the difference in the results is that he compared genetic covariance among a small set of traits primarily related to feeding in a highly derived population with the distribution of a set of populations, whereas we compared a large set of traits related to overall body shape in an ancestral population against the evolutionary trajectory of a lineage. Given all the methodological differences, it is not too surprising that the results of the analyses differed.

Loberg Lake as a Case Study of Adaptation to Novel Environments:

As is the case for lateral plates and gill rakers (Bell et al., 2004; Aguirre and Bell, in prep.), body shape is evolving in the newly established Loberg Lake population in the general direction of the population that originally inhabited the lake. This is consistent with widespread parallelism in the evolution of freshwater stickleback populations (e.g., Bell 1987; Bell and Foster 1994b; Schluter, 2000; Boughman et al., 2005; Colosimo et al., 2005; Marchinko and Schluter, 2007), and observations from other well documented microevolutionary studies (see Hendry and Kinnison, 2001b). The aspects of body shape that have evolved most in the Loberg Lake population were generally the same ones that vary most among resident lake stickleback populations in Cook Inlet, indicating that the same selective factors that act on lake populations in the region have played a major role in the evolution of the Loberg Lake population.

The rate of body shape evolution of the Loberg Lake population was initially high but became lower after 1992. The magnitude of change between 1990 and 1992 is

unclear because there is considerable uncertainty about the position of the 1990 sample mean (Fig 10a). Nonetheless, by 1992 the population had diverged substantially from the ancestral phenotype. One possible explanation for rapid evolution early in the time series is imposition of intense directional selection between 1990 and 1992. Although the sample means are relatively distant in shape space, individuals from the RS samples and the 1990 and 1992 samples from Loberg Lake overlap broadly (Fig. 12). Consequently, the shift in sample means could be produced if the most extreme, existing "lake-like" phenotypes were favored between 1990 and 1992; the range of phenotypes would not have to expand. The selection agents responsible for this shift are unknown, but hydrodynamic performance and shifts in armor structure could be responsible. Hydrodynamic performance based on body shape can have direct fitness consequences (Swain, 1992; Taylor and McPhail, 1986; Walker, 1997) and produce non-random associations between morphology and vulnerability to predators (Reimchen, 1994) and parasites (Reimchen and Nosil, 2001), including especially *Schistocephalus solidus* worms which had very high infection rates early in the time series (unpubl. data). Selection for armor reduction is intense in Loberg Lake (Bell et al., 2004). The displacement of landmarks associated with armor structures between 1990 and 1992 (Fig. 7b) suggests that selection for armor reduction, perhaps due to shifts in predation regimes (Reimchen, 1980) or selection for higher growth rates (Marchinko and Schluter, 2007), may have caused rapid evolution from 1990 to 1992. High growth rates are crucial for overwinter survival in north temperate and boreal freshwater habitats (e.g., Oliver et al., 1979; Toneys and Coble, 1979; Cargnelli and Gross, 1996). Growth rate is retarded in highly armored fish (complete morphs) compared to low armored fish (low morphs) from

the same population in fresh water (Marchinko and Schluter, 2007), suggesting a major fitness cost to armor expression in freshwater environments. Alternatively, if the 1990 sample is the first generation that developed in the lake, maternal effects (Mousseau and Fox, 1998) could be involved, but we are not aware of research on the influence of maternal effects on stickleback body shape.

Although Loberg Lake is relatively deep, the grouping of most Loberg Lake samples (1992-2006) with stream and shallow lake populations is consistent with evolution of the extant population towards the phenotype of the original population (Fig. 5). Means of deep lakes (e.g., Stormy [11], Nancy [20], Big [16], and Long [8]), and of the extinct Loberg Lake sample differed substantially on PC II, and the downward shift in the PC space of the present Loberg time-series between 1992 and 2006 is expected if the population is evolving towards the original phenotype in the lake and not towards that characteristic of deep lakes. In addition, benthic food resources are thought to be exploited first by colonizing anadromous stickleback (McPhail, 1993), which may have contributed to selection for a "benthic" body shape in the newly formed Loberg Lake population. Grouping of Loberg Lake stickleback with samples from shallow lakes and streams suggests the interesting possibility that postglacial populations may go through intermediate phases resembling other freshwater ecomorphs in body shape as they adapt.

Availability of extant anadromous threespine stickleback populations allows both genetic and phenotypic characterization of representatives of the form that was ancestral to postglacial stickleback radiations. Although ancestral phenotypic variation did not constrain or substantially bias evolution of the extant Loberg Lake population, ancestral properties affect adaptation to postglacial environments in several ways. Anadromous

stickleback easily adapt to freshwater conditions because they are born and breed in freshwater. That is, the propensity to establish freshwater populations results from exaptations (Gould and Vrba, 1982) that are adaptations for anadromy and give them ecological access (e.g., Simpson, 1953) to newly created lakes and streams. This is not true for the vast majority of near-shore marine fishes in the northern hemisphere. Moreover, not only can anadromous stickleback colonize freshwater habitats, but the establishment of a resident population in Loberg Lake within seven years of the extermination of the native population, highlights the speed with which novel stickleback populations can be established in postglacial environments.

The abundance of phenotypic and genetic variation in ancestral anadromous stickleback is also an important component of the process. Although oceanic populations are generally uniform in morphology compared to derived freshwater populations (e.g., Walker and Bell, 2000), our study and a more detailed analysis of the Rabbit Slough population (Aguirre et al. in prep.) indicate that anadromous stickleback possess abundant intrapopulation, phenotypic variation in body shape. If this variation is heritable, it provides plentiful raw material for evolutionary change that would facilitate postglacial adaptive radiation of lake populations responding to diverse selective pressures. The abundance of phenotypic variation within anadromous populations is consistent with their abundant genetic variation and probably reflects their life history; anadromous stickleback occur in enormous numbers in the ocean resulting in great mutational input and low rates of drift-based loss of genetic variation (e.g., Hedrick, 2000). Thus ancestral variation is an important component of adaptive radiation.

Adaptation to novel environments, as was the case for the Loberg Lake population, is a major source of evolutionary diversity in nature (Simpson, 1953; reviewed in Reznick and Ghalambor, 2001), and our data are consistent with current knowledge on associated rates and patterns of evolution in such situations (see Kinnison and Hendry, 2001). Postglacial stickleback radiations are possible largely because of a historical contingency; glaciation and deglaciation has created new freshwater habitats that are rich in resources but devoid of native competitors, and stickleback have taken advantage of this opportunity throughout the northern hemisphere (Bell and Foster, 1994b; Schluter, 2000; Taylor and McPhail, 2000; McKinnon and Rundle, 2002). In the case of the Loberg Lake population, it has evolved approximately 71% of the distance to the phenotype of the extinct Loberg Lake population within 25 years of being established (as measured by Procrustes distances between consensus configuration of the RS sample, and the extinct 1982 and modern 2006 Loberg Lake samples). Thus postglacial stickleback populations appear capable of evolving within a matter of decades. Although these habitats have existed for thousands of years, our results suggest that much of the evolutionary change occurs very early in the process. The relative temporal stability observed among populations sampled over multiple years in our study, and the association between morphology and the environment typically observed in postglacial stickleback populations (e.g., Walker, 1997; Spoljaric and Reimchen, 2007), indicates 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 one of evolution proceeding rapidly upon occupation of a novel adaptive zone, followed by relative stasis once an adaptive peak is reached. This scenario is consistent with the

characterization of stickleback diversity as a phylogenetic raceme (Bell 1987; Bell and Foster 1994), and with observations in the fossil record of stasis punctuated by bouts of rapid of evolution (Gould and Eldredge, 1993). It is also consistent with Kinnison and Hendry's (2001) contention that macroevolution results from microevolution "writ in fits and starts". Evolution of major armor phenotypes is possible largely because of the existence of standing variation for armor reduction in ancestral anadromous populations (Colosimo et al., 2005). Selection on standing variation probably also accounts for the rapid evolution of other traits, including body shape, when freshwater habitats are colonized. The reasons for relative stasis in many populations over thousands of years afterwards are unclear. The mechanisms for stasis are a topic of current research (Eldredge et al., 2005; Weins and Graham, 2005; Estes and Arnold, 2007) and beyond the scope of this study, but understanding morphological stasis of postglacial stickleback populations constitutes an important opportunity for future research.

LITERATURE CITED

Adams, D.C., F.J. Rohlf, and D.E. Slice. 2004. Geometric morphometrics: ten years of progress following the "revolution". Italian Journal of Zoology 71:5-16.

Aguirre, W.E., P. Doherty, and M.A. Bell. 2004. Genetics of lateral plate and gill raker phenotypes in a rapidly evolving population of threespine stickleback. Behaviour 141:1465-1483.

Albert, A.Y.K., S. Sawaya, T.H. Vines, A.K. Knecht, C.T. Miller, B.R. Summers, S. Balabhadra, D.M. Kingsley, and D. Schluter. 2007. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. Evolution, in press.

Baker, J.A. 1994. Life history variation in female threespine stickleback. Pages 144-187 in in M.A. Bell, and S.A. Foster (eds.), The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Baumgartner, J.V. 1992. Spatial variation of morphology in a freshwater population of

the threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 70:1140-1148.

Bégin, M., and D.A. Roff. 2004. From micro-to macroevolution through quantitative genetic variation: positive evidence from field crickets. Evolution 58:2287-2304.

Bell, M.A. 1982. Differentiation of adjacent stream populations of threespine sticklebacks. Evolution 36:189-199.

Bell, M.A. 1987. Interacting evolutionary constraints in pelvic reduction of threespine sticklebacks, Gasterosteus aculeatus (Pisces, Gasterosteidae). Biological Journal of the Linnean Society 31:347-382.

Bell, M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. Genetica 112-113:445-461.

Bell, M.A., and K.E. Richland. 1981. Clinal variation of lateral plates in threespine stickleback fish. American Naturalist 117:113-132.

Bell, M.A., and Foster, S.A. 1994a. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M.A., and Foster, S.A. 1994b. Introduction to the evolutionary biology of the threespine stickleback. Pages 1-27 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M.A., G. Ortí, J.A. Walker, and J.P. Koenings. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution 47:906-914.

Bell. M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Boughman, J.W., H.D. Rundle, and D. Schluter. 2005. Parallel evolution of sexual isolation in sticklebacks. Evolution 59:361-373.

Buth, D.G., and T.R. Haglund. 1994. Allozyme variation in the *Gasterosteus aculeatus* complex. Pages 61-84 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Cargnelli, L.M., and M.R. Gross. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). Canadian Journal of Fisheries and Aquatic Sciences 53:360-367.

Chevarud, J.M. 1988. A comparison of genetic and phenotypic correlations. Evolution

42:958-968.

Colosimo, P.F., K.E. Hoseman, S. Balabhadra, G. Villareal Jr., M. Dickson, J. Grimwood, J. Schmtz, R.M. Myers, D. Schluter, and D.M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928-1933.

Eldredge, N., J.N. Thompson, P.M. Brakefield, S. Gavrilets, D. Jablonski, J.B.C. Jackson, R.E. Lenski, B.S. Lieberman, M.A. McPeek, and W. Miller III. 2005. The dynamics of evolutionary stasis. Paleobiology 31:133-145.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76-91.

Endler, J.A. 1986. Natural selection in the wild. Princeton University Press, Princeton.

Estes, S., and S.J. Arnold. 2007. Resolving the paradox of stasis: Models with stabilizing selection explain evolutionary divergence on all time scales. The American Naturalist 169:227-244.

Gibbs, H.L., and P.R. Grant. 1987. Oscillating selection on Darwin's finches. Nature 327:511-513.

Gould, S.J., and N. Eldredge. 1993. Punctuated equilibrium comes of age. Nature 366:223-227.

Gould, S.J., and E.S. Vrba. 1982. Exaptation- a missing term in the science of form. Paleobiology 8:4-15.

Gross, H.P., and J.M. Anderson. 1984. Geographic variation in the gillrakers and diet of European threespine sticklebacks, *Gasterosteus aculeatus*. Copeia 1984:87-97.

Hagen, D.W., and L.G. Gilbertson. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. Evolution 26:32-51.

Hedrick, P.W. 2000. Genetics of populations. Jones and Bartlett Publishers, Sudbury, MA.

Hendry, A.P., and M.T. Kinnison. 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution 53:1637-1653.

Hendry, A.P., and M.T. Kinnison. 2001a. Microevolution. Rate, pattern, process. Genetica (special issue) 112-113:1-535.

Hendry, A.P., and M.T. Kinnison. 2001b. An introduction to microevolution: rate, pattern

process. Genetica 112-113:1-8.

Hendry, A.P., E.B. Taylor, and J.D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:1199-1216.

Karve, A.D., F.A. von Hippel, and M.A. Bell. 2007. Isolation between sympatric anadromous and resident threespine stickleback species in Mud Lake, Alaska. Environmental Biology of Fishes, in press.

Kettlewell, H.B.D. 1973. The evolution of melanism. Clarendon Press, Oxford.

Kinnison, M.T., and A.P. Hendry. 2001. The pace of modern life: from rates of contemporary microevolution to pattern and process. Genetica 112-113:145-164.

Klepaker, T. 1993. Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1231-1258.

Kristjánsson, B.K., S. Skúlason, and D.L.G. Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659-672.

Leinonen, T. J.M. Cano, H. Mäkinen, and J. Merilä. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. Journal of Evolutionary Biology 19:1803-1812.

Lewontin, R.C. 1974. The genetic basis of evolutionary change. Columbia University Press, New York.

Liem, K.F., and L.S. Kaufman. 1984. Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. Pages 203-215 in A.A. Echelle and I. Kornfield (eds.). Evolution of fish species flocks. University at Maine, Orono Press, Orono.

Losos, J.B., K.I. Warheit, and T.W. Schoener. 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387:70-73.

Mäkinen, H.S., J.M. Cano, and J. Merilä. 2006. Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. Molecular Ecology 15:1519-1534.

Marchinko, K.B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution 61:1084-1090.

McGuigan, K., S.F. Chenoweth, and M. W. Blows. 2005. Phenotypic divergence along

lines of genetic variance. The American Naturalist 165:32-43.

McKinnon, J.S., and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model system. Trends in Ecology and Evolution 17:480-488.

McPhail, J.D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pair. Canadian Journal of Zoology 71:515-523.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. Pages 399-437 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Moodie, G.E.E., and T.E. Reimchen. 1976. Phenotypic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25: 49-61.

Mousseau, T.A., and C.W. Fox. 1998. Maternal effects as adaptations. Oxford University Press, Oxford.

Oliver, J.D., G.F. Holeton, and K.E. Chua. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. Transactions of the American Fisheries Society 108:130-136.

Östlund-Nilsson, S., I. Mayer, and F. A. Huntingford (eds.). 2006. Biology of the three-spined stickleback. CRC Press, Boca Raton.

Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 58:1232-1244.

Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. Pages 240-276 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Reimchen, T.E., and P. Nosil. 2001. Ecological causes of sex-biased parasitism in threespine stickleback. Biological Journal of the Linnean Society 73:51-63.

Reimchen, T.E., and P. Nosil. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58:1274-1281.

Reimchen, T.E., and P. Nosil. 2006. Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipielago on the west coast of Canada. Canadian Journal of Zoology 84:643-654.

Reimchen, T.E., E.M. Stinton, and J.S. Nelson. 1985. Multivariate differentiation of parapatric and allopatric threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Canadian Journal of Zoology 63:2944-2951.

Reusch, B.H., K.M. Wegner, and M. Kalbe. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographic proximity. Molecular Ecology 10:2435-2445.

Revell, L.J., L.J. Harmon, R.B. Langerhans, and J.J. Kolbe. 2007. A phylogenetic approach to determining the importance of constraints on phenotypic evolution in the neotropical lizard *Anolis cristatellus*. Evolutionary Ecology Research 9:261-282.

Reznick, D.N., and C.K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112-113:183-198.

Reznick, D.N., F.H. Shaw, F.H. Rodd, and R.G. Shaw. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357-359.

Rohlf, F.J. 2002. BIOMstat: statistical software for biologists, version 3.30o. 2002. Applied Biostatistics Inc., Port Jefferson, NY.

Rohlf, F.J. 2004a. TpsDig version 1.40. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2004b. TpsSplin version 1.20. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2005. TpsRegr software version 1.31. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2006. TpsRelw version 1.44. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J., and L. Marcus. 1993. A revolution in morphometrics. Trends in Ecology and Evolution 8:129-132.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766-1774.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Simpson, G.G. 1953. The major features of evolution. Columbia University Press, New York.

Slice, D.E., F.L. Bookstein, L.F. Marcus, and F. J. Rohlf. 1996. Appendix I. A glossary for geometric morphometrics. Pages 531-551 in L.F. Marcus et al. Advances in morphometrics. Plenum Press, New York.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry. W.H. Freeman and Company, New York.

Spoljaric, M.A., and T.E. Reimchen. 2007. 10,000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. Journal of Fish Biology 70:1484-1503.

Steppan, S.J. 1997. Phylogenetic analysis of phenotypic covariance structure. I. Contrasting results from matrix correlation and common principal component analyses. Evolution 51:571-586.

Swain, D.P. 1992. The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. Evolution 46:987-997.

Tabachnick, B.G., and L.S. Fidell. 1996. Using multivariate statistics. Harper Collins College Publishers, New York.

Taylor, E.B., and J.D. McPhail. 1986. Prolonged and burst swimming in anadromous freshwater threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 64:416-420.

Taylor, E.B., and J.D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proceedings of the Royal Society of London B 267:2375-2384.

Toneys, M.L., and D.W. Coble. 1979. Size-related, first winter mortality of freshwater fishes. Transactions of the American Fisheries Society 108:415-419.

von Hippel, F.A., and H. Weigner. 2004. Sympatric and anadromous-resident pairs of threespine stickleback species in young lakes and streams at Bering Glacier, Alaska. Behaviour 141:1441-1464.

Waitt, D.E., and D.A. Levin. 1998. Genetic and phenotypic correlations in plants: a botanical test of Chevarud's conjecture. Heredity 80:310-319.

Walker, J.A. 1993. Ontogenetic allometry of threespine stickleback body form using landmark-based morphometrics. Pages 193-214 in L.F. Marcus, E. Bello, A. García-Valdecasas (eds.). Contributions to Morphometrics. Madrid: *Museo de Ciencias Naturales*.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Walker, J.A., and M.A. Bell. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). Journal of Zoology 252: 293-302.

Weins, J.J., and C.H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36:519-539.

West-Eberhard, M.J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Willis, J.H., J.A. Coyne, and M. Kirkpatrick. 1991. Can one predict the evolution of quantitative characters without genetics? Evolution 45:441-444.

Zelditch, M.L., D.L. Swiderski, H.D. Sheets, and W.L. Fink. 2004. Geometric morphometrics for biologists, a primer. Elsevier Academic Press, Oxford.

Table 1. MANOVA and partial η^2 estimates of body shape variation in Loberg Lake 1994 and 2006 samples related to centroid size (Cent Size), year of collection (Year), lateral plate morph (LPM), site of collection (Site), and interactions among the main factors. Lower Wilks' λ values imply higher significance and higher partial η^2 values imply greater effect strengths for the particular factor.

Factor	Wilks' λ	F	DF1	DF2	P	partial η^2
Cent Size	0.7704	3.012	28	283	<0.001	0.2296
Year	0.3760	16.78	28	283	<0.001	0.6240
LPM	0.7563	3.257	28	283	<0.001	0.2437
Site	0.5479	1.645	112	1126.6	<0.001	0.1406
Year X LP	0.9255	0.813	28	283	0.7384	NS
Year X Site	0.6474	1.164	112	1126.6	0.1265	NS
LP X Site	0.6220	1.277	112	1126.6	0.0327	0.1127

Table 2. MANOVA and partial η^2 estimates of the influence of population of origin and year collected on body shape of the 13 lake samples collected two years. Lower Wilks' λ values imply higher significance and higher partial η^2 values imply greater effect strengths for the particular factor.

Factor	Wilks' λ	F	DF1	DF2	P	partial η^2
Population	0.00058	14.638	336	5113.9	<0.001	0.4903
Year	0.08549	3.475	364	5469.9	<0.001	0.1878

Table 3. Average levels of body shape variation (multiplied by 10^4) in the Loberg Lake population and neighboring populations. Min and Max are the minimum and maximum variances for groups. Variances are sums of variances of PCs I and II (Var. PCs 1-2), and all PCs (Var. PCs All). * The Zero Lake and Jean Lake populations both had unusually high levels of body shape variance so they were separated from the rest of the lake populations.

Sample	Var. PCs 1-2	Min	Max	Var. PCs All	Min	Max
Lob 82	0.911	-	-	6.638	-	-
Lob 90	0.956	-	-	10.865	-	-
Lob 91	0.921	-	-	6.466	-	-
Lob 92-06	0.771	0.530	1.055	6.649	5.574	8.083
Anad.	0.660	0.382	1.011	6.668	6.168	7.636
Lake	0.740	0.357	1.377	6.326	4.625	8.311
Stream	0.950	0.404	1.346	6.795	5.420	8.215
Jean*	4.307	-	-	10.045	-	-
Zero*	2.644	2.249	3.039	15.752	15.159	16.345

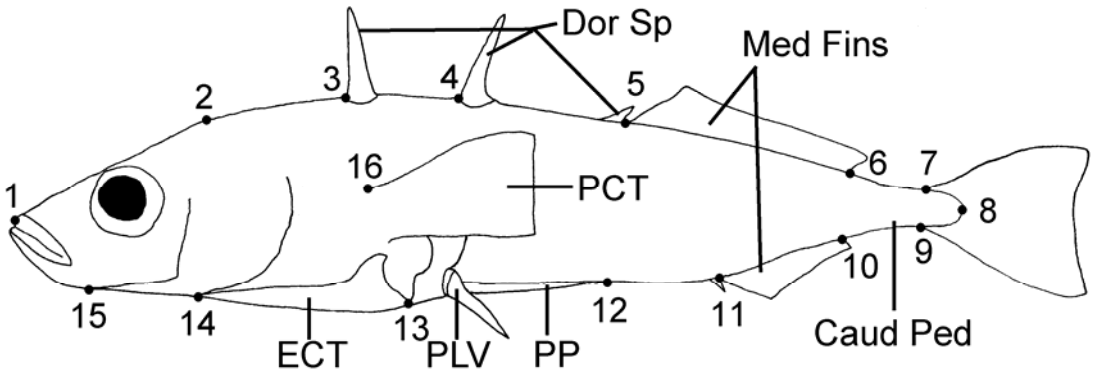


Fig. 1. Landmarks used in this study (numbers 1-16). Anatomical structures mentioned in text are labeled. ECT = ectocoracoid, PLV = pelvis, which includes the pelvic spine, anterior process, ascending branch, and posterior process, PP = posterior process of the pelvis, Caud Ped = caudal peduncle, Med Fins = median fins, Dor Sp = dorsal spines, and PCT = pectoral fin.

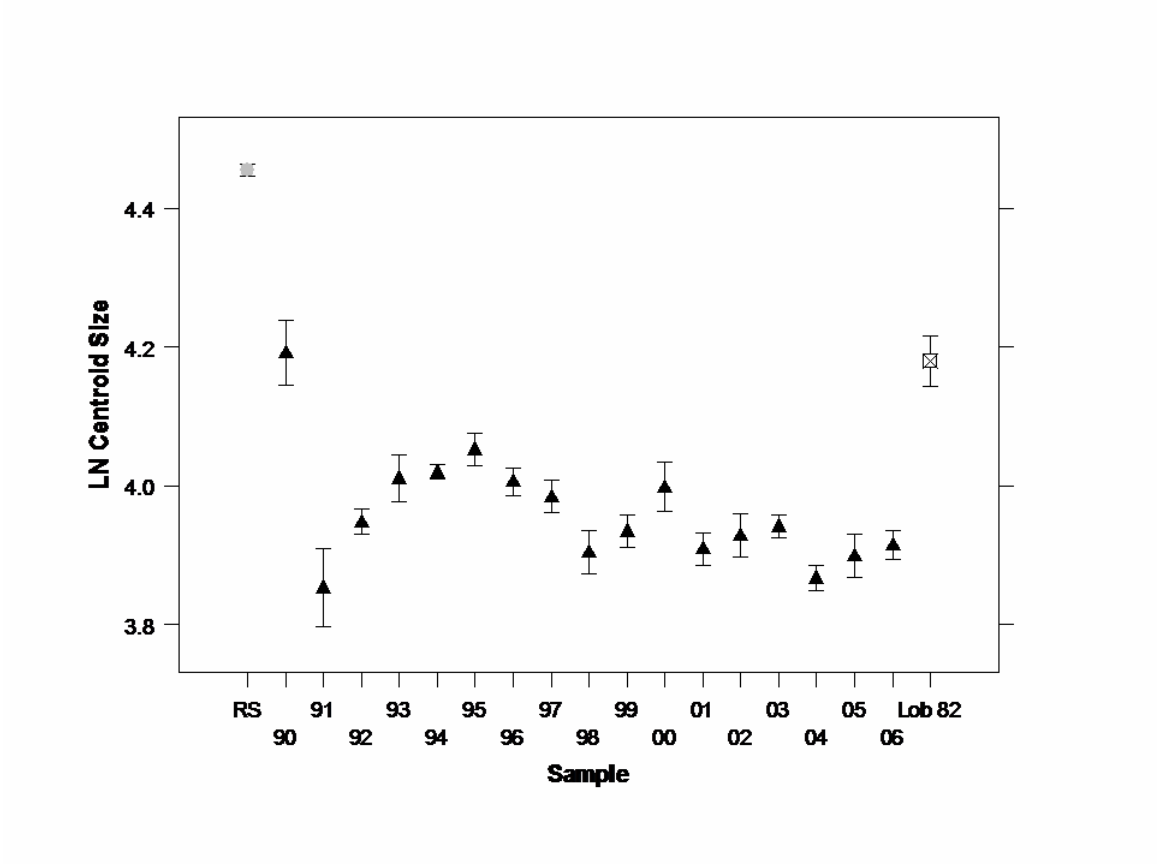


Fig. 2. Natural logarithm (LN) of mean centroid size of andromous Rabbit Slough population (gray circle, far left), Loberg Lake time series (black triangles), and a sample from the extinct Loberg Lake 1982 sample (Lob 82, X in square, far right). Bars around mean are 95% confidence intervals. Excluding the 1991 sample which was collected at a different time of year, centroid size declined significantly over time in the modern Loberg Lake population (regression analysis, $r^2 = 0.580$, $F = 19.316$, $P = 0.001$).

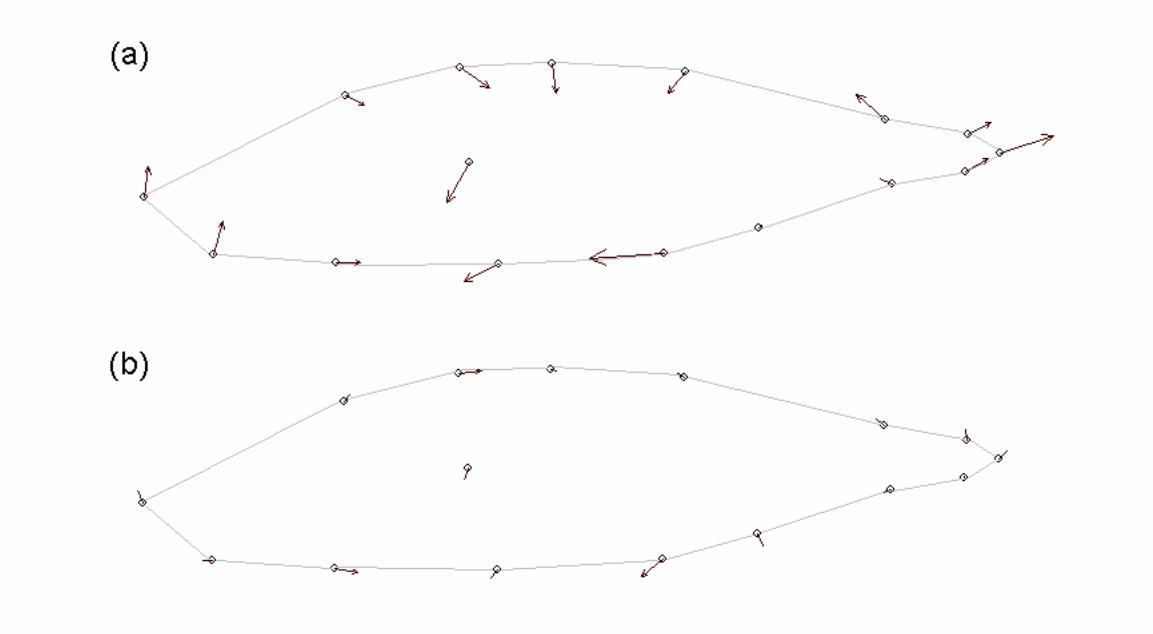


Fig. 3. (a) Divergence of landmarks of the 2006 complete morph consensus from the 1994 complete morph consensus configuration indicating evolution over time within morphs (vectors indicate direction and magnitude of landmark displacements in 2006 compared to their position in 1994). Divergence of lows between 1994 and 2006 was similar and is not shown. (b) Divergence of landmarks of the low morph consensus configuration from the complete morph consensus configuration in 1994. Divergence of morph consensus configurations was almost unperceivable in 2006 and is not shown. The deviations between consensus configurations were small so vector lengths are exaggerated by a factor of 5 to facilitate visualization.

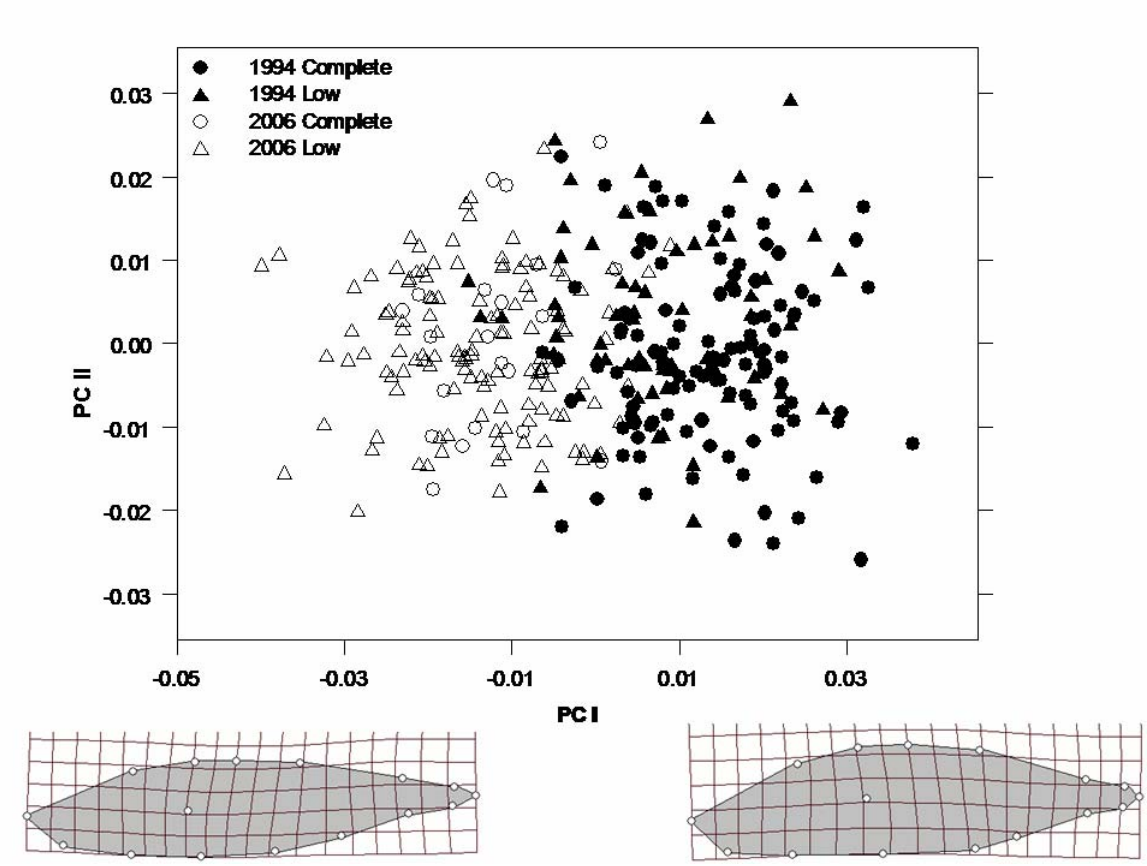


Fig. 4. Principal components analysis plot of Loberg Lake 1994 and 2006 specimens. Deformation grids are the predicted shapes at the extremes of PC I.

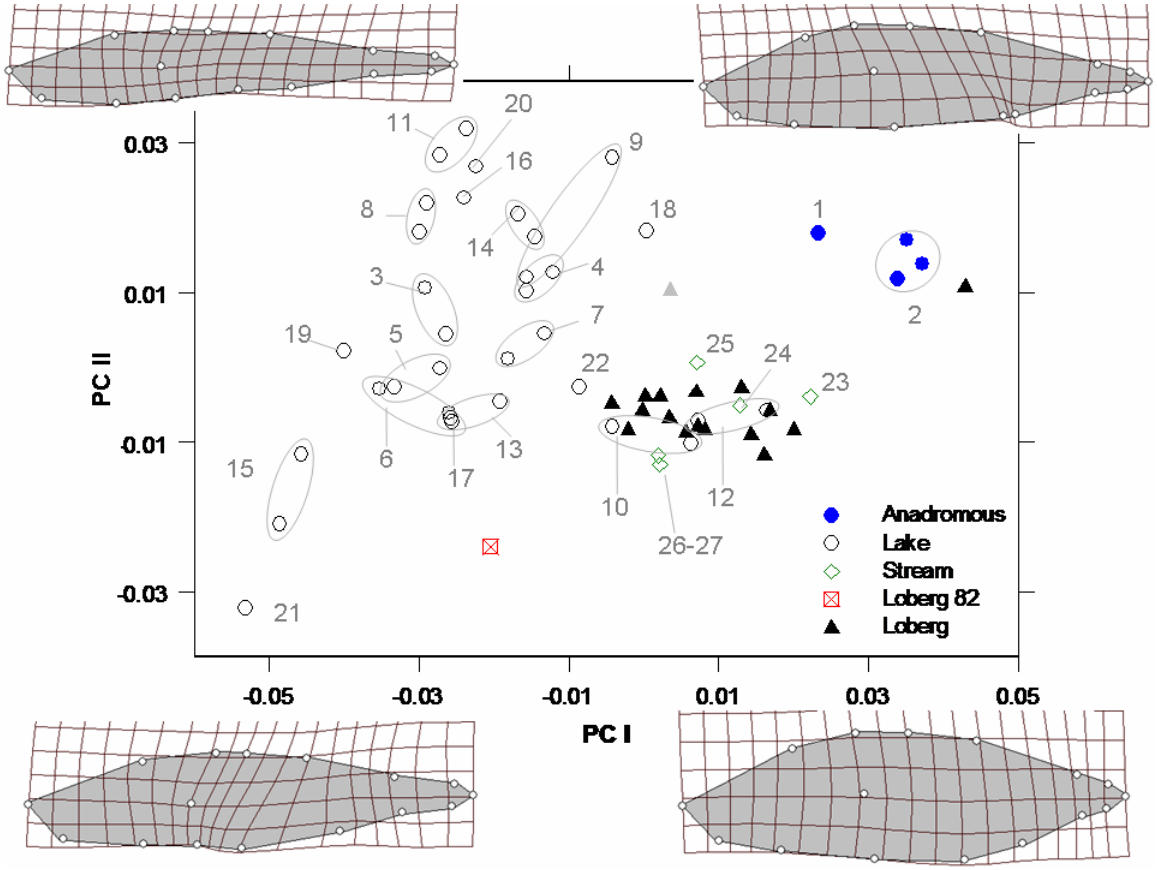


Fig. 5. Principal components analysis plot depicting the two major axes of body shape variation. Points are sample means. The gray triangle is the 1991 Loberg Lake sample. Ellipses connect samples from the same populations in different years (except for the Loberg Lake population). Numbers are population codes (see Appendix 2). Deformation grids are predicted body shapes in four corners of shape space.

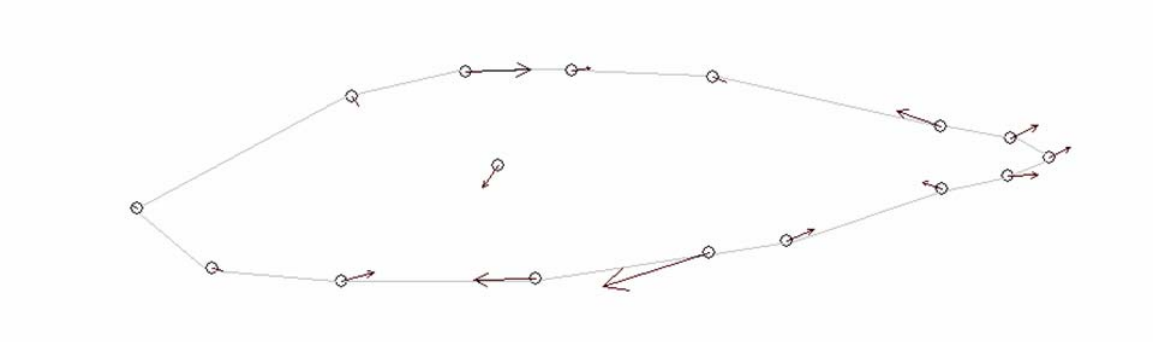


Fig. 6. Body shape of extinct 1982 Loberg Lake population compared to its most likely ancestor, the anadromous Rabbit Slough (RS) population. Landmark displacements of consensus configurations of the extinct Loberg 1982 sample from the RS sample. Vectors are exaggerated by a factor of two to facilitate visualization.

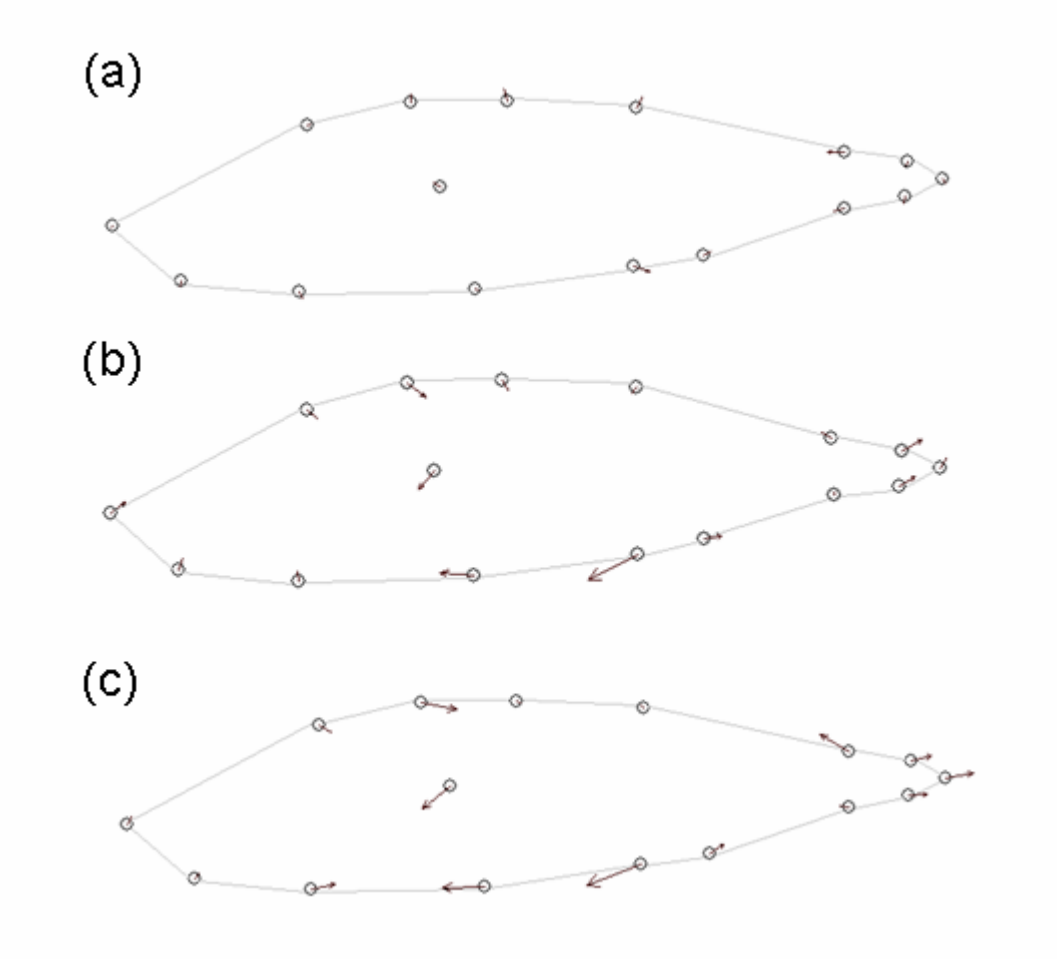


Fig. 7. Body shape evolution of the extant Loberg Lake population. Landmark displacement of the consensus configurations of (a) the 1990 Loberg Lake sample from the Rabbit Slough (RS) sample, (b) the 1992 Loberg Lake sample from the 1990 sample, and (c) the 2006 Loberg Lake sample from the RS sample. Vectors are exaggerated by a factor of two to facilitate visualization.

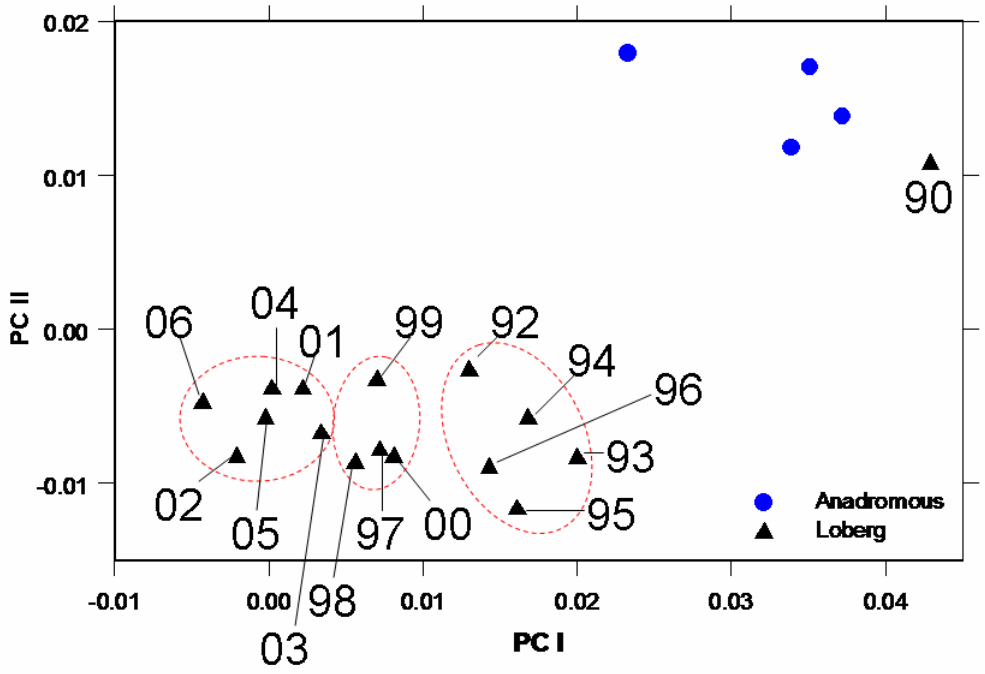


Fig. 8. Close up of Loberg Lake annual sample distribution in shape space. Numbers indicate the year of collection of each of the Loberg Lake samples. See Figure 4 for a broader context.

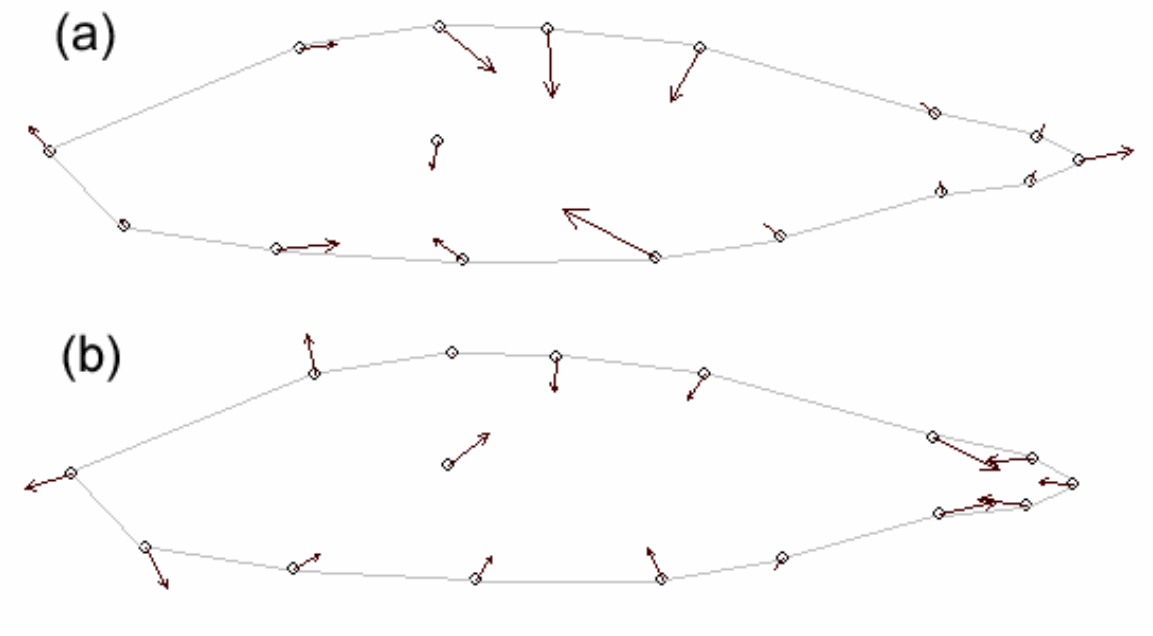


Fig. 9. Contributions of year, i.e., temporal evolution (a), and allometry (b) to variation in Loberg Lake body shape (between 1990 and 2006). Vectors indicate direction and magnitude of displacement of landmark positions (a) over time and (b) as specimens increase in size. The magnitude of the variation in body shape accounted for by these factors was small (<11% combined), so vector lengths are exaggerated by a factor of 10 to facilitate visualization.

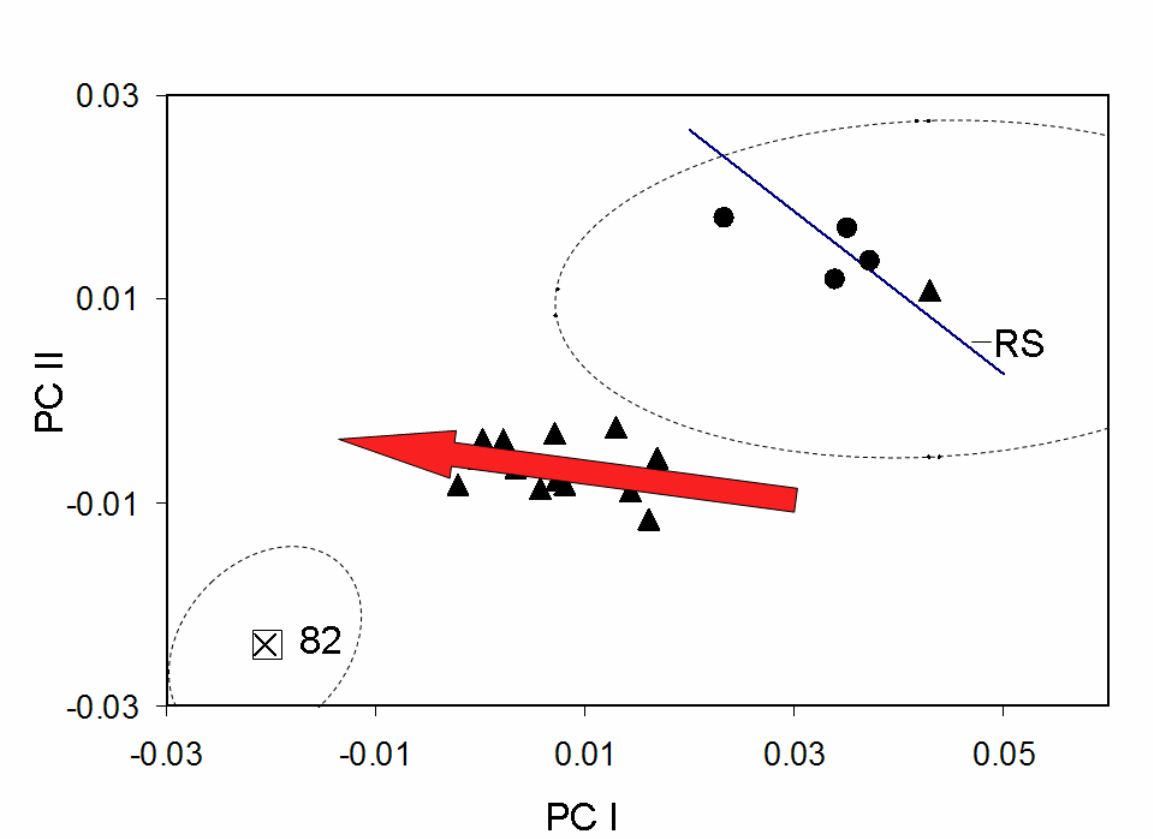


Fig. 10. Principal axes for annual samples from Loberg time series in the shape space. Circles are anadromous means, triangles are extant Loberg Lake means, and the x in the square is the extinct Loberg Lake mean. Ellipses are 95% confidence ellipses of bivariate means for 1990 (top right) and 1982 (bottom left) samples. The thin line is the principal axis of the anadromous Rabbit Slough sample across all three years and the large arrow is the principal axis through the Loberg Lake population 1992-2006 time series of annual means.

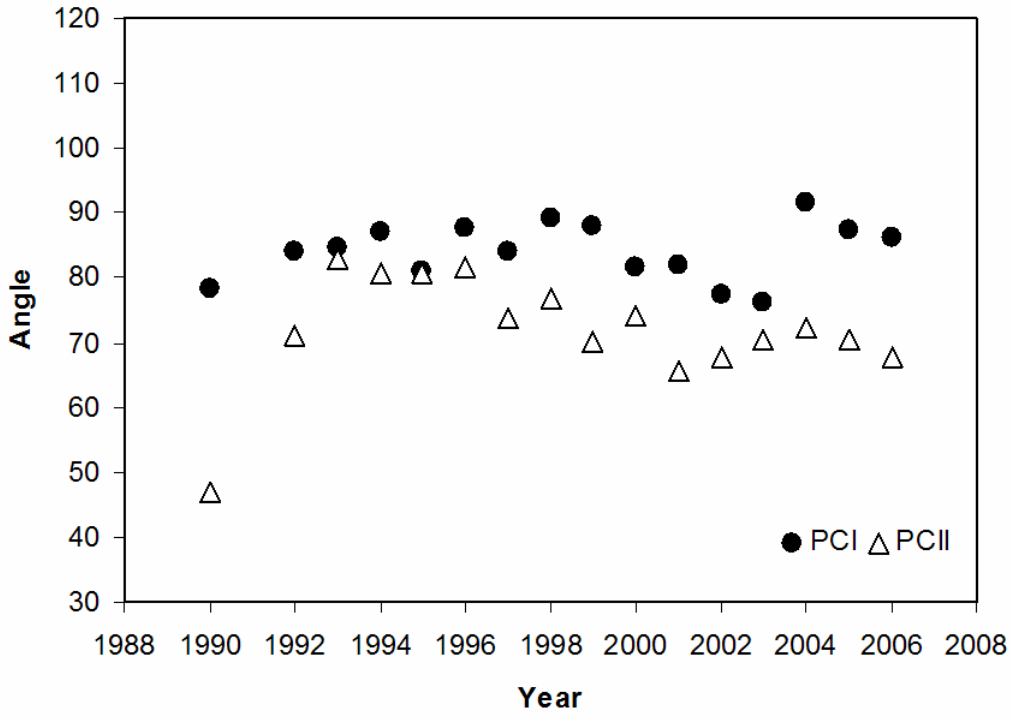
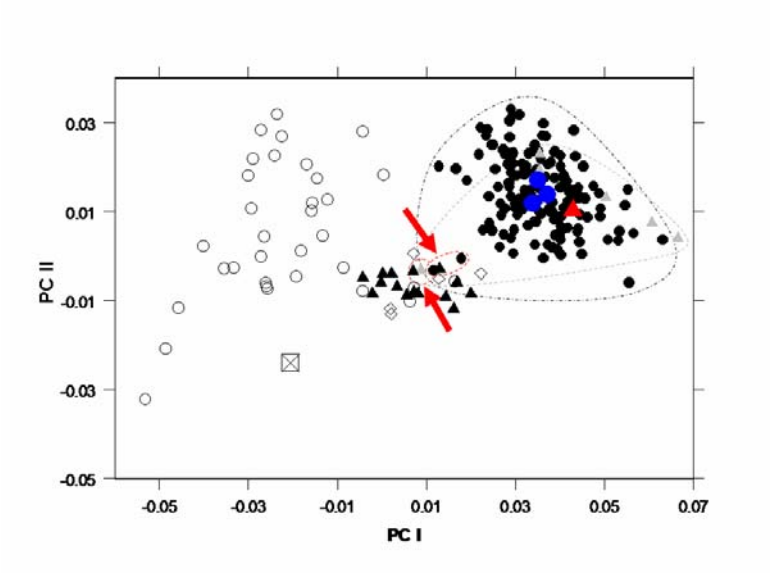


Fig. 11. Angles (θ) between $\mathbf{z}_i$, representing the direction of the line separating the anadromous Rabbit Slough (RS) and the Loberg Lake population annual means, and PC I ($\mathbf{p}_{max}$) and PC II ($\mathbf{p}_2$), the two major axes of variation in the anadromous RS population.

(a)



(b)

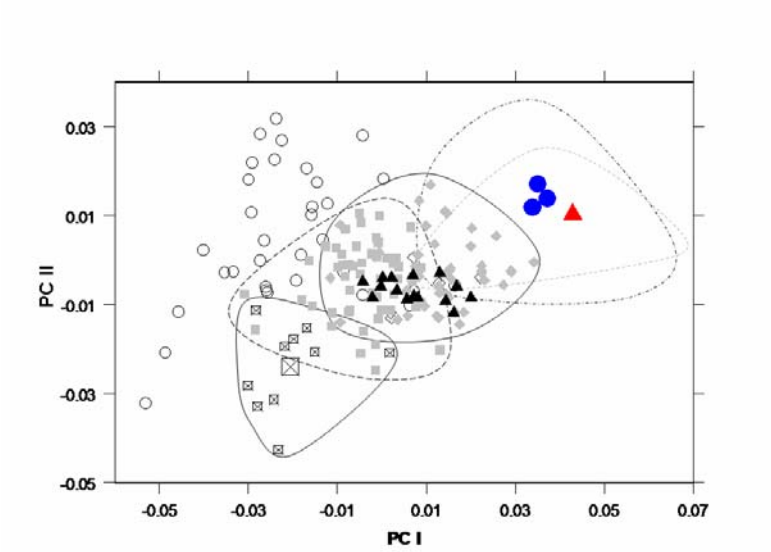


Fig. 12. Scatter plot of individuals from selected samples in PC space. Unless otherwise indicated, symbols follow Fig. 5 and larger symbols are sample means. (a) Scatter of RS (black circles) and Loberg Lake 1990 individuals (gray triangles). Both samples were outlined to facilitate visualization of boundaries. The arrows and small ellipses indicate two RS specimens and one Loberg Lake 1990 specimen that were highly divergent. (b) Scatter of Loberg Lake 1992 (gray diamonds), 2006 (gray squares), and 1982 sample from extinct population (X in squares) outlined to indicate sample boundaries. The outlines for the Loberg Lake 1990 and RS samples are preserved to facilitate visualization.

Chapter 4

Contemporary Evolution and Genetic Diversity of a Recently Established Threespine Stickleback Lake Population

ABSTRACT

Analyses of contemporary evolution can increase our understanding of evolutionary diversification in nature because they allow us to study evolution as it happens. We present rates and patterns of contemporary evolution of a recently established lake stickleback population. The native stickleback population in Loberg Lake, Alaska, was exterminated in 1982, and the lake was recolonized by anadromous stickleback sometime between 1983 and 1989. Since then, the extant population has rapidly evolved in the direction of the exterminated population for several traits. We build on our previous work on the evolution of armor reduction in this population, adding data on lateral plate evolution between 2002 and 2006 and trophic morphology. We also examine the genetic structure of this population using microsatellite markers to infer whether complete and low lateral plate morphs form a single deme and whether the population was bottlenecked when it was founded. The frequency of the ancestral "complete" lateral plate morph has continued to decline, and by 2006 it had dropped to 3.2%, while frequencies of three intermediate lateral plate morphs have remained relatively stable. The frequency of "low" morphs has risen from 0% in 1990 to 88%. Low morph lateral plate number and gill raker number have also declined significantly in the direction of the extinct population, and the rates of evolution of these traits were comparable. These rates are not unusual for populations colonizing novel environments

and suggest that phenotypes typical of postglacial stickleback populations evolved within decades of establishment. In accordance with our morphological data, variation at microsatellite loci indicates that Loberg Lake is inhabited by a single interbreeding population that is polymorphic for lateral plate phenotypes. Genetic diversity was lower than that of ancestral anadromous populations but comparable to that of neighboring lake populations, indicating that the demographic history of the Loberg Lake population is similar to that of neighboring lake populations. Consequently, the Loberg Lake population can probably be used as a model for studying the evolution of postglacial lake populations in general.

INTRODUCTION

Adaptive radiations of postglacial threespine stickleback, *Gasterosteus aculeatus*, are among our most illuminating examples of evolutionary diversification and parallelism (Bell and Foster, 1994a; Schluter, 2000; McKinnon, and Rundle, 2002; Östlund-Nilsson et al., 2006). Threespine stickleback are primitively anadromous (live in the ocean and migrate to freshwater to reproduce) but have established countless resident freshwater populations in postglacial areas throughout the northern hemisphere. Within the last 21,000 years, resident freshwater populations have diverged substantially from their anadromous ancestors and from one another in response to divergent natural selection. Armor structure and trophic morphology are among the most conspicuous features to evolve.

Reduction of armor in freshwater environments is usually dramatic and includes dorsal and pelvic spines, the pelvic girdle, and lateral plate (LP) phenotypes. Divergence

of the last trait is particularly striking. Anadromous stickleback are typically "complete" morphs; they have a large number (modally 33) of superficial bony LP covering the entire flank. Resident lake and stream stickleback are typically "low" morphs; they generally have a few LP (< 10) restricted to the anterior part of the body. There are also "partial" morphs in both freshwater and estuarine populations, which have a gap near the center of the series (Hagen and Gilbertson, 1972; Bell and Foster, 1994b; Klepaker 1996). We recognize two additional intermediate phenotypes in populations exhibiting unusual levels of LP variability (Francis et al., 1985; Bell et al., 2004). The "intermediate low" phenotype has 10 or more abdominal plates but lacks posterior plates. The "intermediate partial" phenotype resembles the partial morph but has isolated plates in the gap between the continuous anterior and posterior plate rows, separated by unplated body segments. The evolution of LP phenotypes depends on variation at the *Ectodysplasin* (*Eda*) locus, which can have a large effect on LP number (Colosimo et al., 2005). An *Eda* allele for LP reduction that has low penetrance in heterozygotes, is carried at low frequencies in anadromous populations and has been selected in parallel throughout the world (Colosimo et al., 2005). Its frequent fixation in fresh water may result from a shift in predation regimes (Reimchen, 1994; 2000) and increased cost of armor expression (Marchinko and Schluter, 2007).

Lateral plate number within the low morph exhibits adaptive variation among resident freshwater populations (e.g., Hagen and Gilbertson, 1972, 1973; Moodie and Reimchen, 1976; Bell and Richland, 1981; Bell, 1984; Reimchen, 1983, 1992, 1994). Populations subjected to high rates of predation by fish tend to have strong modal counts of 7 LP per side, whereas those in environments lacking fish predators have lower means.

The function of the lateral plates varies by position (Reimchen, 1983, 1992, 1994). The plates located between the first and second dorsal spines and the ascending branch of the pelvis buttress the dorsal and pelvic spines against manipulation by predators. The anterior most plates protect against puncture by toothed predators and tend to be lost in lakes lacking predacious fish. Adverse effects on acceleration (Reimchen, 1994) and the metabolic cost of armor expression in freshwater (see Marchinko and Schluter, 2007) may be responsible for extremely low plate counts in low plate morphs.

The evolution of trophic morphology in freshwater populations is also substantial. They evolve along a phenotypic continuum based on specialization on larger benthic food items associated with vegetation in shallow habitats or smaller planktonic prey in the water column. This divergence is most dramatic in several lakes in British Columbia where sympatric "benthic" and "limnetic" species pairs exist (e.g., McPhail, 1984; 1994; Schluter, 2000; McKinnon and Rundle, 2002). Divergence between benthics and limnetics involves numerous traits, including body size and shape, head size, eye diameter, snout length, gape width, and the length and number of gill rakers (GR), with the latter often serving as an indicator of the level of trophic specialization (McPhail, 1984). Gill rakers are small bony structures on the gill arches that prevent captured prey from leaving the pharynx (and damaging the gill filaments) when water is expelled through the gill chamber (Moyle and Cech, 1996). Number and length of GR tend to increase with prey size in diverse fishes (Moyle and Cech, 1996), including stickleback (Gross and Anderson, 1984; Bentzen and McPhail, 1984; Lavin and McPhail, 1986; Hart and Gill, 1994).

Recent studies of contemporary evolution in stickleback suggest that major morphological changes, comparable to differences among species of many other groups, can evolve in a matter of decades (Klepaker, 1993; Bell, 2001; Kristjánsson et al., 2002; Bell et al, 2004; von Hippel and Weigner, 2004). One well documented case involves a recently established population in Loberg Lake, Alaska (Bell, 2001; Aguirre et al., 2004; Bell et al., 2004). The native Loberg Lake population was exterminated in 1982 and was typical of lake populations in the area for armor structure (Bell et al., 2004). The extinct Loberg Lake population exhibited significant armor reduction (mean LP = 5.08, n = 100) and exhibited a lower GR count (mean=20.82, n=50) than anadromous stickleback from Rabbit Slough (RS), in the same drainage as Loberg Lake (mean GR=22.36, n=202). Anadromous stickleback must have recolonized the lake sometime between 1983, after the native population was exterminated, and 1989, the year before a sample resembling anadromous stickleback was made (Bell et al., 2004). Annual sampling between 1990 and 2001 revealed that the frequency of complete morph fish in Loberg Lake declined from 95.9 to 11.2% (Bell et al., 2004), and LP number in the low morph also declined significantly from 6.82 in 1992 (n=103) to 6.37 in 2001 (n=200). Other traits such as body shape also are evolving in the direction of the extinct population originally inhabiting the lake (Aguirre et al., in prep.).

In this study, we build on our previous results for evolution of armor in the Loberg Lake population (Bell et al., 2004), and add data on LP evolution between 2002 and 2006 and GR number from 1990 and 2006. The rates and patterns of evolution of these traits are compared. We also use five unlinked microsatellite loci to examine population genetic structure and genetic diversity of the Loberg Lake population

compared to neighboring lake, stream and anadromous populations. All previous morphological data examined (Bell et al., 2004; Aguirre and et al., in prep.) suggest that Loberg Lake is inhabited by a single deme that is polymorphic for LP phenotypes and has not been seriously bottlenecked. We carry out the first genetic tests of these hypotheses.

MATERIALS AND METHODS

Site Description, Sampling Methods, and Phenotypic Scoring:

Loberg Lake is a small lake (~4.45 ha surface area) in the Matanuska - Susitna Borough of Cook Inlet, Alaska (61˚ 33' 35"N, 149˚ 15' 30"W). A map of the lake, collection sites, physical and biological aspects of the lake, and the sampling and preservation methods employed can be found in Bell et al. (2004). Briefly, *G. aculeatus* were sampled from five sites around the perimeter of Loberg Lake with minnow traps in late spring or early summer most years between 1990 and 2006. Fish were seined in 1990 and trapped through the ice in November in 1991. Between 1990 and 1993 stickleback were sampled from only at Site A. Preserved stickleback were stained with alkaline Alazarin Red S to make bony structures visible. Specimens collected from the extinct population in 1982 (when the lake was poisoned), and from an anadromous population in Rabbit Slough (RS) in 1997, 2000, and 2003 were also incorporated into this study for comparison.

Lateral plate morph (LPM) and LP number were scored for specimens collected between 2002 and 2006 and $\geq$32 mm standard length (SL), by which size all plates have ossified in most populations (e.g., Hagen and Gilbertson 1972; Bell 2001). Lateral plate phenotype was scored following Bell et al. (2004), and all specimens from sites A-E were

assigned to a morph (complete, intermediate partial, partial, intermediate low, or low) based on maximal plate expression on either side. Scores for these traits between 1990 and 2001 and for the extinct population collected in 1982 are available in Bell et al. (2004). Plate morph frequencies reported are weighted means from the five sites. Annual sample sizes across all sites ranged from 2,125 to 7,245 and averaged 4,696. Plates were counted in subsamples of 200 low morphs. Gill rakers are considerably more tedious to count than LP, so GR counts were generally taken from samples of 50 specimens. Sexual dimorphism of trophic structures and GR length has been reported for stickleback from some populations (e.g., Reimchen and Nosil, 2006), but there is no evidence of sexual dimorphism in GR number in the Loberg Lake population or the anadromous Rabbit Slough population, which is its most likely ancestor (Aguirre, in prep.). Nonetheless, we opted to be conservative and counted GR only from male specimens for this analysis. Counts from random samples and samples only based on males were very similar and did not differ significantly in the anadromous Rabbit Slough population (random mean ($\pm$ standard error of the mean) = 22.36$\pm$0.101, n=202; males mean = 22.31$\pm$0.112, n=150) or the Loberg Lake extinct 1982 (random mean = 20.82$\pm$0.193, n=50; males mean = 20.94$\pm$0.112, n=17) or extant 1990 (random mean = 22.34$\pm$0.189, n=50; males mean = 22.29$\pm$0.329, n=17) samples (anova, $P \geq 0.751$ in all cases). Gill rakers were counted from the first right gill arch of 50 male specimens from Loberg Lake most years between 1990 and 2006. Sample sizes in 1990, 1991, 1992, 1993, were 17, 14, 45, and 25 respectively. Gill raker counts were also collected from a sample of 17 male specimens from the extinct 1982 Loberg population and 150 male specimens from RS collected in 1997, 2000, and 2003 (50 specimens/year).

Estimates of Temporal Trends, Evolutionary Rates, and Selection:

Linear regression was used to analyze temporal trends in low morph mean LP number between 1991 and 2006, and in GR number between 1990 and 2006. Residuals were tested for serial autocorrelation using the Durbin-Watson test (Draper and Smith, 1998) and none was found for either trait (low morph LP number: $d = 2.615$, n = 16, $P > 0.1$; GR number: $d = 2.689$, n = 17, $P > 0.05$).

Time series rates of low morph plate number (between 1991 and 2006) and GR number (between 1990 and 2006) evolution were calculated following Hendry and Kinnison (1999). Briefly, darwins were calculated as the slope of the regression of ln(x) against the number of years (in millions) since the first annual sample. Haldanes were calculated as slope of the regression of x/s_p against the number of generations since the first annual sample, where x is the trait value for a particular year and s_p is the pooled standard deviation (s_p) across years. The pooled standard deviation was calculated as $\sqrt{[(SS_1 + SS_2 \ldots + SS_k)/((n_1 - 1) + (n_2 - 1) \ldots + (n_k - 1))]}$, where SS and n are the sum of squares and sample sizes for k annual samples, respectively. Generation times were assumed to be two years, which is typical for Cook Inlet stickleback (e.g., Havens et al., 1984; Baker, 1994). Standard errors and 95% confidence intervals for the evolutionary rates were calculated using univariate methods for slopes (Sokal and Rohlf, 1995).

We estimated the average strength of selection on low morph LP number and GR number following Hendry and Kinnison (1999). The intensity of selection, which is the standardized selection differential (Falconer and Mackay, 1996), was obtained by dividing the rate of evolution in haldanes by the trait's heritability. We used our narrow

sense heritability estimates of 0.879 and 0.511 for low morph LP number and GR number, respectively, for crosses of Loberg Lake fish (Aguirre et al., 2004). These estimates are similar to Hagen's (1973) estimates of 0.83 and 0.84 at 16 C for low morph LP number (however, his heritability estimate for fish reared at 21 C was 0.50) and 0.58 for GR number from other populations. The selection differential, which is an estimate of the mean phenotypic value of the individuals selected as parents (averaged over the time series in this case) expressed as a deviation of the population mean prior to selection (Falconer and Mackay, 1996), was estimated by multiplying the selection intensity by the pooled standard deviation of the trait.

DNA Extraction and Microsatellite Genotyping Methods:

A total of 94 Loberg Lake stickleback (52 complete morphs and 42 low morphs) were collected at site A in June of 2003 and separated for genetic analysis. The specimens were frozen at -80˚C until DNA was extracted from right pectoral and caudal fin clips through phenol-chloroform extraction. Stickleback from 15 other sites including 12 lakes, one stream, and two sites harboring anadromous populations in the Matanuska-Susitna Borough and surrounding areas (Appendix 3) were included for comparative analysis of genetic diversity. Most samples (12 of 15) were from the Fish Creek Drainage immediately west of the Spring Creek drainage, which includes Loberg Lake, and one sample (Mud Lake) was from a drainage east of Spring Creek. Samples generally consisted of 32 specimens per site collected between 2003 and 2005 using methods and storage procedures described above.

Five microsatellite markers, Gac4170PBBE (linkage group, LG, 1) and Gac7033PBBE (LG 11) from Largiadèr et al. (1999), Cier62 (LG4) from Rico et al. (1993), and Gacμ9 (LG19) and Gacμ10 (LG 7) from Taylor (1998), were used to examine population genetic structure and genetic diversity (Appendix 4). Linkage group positions are from Peichel et al. (2001), except Gacμ9, which was provided by Peichel (pers. comm.). One of three fluorescent tags (FAM, HEX, or NED) was attached to each forward primer for visualization and scoring of PCR products, which was carried out on an Applied Biosystems 3730 DNA Analyzer at the University of Arizona Fragment Analysis Facility (http://gatc.arl.arizona.edu/). PCR reactions were carried out in 10 μl volumes consisting of 1x PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 2 mM $MgCl_2$, 0.25 mM dNTP (Invitrogen), 0.3-0.4 μM primers, 0.25-0.5 units of Taq DNA polymerase (Invitrogen), and approximately 20 ng of template DNA. PCR conditions consisted of one cycle at 95 C for 1 min 45 s, the locus specific annealing temperature (anneal temp) for 45 s, and 72C for 45 s; followed by four cycles of 94 C for 45 s, anneal temp for 45 s, and 72 C for 45 s; then 30 cycles of 92 C for 30 s, anneal temp for 45 s, and 72 C for 45 s; and a final extension of 72 C for 7 min. The annealing temperatures were 56 C for Gacμ9, 62 C for Gacμ10, 65 C for Gac4170PBBE, 58 C for Gac7033PBBE, and 65 C for Cier62.

Analysis of Genetic Data:

Low and complete morphs in Loberg Lake were tested for significant heterogeneity in allele frequencies using Fisher's exact test as implemented in Genepop version 3.4 (Raymond and Rousset, 1995), G-tests as implemented in BIOMstat vers.

3.30o (Applied Biostatistics, 2002), and by testing whether the pairwise F_{st} value between completes and lows was significantly different from zero. The variance between lows and completes, F_{st}, was calculated as a weighted analysis of variance following Weir and Cockerham (1984) using Genepop version 3.4 (Raymond and Rousset, 1995). We also tested for significant heterogeneity in allele frequencies between the Loberg Lake population and the Rabbit Slough population. Pairwise F_{st} values calculated as above, Reynolds' coancestry coefficient (Reynolds et al., 1983) as calculated in Arlequin version 2.0 (Schneider et al., 2000), Nei's unbiased genetic distance (Nei, 1978), Cavalli-Svorza's Chord distance (Cavalli-Svorza and Edwards, 1967), and Rogers Genetic distance (Rogers, 1972) as calculated in NTSYSpc (Rohlf, 2004), were used to examine levels of genetic divergence between Loberg Lake and the other populations included in this study. To contrast levels of genetic diversity in the Loberg Lake population with those of the other populations included in the study, we calculated average observed heterozygosity as the proportion of heterozygotes at each locus averaged across loci, the expected heterozygosity as $H_e = 1 - \sum p_i^2$, where p is the frequency of the ith allele, the effective number of alleles as $n_e = 1/\sum p_i^2$, and allelic diversity as the average number of alleles per locus for each sample following Frankham et al. (2002). The sample size for the Loberg Lake population was substantially larger than those of other populations, so we used the first 32 specimens in the sample to calculate its allelic diversity. The other measures used are relatively robust to differences in sample size, and the difference between estimates from all specimens and the first 32 specimens was relatively small and did not affect the pattern described. Nonetheless, we used values from the reduced sample to be conservative.

GENPOP (version 3.4, Raymound and Rousset, 1995) was used to test loci for linkage disequilibrium (10,000 batches, 10,000 iterations per batch) and departures from Hardy-Weinberg equilibrium frequencies (1,000 batches, 1,000 iterations per batch). None of the loci were in linkage disequilibrium within populations, and only four of the 160 tests within populations were individually significant ($\alpha = 0.05$). These did not correspond to the same pair of loci and are fewer than expected by chance given the multiple tests performed. Pairs of loci tested across all populations were not significantly linked either ($P \geq 0.575$). One of the loci, Gacμ9 exhibited significant departures from Hardy Weinberg in 15 of the 16 populations surveyed. This locus is located on linkage group 19, which appears to be an evolving sex chromosome (Peichel et al., 2004), and 38 of the 40 Loberg Lake males were homozygous at this locus, which is consistent with the presence of a null alleles in male chromosomes. Although none of the tested populations deviated significantly from Hardy-Weinberg expectations at Gacμ10, this locus deviated significantly in other populations in the area (Chapter 5). To be conservative, we calculated the measures of genetic diversity and divergence in several ways to assure that results were not biased by a particular locus. First, we calculated all estimates of genetic diversity and genetic divergence between the Loberg Lake population and neighbors using only Gac4170PBBE, Gac7033PBBE, and Cier62 (the three loci for which there is no evidence of deviations from Hardy-Weinberg) and compared the results to estimates using all five loci; the pattern observed was the same. Second, we calculated expected heterozygosity (as our measure of genetic diversity) and pairwise F_{st} values (as our measure of genetic divergence) excluding Gac4170PBBE, Gac7033PBBE, and Cier62 one at a time from the set of five loci to ensure that none of these loci had an inordinate

effect on the result. Once again, the pattern was the same for all sets indicating that the results are robust to the effects of individual loci. Therefore, we present the results including all five loci.

RESULTS

Temporal Evolution of Lateral Plates and Gill Rakers:

Both LPM frequencies and low morph left LP number continued to evolve between 2002 and 2006 in similar fashion to 1990 – 2006 (Bell et al., 2004). The complete morph declined from 11.2% in 2001 to 8.4% in 2002, and continued to decline steadily thereafter to 3.2% in 2006 (Table 1). Lows increased from 0% in 1990 to 75.2% in 2001, and to 87.8% in 2006, whereas the intermediate partials, partials, and intermediate lows remained relatively stable in frequency (Fig. 1). By 2004, the frequency of partials was greater than that of completes. Within 25 years of establishment, the population has gone from being almost monomorphic for the complete morph (frequency = 95.9%) and lacking low morphs (measurable frequency = 0%) in 1990, to being dominated by the low morph in 2006, with completes comprising only about 3% of the fish.

The mean low morph left LP number in 1991, the first year that they appear in Loberg Lake, was 6.87 (Fig. 2). This mean is based on a small number of specimens (15) and is included here for completeness (it was not included in Bell et al., 2004). It is similar to the mean for the 1992 sample, 6.82. By 2001, LP number had declined to 6.37, and in 2006 the mean was 5.91 (Fig. 2). The decline in left LP number across the entire time series (1991-2006) was significant (regression analysis, $F_{1, 14} = 45.552$, $P < 0.001$).

Despite the significant decrease, LP number in the modern population (mean = 5.91) is still much higher than that of the extinct Loberg Lake population (mean = 5.08).

Mean gill raker number also generally declined significantly over the time series (Fig. 3; regression analysis, $F_{1, 15} = 15.449$, P = 0.001). The mean for the 1990 sample (22.29) was very similar to that of the ancestral anadromous population (22.31), and did not differ significantly from it (anova: F = 0.002, DF = 1, 165, P = 0.965). There was irregularity in the pattern of change, particularly early in the time series, but this is expected in natural populations subjected to stochastically changing environmental conditions over time (e.g., Grant and Grant, 2002). In addition, the 1991 sample is relatively small in number, was collected a different time of year, and was aberrant for other phenotypic traits like body shape (Aguirre, in prep.), so we avoid making too much of this early heterogeneity. Counts declined to a mean of 20.66 in 2006, below the mean GR count for the extinct population (20.94), but not significantly different from it (anova: F = 0.614, DF = 1, 65, P = 0.436). Consequently, the modern Loberg Lake population has evolved a GR count similar to that of the original population inhabiting the lake within 25 years of colonization.

The rates of evolution, and selection differential and intensity for the low morph LP number over the complete time series between 1991 and 2006 (Table 2), were similar to the previous estimates based on samples collected between 1992 and 2001 (Bell et al., 2004). Rates of evolution for GR number were approximately the same in haldanes, in which the confidence intervals overlapped, or slightly lower in darwins than the evolutionary rate for low morph LP number. However, the selection differential and the

intensity of selection for GR number were slightly higher than for low morph LP number, despite the lower evolutionary rates.

Population Genetic Structure, Genetic Divergence, and Genetic Diversity:

Allele frequencies did not differ significantly between low and complete morph stickleback in Loberg Lake for any of the five loci tested (Fisher's exact test, $P \geq 0.178$ for all loci; G-test, $P \geq 0.108$ for all loci), and there was no evidence of significant population structure either, F_{st} values between lows and completes were 0 for all loci (actual values ranged from -0.0053 to -0.0027). Complete and low morphs in Loberg Lake were pooled for the remaining analyses. The Loberg Lake population differed significantly from its most likely ancestor, the anadromous RS population, at all loci (Fisher's exact test, $P < 0.0001$ for all loci). The F_{st} value between the Loberg Lake and RS populations was 0.0713, which falls into the "moderate differentiation" category on Wright's scale (Conner and Hartl, 2004). Comparing the Loberg Lake population to the other anadromous population included (Mud Lake) yielded similar results; the two anadromous populations did not differ significantly from one another (Fisher's exact test, $P < 0.0001$ for all loci, pairwise $F_{st} = -0.0033$).

Genetic diversity in the Loberg Lake population was comparable to neighboring resident freshwater populations; the pattern was same for the four measures assessed, so we only present the expected heterozygosity and the effective number of alleles (Fig. 4). Anadromous populations exhibited the highest levels of genetic diversity. The expected heterozygosity and the effective number of alleles for the Loberg Lake population were lower than for the anadromous samples, indicating some loss of genetic diversity but

within the range of the freshwater populations, indicating that the Loberg Lake population was not substantially bottlenecked compared to neighboring lake populations. The pattern of genetic divergence between the Loberg Lake population and the other populations was also similar across all measures examined (except Cavalli-Sforza's Chord distance which exhibited no pattern) so we only present the pairwise F_{st} values and Nei's unbiased genetic distance (Fig. 5). The Loberg Lake population was generally less divergent from the anadromous populations than from the lake and stream populations, which is consistent with its recent descent from anadromous stickleback.

DISCUSSION

Evolution following entry into a new adaptive zone represents a major source of evolutionary diversity (Simpson, 1953). Anadromous stickleback established resident freshwater populations that diverged considerably in response to novel selection pressures after the retreat of glaciers throughout the northern hemisphere over the last 20,000 years (Bell and Foster, 1994a; Schluter, 2000; McKinnon and Rundle, 2002). The Loberg Lake population, as well as other documented cases of contemporary colonization of freshwater habitats by *G. aculeatus* (e.g., Klepaker, 1993; Bell et al., 2004; Kristjánsson et al., 2002; von Hippel and Weigner, 2004), indicate that much of the evolutionary change following freshwater colonization may occur within a matter of decades. Coupled with frequent observations of phenotype-environment correlations, this suggests that individual postglacial stickleback populations are generally located near adaptive peaks. Evolution of postglacial stickleback populations is consistent with emerging conclusions from microevolutionary studies (Reznick et al., 1997; Hendry and

Kinnison, 1999; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001). Relatively high evolutionary rates are associated with adaptation to new environmental conditions (Hendry and Kinnison, 1999; Reznick and Ghalambor, 2001), as when anadromous stickleback populations colonize postglacial lakes and streams, and decline over time (Kinnison and Hendry, 2001), as occurs in postglacial stickleback populations as they approach an adaptive peak. Stasis, punctuated by rare bouts of rapid evolution in response to new environmental conditions, is common (Reznick et al., 1997; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001; Hendry et al., 2007), and is consistent with patterns in the fossil record (Gould and Eldredge, 1977).

The evolution of LPM frequencies in the Loberg Lake population is particularly striking. Although the frequency of the three intermediate phenotypes has remained relatively stable since they declined from transient maxima in 1993, the frequency of complete morphs has plummeted to 3.2% in 2006, and most stickleback in Loberg Lake now resemble typical resident lake stickleback for armor structure. Since our previous report on LP evolution in Loberg Lake (Bell et al., 2004), considerable progress has been made on the genetic basis of LPM polymorphism (Colosimo et al., 2004, 2005; Cresko et al., 2004; Kinsgley et al., 2004, 2007). The *Ectodysplasin* (*Eda*) locus, which regulates the development of ectodermal derivatives in vertebrates, plays a major role in regulating stickleback LP polymorphism throughout the world (Colosimo et al., 2005). Oceanic stickleback carry the low *Eda* allele as recessive variation at low frequencies (Colosimo et al., 2005). Consequently, evolution of LPM phenotypes in Loberg Lake does not require a special mechanism to account for the origin of the variation in such a short time period. The anadromous stickleback that founded the extant Loberg Lake population

probably carried the low *Eda* allele at low frequency. Selection on standing variation is likely also driving the evolution of low morph LP number, GR number, and body shape (Aguirre et al., in prep.). The presence of abundant genetic variation in ancestral taxa appears to be crucial for adaptation and the rapid evolutionary diversification that follows when they encounter new environmental conditions.

The comparable levels of genetic diversity between the Loberg Lake and neighboring freshwater populations is consistent with the abundant phenotypic variation that the population exhibits (Aguirre et al., in prep.). Although several studies have documented contemporary evolution of recently established freshwater stickleback populations (Klepaker, 1993; Kristjánsson et al., 2002; von Hippel and Weigner, 2004), none have documented levels of genetic diversity. Our results indicate that newly established postglacial populations probably do not go through severe genetic bottlenecks when founded, and retain substantial ancestral genetic variation. Assuming that neutral genetic variation serves as an indicator of long-term evolutionary potential (e.g., Frankham et al., 2002; McKay and Latta, 2002; but see Reed and Frankham, 2001), postglacial stickleback populations may retain considerable evolutionary potential, which is consistent with the evolutionary flexibility they exhibit in response to divergent selective pressures in freshwater environments (Bell and Foster, 1994b; McPhail, 1994; Schluter, 2000). Our results also indicate the populations invading novel adaptive zones can diverge genetically from their ancestors within decades, which is consistent with data from other studies of contemporary evolution (e.g., Hendry et al., 2000; Hendry, 2001; Quinn et al., 2001; Kinnison et al., 2002; Rasner et al., 2004). High levels of genetic diversity and lack of population genetic structure between the anadromous populations is

in accordance with previous research on the topic (e.g., Buth, 1994; Cresko, 2000; Reusch et al., 2001; Mäkinen et al., 2006).

We do not know the specific selective mechanisms driving the evolution of LP and GR in the Loberg Lake population, but the evolutionary trajectory of these traits is consistent with differences between anadromous and older postglacial resident freshwater populations. The first low morphs to appear in Loberg Lake in 1991 and 1992 had relatively high LP counts with means close to 7. Low morph plate counts of 7 are thought to be adaptive as a defense against fish predation (Hagen and Gilbertson, 1972; Reimchen, 1983, 1992), and appear to be the ancestral condition for low LP morphs when they first evolve (Bell et al., 2004). The mean low morph LP number of the original population inhabiting the lake was 5.08 (Bell et al., 2004), which is relatively low and consistent with the lack of native piscivorous fishes in the lake. Although Loberg Lake is stocked annually with rainbow trout and/or coho salmon, recreational fishing pressures are intense, and most are probably removed before they become a major source of mortality for adult stickleback. The significant decline in low morph LP number is consistent with evolution towards the value of the extinct population that originally inhabited the lake. However, despite already having declined 1.03 standard deviation units within 25 years of establishment (using the pooled standard deviation of 0.8808 for the extant population), low morph LP number has changed only 52.2% of the difference between its original value and that of the extinct population (taking the large 1992 sample as the starting point). If the population continues to evolve at the same rate, we estimate that it will take about 20 more years for low morphs to evolve a mean of 5.08 LP (using the regression equation for calculating rates in haldanes). However, it will take longer if

the rate decreases (Kinnison and Hendry, 2001) and presence of planted salmon and trout may halt divergence at a higher value.

Gill raker number also declined significantly, and by 2006 had reached the value of the extinct population. Evolution of trophic structures in general, and gill rakers in particular, is a major source of morphological diversity among postglacial fish populations (e.g., Bentzen and McPhail, 1984; Hart and Gill, 1994; Lu and Bernatchez, 1999; Bernatchez, 2004), so rapid evolution of gill raker number in the Loberg Lake population is not surprising either. Rates of evolution for low morph LP number and GR number were similar, especially in haldanes which are preferable to darwins for microevolutionary studies (Hendry and Kinnison, 1999). The selection estimates were higher for GR number, and the similar evolutionary rates despite higher selection estimates for GR number, reflect differences between traits in heritability estimates. The heritability for GR number is lower than that of low morph LP number (i.e., 0.511 and 0.879, respectively), and greater selection is necessary to cause comparable shifts is means for less heritable traits. Despite the similar evolutionary rates, mean LP number in 2006 is still about 0.94 standard deviation units from the value of the extinct population, whereas GR number has already reached it. The difference in the values for these two traits at the beginning of the time series for the extant population compared to the extinct population appears to be responsible. Low morph LP number in the extant population in 1992 was 1.97 standard deviation units from the value for the extinct population, whereas GR number in 1990 was only 1.055 standard deviation units from that of the extinct population (based on the pooled standard deviation of 1.283 for the extant population). The extinct Loberg Lake population had a mean GR count (20.94) that was slightly

higher than the average of 40 lake populations in Cook Inlet (mean = 20.51, range = 17.97 to 23.20) surveyed by Walker (1997). Loberg Lake is a relatively deep lake; the area of the lake bottom capable of supporting macrophyte growth, its relative littoral area (see Walker, 1997), is 27.05, which means that most of the lake bottom is too deep for vegetation to grow. Under these conditions stickleback tend to occur in the water column where they feed on relatively small zooplankton, which selects for longer, more numerous GR (Bentzen and McPhail, 1984; Gross and Anderson, 1984; Lavin and McPhail, 1986; Hart and Gill, 1994). Little is known about the ecology of oceanic stickleback, but they have high GR counts (Hagen, 1967; Gross and Anderson, 1984; Aguirre et al., in prep.) which are thought to be adaptive for feeding on planktonic prey items in the open ocean. The relatively small difference in GR counts between ancestral anadromous stickleback and the extinct 1982 sample of the Loberg Lake population is thus consistent with their presumed ecological habits.

Rates of evolution and selection estimates for low morph LP and GR number in the Loberg Lake population are not unusually high; they are among the more common rates for microevolutionary studies carried out over short time scales (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001), and far lower than the highest rates documented even for stickleback fish (Kristjánsson, 2002). Thus, although the Loberg Lake population is evolving measurably toward the phenotype of the original population inhabiting the lake, evolutionary rates and selection intensities are moderate compared to previously documented cases.

In summary, we present evidence that both armor structure and trophic morphology have evolved dramatically in the recently established Loberg Lake

population in the direction of the extinct population that originally inhabited the lake. This study adds to the growing list of cases of parallel evolution documented for threespine stickleback (e.g., Bell 1987; Bell and Foster 1994b; Schluter, 2000; Boughman et al., 2005; Colosimo et al., 2005; Marchinko and Schluter, 2007) and to the notion that parallel evolution is a common process in nature. Evolution of the Loberg Lake population indicates that phenotypes typical of resident freshwater populations evolve within decades. However, the rates of evolution and selection intensities associated with this rapid adaptation are not unusual for populations colonizing new environments, supporting the notion that adaptation to novel or changing environmental conditions is a major source of evolutionary diversity. Microsatellite variation indicates that Loberg Lake is inhabited by a single deme that has been polymorphic for lateral plate morphs, and that the population was not bottlenecked beyond levels typical for resident lake populations. Thus, the Loberg Lake population can be used as a model for adaptation of natural populations of threespine stickleback and other species to freshwater. Continued monitoring of rates and patterns of morphological evolution, and more thorough assessment of the population genetic structure and genetic diversity of this population, can expand our understanding of how postglacial stickleback adaptive radiations originate in nature.

LITERATURE CITED

Aguirre, W.E., P. Doherty, and M.A. Bell. 2004. Genetics of lateral plate and gill raker phenotypes in a rapidly evolving population of threespine stickleback. Behaviour 141:1465-1483.

Baker, J.A. 1994. Life history variation in female threespine stickleback. Pages 144-187 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback, Oxford University Press, Oxford.

Bell, M.A. 1984. Evolutionary genetics and phenetics, the threespine stickleback, *Gasterosteus aculeatus*, and related species. Pages 431-527 in B.J. Turner (ed.). Evolutionary genetics of fishes. Plenum Publishing Corporation.

Bell, M.A. 1987. Interacting evolutionary constraints in pelvic reduction of threespine sticklebacks, Gasterosteus aculeatus (Pisces, Gasterosteidae). Biological Journal of the Linnean Society 31:347-382.

Bell, M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica* 112/113:45-461.

Bell, M.A. and S.A. Foster (eds.). 1994a. The Evolutionary Biology of the Threespine Stickleback. Oxford University Press, Oxford.

Bell, M.A., and S.A. Foster. 1994b. Introduction to the evolutionary biology of the threespine stickleback. Pages 1-27 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press.

Bell, M.A., and K.E. Richland. 1981. Clinal variation of lateral plates in threespine stickleback fish. American Naturalist 117:113-132.

Bell, M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Bentzen, P., and J.D. McPhail. 1984. Ecology and evolution of sympatric sticklebacks: specialization for alternative trophic niches in the Enos Lake species pair. Canadian Journal of Zoology 62:2280-2286.

Bernatchez, L. 2004. Ecological theory of adaptive radiation, an empirical assessment from coregonine fishes (Salmoniformes). Pages 175-207 in A.P. Hendry and S.C. Stearns. Evolution illuminated, salmon and their relatives. Oxford University Press, Oxford.

Boughman, J.W., H.D. Rundle, and D. Schluter. 2005. Parallel evolution of sexual isolation in sticklebacks. Evolution 59:361-373.

Buth, D.G., and T.R. Haglund. 1994. Allozyme variation in the *Gasterosteus aculeatus* complex. Pages 61-84 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press.

Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Colosimo, P.F., C.L. Peichel, K. Nereng, B.K. Blackman, M.D. Shapiro, D. Schluter, & D.M. Kingsley. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PloS Biology 2:635-641.

Colosimo, P.F., K.E. Hoseman, S. Balabhadra, G. Villareal Jr., M. Dickson, J. Grimwood, J. Schmtz, R.M. Myers, D. Schluter, and D.M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928-1933.

Conner, J.K., and D.L. Hartl. 2004. A primer of ecological genetics. Sinauer Associates, Inc., Sunderland, MA.

Cresko, W.A. 2000. The ecology and geography of speciation: a case study using an adaptive radiation of threespine stickleback in Alaska. Ph.D. Dissertation, Clark University, Worcester, MA, USA.

Cresko, B., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. Bell, C. Kimmel, & J. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. PNAS 101:6050-6055.

Draper, N.R., and H. Smith. 1998. Applied regression analysis. John Wiley & Sons, Inc. New York.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Longman Group Ltd, Harlow, UK.

Francis, R.C., A.C. Havens, and M.A. Bell. 1985. Unusual lateral plate variation of threespine sticklebacks (*Gasterosteus aculeatus*) from Knik Lake, Alaska. Copeia 1985:619-624.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge.

Gould, S.J., and N. Eldredge. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3:115-151.

Grant, P.R., and B.R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296:707-711.

Gross, H.P., and J.M. Anderson. 1984. Geographic variation in gillrakers and diet of European threespine sticklebacks, *Gasterosteus aculeatus*. Copeia 1984:87-97.

Hagen, D.W. 1967. Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). Journal of the Fisheries Research Board of Canada 24:1637-1692.

Hagen, D.W. 1973. Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculeatus*. Heredity 30:303-312.

Hagen, D.W., and L.G. Gilbertson. 1972. Geographic variation and environmental selection in *Gatserosteus aculeatus* L. in the Pacific northwest, America. Evolution 26:32-51.

Hagen, D.W., and L.G. Gilbertson. 1973. Selective predation and the intensity of selection acting upon the lateral plates of threespine sticklebacks. Heredity 30:273-287.

Hart, P.J.B., and A.B. Gill. 1994. Evolution of foraging behaviour in the threespine stickleback. Pages 207-239 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press.

Havens, A.C., D.E. Sweet, C.L. Baer, and T.J. Bradley. 1984. Investigation of threespine stickleback abundance in landlocked Matanuska-Susitna Valley lakes. Alaska Department of Fish and Game, Juneau, AK.

Hendry, A.P., 2001. Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. Genetica 112-113:515-534.

Hendry, A.P., and M.T. Kinnison. 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution 53:1637-1653.

Hendry, A.P., J.K. Wenburg, P. Bentzen, E.C. Volk, and T.P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516-518.

Hendry, A.P., T.J. Farrugia, and M.T. Kinnison. 2007. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology, in press.

Kingsley, D., B. Zhu, K. Osoegawa, P. J. de Jong, J. Schein, M. Marra, C. Peichel, C. Amemiya, D. Schluter, S. Baldhadra, B. Friedlander, Y. M. Cha, M. Dickson, J. Grimwood, J. Schmutz, W. S. Talbot, and R. Myers. 2004. New genomic tools for molecular studies of evolutionary change in sticklebacks. Behaviour 141:1331-1344.

Kingsley, D. M. and C. L. Peichel. 2007. The molecular genetics of evolutionary change in sticklebacks. Pages 41-81in S. Östlund-Nilsson, I. Mayer, and F. A. Huntingford (eds.), Biology of the three-spined stickleback, CRC Press, Boca Raton.

Kinnison, M.T., and A.P. Hendry. 2001. The pace of modern life: from rates of contemporary microevolution to pattern and process. Genetica 112-113:145-164.

Kinnison, M.T., P. Bentzen, M.J. Unwins, and T.P. Quinn. 2002. Reconstructing recent divergence: evaluating nonequilibrium population structure in New Zealand Chinook salmon. Molecular Ecology 11:739-754.

Klepaker, T. 1993. Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1231-1258.

Klepaker, T. 1996. Lateral plate polymorphism in marine and estuarine populations of the threespine stickleback (*Gasterosteus aculeatus*) along the coast of Norway. Copeia 1996:832-838.

Kristjánsson, B.K., S. Skúlason, and D.L.G. Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659-672.

Largiadèr, C.R., V. Fries, B. Kobler, and Th.C.M. Bakker. 1999. Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.). Molecular Ecology 342-344.

Lavin, P.A., and McPhail. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 43:2455-2463.

Lu, G., and L. Bernatchez. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution 53:1491-1505.

Mäkinen, H.S., J.M. Cano, and J. Merilä. 2006. Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. Molecular Ecology 15:1519-1534.

Marchinko, K.B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution 61:1084-1090.

McKay, J.K., and R.G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution 17:285-291.

McKinnon, J.S., and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model system. Trends in Ecology and Evolution 17:480-488.

McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62:1402-1408.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the

sticklebacks (*Gasterosteus*) of south-western British Columbia. Pages 399-437 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Moodie, G.E.E., and T.E. Reimchen. 1976. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25:49-61.

Moyle, P.B., and J.J. Cech, Jr. 1996. Fishes, an introduction to ichthyology. Prentice Hall, Upper Saddle River, NJ.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Östlund-Nilsson, S., I. Mayer, and F. A. Huntingford (eds.). 2006. Biology of the three-spined stickleback. CRC Press, Boca Raton.

Peichel, C.L., C.S. Nereng, K.A. Ohgi, B.L.E. Cole, P.F. Colosimo, C.A. Buerkle, D. Schluter, and D.M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901-905.

Peichel, C.L., J.A. Ross. C.K. Matson, M. Dickson, J. Grimwood, J. Schmutz, R.M. Myers, S. Mori, D. Schluter, and D. Kingsley. 2004. The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Current Biology 14:1416-1424.

Quinn, T.P., M.T. Kinnison, and M.J. Unwin. 2001. Evolution of Chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. Genetica 112-113:493-513.

Rasner, C.A., P. Yeh, L.S. Eggert, K.E. Hunt. D.S. Woodruff, and T.D. Price. 2004. Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. Molecular Ecology 13:671-681.

Raymond M., and Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Reed, D.H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095-1103.

Reimchen, T.E. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). Evolution 37:931-946.

Reimchen, T.E. 1992. Injuries on stickleback from attacks by a toothed predator

(*Oncorhynchus*) and implications for the evolution of lateral plates. Evolution 46:1224-1230.

Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. Pages 240-276 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Reimchen, T.E. 2000. Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition.

Reimchen, T.E., and P. Nosil. 2006. Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipielago on the west coast of Canada. Canadian Journal of Zoology 84:643-654.

Reusch, B.H., K.M. Wegner, and M. Kalbe. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographic proximity. Molecular Ecology 10:2435-2445.

Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for short-term genetic distance. Genetics 105:767-779.

Reznick, D.N., and C.K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112-113:183-198.

Reznick, D.N., F.H. Shaw, F.H. Rodd, and R.G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934-1937.

Rico, C., D. Zadworny, U. Kuhnlein, and G.J. Fitzgerald. 1993. Characterization of hypervariable microsatellite loci in the threespine stickleback *Gasterosteus aculeatus*. Molecular Ecology 2:271-272.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Publication 7213:145-153.

Rohlf, F.J. 2004. NTSYSpc version 2.11w. Applied Biostatistics, Inc. Port Jefferson, NY.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press. Oxford.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Simpson, G.G. 1953. The major features of evolution. Columbia University Press, New

York.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry. W.H. Freeman and Company, New York.

Taylor, E.B. 1998. Microsatellites isolated from the threespine stickleback *Gasterosteus aculeatus*. Molecular Ecology 7:930-931.

von Hippel, F.A., and H. Weigner. 2004. Sympatric and anadromous-resident pairs of threespine stickleback species in young lakes and streams at Bering Glacier, Alaska. Behaviour 141:1441-1464.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Table 1. Sample size and lateral plate morph (LPM) frequencies for *Gasterosteus aculeatus* in Loberg Lake from 2002-2006. Frequencies for 1990-2001 are available in Bell et al. (2004). Lateral plate morph frequencies are weighted averages of all sites. Int. Partial and Int. Low are the intermediate partial and intermediate low lateral plate phenotypes, respectively.

Year	n	Complete	Int. Partial	Partial	Int. Low	Low
2002	2121	0.084	0.030	0.100	0.014	0.773
2003	7254	0.087	0.011	0.073	0.006	0.824
2004	5738	0.065	0.012	0.070	0.005	0.824
2005	3241	0.034	0.013	0.065	0.014	0.873
2006	5125	0.032	0.019	0.050	0.021	0.878

Table 2. Time-series rates of evolution in haldanes and darwins (± standard error), and selection intensities and differentials, for low morph left lateral plate number (LP) and gill raker number (GR) in *Gasterosteus aculeatus* from Loberg Lake. CI is confidence interval, Sel. Int. is the selection intensity and Sel. Diff. is the selection differential.

Rates	LP	GR
haldanes	-0.116 ± 0.017	-0.087 ± 0.022
95% CI	-0.153 to -0.079	-0.134 to -0.040
darwins	-7949.7 ± 1193.7	-2598.6 ± 657.6
95% CI	-10509.8 to -5389.5	-4000.3 to – 1196.9
Selection estimates		
Sel. Int.	-0.132	-0.170
Sel. Diff.	-0.116	-0.218

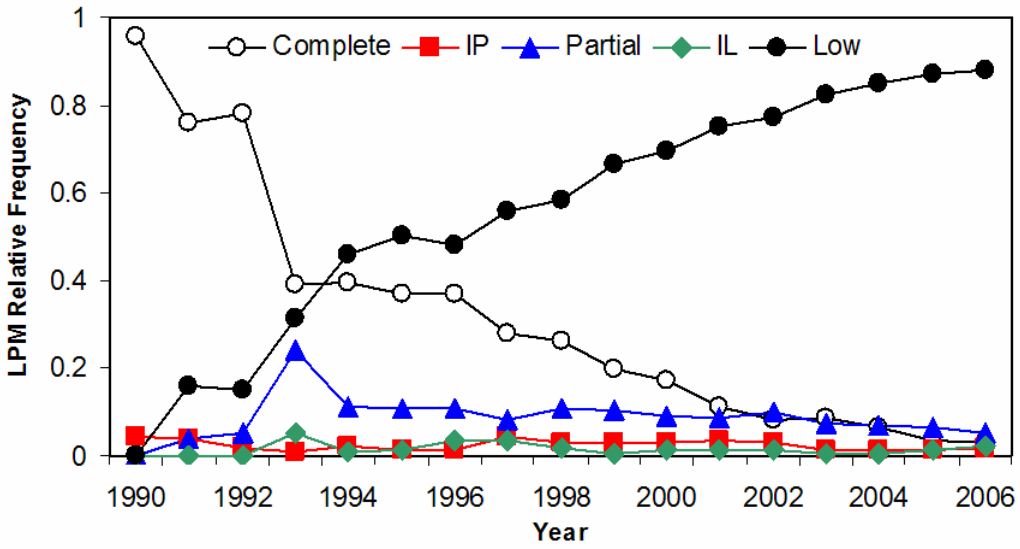


Fig. 1. Temporal variation of lateral plate morph (LPM) frequencies of *Gasterosteus aculeatus* from Loberg Lake based on complete samples from all sites. IP is the intermediate partial lateral plate phenotype, and IL is the intermediate low lateral plate phenotype.

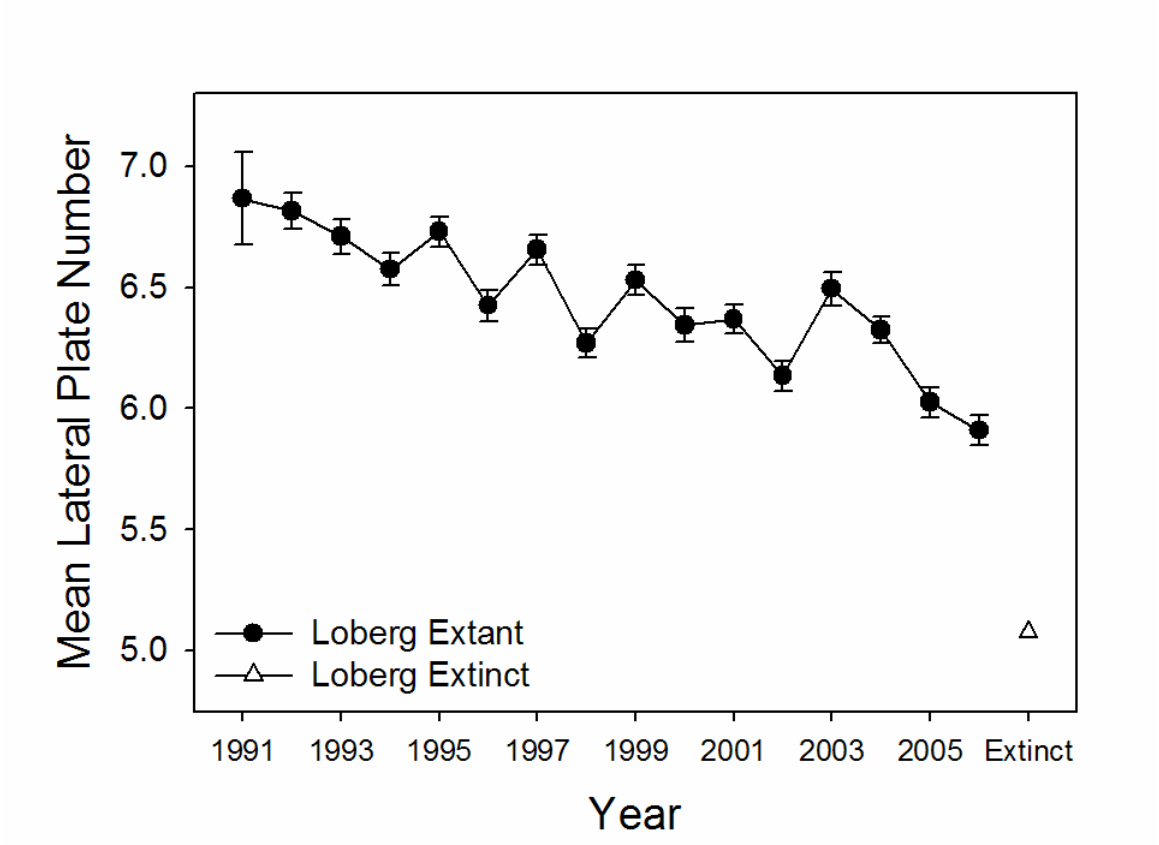


Fig. 2. Temporal variation of mean left lateral plate (LP) number of low morph *Gasterosteus aculeatus* from Loberg Lake. The error bars are standard error of the mean. Extinct is the mean for the extinct Loberg Lake population sampled in 1982. Sample sizes are 200 specimens except for the 1991, 1992, 1993, and extinct 1982 samples (see methods).

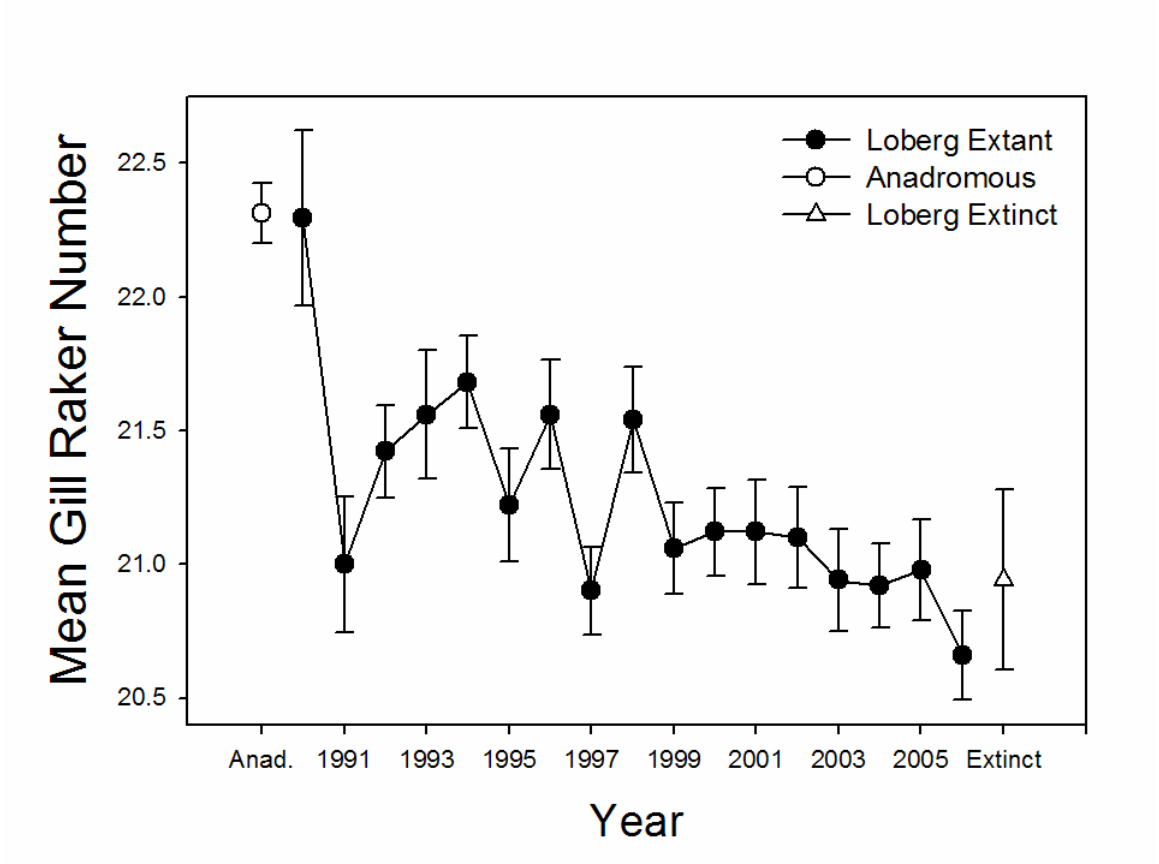


Fig. 3. Temporal variation of mean right gill raker (GR) number of *Gasterosteus aculeatus* from Loberg Lake and Rabbit Slough (Anad.). The error bars are standard error of the mean. Extinct is the mean for the extinct Loberg Lake population sampled in 1982. Sample sizes are generally 50 specimens, except the Rabbit Slough, 1990, 1991, 1992, 1993, and extinct 1982 samples (see methods).

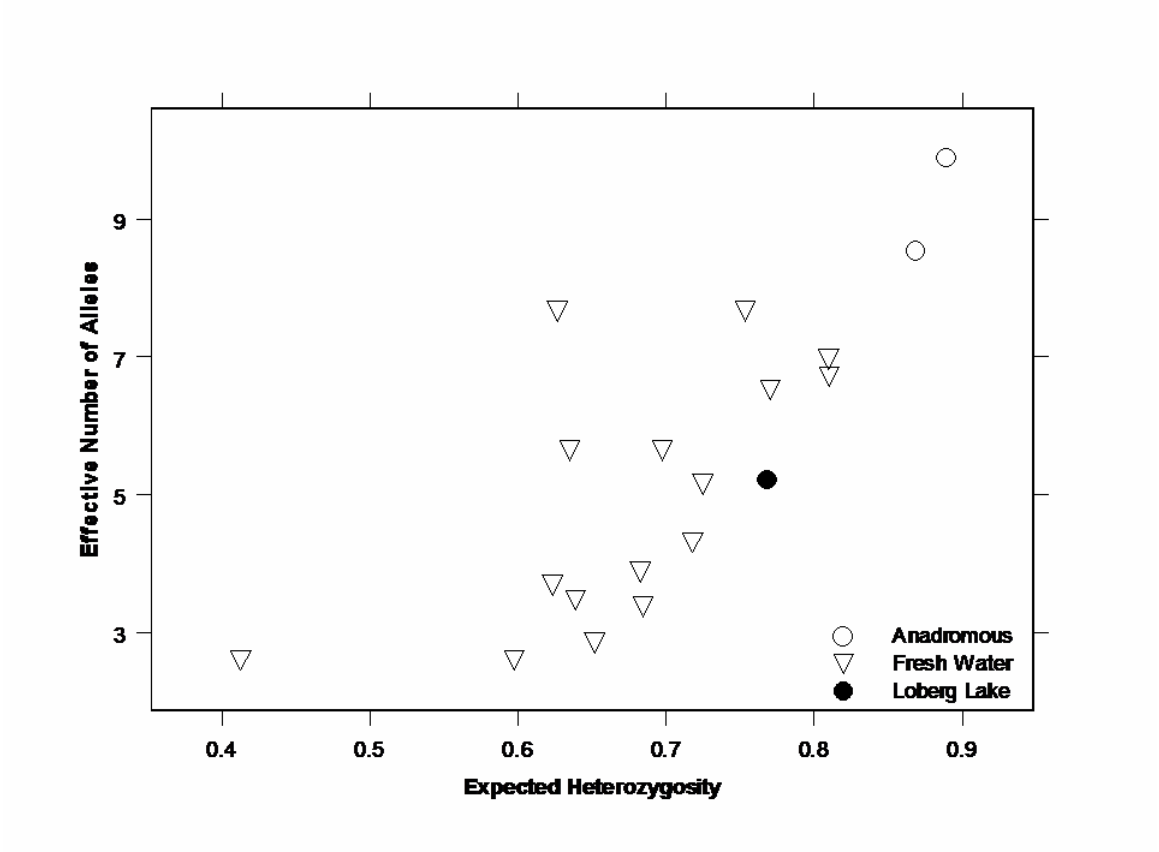


Fig. 4. Genetic diversity, as measured by the expected heterozygosity and the effective number of alleles, of the recently established Loberg Lake population compared to neighboring anadromous and resident freshwater populations.

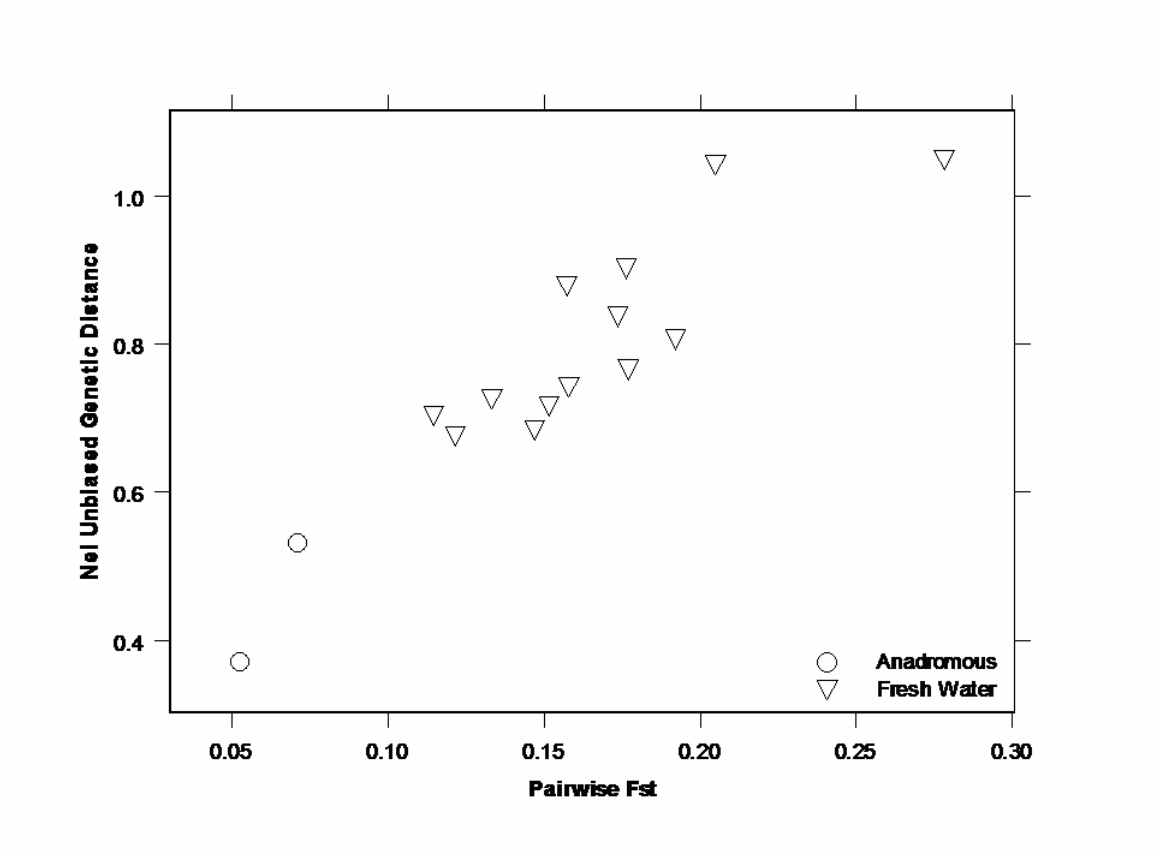


Fig. 5. Genetic divergence, as measured by pairwise F_{st} and Nei's Unbiased genetic distance, between the recently established Loberg Lake population and neighboring anadromous and resident freshwater populations. Each point reflects the distance between a particular population and Loberg Lake.

Chapter 5

The Geography of Adaptive Radiation: Gene Flow and Natural Selection Acting on Alaskan Threespine Stickleback

ABSTRACT

Understanding the factors that influence the early stages of adaptive radiation is an important problem in evolutionary biology. In this study, we used an ecologically diverse postglacial stickleback radiation in the upper Fish Creek drainage of Cook Inlet, Alaska to investigate the relationships of habitat type and geographic distance among sites with phenotypic and quasi-neutral genetic (microsatellite) variation. We also contrasted phenotypic divergence among sites to divergence from anadromous populations and tested whether phenotypic and genetic distances among sites are associated. Phenotypic divergence between resident freshwater and anadromous populations was greater than among resident freshwater populations, indicating that environmental differences between freshwater and marine habitats have a greater effect on fitness than differences among freshwater habitats. Phenotypic distances among samples were associated with habitat type but not with geographic distances, and phenotypic divergence was consistent with expectations based on the relative depth of the habitats. The forms most divergent phenotypically, deep lake and stream stickleback, were often separated by small geographic distances. Genetic distances among samples were associated with geographic distances but not morphological distances or habitat type. Thus divergent natural selection appears to have caused parapatric phenotypic

differentiation despite gene flow among adjacent constrasting populations. Genetic diversity was lowest among headwater lakes and highest in streams, indicating a downstream bias to gene flow. Headwater lakes also tend to be the most divergent genetically, indicating that they harbor significant evolutionary potential. Our study provides insight into the mechanisms regulating evolutionary diversification during the early stages of adaptive radiation and lays the foundation for future research on this ecologically diverse postglacial system.

INTRODUCTION

Understanding the factors responsible for adaptive radiation is a central topic in evolutionary biology. Divergent natural selection plays a key role during adaptive radiation, and its influence has been documented in numerous empirical studies (reviewed in Endler, 1986; Schluter, 2000). Indeed, given the demonstrated potential for natural selection to cause rapid adaptation (Hendry and Kinnison 1999), it is surprising that organisms are not evolving more (Merilä et al., 2001; Eldredge et al., 2005; Wiens and Graham, 2005; Estes and Arnold, 2007). "Ecological opportunity" (e.g., Schluter, 2000; Nosil and Reimchen, 2005) appears to be a major factor associated with divergent natural selection in nature. Ecological opportunity refers to the availability of underutilized resources. Invasion of a new habitat or "adaptive zone" (Simpson, 1953), particularly one that allows rapid population growth, can result in substantial divergence between ancestral and derived taxa over short time periods (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001). Diversification within the adaptive zone can also proceed rapidly, as evidenced by phylogenetic studies indicating

relatively rapid bursts of speciation during the initial stages of adaptive radiation (e.g., Hodges, 1997; Danley and Kocher, 2001; López-Fernández et al., 2005) and the existence of young adaptive radiations in isolated habitats (e.g., Echelle and Kornfield, 1984; Bell and Foster, 1994b; Skúlason et al., 1999; McKinnon and Rundle, 2002). Divergent natural selection is implicit during the early stages of adaptive radiation, but the impact of other factors, like gene flow between ecologically contrasting sites, is less clear. Gene flow can inhibit adaptive divergence by reducing the fitness of individuals adapted to ecologically contrasting habitats (Lenormand, 2002; Riechert, 1993; King and Lawson, 1995; Hendry et al., 2002; Hendry and Taylor, 2004; Moore and Hendry, 2005), or it can enhance it by providing adaptive genetic variation that had previously been absent from populations in contrasting habitats. However, its relative importance may vary among traits and taxa and depend on the geographic details of the system (e.g., Hendry and Taylor, 2004; Moore et al., 2007).

Postglacial threespine stickleback, *Gasterosteus aculeatus*, are among the most celebrated examples of adaptive radiation (e.g., Bell and Foster, 1994a; Schluter, 2000; McKinnon and Rundle, 2002; Östlund-Nilsson et al., 2006). Threespine stickleback are primitively oceanic but enter freshwater to reproduce. Thousands of new lakes and streams formed when glaciers retreated in coastal areas of the northern hemisphere beginning approximately 20,000 years ago. Anadromous (sea-run) stickleback move between marine and fresh water during their normal life cycle, and they have established resident populations that have adapted to these new environments, diverging from their oceanic ancestors and from one another in response to different selective pressures in contrasting habitats. Adaptation of threespine stickleback to local conditions is well

documented; stickleback often vary considerably in morphology even over short geographic distances based on divergent selective pressures (e.g., Bell and Richland, 1981; Bell, 1982; Baumgartner 1986, 1992; Reimchen et al., 1985; Hendry et al., 2002; Spoljaric and Reimchen, 2007). Divergent natural selection for the exploitation of different food resources is one of the most common diversifying mechanisms affecting both resident freshwater stickleback (Gross and Anderson, 1984; Bentzen and McPhail, 1984; Lavin and McPhail, 1986; Hart and Gill, 1994) and boreal fishes in general (Robinson and Wilson, 1994; Bell and Andrews, 1997; Robinson and Schluter, 2000).

Resident freshwater stickleback populations have evolved along a phenotypic continuum based on specialization on larger benthic food items associated with vegetation in shallow habitats or smaller planktonic prey found in open water in deeper habitats. This divergence is most dramatic in several lakes in British Columbia where sympatric "benthic" and "limnetic" species pairs exist (e.g., McPhail, 1984; 1994; Schluter, 1995; 2000; McKinnon and Rundle, 2002). Divergence between benthics and limnetics involves numerous traits, including body shape. Benthics are adapted to exploit larger food items associated with vegetation in shallow water and have deeper bodies better suited for maneuvering and burst swimming. Limnetics have narrower bodies that are better suited for sustained swimming in open water where they feed on small plankton. Although evolution along the benthic-limnetic continuum is common for resident stickleback, sympatric species pairs exist only in a few lakes in British Columbia (McPhail, 1994; McKinnon and Rundle, 2002; but see Cresko and Baker, 1996).

In this study, we explore phenotypic and genetic variation of threespine stickleback in a small, ecologically diverse Alaskan drainage to identify the major factors

influencing the partitioning of phenotypic and genetic variation. The upper Fish Creek drainage contains several lakes that differ substantially in relative depth and are connected by streams containing stickleback. These populations have been included in numerous evolutionary, ecological and behavioral studies, which have indicated that there is substantial phenotypic variability in the system (e.g., Francis et al., 1986; Bell and Orti, 1994; Baker et al., 1995; Walker, 1997; Baker and Foster, 2002; Cresko et al., 2004; Kimmel et al., 2005; Patankar et al., 2006; Purnell et al., 2006; Bell et al., 2007), but no previous study has systematically screened phenotypic and genetic variation within the drainage. We investigate phenotypic and genetic variation throughout this drainage in relation to habitat type and geographic position. Specifically, we address the following questions: (1) Is phenotypic variation associated with habitat type, geographic location within the drainage, or both? (2) How does phenotypic divergence among freshwater populations compare with divergence between freshwater and anadromous populations? (3) Are phenotypic distances among sites associated with genetic distances? (4) How is genetic variation structured among local populations within the drainage? This study explicitly takes into account the geographic context in which stickleback populations occur and is intermediate in scale in comparison with similar studies that have typically examined variation between lake and adjacent stream sites (e.g., Hendry et al., 2002; Hendry and Taylor, 2004; Moore and Hendry, 2005) or at larger regional or continental scales (Ortí et al. 1994; Reusch et al., 2001; Leinonen et al., 2006; Mäkinen et al., 2006). We use microsatellites as our measure of genetic variation and focus on body shape as our measure of phenotypic differentiation. Body shape is a composite trait that captures morphological variation throughout the body and is strongly associated with

multiple ecological variables that have important fitness consequences, including adaptation to different feeding regimes in shallow littoral and deeper open-water habitats (e.g., Taylor and McPhail, 1986; Walker, 1997; Spoljaric and Reimchen, 2007). Although phenotypic plasticity is common in fishes and is probably important during the initial stages of adaptation to new environments (West-Eberhard, 2003; Hendry et al., 2007), it seems to account for relatively little body shape variation among ecologically diverse postglacial stickleback populations (Hendry et al., 2002; Spoljaric and Reimchen, 2007; Aguirre and Caldecutt, unpublished data).

MATERIALS AND METHODS

Sampling Procedures:

Fish Creek is a small postglacial drainage in the Matanuska-Susitna Borough, Cook Inlet, Alaska. It includes Meadow, Little Meadow, and Lucille creeks in its upper portion (Fig. 1, Appendix 5). These creeks flow generally southwest into Big Lake, which in turn drains south into Fish Creek proper. Fish Creek runs south approximately 20.1 km before discharging into the Knick Arm of Cook Inlet, and there are few lakes associated with it south of Big Lake. We sampled 20 sites, including 12 lakes and eight stream locations in the upper Fish Creek drainage (i.e., above Big Lake). Our study did not include the full morphological diversity within the drainage because lakes lacking a stream connection, known to harbor stickleback populations with extreme armor reduction (Bell et al., 1985; Bell and Orti, 1994), were not included. Fish were collected in the spring (generally in June) between 2003 and 2005 with 6 to 20 unbaited 1/4 and/or 1/8 inch mesh minnow traps set overnight. Specimens were anesthetized with MS-222,

and either fixed in 10% formalin for morphological analysis or placed on dry ice and stored at -80˚C for DNA analysis. Eventually, specimens were transferred to 50% isopropanol for storage, and stained with Alizarin Red S to facilitate visualization of landmarks associated with bones. We included samples from two anadromous populations occurring in neighboring drainages collected in 2003 for comparison (Appendix 5), and samples collected in 1997 and 2000 from one of these populations (Rabbit Slough) were also included in the morphological analysis.

Collection of Body Shape Data:

We used geometric morphometric methods (Rohlf and Marcus, 1993; Adams et al., 2004; Zelditch et al., 2004) to study variation in body shape. 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. 2) using tpsDig version 1.40 (Rohlf, 2004b). The landmarks are based on Walker (1997), with the addition of a sixteenth landmark at the dorsal origin of the pectoral fin (Fig. 1). The landmark data were aligned using Procrustes superimposition implemented in the program tpsRelw version 1.44 (Rohlf, 2006) to eliminate variation related to rotation, translation, and size. All specimens in the study were included in a single alignment from which the shape variables were generated. We only included adult male specimens to minimize variation related to allometry and sexual dimorphism. Specimens infected with *Schistocephalus solidus* worms were also excluded because of the worms may distort body shape. Samples for morphological analysis generally consisted of 20 specimens per site (see Appendix 5 for details).

DNA Extraction and Microsatellite Genotyping Methods:

DNA was extracted from right pectoral and caudal fin clips of specimens using a phenol-chloroform DNA extraction method. Briefly, fins were digested overnight in 600 µl solution of 10 mM Tris pH 8.0, 100 mM NaCl, 10 mM EDTA, 0.5% SDS and 10 µl proteinase K (20mg/ml). An equal volume of 1:1 phenol-chloroform solution was added and DNA was separated by centrifugation at 12,100 rpm, washed with ethanol, and suspended in 100 µl of TE. Our working stock was 1:25 dilution in H_20. Samples generally consisted of 32 specimens per site (see Appendix 5 for details). We used five microsatellite markers, Gac4170PBBE and Gac7033PBBE from Largiadèr et al. (1999), Cier62 from Rico et al. (1993), and Gacµ9and Gacµ10 from Taylor (1998), to examine population genetic structure and genetic diversity (Appendix 7). One of three fluorescent tags (FAM, HEX, or NED) were attached to each forward primer for visualization and scoring of PCR products. PCR reactions were carried out in 10 µl volumes consisting of 1x PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 2 mM $MgCl_2$, 0.25 mM dNTP (Invitrogen), 0.3-0.4 µM primers, 0.25-0.5 units of Taq DNA polymerase (Invitrogen), and approximately 20 ng of template DNA. PCR conditions consisted of one cycle at 95 C for 1 min 45 s, the locus specific annealing temperature (anneal temp) for 45 s, and 72C for 45 s; followed by four cycles of 94 C for 45 s, anneal temp for 45 s, and 72 C for 45 s; then 30 cycles of 92 C for 30 s, anneal temp for 45 s, and 72 C for 45 s; and a final extension of 72 C for 7 min. The annealing temperatures were 56 C for Gacµ9, 62 C for Gacµ10, 65 C for Gac4170PBBE, 58 C for Gac7033PBBE, and 65 C for Cier62.

Genotyping based on repeat number was carried out on an Applied Biosystems 3730 DNA Analyzer at the University of Arizona Fragment Analysis Facility.

We used GENPOP (vers. 3.4, Raymound and Rousset, 1995) to test loci for linkage disequilibrium (10,000 batches, 10,000 iterations per batch) and departures from Hardy-Weinberg equilibrium (HW) frequencies (1,000 batches, 1,000 iterations per batch). None of the loci were in linkage disequilibrium; only six of the 220 tests within populations were individually significant ($\alpha = 0.05$). These did not correspond to the same pair of loci and are fewer than expected by chance. Two of the loci exhibited substantial departures from Hardy Weinberg equilibrium frequencies (HWE) within samples, however. Gacμ9 exhibited significant departures in 19 of the 22 populations, and Gacμ10 exhibited departures in 9 populations. Gacμ9 is located on linkage group 19 (C.L. Peichel, pers. comm.), which appears to be an evolving sex chromosome (Peichel et al., 2004). The deviation from HWE at this locus seems to be due to the presence of null alleles on the male chromosome. We carried out the Mantel tests (described below) several ways to ensure that our results are not an artifact of deviations from HWE or due to the influence of a single locus. We calculated genetic distances using only the three loci that were in HW, to ensure that the loci deviating from HW were not driving the results. We also excluded Gac4170PBBE, Gac7033PBBE, and Cier62 (the three loci in HW) one at a time from the set of five loci to ensure that none of these had an inordinate effect on the result. The outcome of the Mantel tests was the same in all cases indicating that the results are robust to the effects of individual loci. Similarly, the matrix correlation between genetic distance matrices including all five loci vs. the three loci in HW was 0.921. Therefore, we include all five loci in the analysis.

Multivariate Analyses:

Principal components analysis (PCA) was implemented in tpsRelw version 1.44 (Rohlf, 2006) to explore body shape variation among sample means. The PCA was carried out on all individuals with sample means calculated afterwards. We also pooled populations by habitat type (see below) and calculated the Procrustes distance between their consensus configurations using tpsSplin ver. 1.20 (Rohlf, 2004c) to evaluate levels of body shape divergence. A discriminant function analysis (DFA) in SPSS vers. 11.0.0 (SPSS Inc., 2001) was used to assess population and habitat level differentiation in body shape. A leave-one-out classification procedure was used such that each case in the analysis is classified by the functions derived from all cases other than that case. Two analysis were performed. In the first, the analysis determined rates of correct classification based on the collection site, and in the second, it determined rates of correct classification based on habitat type. Shape variables (partial warps and uniform component) obtained from a single alignment of all specimens done using tpsRelw vers. 1.44 (Rohlf, 2006) were used in the DFA.

The Mantel tests (Mantel, 1967; Sokal, 1979) implemented in PASSAGE version 1.1 (Rosenberg, 2001) were used to evaluate the association between geographic distances, habitat type distances, phenotypic distances, and genetic distances. The Mantel Test is a test of the association between corresponding elements of two distance matrices. Significance was assessed using the permutation procedure, with 99,999 permutations. We created four distance matrices: a geographic distance matrix, a habitat dissimilarity matrix, a phenotypic distance matrix, and a genetic distance matrix. The geographic

distance matrix consisted of ln-transformed distances in km measured along the stream between all sampling sites. The habitat distance matrix used four categories: stream (8 samples), shallow lake (seven samples), intermediate depth lakes (two samples), and deep lakes (three samples). Lake categories were based on their relative littoral area, a measure of the area of the bottom of the lake capable of supporting macrophyte growth (Walker 1997). Lakes < 40% relative littoral area were classified as deep, lakes between 40 and 60% as intermediate depth lakes, and lakes greater than 60% as shallow lakes. Scores of 0 and 1 were assigned to cells for pairwise comparisons of samples from the same and different habitat types respectively (e.g., cells corresponding to two samples from the same habitat were given a score of 0 and cells for samples from two different habitats were given a score of 1.), with the exception of intermediate depth lakes, which were given a value of 0.5 with deep and shallow lake samples. Procrustes distances between sample means were used for the phenotypic distance matrix. 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). We performed the Mantel tests using genetic distance matrices (calculated from the five loci) using Nei's unbiased genetic distance (Nei, 1978), Cavalli-Svorza's Chord distance (Cavalli-Svorza and Edwards, 1967), and Rogers Genetic distance (Rogers, 1972), calculated in NTSYSpc (Rohlf, 2004a), pairwise F_{st} values calculated following Weir and Cockerham (1984) using GENPOP vers. 3.4 (Raymound and Rousset, 1995), and Reynolds' coancestry coefficient (Reynolds et al., 1983) from Arlequin vers. 3.11 (Excoffier and Schneider, 2005) because there is little consensus on the best measure of

genetic distance to use with microsatellite data. The different genetic distance measures generally gave the same results so we used pairwise F_{st} values.

MANCOVA implemented in tpsRegr ver. 1.31 (Rohlf, 2005) was used to test the association between body shape and habitat type after obtaining the results of the Mantel test between body shape and geographic distances. Centroid Size was included as a covariate and the probability was computed through a permutation test (1000 permutations).

F_{st} values calculated following Weir and Cockerham (1984) with GENPOP (version 3.4, Raymound and Rousset, 1995) were used to evaluate population genetic structure. Genetic relationships among samples were also examined through cluster analysis of allele frequencies using PHYLIP vers. 3.66 (Felsenstein, 2005). One hundred bootstrapped allele frequency matrices were created and Reynolds, Weir and Cockerham's genetic distance was calculated between samples for all bootstrapped data sets. Neighbor-joining trees were obtained from each of these, and the program Consense was used to create a majority rule consensus tree. We used observed heterozygosity to examine differences in genetic diversity among sites throughout the drainage. Probability of pairwise F_{st} values was computed with Arlequin vers. 3.11 (Excoffier and Schneider, 2005). Finally, we used the model-based clustering program Structure (Pritchard et al., 2000) to further examine population genetic structure in the drainage. We used it to obtain an *ad hoc* approximation of the number of genetically distinct clusters present in the drainage, without providing *a priori* information about group membersip, and examined the assignment of our samples to these groups. Our data are probably at the limit of applicability of this method because we only have data for five loci, two of which

deviated from HWE (one of the criteria used for assignments), the radiation is relatively young, there is evidence of isolation by distance in the data (for which the program is not well suited), and genetic diversity and gene flow seem to be relatively high (see below),. The methods implemented in Structure can also be less powerful than traditional tests when probable populations can be predefined, as is the case here (Pritchard et al., 2007). Therefore, we use results from this analysis only in comparison with other results. We used an admixture model, with allele frequencies correlated, and burnin and Monte Carlo Markov chain values of 100,000 each. We ran the model for each value of k between 1 and 20 (where k is the number of possible clusters) three times and averaged the results to obtain the most likely number of genetic clusters.

RESULTS

Populations differed in body shape in the space spanned by principal components I and II, and there was a clear segregation among sample means based on habitat type (Fig. 3). The anadromous populations differed substantially from resident lake and stream populations and segregated along PC I. The difference in body shape between anadromous and freshwater populations was greater than differences among freshwater populations. This was confirmed by examining Procrustes distances between the consensus configurations of populations pooled by habitat type (Table 1). Differences in body shape between anadromous and freshwater populations were generally consistent with previous comparisons (Aguirre et al., in prep.). Anadromous stickleback tended to have a larger pelvis and ectocoracoid, a shorter caudal peduncle, anteriad positioned first dorsal spine, posteriodorsally displaced pectoral fins, and slightly longer median fins

(Fig. 4A). The freshwater resident populations also differed along PC II from stream to shallow, intermediate, and deep-lake samples, with broad overlap between stream and shallow-lake sample means and between shallow-lake and intermediate-depth lake sample means. Divergence in body shape along this gradient was largely associated with body depth. Shallow-lake and stream samples were relatively similar in body shape, with the largest landmark displacement occurring at the posterior tip of the pelvis (Fig. 4B). Stream and deep-lake samples were the most divergent phenotypically (Table 1), with fish in deep lakes having more elongate bodies (Fig. 4C).

The discriminant function analysis based on site of collection indicated significant phenotypic divergence among sites. Of 451 resident freshwater specimens, 64.7% were correctly assigned to their collection site, and 54.7% of the "misclassified" fish were assigned to other samples from the same habitat type (Appendix 6). Thus, only 16.0% of specimens were assigned to the wrong habitat type. Deep lake samples had the lowest misclassification percentage (14.5%) and shallow lake and stream samples had the highest (38.0 and 42.2% respectively). The same analysis carried out on habitat type yielded better classification results results, 78.3% of individuals were correctly assigned to their original habitat. Once again, deep lakes had the lowest misclassification rates, but intermediate depth lakes had the highest misclassification results instead of shllow lakes and streams. Misclassification percentages were generally lowest between ecologically similar habitat types except for deep lakes for which the greatest number of misclassified fish were assigned to shallow lakes (Table 2).

Variation in body shape among lake and stream samples was not associated with the geographic distances among them (Mantel Test, $r = -0.0518$, $Z = 195.64$, $P = 0.659$),

suggesting that geographic structure was not responsible for the pattern of phenotypic divergence observed. Phenotypic distances among lake and stream samples were significantly associated with the habitat type from which they were collected (Mantel Test, r = 0.353, Z = 7.455, P = 0.00024), suggesting that natural selection associated with the habitat occupied is the primary factor structuring phenotypic variation in this system of populations. MANCOVA (with centroid size included as a covariate) confirmed that body shape of resident lake and stream populations differed significantly among habitat types (Wilks $\lambda = 0.180$, $F_s = 11.54$, df1 = 84, df2 = 1254.3, $P < 0.001$).

Population genetic structure was moderate, with an F_{st} value of 0.0866, ranging from 0 to 0.233 between pairs of samples (the anadromous samples were not included in the calculations), and pairwise F_{st} values were significantly different from zero between most pairs of sites (Table 4). Lack of significant differences occurred only between neighboring sites (Fig. 5). Four sets of neighboring samples exhibited non-significant or marginally significant ($P > 0.01$) F_{st} values, forming sets with mixed habitat types: Lucille Lake and the three Lucille Creek samples; Big Lake, MC1 and LMC 1 and 2 samples; Stepan and Big Beaver lakes; and the LMC3 sample with both Cloudy and Rainbow lakes. Results of the program Structure yielded comparable results (Table 3). During the initial set of runs, the most likely number of genetic clusters was k = 6, with k = 5 being the second most likely value. However, two of the three values for k = 7 were quite similar to those of k = 6, and one was highly aberrant suggesting a longer number of runs was required. Consequently we increased burnin and Monte Carlo Markov chain values to 200,000 (400,000 total) for values of k between 5 and 8 and carried out three additional runs for each. The most likely number of genetic clusters was 7 (ln PR= -

12,5471, -12,513.1, and -12,518.5, avg. = -12,526.2), followed closely by k = 6 (ln PR= -12,578.5, -12,575.3, -12,531.4, avg. = -12,561.7). However, the proportion of samples falling into different genetic clusters were quite similar for the samples that clustered the best at both values of k. Averages for k = 5 and k = 8 were higher (-12,609.4 and -12,851.4 respectively). Clustering of some samples into the same genetic groups, especially the Meadow Creek, Little Meadow Creek, and shallow lake samples in the lower portions in the streams, was quite poor suggesting substantial genetic heterogeneity among these samples. Grouping of samples based on lack of significant F_{st} values described above tended to be reflected in the results of the cluster analysis as well. For example Lucille Lake and the three Lucille Creek samples largely clustered in the same group, as was the case for Stepan and Beaver Lakes, and Cloudy, Rainbow, and LMC3. The highest clustering values within populations occurred in samples located together toward the head waters of the same tributary (e.g., Beverley and Seymour).

Genetic distances among samples were significantly associated with geographic distances (Mantel test: r = 0.429, P = 0.011), but were not significantly associated with phenotypic distances (Mantel test: r = 0.052, P = 0.330) or habitat type (Mantel test: r = 0.093, P = 0.075), indicating that gene flow among sites based on their geographic location is the primary factor structuring genetic variation but that this gene flow does not prevent divergence of ecologically important traits. This was also supported by the neighbor-joining cluster analysis of samples (Fig. 6). Except for segregation of the anadromous samples from the freshwater samples, there was little evidence of clustering based on habitat type. Samples from different habitat types were interspersed throughout the tree, and higher bootstrap values were again associated with geographically adjacent

samples. Consequently, the major factors influencing phenotypic and neutral genetic variation in the upper Fish Creek Drainage differ.

Genetic diversity (as measured by observed heterozygosity) varied considerably among samples ranging from 0.48 to 0.78 for the freshwater populations (Fig. 5). Heterozygosity was highest in the anadromous samples as previously documented for stickleback (e.g., Buth, 1994; Cresko, 2000; Taylor and McPhail, 2000; Reusch et al., 2001; Mäkinen et al., 2006). The lowest heterozygosity among the Fish Creek samples tended to occur in headwater lakes (e.g., Frog, 0.48; Beverley, 0.52; Seymour, 0.60; and Lucille, 0.60), whereas stream sites tended to have high levels of heterozygosity.

DISCUSSION

The major axis of phenotypic variation in this study was largely associated with divergence between ancestral anadromous and derived resident freshwater populations, which has been documented previously (e.g., Walker and Bell, 2000; Leinonen et al., 2006) and is consistent with large phenotypic transitions occurring early in the process of adaptive radiation. Indeed, empirical studies indicate that high evolutionary rates are often associated with adaptation to novel conditions (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001), suggesting that environmental differences between freshwater and marine habitats have a greater effect on fitness than differences among freshwater habitats. This makes the stickleback system all the more remarkable, given recent findings that anadromous populations may evolve phenotypes characteristic of freshwater environments within decades after establishing resident populations

(Klepaker, 1993; Bell, 2001; Kristjánsson et al., 2002; Bell et al., 2004; von Hippel and Weigner, 2004; Aguirre et al., in prep.).

The magnitude of phenotypic evolution within the upper Fish Creek drainage matched expectations. Sample means tended to group by habitat type and phenotypic distances among sample means were associated with the type of habitat occupied, not with geographic distances among sites. In addition, body shape variation by habitat type was consistent with previous knowledge of evolutionary divergence associated with adaptation to shallow versus deep freshwater environments (McPhail, 1984, 1994; Reimchen et al., 1985; Schluter, 1993; Walker, 1997; Spoljaric and Reimchen, 2007). The stream and shallow lake sites were inhabited by deeper-bodied stickleback, while deep lakes were inhabited by more elongate stickleback. Thus, body shape in stickleback populations in the Upper Fish Creek drainage appears to be evolving along the "benthic" - "limnetic" continuum in response to site-specific selection, despite gene flow from ecologically divergent neighboring sites. Along this continuum, stream samples tended to be most similar to shallow lake samples, which is consistent with similarities in the environmental characteristics of these habitats and how stickleback feed within them. The stream habitats in which stickleback are most common are shallow, slow moving streams rich in vegetation, akin to habitats found in shallow lakes, and stickleback tend to eat benthic prey in both habitats. The greatest phenotypic divergence was between deep-lake and stream populations, which often occur parapatrically in this system, and between which gene flow is high.

Genetic variation did not exhibit the same pattern seen in phenotypic variation. Genetic distances among samples were not associated with morphological distances,

indicating that the system does not possess reproductively isolated ecomorphs. Genetic distances were significantly associated with geographic distances among samples, indicating that the geography of the drainage is the most important factor influencing segregation of neutral genetic variation. The prevalence of geographic features in the structuring of genetic variation among populations is common and expected (e.g., Bell and Richkind, 1981; Baumgartner, 1986; Taylor and McPhail, 1999; Crispo et al., 2006; Mäkinen et al., 2006) but not universal, even in stickleback (e.g., Reusch et al., 2001). Our result was supported by several lines of evidence including Mantel tests, pairwise F_{st} values, the neighbor-joining cluster analysis, and the cluster analysis carried out with the program Structure. In the upper Fish Creek drainage, natural selection appears to be pulling populations apart phenotypically based on the characteristics of the habitats that they occupy, while neutral genetic variation is homogenized based on the geographic distances. By implication, natural selection is acting on adaptively important genes, and we anticipate that allele frequencies at loci with adaptive functions will differ significantly among sites based on habitat type. Our results indicate that speciation is not a necessary prerequisite for adaptive radiation in geographically structured systems if selection is strong relative to gene flow. In addition, although populations in the system may go extinct occasionally, the rate of extinction must be low compared to rates of evolution to allow the substantial population differentiation observed (e.g., Harrison and Hastings, 1996).

Evolution and maintenance of phenotypic differentiation despite gene flow from ecologically divergent populations is not uncommon and depends on the interaction between the strength of selection and rate of migration (Endler, 1977; Slatkin, 1985;

Lenormand, 2002). Indeed, permanent clines are thought to result from the balance between gene flow and selection (Haldane 1948; Fisher, 1950; Endler, 1977; Slatkin, 1985). Empirical studies documenting morphological divergence in the face of gene flow have been reported previously (e.g., Thoday and Boam, 1959; Saint-Laurent et al., 2003) including in threespine stickleback (e.g., Baumgartner, 1992; McPhail, 1994). For example, Baumgartner (1992) found significant phenotypic differentiation in armor structure and body shape in stickleback from a small coastal drainage in California despite relatively high gene flow. Our results add to the growing list of such cases and provide a basis for exploring how gene flow and divergent natural selection interact in this system in much greater detail. It would be particularly interesting to examine the interaction between pairs of ecologically divergent, neighboring populations, like the parapatric deep-lake and stream populations. This system seems to present favorable conditions for the evolution of reinforcement (e.g., Rundle and Schluter, 1998). Deep and intermediate depth lakes possess shallow water habitat along their margins, which presumably is not occupied by individuals well adapted to exploit it. Research aimed at understanding why species pairs have not evolved, given that the phenotypic and ecological variation necessary for their occurrence seems to be present within the drainage and sympatry of resident lake and anadromous stickleback in the region (Karve 2007), would be an interesting direction for future research.

The geographic structuring of genetic variation was consistent with observations form other systems. Genetic diversity was lower and genetic differentiation was greater in headwater lake populations than in downstream lake and stream populations. The lower genetic diversity at headwater lakes is consistent with a downstream bias to gene

flow which is common in lake/stream systems (Bell and Richkind, 1981; Hendry et al., 2002; Moore and Hendry, 2005; Crispo et al., 2006; Moore et al., 2007). Reduced rates of gene flow upstream would also allow greater genetic differentiation of headwater lakes, as was observed. Consequently, although the greatest differentiation within the drainage occurred between stream and deep lake sites, which tended to be lower in the system, there seems to be significant potential for evolutionary divergence at upstream sites. Because they appear to receive genes from lakes throughout the system, stream populations in the upper Fish Creek drainage exhibit high genetic diversity, though they probably harbor much smaller populations than lakes. Stream stickleback are also relatively homogeneous genetically over long geographic distances, indicating high levels of gene flow between adjacent sites. Thus stream populations seem to act as a reservoir of the genetic diversity occurring throughout the drainage, which has interesting evolutionary implications. Assuming that stream stickleback are the most likely to colonize newly formed or empty lakes (e.g., after a winter-kill), the newly established population will likely already contain abundant adaptive genetic variation from throughout the drainage for selection to act on. In addition, most anadromous populations enter streams and if hybridization occurs, it is most likely to occur with resident stream fish. Thus as a consequence of the effect of geographic setting on patterns of gene flow, anadromous fish would have access to much of the genetic diversity found throughout the drainage, if they hybridize with stream fish. This could be significant if introgression of freshwater alleles into anadromous populations is an important source of adaptive genetic variation for establishment of new resident freshwater populations. Standing genetic variation in anadromous populations is clearly an important source of variation for

selection to act on in postglacial environments, and mechanisms increasing that variation would facilitate rapid responses to new or changing environmental conditions. For example, hybridization between freshwater resident and anadromous stickleback may play a role in increasing the frequency of the *Ectodysplasin* low allele in anadromous populations. This allele exhibits low penetrance, occurs at low frequencies in oceanic stickleback populations, and has been selected in parallel throughout the world for its role in armor reduction (Colosimo et al., 2005).

In summary, both divergent natural selection and gene flow among geographically neighboring sites are important during the early stages of adaptive radiation, but these two processes affect phenotypic and genetic variation in different ways. Gene flow is probably sufficient to provide ample variation at loci influencing adaptive phenotypes but low enough to prevent swamping of local genetic differentiation. In addition, rates of adaptation to local conditions are high enough to produce population differentiation even if lake populations occasionally go extinct and are recolonized by stream fish; selection is rapid compared to the rate of extinction. This study lays the foundation for future research on the factors regulating adaptive radiation in this ecologically diverse postglacial drainage.

LITERATURE CITED

Adams, D.C., F.J. Rohlf, and D.E. Slice. 2004. Geometric morphometrics: ten years of progress following the “revolution”. Italian Journal of Zoology 71:5-16.

Baker, J.A., and S.A. Foster. 2002. Phenotypic plasticity of life history traits in a stream population of the threespine stickleback, *Gasterosteus aculeatus* L. Ecology of Freshwater Fishes 11:20-29.

Baker, J.A., S.A. Foster, and M.A. Bell. 1995. Armor morphology and reproductive output in threespine stickleback, *Gasterosteus aculeatus*. Environmental Biology of Fishes 44:225-233.

Baumgartner, J.V. 1986. The genetics of differentiation in a stream population of the threespine stickleback, *Gasterosteus aculeatus*. Heredity 57:199-208.

Baumgartner, J.V. 1992. Spatial variation of morphology in a freshwater population of the threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 70:1140-1148.

Bell, M.A. 1982. Differentiation of adjacent stream populations of threespine sticklebacks. Evolution 36:189-199.

Bell, M.A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica* 112/113:45-461.

Bell, M.A. and C.A. Andrews. 1997. Evolutionary consequences of postglacial colonization of fresh water by primitively anadromous fishes. Pages 323-363 in B. Streit, T. Strädler, and C.M. Lively (eds.). Evolutionary ecology of freshwater animals. Birkhäuser Verlag Basel, Switzerland.

Bell, M.A. and S.A. Foster (eds.). 1994a. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M.A., and S.A. Foster. 1994b. Introduction to the evolutionary biology of the threespine stickleback. Pages 1-27 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell., M.A., and G. Ortí. 1994. Pelvic reduction in threespine stickleback from Cook Inlet lakes: geographic distribution and intrapopulation variation. Copeia 1994:314-325.

Bell, M.A., and K.E. Richland. 1981. Clinal variation of lateral plates in threespine stickleback fish. The American Naturalist 117:113-132.

Bell, M.A., R.C. Francis, and A.C. Havens. 1985. Pelvic reduction and its directional asymmetry in threespine sticklebacks from the Cook Inlet region, Alaska. Copeia 1985:437-444.

Bell, M.A., W.E. Aguirre, and N.J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58:814-824.

Bell, M.A., V. Khalef, and M.P. Travis. 2007. Directional asymmetry of pelvic vestiges in threespine stickleback. Journal of Experimental Zoology (Mol. Dev. Evol.)

308B:189-199.

Bentzen, P., and J.D. McPhail. 1984. Ecology and evolution of sympatric sticklebacks: specialization for alternative trophic niches in the Enos Lake species pair. Canadian Journal of Zoology 62:2280-2286.

Buth, D.G., and T.R. Haglund. 1994. Allozyme variation in the *Gasterosteus aculeatus* complex. Pages 61-84 in M.A. Bell, and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Colosimo, P.F., K.E. Hoseman, S. Balabhadra, G. Villareal Jr., M. Dickson, J. Grimwood, J. Schmtz, R.M. Myers, D. Schluter, and D.M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928-1933.

Cresko, W.A. 2000. The ecology and geography of speciation: a case study using an adaptive radiation of threespine stickleback in Alaska. Ph.D. Dissertation, Clark University, Worcester, MA, USA.

Cresko, W.A., and J.A. Baker. 1996. Two morphotypes of of lacustrine threespine stickleback, *Gasterosteus aculeatus*, in Benka Lake, Alaska. Environmental Biology of Fishes 45:343-350.

Cresko, B., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. Bell, C. Kimmel, & J. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. PNAS 101:6050-6055.

Crispo, E., P. Bentzen, D.N. Reznick, M.T. Kinnison, and A.P. Hendry. The relative influence of natural selection and geography on gene flow in guppies. Molecular Ecology 15:49-62.

Danley, P.D., and T.D. Kocher. 2001. Speciation in rapidly diverging systems: Lessons from Lake Malawi. Molecular Ecology 10:1075-1086.

Echelle, A.A., and I. Kornfield (eds.). 1984. Evolution of fish species flocks. University of Maine at Orono Press, Orono.

Eldredge, N., J.N. Thompson, P.M. Brakefield, S. Gavrilets, D. Jablonski, J.B.C. Jackson, R.E. Lenski, B.S. Lieberman, M.A. McPeek, and W. Miller III. 2005. The dynamics of evolutionary stasis. Paleobiology 31:133-145.

Endler, J.A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton.

Endler, J.A. 1986. Natural selection in the wild. Monographs in Population Biology 21. Princeton University Press, Princeton.

Estes, S., and S.J. Arnold. 2007. Resolving the paradox of stasis: Models with stabilizing selection explain evolutionary divergence on all time scales. The American Naturalist 169:227-244.

Excoffier, L.G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Fisher, R.A. 1950. Gene frequencies in a cline determined by selection and diffusion. Biometrics 6:353-361.

Francis, R.C., J.V. Baumgartner, A.C. Havens, and M.A. Bell. 1986. Historical and ecological sources of variation among lake populations of threespine sticklebacks, *Gasterosteus aculeatus*, near Cook Inlet, Alaska. Canadian Journal of Zoology 64:2257-2265.

Gross, H.P., and J.M. Anderson. 1984. Geographic variation in gillrakers and diet of European threespine sticklebacks, *Gasterosteus aculeatus*. Copeia 1984:87-97.

Haldane, J.B.S. 1948. The theory of a cline. Journal of Genetics 48:277-284.

Harrison, S., and A. Hastings. 1996. Genetic and evolutionary consequences of metapopulation structure. Trends in Ecology and Evolution 11:180-183.

Hart, P.J.B., and A.B. Gill. 1994. Evolution of foraging behaviour in the threespine stickleback. Pages 207-239 in M.A. Bell, and S.A. Foster (eds.), The evolutionary biology of the threespine stickleback. Oxford University Press.

Hendry, A.P., and M.T. Kinnison. 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution 53:1637-1653.

Hendry, A.P., and E.B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319-2331.

Hendry, A.P., E.B. Taylor, and J.D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:1199-1216.

Hendry, A.P., T.J. Farrugia, and M.T. Kinnison. 2007. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology, in press.

Hodges, S.A. 1997. Rapid radiation due to a key innovation in columbines (Ranunculaceae: *Aquilegia*). Pages 391-405 in T.J. Givnish and K.J. Sytsma (eds.). Molecular evolution and adaptive radiation. Cambridge University Press, Cambridge.

Karve, A.D., F.A. von Hippel, and M.A. Bell. 2007. Isolation between sympatric anadromous and resident threespine stickleback species in Mud Lake, Alaska. Environmental Biology of Fishes, in press.

Kimmel, C.B., B. Ullmann, C. Walker, C. Wilson, M. Currey, P.C. Phillips, M.A. Bell, J.H. Postlethwait, and W. A. Cresko. 2005. Proceedings of the National Academy of Sciences, USA 102:5791-5796.

King, R.B., and R. Lawson. 1995. Color-pattern variation in lake Erie water snakes: the role of gene flow. Evolution 49:885-896.

Kinnison, M.T., and A.P. Hendry. 2001. The pace of modern life: from rates of contemporary microevolution to pattern and process. Genetica 112-113:145-164.

Klepaker, T. 1993. Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. Canadian Journal of Zoology 71:1231-1258.

Kristjánsson, B.K., S. Skúlason, and D.L.G. Noakes. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659-672.

Largiadèr, C.R., V. Fries, B. Kobler, and Th.C.M. Bakker. 1999. Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.). Molecular Ecology 342-344.

Lavin, P.A., and McPhail. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 43:2455-2463.

Leinonen, T., J.M. Cano, H. Mäkinen, and J. Merilä. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. Journal of Evolutionary Biology 19:1803-1812.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology and Evolution 17:183-189.

López-Fernández, H., R.L. Honeycutt, and K.O. Winemiller. 2005. Molecular phylogeny

and evidence for an adaptive radiation of Geophagine cichlids from South America (Perciformes: Labroidei). Molecular Phylogenetics and Evolution 34:227-244.

Mäkinen, H.S., J.M. Cano, and J. Merilä. 2006. Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. Molecular Ecology 15:1519-1534.

Mantel, N.A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209-220.

McKinnon, J.S., and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model system. Trends in Ecology and Evolution 17:480-488.

McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62:1402-1408.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. Pages 399-437 in M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Merilä, J., B.C. Sheldon, and L.E.B. Kruuk. 2001. Explaining stasis: microevolutionary studies in natural populations. Genetica 112-113:199-222.

Moore, J.S., and A.P. Hendry. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. Evolutionary Ecology Research 7:871-886.

Moore, J.S., J.L. Gow, E.B. Taylor, and A.P. Hendry. 2007. Quantifying the constraining influence of gene flow on adaptive divergence in the lake-stream threespine stickleback system. Evolution, in press.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-589.

Nosil, P., and T.E. Reimchen, 2005. Ecological opportunity and levels of morphological variance within freshwater stickleback populations. Biological Journal of the Linnean Society 86:297-308.

Orti, G., M.A. Bell, T.E. Reimchen, and A. Meyer. 1994. Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. Evolution 48:608-622.

Östlund-Nilsson, S., I. Mayer, and F. A. Huntingford (eds.). 2006. Biology of the three-spined stickleback. CRC Press, Boca Raton.

Patankar, R., F.A. von Hippel, and M.A. Bell. 2006. Extinction of a weakly armoured threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. Ecology of Freshwater Fish 15:482-487.

Peichel, C.L., J.A. Ross. C.K. Matson, M. Dickson, J. Grimwood, J. Schmutz, R.M. Myers, S. Mori, D. Schluter, and D. Kingsley. 2004. The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Current Biology 14:1416-1424.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using mutlilocus genotype data. Genetics 155:945-959.

Pritchard, J.K., X. Wen, and D. Falush. 2007. Documentation for structure software: version 2.2. http://pritch.bsd.uchicago.edu/software.html.

Purnell, M.A., P.J.B. Hart, D.C. Baines, and M.A. Bell. 2006. Quantitative analysis of dental microwear in threespine stickleback: a new approach to analysis of trophic ecology in aquatic vertebrates. Journal of Animal Ecology 75:967-977.

Raymond M., and Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Riechert, S. 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. Behavioral Ecology and Sociobiology 32:355-363.

Reimchen, T.E., E.M. Stinson, and J.S. Nelson. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Canadian Journal of Zoology 63: 2944-2951.

Reusch, B.H., K.M. Wegner, and M. Kalbe. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographic proximity. Molecular Ecology 10:2435-2445.

Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for short-term genetic distance. Genetics 105:767-779.

Reznick, D.N., and C.K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112-113:183-198.

Rico, C., D. Zadworny, U. Kuhnlein, and G.J. Fitzgerald. 1993. Characterization of hypervariable microsatellite loci in the threespine stickleback *Gasterosteus*

aculeatus. Molecular Ecology 2:271-272.

Robinson, B.W., and D. Schluter. 2000. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. Pages 65-94 in Adaptive genetic variation in the wild. T.A. Mousseau, B. Sinervo, and J.A. Endler (eds.). Oxford University Press, Oxford.

Robinson, B.W., and D.S. Wilson. 1994. Character release and displacement in fishes: a neglected literature. The American Naturalist 144:596-627.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Publication 7213:145-153.

Rohlf, F.J. 2004a. NTSYSpc version 2.11w. Applied Biostatistics, Inc. Port Jefferson, NY.

Rohlf, F.J. 2004b. TpsDig version 1.40. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2004c. Thin-plate spline version 1.20. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2005. TpsRegr software version 1.31. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2006. Relative warps version 1.44. http://life.bio.sunysb.edu/morph/.

Rohlf, F.J., and L. Marcus. 1993. A revolution in morphometrics. Trends in Ecology and Evolution 8:129-132.

Rosenberg, M.S. 2001. PASSAGE. Pattern analysis, spatial statistics and geographic exagesis, version 1.1, release 3.4. Department of Biology, Arizona State University, Tempe, AZ.

Rundle, H.D., and D. Schluter. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. Evolution 52:200-208.

Saint-Laurent, R., M. Legault, and L. Bernatchez. 2003. Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). Molecular Ecology 12:315-330.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology 74:699-709.

Schluter, D. 1995. Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. Ecology 76:82-90.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Simpson, G.G. 1953. The major features of evolution. Columbia University Press, New York.

Skúlason, S., S.S. Snorrason, and B. Jonsson. 1999. Sympatric morphs, populations and speciation in freshwater fish with an emphasis on arctic charr. Pages 160-183 in A.E. Magurran and R.M. May (eds.). Evolution of biological diversity. Oxford University Press, Oxford.

Slice, D.E., F.L. Bookstein, L.F. Marcus, and F. J. Rohlf. 1996. Appendix I. A glossary for geometric morphometrics. Pages 531-551 in L.F. Marcus et al. Advances in morphometrics, Plenum Press, New York.

Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics. 16: 393-430.

Sokal, R.R. 1979. Testing statistical significance of geographic variation patterns. Systematic Zoology 28:227-232.

Spoljaric, M.A., and T.E. Reimchen. 2007. 10,000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. Journal of Fish Biology 70:1484-1503.

Taylor, E.B. 1998. Microsatellites isolated from the threespine stickleback *Gasterosteus aculeatus*. Molecular Ecology 7:930-931.

Taylor, E.B., and J.D. McPhail. 1986. Prolonged and burst swimming in anadromous freshwater threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 64:416-420.

Taylor, E.B., and J.D. McPhail. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. Biological Journal of the Linnean Society 66:271-291.

Taylor, E.B., and J.D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proceedings of the Royal Society of London B 267:2375-2384.

Thoday, J.M., and T.B. Boam. 1959. Effects of disruptive selection, 2. Polymorphism and divergence without isolation. Heredity 13:205-218.

von Hippel, F.A., and H. Weigner. 2004. Sympatric and anadromous-resident pairs of threespine stickleback species in young lakes and streams at Bering Glacier, Alaska. Behaviour 141:1441-1464.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Walker, J.A., and M.A. Bell. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). Journal of Zoology 252: 293-302.

Weins, J.J., and C.H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36:519-539.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

West-Eberhard, M.J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Zelditch, M.L., D.L. Swiderski, H.D. Sheets, and W.L. Fink. 2004. Geometric morphometrics for biologists, a primer. Elsevier Academic Press, Oxford.

Table 1. Procrustes distances between consensus configurations for populations pooled by habitat type.

	Deep	Interm.	Shallow	Stream	Anadr.
Deep	0				
Interm.	0.018	0			
Shallow	0.024	0.017	0		
Stream	0.030	0.026	0.021	0	
Anadr.	0.066	0.065	0.063	0.050	

Table 2. Classification results from discriminant function analysis of body shape data by habitat type; s is the number of sites sampled per habitat type, n is the number of specimens included per habitat type, % Classified as indicates the percentage of fish assigned to each habitat type.

Collected in:			% Classified as:			
	s	n	Deep Lake	Interm. Lake	Shallow Lake	Stream
Deep Lake	3	69	**82.6**	2.9	8.7	5.8
Interm. Lake	2	40	12.5	**65.0**	15.0	7.5
Shallow Lake	7	150	1.33	2.9	**78.0**	18.7
Stream	8	192	1.6	2.1	16.7	**79.7**

Table 3. Inferred genetic clusters (k=7) from Structure program. Numbers are proportion of fish at each site assigned to a particular cluster for the highest value of ln Pr(k=7).

	Inferred Cluster						
	1	2	3	4	5	6	7
Big	0.176	0.053	0.217	0.094	**0.356**	0.045	0.059
Long	0.228	0.093	**0.298**	0.107	0.194	0.036	0.043
Stepan	0.095	0.070	**0.631**	0.042	0.086	0.043	0.033
Big Beaver	0.065	0.080	**0.573**	0.059	0.135	0.044	0.044
Beverley	0.018	**0.899**	0.013	0.020	0.018	0.018	0.013
Seymour	0.015	0.013	0.014	0.017	0.015	**0.912**	0.014
Corcoran	0.074	0.030	0.179	0.114	**0.484**	0.040	0.079
Cloudy	0.069	0.091	0.046	**0.587**	0.067	0.089	0.051
Lucille	0.063	0.012	0.088	0.030	0.064	0.023	**0.721**
Rainbow	0.064	0.212	0.050	**0.448**	0.106	0.049	0.071
Frog	**0.637**	0.040	0.034	0.086	0.101	0.074	0.029
Visnaw	0.049	0.045	0.095	0.056	0.039	**0.697**	0.020
LMC3	0.071	0.098	0.069	**0.568**	0.074	0.080	0.040
LMC2	0.234	0.055	0.125	0.083	**0.325**	0.044	0.135
MC1	0.176	0.026	0.110	0.166	**0.386**	0.051	0.086
LMC1	0.159	0.029	0.123	0.120	**0.387**	0.056	0.125
LC2	0.058	0.018	0.039	0.038	0.043	0.037	**0.767**
LC1	0.057	0.016	0.037	0.032	0.038	0.020	**0.800**
LC3	0.051	0.035	0.054	0.032	0.032	0.028	**0.768**
MC2	**0.276**	0.078	0.135	0.164	0.272	0.035	0.040

Table 4 Pairwise F_{st} estimates below the diagonal. P values for pairwise F_{st} estimates above the diagonal.

	Big	Long	Step	Bbeav	Bev	Seym	Corc	Cloud	Luc	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2
Big	*	0	0	0	0	0	0	0	0	0	0	0	0	0.32	0.07	0.14	0	0	0	0
Long	0.034	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Step	0.060	0.049	*	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BBea	0.049	0.042	0.005	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bev	0.136	0.137	0.200	0.165	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seym	0.121	0.157	0.186	0.166	0.198	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corc	0.029	0.045	0.081	0.083	0.155	0.151	*	0	0	0	0	0	0	0.01	0	0	0	0	0	0
Cloud	0.062	0.051	0.108	0.099	0.121	0.129	0.045	*	0	0.01	0	0	0.12	0	0	0	0	0	0	0
Luc	0.052	0.100	0.115	0.109	0.233	0.180	0.046	0.099	*	0	0	0	0	0	0	0	0.59	0.56	0.14	0
Rainb	0.052	0.035	0.088	0.083	0.079	0.137	0.037	0.014	0.103	*	0	0	0.30	0	0	0	0	0	0	0
Frog	0.087	0.056	0.104	0.129	0.199	0.182	0.082	0.105	0.131	0.069	*	0	0	0	0	0	0	0	0	0
Visn	0.128	0.119	0.137	0.121	0.211	0.139	0.128	0.119	0.176	0.113	0.149	*	0	0	0	0	0	0	0	0
LMC3	0.051	0.032	0.093	0.089	0.117	0.148	0.030	0.006	0.087	0.002	0.070	0.107	*	0	0	0	0	0	0	0
LMC2	0.006	0.048	0.072	0.069	0.147	0.139	0.015	0.062	0.029	0.046	0.072	0.142	0.042	*	0.06	0.28	0	0	0	0
MC1	0.003	0.029	0.070	0.058	0.141	0.132	0.031	0.056	0.054	0.051	0.087	0.153	0.049	0.010	*	0.02	0	0	0	0
LMC1	0.006	0.034	0.074	0.062	0.151	0.135	0.018	0.058	0.032	0.045	0.067	0.131	0.037	0.000	0.010	*	0	0	0	0
LC2	0.056	0.100	0.109	0.108	0.214	0.177	0.036	0.087	0.000	0.085	0.131	0.181	0.078	0.031	0.057	0.035	*	0.12	0.03	0
LC1	0.036	0.086	0.103	0.098	0.202	0.176	0.042	0.091	0.000	0.082	0.116	0.176	0.079	0.019	0.040	0.024	0.005	*	0.41	0
LC3	0.037	0.085	0.098	0.083	0.180	0.153	0.052	0.087	0.006	0.084	0.132	0.167	0.082	0.022	0.038	0.031	0.014	0.000	*	0
MC2	0.027	0.019	0.066	0.066	0.166	0.169	0.044	0.074	0.084	0.058	0.069	0.156	0.051	0.031	0.030	0.026	0.088	0.065	0.074	*

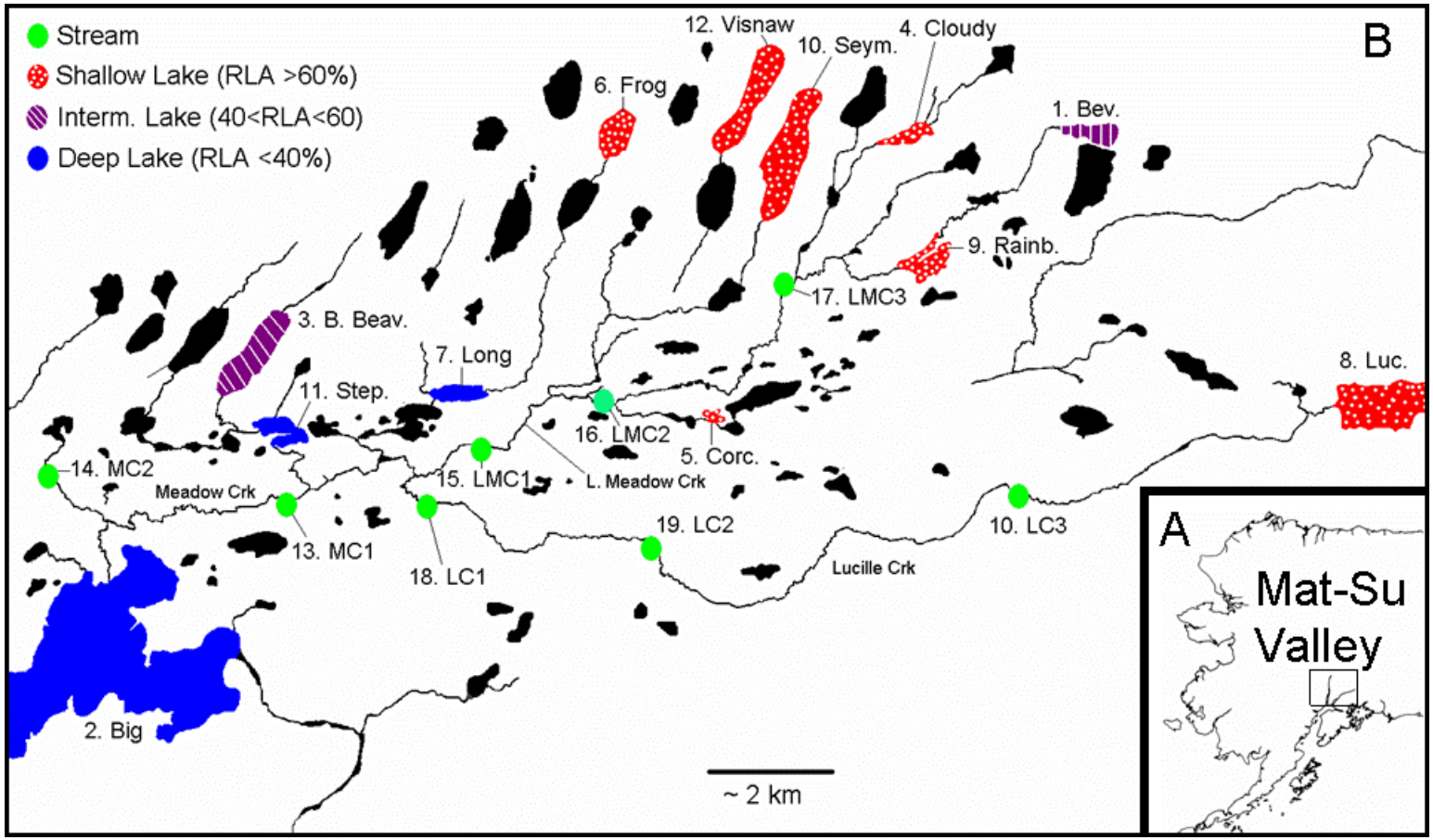


Fig. 1. Map of collection sites. A. Approximate position of Matanuska-Susitna Valley. B. Lake and stream sites sampled. See Appendix 5 for specific site information.

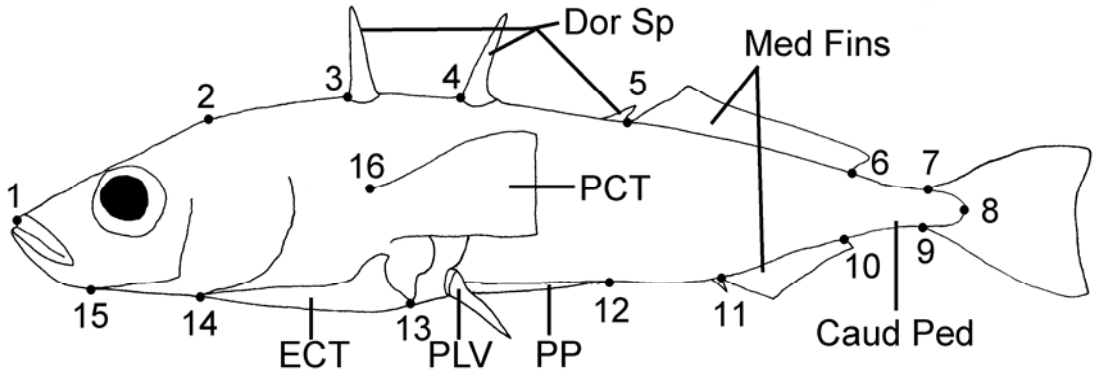


Fig. 2. Landmarks used in this study (numbers 1-16). Anatomical structures mentioned in text are labeled. ECT = ectocoracoid, PLV = pelvis, PP = posterior process of the pelvis, Caud Ped = caudal peduncle, Med Fins = median fins, Dor Sp = dorsal spines, and PCT = pectoral fin.

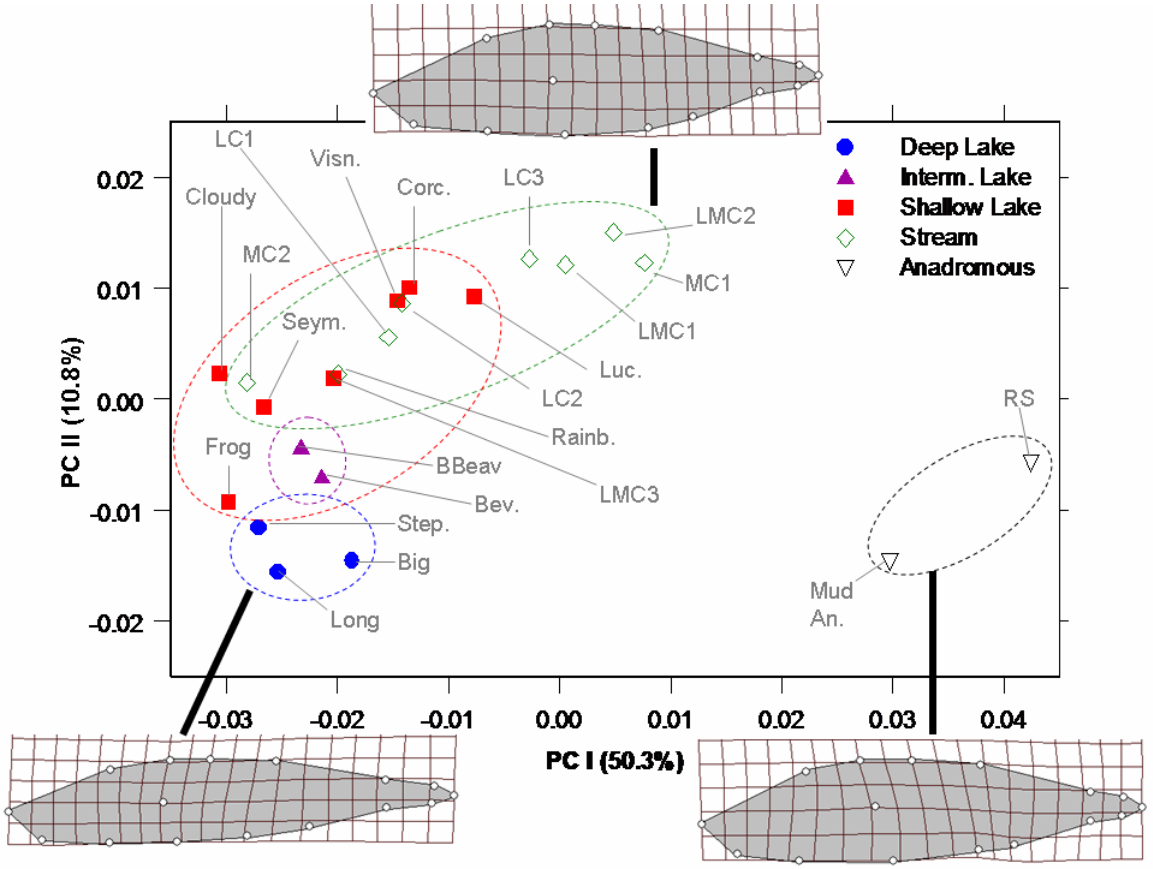


Fig. 3. Principal components plot of sample means. Deformation grids are predicted shapes at the extremes of PC space.

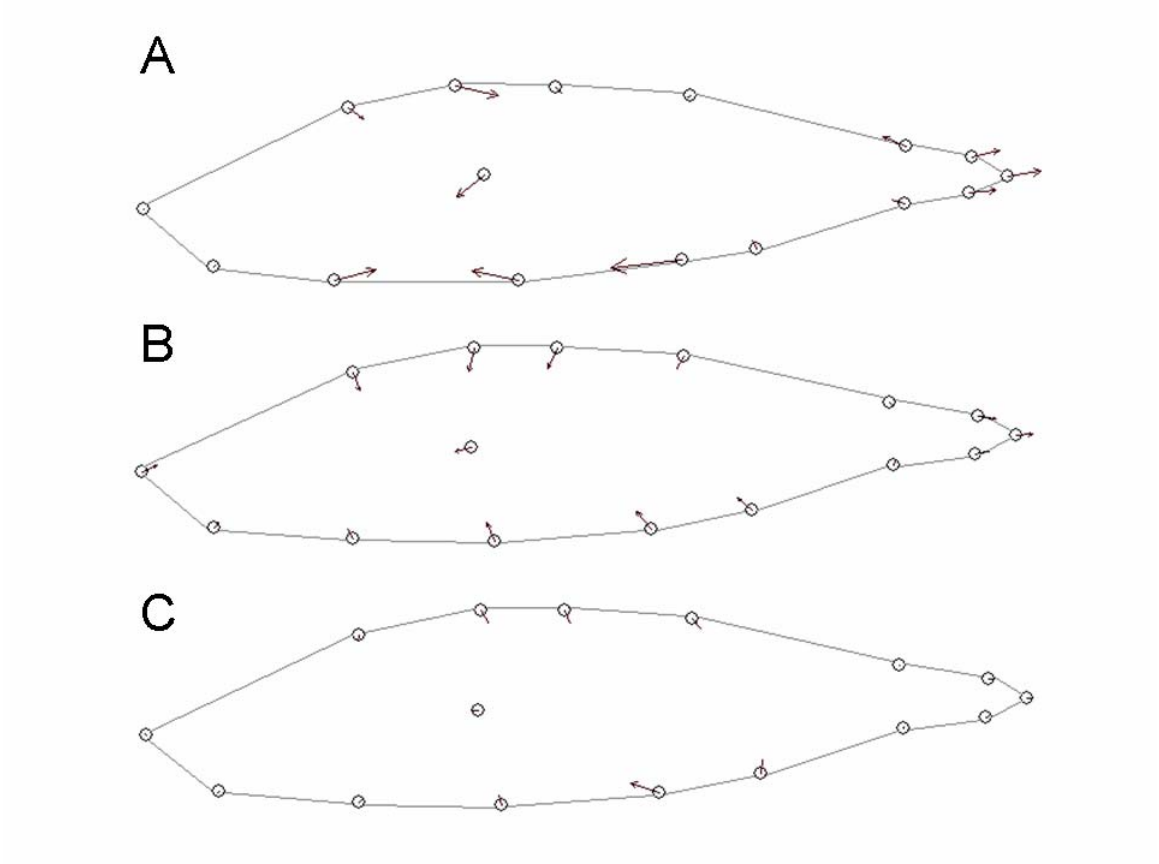


Fig. 4. Body shape variation among consensus configurations for populations pooled by habitat type. A Change in position of landmarks of consensus freshwater configuration from consensus anadromous configuration; B consensus deep lake from consensus stream configuration; and C, consensus shallow lake from consensus stream configuration. Landmark displacements were exaggerated by a factor of two for visualization.

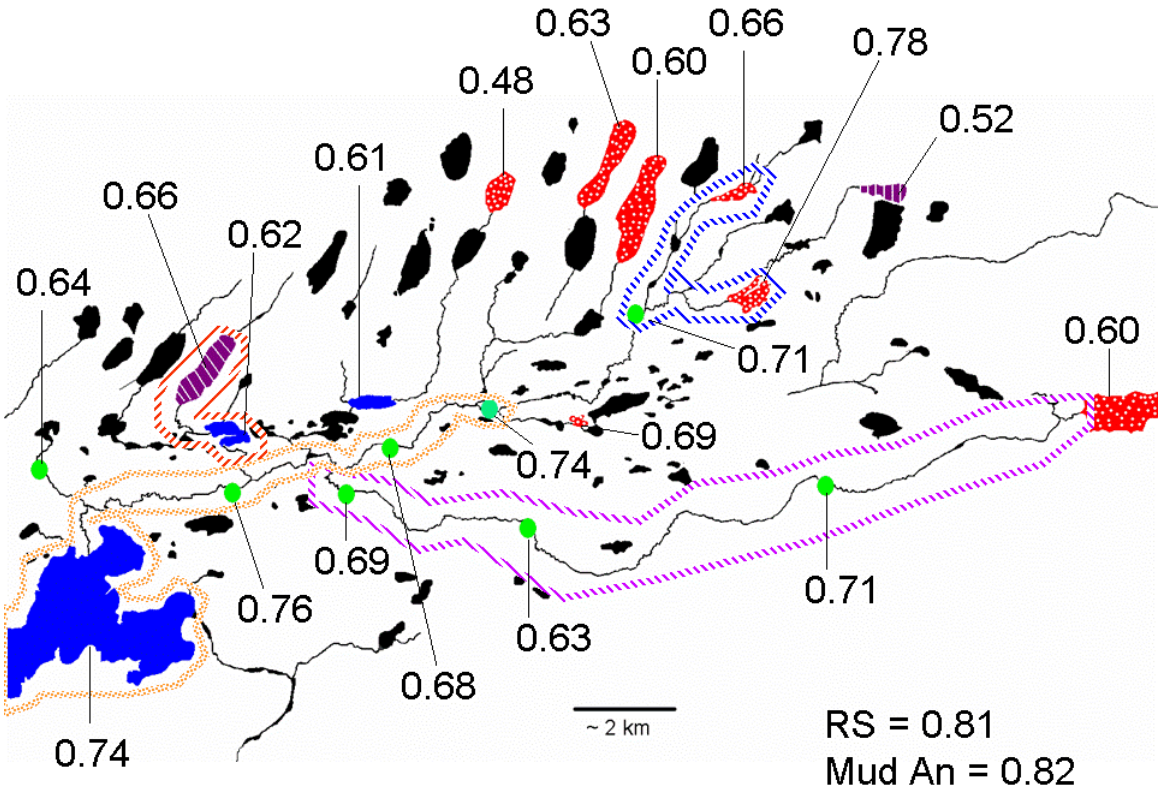


Fig. 5. Population genetic structure and diversity in the upper Fish Creek drainage. Numbers indicate observed heterozygosity. The lines surround four portions of the drainage (each indicated by different patterns) lacking strong genetic structure (i.e., pairwise F_{st} values were either not significantly different from 0 or only marginally significant).

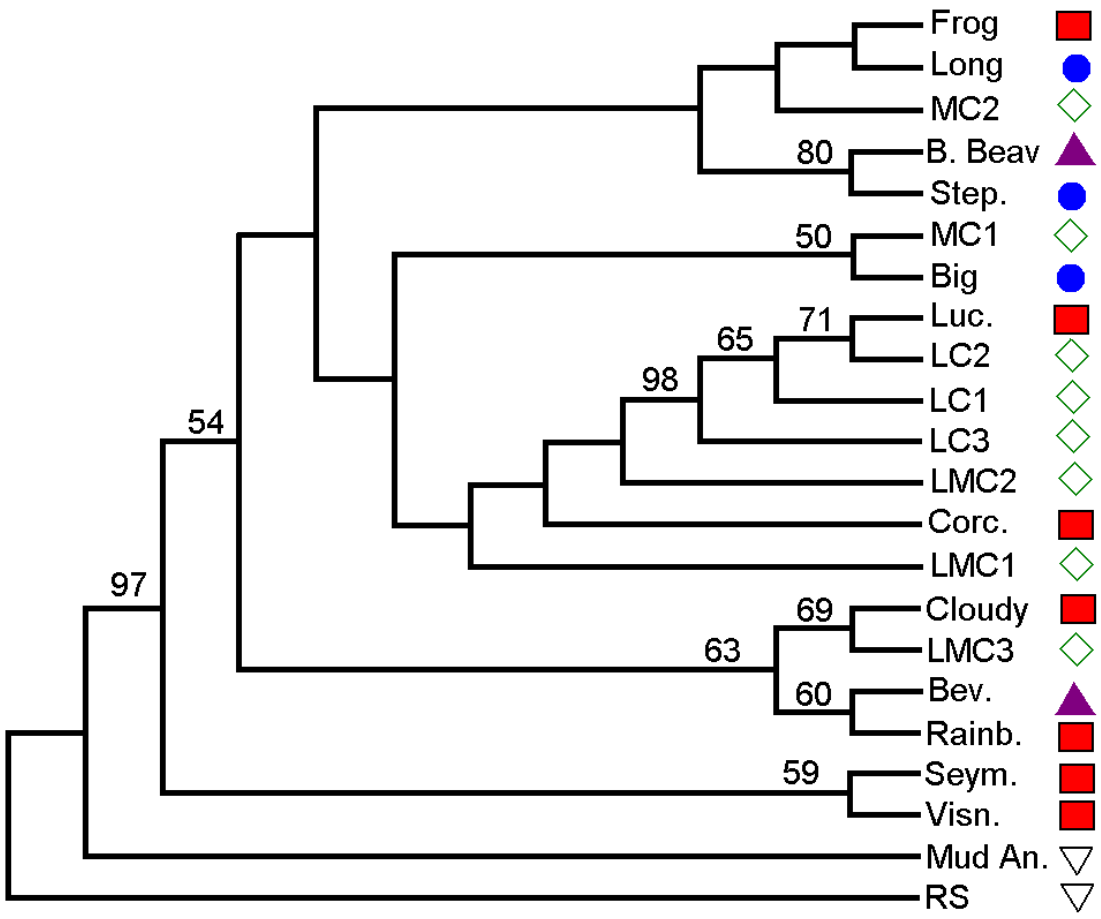


Fig. 6. Neighbor-joining tree of genetic relationships among samples. Numbers are bootstrap values; only bootstrap values of 50 or higher are displayed. Circles are deep lakes, triangles are intermediate depth lakes, squares are shallow lakes, diamonds are stream sites, and inverted triangles are anadromous populations.

Appendices

Appendix 1

Attribution of Effort: My dissertation research has been largely a collaborative effort and all my chapters include coauthors. Below I list the contributions of the coauthors to my dissertation research. I designed, analyzed, and wrote all chapters.

Chapter 2: This chapter is coauthored with Kaitlyn Ellis, Mary Kusenda, and Michael A. Bell. Kaitlyn Ellis collected some of the body shape data, Mary Kusenda collected preliminary body shape data used in a pilot study, and Michael A. Bell collected most of the samples and provided guidance throughout. I collected most of the body shape data and all of the linear measurements.

Chapter 3: This chapter is coauthored with Michael A. Bell. He collected most of the samples included in this study. I collected all of the data.

Chapter 4: This chapter is coauthored with Michael A. Bell. He collected most of the Loberg Lake samples and some of the other populations included for genetic comparison. I collected all of the morphological and genetic data used in this study.

Chapter 5: This chapter is coauthored with Joanne Soong. She extracted DNA from some of the populations and collected part of the microsatellite data. I collected all of the morphological data and part of the microstaellite data.

Appendix 2

Populations used in Chapter 3. The latitudes and longitudes are in decimal degrees.

Anadromous: 1. Mud Lake, (61.563N, 148.949W), 2005 (n=18); 2. Rabbit Slough (61.534N, 149.268W), 1997 (n=50), 2000 (n=50), 2003 (n=46).

Lakes sampled twice: 3. Beaverhouse (61.574N, 149.863W), 1990 (n=7), 2004 (n=20); 4. Blodgett (61.578N, 149.672W), 1990 (n=20), 2004 (n=20); 5. Cloudy (61.619N, 149.626W), 1990 (n=20), 2005 (n=30); 6. Dollar (61.354N, 149.952W), 1990 (n=20), 2004 (n=20); 7. Kashwitna (61.833N, 150.076W), 1990 (n=20), 2004 (n=20); 8. Long (61.578N, 149.764W), 1990 (n=21), 2004 (n=20); 9. Lower Ohmer (60.456N, 150.315W), 1990 (n=8), 2005 (n=20); 10. Mud, (61.563N, 148.949W), 1990 (n=20), 2004 (n=20); 11. Stormy (60.771N, 151.047W), 1990 (n=17), 2005 (n=31); 12. Tern (60.533N, 149.550W), 1990 (n=15), 2004 (n=20); 13. Visnaw (61.619N, 149.677W), 1990 (n=20), 2004 (n=20); 14. Wasilla (61.586N, 149.396W), 1990 (n=20), 2005 (n=20); 15. Zero (61.647N, 149.814W), 1992 (n=20), 2004 (n=24).

Lakes sampled once: 16. Big (61.533N, 149.888W), 2005 (n=30); 17. Diamond (61.501N, 150.019W), 1990 (n=20); 18. Jean (60.506N, 150.171W), 1990 (n=14); 19. Little Beaver (61.586N, 149.862W), 1990 (n=20); 20. Nancy (61.685N, 150.000W), 1990 (n=20); 21. Nowack (60.771N; 151.134W), 1990 (n=20); 22. Suneva (60.763N, 151.197W), 1990 (n=20).

Streams: 23. Crocker Creek (61.512N, 149.628W), 2004 (n=20); 24. Little Meadow Creek (61.563N, 149.826W), 2005 (n=30); 25. Obrien Creek (61.484N, 149.683W), 2004 (n=21); 26. Spring Creek (61.548N, 149.229W), 1992 (n=20); 27. Swanson River (60.745N, 150.794W), 2004 (n=20).

Appendix 3

Populations from Cook Inlet used in genetic comparisons in Chapter 4. Samples consisted of 32 specimens. The latitudes and longitudes are in decimal degrees.

Anadromous: Rabbit Slough (RS- 61.534N, 149.268W), Mud Lake (MudAn- 61.565N, 148.947W).

Lakes: Bear Paw (BP- 61.614N, 149.753W), Beverley (Bev- 61.613N, 149.569W), Big (Big- 61.535N, 149.826W), Big Beaver (BBeav- 61.578N, 149.842W), Bruce (Bruce- 61.610N, 149.556W), Corcoran (Corc- 61.573N, 149.693W), Long (Long- 61.576N, 149.774W), Meadow Creek (MCrk- at intersection with N. Big Lake Rd., just south of Orchid Lake, 61.566N, 149.893W) Mud Lake resident (MudL- 61.565N, 148.947W), Rainbow (Rainb- 61.594N, 149.632W), Seymour (Seym- 61.614N, 149.670W), Stepan (Step- 61.570N, 149.816W), Visnaw (Visn- 61.614N, 149.680W).

Appendix 4

Allele frequencies for populations included in Chapter 4. See Appendix 3 for abbreviations.

Gac4170PBBE

	Lob	RS	MudAn	BP	Bev	Big	BBeav	Bruce	Corc	Long	MCrk	Mud L	Rainb	Seym	Step	Visn
101	-----	-----	-----	-----	-----	-----	-----	-----	0.156	-----	-----	-----	-----	-----	-----	-----
105	-----	0.027	0.052	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----
107	-----	0.014	-----	-----	-----	-----	-----	-----	-----	-----	0.048	0.047	-----	-----	-----	-----
109	0.242	0.230	0.310	0.078	0.359	0.333	0.234	-----	0.047	0.222	0.274	0.250	0.063	0.391	0.065	0.109
111	0.159	0.108	0.121	-----	0.031	0.017	0.016	-----	-----	0.019	0.129	0.172	-----	-----	-----	-----
113	0.132	0.149	0.069	0.047	0.047	0.117	0.266	-----	0.031	0.222	0.226	0.328	0.141	0.219	0.500	0.078
115	-----	0.041	0.103	-----	-----	0.017	-----	-----	0.031	0.056	-----	-----	0.109	-----	-----	-----
117	-----	0.095	0.069	-----	-----	-----	-----	-----	0.016	-----	-----	0.031	-----	-----	-----	-----
119	-----	0.041	0.017	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
121	0.033	0.014	0.017	-----	-----	-----	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----
123	-----	0.014	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
131	-----	0.014	0.034	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
133	-----	0.014	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
135	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	0.016	-----	0.031	-----	-----	-----
141	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----
143	0.121	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
145	-----	0.014	0.017	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
147	-----	0.027	0.034	-----	-----	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
149	-----	0.014	0.052	-----	-----	0.167	-----	-----	0.078	0.019	0.097	0.031	0.016	-----	-----	-----
151	-----	0.027	0.017	-----	-----	0.017	0.016	-----	-----	-----	-----	-----	-----	-----	0.016	-----
153	0.143	0.014	0.017	0.156	0.531	0.067	0.250	0.969	0.156	0.389	0.145	0.016	0.484	0.328	0.194	0.688
155	-----	0.068	-----	-----	-----	-----	0.016	0.016	-----	-----	-----	-----	0.016	-----	0.032	-----
157	-----	0.014	0.017	-----	-----	0.050	0.031	-----	0.016	-----	-----	-----	-----	-----	-----	-----
159	-----	0.014	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016
161	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
187	-----	-----	-----	-----	-----	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
197	0.038	0.054	0.052	0.719	-----	0.167	0.156	-----	0.328	0.074	0.065	0.063	0.141	0.063	0.161	0.047
199	0.132	-----	-----	-----	-----	0.017	0.016	-----	0.094	-----	-----	-----	-----	-----	0.032	-----
203	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----
205	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.063
n=	8	21	16	4	6	12	9	3	13	7	8	11	8	4	7	6

Gac7033PBBE

	Lob	RS	MudAn	BP	Bev	Big	BBeav	Bruce	Corc	Long	MCrk	Mud L	Rainb	Seym	Step	Visn
180	-----	-----	0.032	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
184	0.011	0.350	0.226	0.016	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	0.016	-----
190	-----	-----	0.016	-----	-----	-----	-----	-----	0.016	-----	-----	-----	0.047	-----	-----	-----
192	0.050	0.050	0.065	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
194	0.578	0.250	0.274	-----	0.078	0.016	0.016	-----	0.031	0.017	-----	0.016	0.078	0.109	0.047	0.177
196	-----	0.100	0.032	0.065	0.141	0.078	0.097	0.016	0.078	-----	-----	0.016	0.016	0.594	0.063	0.516
198	0.072	0.067	0.065	0.145	-----	0.188	0.177	-----	0.109	0.017	0.207	-----	0.094	-----	0.219	0.016
200	-----	0.033	0.048	-----	-----	0.016	0.032	-----	0.031	-----	-----	-----	0.047	-----	0.016	-----
202	-----	-----	-----	-----	0.031	0.031	-----	-----	-----	-----	-----	-----	0.031	-----	-----	-----
204	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----	0.017	-----	-----	-----	-----	-----
206	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
208	-----	-----	-----	-----	-----	0.016	0.016	0.047	-----	-----	-----	-----	-----	-----	-----	-----
210	-----	-----	-----	-----	-----	0.016	0.016	0.016	0.016	0.017	-----	-----	-----	-----	-----	-----
212	0.178	-----	0.032	0.694	0.719	0.516	0.548	0.781	0.594	0.783	0.741	0.903	0.625	0.281	0.563	0.210
214	0.094	-----	-----	0.081	0.031	0.031	0.065	0.141	0.063	0.033	0.017	0.065	-----	-----	0.031	-----
216	-----	-----	-----	-----	-----	0.078	0.016	-----	-----	0.117	-----	-----	0.016	0.016	0.047	0.081
222	-----	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
224	-----	-----	0.016	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----
226	-----	-----	0.065	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
228	-----	0.033	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----
230	-----	-----	0.032	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
232	-----	0.017	0.016	-----	-----	-----	-----	-----	0.016	0.017	0.017	-----	0.016	-----	-----	-----
234	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----
236	-----	-----	0.016	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----	-----	-----	-----
238	-----	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
240	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
242	0.017	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
244	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
246	-----	0.017	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
248	-----	0.033	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
n=	7	13	18	5	5	11	10	5	11	7	5	4	11	4	8	5

Cier62

	Lob	RS	MudAn	BP	Bev	Big	BBeav	Bruce	Corc	Long	MCrk	Mud L	Rainb	Seym	Step	Visn
94	-----	-----	-----	-----	-----	0.017	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

98	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
100	-----	0.018	0.016	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
102	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.036	-----	-----	-----	-----	-----
104	0.114	-----	0.063	0.031	-----	0.155	0.300	-----	0.155	0.236	0.107	0.097	0.109	0.067	0.246	0.129
106	0.103	-----	0.016	-----	-----	-----	-----	-----	-----	-----	0.071	-----	0.031	-----	0.016	0.032
108	-----	-----	-----	-----	0.031	-----	-----	-----	-----	-----	0.071	-----	-----	-----	-----	-----
110	-----	0.018	0.016	-----	-----	-----	0.017	-----	0.034	-----	-----	-----	-----	-----	-----	-----
112	-----	0.018	0.048	-----	-----	-----	-----	-----	-----	-----	0.036	0.016	-----	-----	0.016	-----
114	0.022	0.161	0.127	-----	-----	-----	-----	-----	0.052	-----	0.018	0.097	-----	-----	-----	-----
116	-----	0.161	0.127	0.234	-----	0.086	0.067	-----	0.017	0.073	0.143	0.048	0.016	-----	0.016	-----
118	0.043	-----	0.032	-----	-----	-----	-----	-----	0.017	0.036	-----	0.048	-----	-----	-----	-----
120	0.386	0.054	0.063	-----	-----	0.034	0.033	-----	0.103	0.055	0.054	0.016	0.063	0.133	0.066	0.048
122	0.065	0.036	0.048	-----	-----	0.034	0.033	-----	0.017	0.055	0.018	-----	-----	-----	0.033	0.016
124	-----	0.018	0.048	-----	0.125	0.069	0.017	-----	-----	0.055	0.071	-----	-----	-----	-----	0.016
126	-----	-----	0.016	-----	-----	-----	0.017	-----	-----	-----	0.018	0.194	-----	-----	0.049	0.032
128	0.054	0.054	0.016	-----	0.609	0.241	0.233	0.609	0.155	0.091	0.107	0.145	0.281	-----	0.230	0.065
130	0.005	0.071	0.032	0.063	0.078	0.034	0.100	0.156	0.138	0.036	0.089	0.032	0.125	0.133	0.098	0.016
132	0.022	0.036	0.048	0.375	0.016	0.069	0.050	-----	0.069	0.091	0.054	0.048	0.047	-----	0.049	0.065
134	0.005	0.036	0.048	0.109	-----	0.103	0.017	-----	0.034	0.036	0.018	-----	0.094	0.167	0.098	0.113
136	0.005	0.018	0.048	0.078	-----	0.017	0.033	0.047	-----	0.036	0.071	0.065	0.063	-----	0.016	0.145
138	0.158	0.089	0.016	0.047	0.094	0.017	0.033	-----	0.069	0.091	-----	0.048	0.016	-----	0.016	0.065
140	-----	0.036	0.032	0.047	0.047	0.034	0.017	0.156	0.017	0.018	-----	0.032	0.063	0.017	0.016	0.194
142	0.016	0.036	0.063	-----	-----	-----	-----	0.031	-----	-----	0.018	-----	0.047	0.167	-----	0.048
144	-----	0.018	0.032	-----	-----	0.034	0.017	-----	0.034	-----	-----	0.097	0.016	0.317	-----	-----
146	-----	-----	-----	-----	-----	0.034	0.017	-----	0.069	0.018	-----	-----	0.031	-----	0.033	0.016
148	-----	-----	-----	-----	-----	0.017	-----	-----	-----	0.018	-----	-----	-----	-----	-----	-----
150	-----	0.036	-----	-----	-----	-----	-----	-----	-----	0.036	-----	-----	-----	-----	-----	-----
152	-----	0.071	0.032	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
154	-----	0.018	-----	-----	-----	-----	-----	-----	0.017	-----	-----	0.016	-----	-----	-----	-----
156	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.018	-----	-----	-----	-----	-----	-----
162	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.018	-----	-----	-----	-----	-----	-----
n=	13	20	23	9	7	16	16	5	16	18	17	15	14	7	15	15

Gacμ10

	Lob	RS	MudAn	BP	Bev	Big	BBeav	Bruce	Corc	Long	MCrk	Mud L	Rainb	Seym	Step	Visn
176	-----	0.010	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
182	-----	0.010	-----	-----	-----	0.063	-----	-----	0.141	-----	0.050	0.016	-----	-----	0.048	-----

1	184	-----	0.010	0.032	-----	-----	0.016	0.032	-----	0.031	0.050	-----	-----	-----	-----	-----	-----
2	186	-----	0.190	0.145	-----	0.266	0.047	0.016	0.016	-----	-----	0.017	-----	0.032	-----	0.016	-----
3	188	0.017	0.060	0.048	-----	0.063	-----	-----	-----	-----	0.017	-----	-----	0.048	-----	-----	-----
4	190	-----	0.030	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
5	192	-----	0.090	0.048	-----	-----	0.016	0.048	0.328	0.016	0.033	-----	-----	0.032	-----	0.032	-----
6	194	0.035	0.040	0.016	-----	-----	-----	0.016	-----	-----	-----	-----	-----	0.032	-----	-----	-----
7	196	0.081	0.040	0.032	-----	-----	0.188	0.710	-----	0.047	0.267	0.167	-----	-----	0.063	0.645	0.391
8	198	0.006	0.070	0.016	-----	-----	0.031	-----	-----	-----	0.017	0.017	-----	-----	-----	-----	0.016
9	200	-----	0.030	0.065	-----	0.047	0.047	-----	-----	0.078	0.017	-----	-----	0.016	0.125	-----	0.344
10	202	0.023	0.030	0.032	0.094	-----	0.016	0.081	-----	0.031	0.167	0.283	-----	0.016	0.031	0.065	0.094
11	204	0.186	0.090	0.097	0.031	0.266	0.016	0.065	-----	0.156	0.100	0.117	-----	0.081	-----	0.065	0.016
12	206	0.006	0.050	0.065	0.156	0.219	0.047	-----	-----	0.047	0.017	-----	-----	0.065	-----	-----	0.016
13	208	-----	0.090	0.032	-----	0.016	0.016	-----	-----	0.047	-----	-----	-----	0.048	-----	-----	0.047
14	210	0.035	0.050	0.048	-----	-----	0.047	-----	-----	-----	0.017	0.033	-----	-----	-----	-----	-----
15	212	0.047	0.030	0.081	-----	-----	0.016	-----	0.078	0.016	0.033	0.017	-----	-----	-----	0.032	-----
16	214	-----	0.030	0.016	-----	-----	0.031	-----	0.016	-----	-----	-----	0.047	-----	-----	-----	-----
17	216	-----	-----	0.048	-----	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----
18	218	-----	0.010	-----	-----	0.016	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
19	220	-----	0.010	0.016	-----	-----	-----	-----	-----	0.109	0.017	-----	-----	0.081	-----	-----	0.063
20	222	-----	0.020	0.032	-----	-----	0.031	-----	0.031	-----	-----	-----	-----	-----	-----	0.016	-----
21	224	0.186	-----	-----	-----	-----	0.016	-----	0.047	-----	-----	-----	-----	0.048	-----	-----	-----
22	226	-----	-----	0.048	0.016	-----	0.016	-----	0.016	-----	-----	0.050	-----	-----	0.188	-----	0.016
23	228	-----	-----	-----	-----	0.016	0.031	-----	-----	0.016	-----	-----	-----	0.016	-----	-----	-----
24	230	0.035	-----	0.016	-----	-----	-----	-----	-----	-----	0.017	-----	0.047	0.048	-----	-----	-----
25	232	-----	-----	-----	0.016	-----	0.016	-----	0.016	0.016	-----	0.100	0.109	0.097	0.109	-----	-----
26	234	0.128	-----	-----	0.031	0.047	-----	-----	-----	-----	-----	0.017	0.016	0.048	-----	-----	-----
27	236	-----	-----	0.016	-----	0.031	-----	-----	-----	0.047	0.017	0.017	0.078	0.016	-----	-----	-----
28	238	0.029	-----	-----	0.016	-----	0.031	0.016	-----	0.063	-----	-----	-----	0.032	0.484	-----	-----
29	240	-----	-----	-----	0.016	-----	0.016	-----	0.047	0.016	-----	0.067	0.063	0.032	-----	-----	-----
30	242	0.093	0.010	-----	-----	0.016	0.094	0.016	0.016	0.031	-----	0.017	0.031	0.032	-----	0.016	-----
31	244	-----	-----	-----	0.016	-----	0.016	-----	0.063	0.031	0.033	0.033	-----	0.081	-----	0.016	-----
32	246	0.052	-----	-----	0.156	-----	0.078	-----	0.141	0.016	-----	-----	-----	-----	-----	-----	-----
33	248	-----	-----	-----	0.016	-----	-----	-----	0.016	0.016	0.050	-----	0.016	0.048	-----	0.016	-----
34	250	-----	-----	-----	0.031	-----	0.031	-----	0.031	-----	-----	-----	0.031	-----	-----	-----	-----
35	252	-----	-----	-----	0.063	-----	0.016	-----	0.031	-----	-----	-----	-----	0.016	-----	-----	-----
36	254	-----	-----	-----	0.047	-----	-----	-----	-----	0.016	-----	-----	0.078	0.016	-----	-----	-----
37	256	0.041	-----	-----	0.078	-----	0.016	-----	0.094	0.016	-----	-----	-----	-----	-----	-----	-----
38	258	-----	-----	-----	0.016	-----	-----	-----	-----	-----	0.017	-----	0.063	-----	-----	-----	-----

260	-----	-----	0.016	0.109	-----	-----	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----
262	-----	-----	-----	0.031	-----	-----	-----	-----	-----	0.017	-----	0.031	-----	-----	0.016	-----
264	-----	-----	-----	0.016	-----	-----	-----	-----	-----	0.050	-----	0.031	-----	-----	-----	-----
266	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.017	-----	0.063	-----	-----	0.016	-----
268	-----	-----	0.016	-----	-----	-----	-----	-----	-----	0.017	-----	0.078	0.016	-----	-----	-----
270	-----	-----	-----	0.031	-----	-----	-----	-----	-----	0.017	-----	-----	-----	-----	-----	-----
272	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
276	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----
278	-----	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	0.047	-----	-----	-----	-----
280	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
282	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----	-----
288	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----
n=	16	22	24	21	11	27	9	17	22	22	15	24	24	6	13	9

Gacμ9

	Lob	RS	MudAn	BP	Bev	Big	BBeav	Bruce	Corc	Long	MCrk	Mud L	Rainb	Seym	Step	Visn
152	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
156	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
158	-----	-----	-----	0.031	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
160	0.141	0.069	0.078	-----	0.016	-----	0.016	-----	-----	0.048	0.016	0.017	0.129	-----	-----	-----
162	0.065	0.103	0.063	-----	-----	0.250	0.094	-----	0.089	0.177	0.129	0.121	0.016	0.323	0.094	0.032
164	-----	0.034	0.016	-----	-----	0.016	-----	0.047	-----	0.032	0.016	-----	-----	-----	-----	-----
166	-----	0.103	0.031	-----	-----	0.016	-----	-----	0.018	0.032	-----	-----	-----	-----	0.031	-----
168	0.266	0.397	0.422	0.656	0.266	0.547	0.531	0.797	0.696	0.597	0.726	0.207	0.484	0.355	0.625	0.726
170	0.152	0.034	0.047	0.250	0.016	0.078	0.031	0.047	0.089	0.065	0.065	0.224	0.032	-----	0.063	0.145
172	0.065	0.052	0.094	0.047	0.547	0.016	0.156	0.047	0.018	0.032	0.016	0.259	0.177	0.323	0.125	0.097
174	0.212	0.034	0.094	-----	0.156	-----	0.094	0.063	-----	0.016	-----	0.034	0.032	-----	-----	-----
176	-----	0.052	0.016	-----	-----	0.031	-----	-----	0.071	-----	-----	-----	-----	-----	0.063	-----
177	-----	-----	-----	-----	-----	-----	-----	-----	0.018	-----	-----	-----	0.016	-----	-----	-----
178	-----	0.034	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
180	-----	-----	0.031	-----	-----	-----	0.078	-----	-----	-----	-----	-----	-----	-----	-----	-----
182	0.060	-----	0.016	0.016	-----	-----	-----	-----	-----	-----	-----	-----	0.016	-----	-----	-----
184	-----	0.086	0.016	-----	-----	0.016	-----	-----	-----	-----	0.032	0.138	0.097	-----	-----	-----
188	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
190	-----	-----	-----	-----	-----	0.031	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
194	-----	-----	0.031	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
204	0.022	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
208	-----	-----	0.016	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

l	n=	9	11	16	5	5	9	7	5	7	8	7	7	9	3	6	4

Appendix 5

Populations included in Chapter 5. Samples consisted of 32 specimens for the genetic analysis, except that LC1 consisted of 31 specimens and MC1, LMC1, and LMC2 consisted of 30 specimens. Sample sizes for morphological analyses (n_M) unless n_M is 20 and year of collection are in parenthesis. G_{yr} indicates the year of collection of samples for genetic analysis in cases in which it differed from the year of collection of the morphological samples. The latitudes and longitudes are in decimal degrees.

Lakes: 1. Beverley (Bev., 61.613N, 149.569W, 2005), 2. Big (Big, 61.535N, 149.826W, n_M=30, 2005, G_{yr}=2003), 3. Big Beaver (BBeav., 61.578N, 149.842W, 2005), 4. Cloudy (Cloud, 61.612N, 149.639W, n_M=30, 2005), 5. Corcoran (Corc., 61.573N, 149.693W, 2004, G_{yr}=2003), 6. Frog (Frog, 61.614N, 149.723W, 2005), 7. Long (Long, 61.576N, 149.774W, 2004, G_{yr}=2003), 8. Lucille (Luc., 61.579N, 149.450W 2004), 9. Rainbow (Rainb., 61.594N, 149.632W, 2005), 10. Seymour (Seym. 61.614N, 149.670W, 2004), 11. Stepan (Step., 61.570N, 149.816W, n_M=19, 2005), 12. Visnaw (Visn., 61.614N, 149.680W, 2004).

Stream Sites: Meadow Creek: 13. MC1 (at intersection with Beaver Lake Rd., just south of N. Big Lake Rd., 61.563N, 149.826W, n_M=30, 2005), 14. MC2 (at intersection with N. Big Lake Rd., just south of Orchid Lake, 61.566N, 149.893W, n_M=11, 2005); Little Meadow Creek: 15. LMC1 (at intersection with Kenlar Rd. just north of Big Lake Rd., 61.569N, 149.760W, n_M=30, 2005), 16. LMC2 (at intersection of Big Lake Rd. and Parks Highway, 61.576N, 149.728W, n_M=30, 2005, G_{yr}=2003), 17. LMC3 (at intersection with Meadow Lakes Rd., 61.592N, 149.666W, n_M=30, 2005, G_{yr}=2004); Lucille Creek: 18. LC1 (at intersection with Big Lake Rd., 61.561N, 149.779W, n_M=17, 2004), 19. LC2 (at intersection with Johnson Rd., 61.553N, 149.708W, n_M=18, 2004), 20. LC3 (at intersection with Vine Rd., 61.562N, 149.602W, n_M=26, 2004).

Anadromous: 21. Mud Lake (MudAn., 61.565N, 148.947W, n_M=18, 2003), 22. Rabbit Slough (RS, 61.534N, 149.268W, n_M=146, 1997, 2000, 2003, G_{yr}=2003).

Appendix 6

Classification results of discriminant function analysis of body shape data by sample carried out in Chapter 5. Rows are population of origin, columns are population of classification.

	Big	Long	Step.	BBeav.	Bev.	Seym.	Corc.	Cloud	Luc.	Rainb.	Frog	Visn.	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2
Big	**93.3**	3.33	*	*	*	*	*	*	*	*	*	*	*	*	*	3.33	*	*	*	*
Long	5	**90**	*	5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Step.	*	*	**68.4**	5.26	*	*	*	*	*	5.26	0	5.26	*	*	*	*	5.26	10.5	*	*
BBeav.	*	*	*	**65**	15	*	*	5	*	10	5	*	*	*	*	*	*	*	*	*
Bev.	*	*	*	5	**80**	*	5	5	*	*	*	*	*	*	*	*	*	5	*	*
Seym.	*	*	*	*	*	**95**	*	*	*	*	5	*	*	*	*	*	*	*	*	*
Corc.	*	*	*	*	5	*	**65**	*	10	*	*	*	*	*	*	*	5	10	5	*
Cloudy	*	*	*	*	3.33	*	3.33	**53.3**	*	13.3	3.33	3.33	6.67	*	*	*	*	3.33	*	10
Luc.	*	*	*	*	*	*	*	*	**45**	5	0	0	0	*	*	20	5	15	10	*
Rainb.	5	*	*	*	5	*	*	10	5	**40**	5	15	15	*	*	*	*	*	*	*
Frog	*	*	*	*	*	*	*	5	*	15	**75**	5	0	*	*	*	*	*	*	*
Visn.	*	*	*	*	0	10	*	15	*	0	5	**65**	0	*	*	*	*	5	*	*
LMC3	*	*	*	*	3.33	*	*	*	*	13.3	*	*	**80**	*	*	3.33	*	*	*	*
LMC2	*	*	*	*	*	*	6.67	*	3.33	*	*	*	*	**53.3**	3.33	23.3	3.33	*	6.67	*
MC1	*	*	*	*	*	*	*	*	*	*	*	*	*	6.67	**63.3**	23.3	*	*	3.33	3.33
LMC1	*	6.67	*	*	*	*	*	*	6.67	0	*	3.33	*	10	30	**43.3**	*	*	*	*
LC2	*	*	5.56	5.56	5.56	*	*	*	5.56	5.56	*	5.56	*	*	*	*	**38.9**	27.8	*	*
LC1	*	*	5.88	11.8	5.88	*	5.88	*	11.8	*	*	*	*	*	*	*	29.4	**29.4**	*	*
LC3	*	*	*	*	*	*	*	*	7.69	*	*	*	*	11.5	*	3.85	3.85	*	**73.1**	*
MC2	*	*	*	*	*	*	*	9.09	*	*	*	*	9.09	*	*	9.09	*	*	*	**72.7**

Appendix 7

Allele frequencies for populations included in Chapter 5. See Appendix 5 for abbreviations.

Gac4170PBBE

Bin	Big	Long	Step	BBeav	Bev	Seym	Corc	Cloud	LucL	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2	MudAn	RS
101	----	----	----	----	----	----	0.156	----	----	----	----	----	----	0.052	0.017	0.018	----	----	----	----	----	----
105	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	0.052	0.027
107	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.048	----	0.014
109	0.333	0.222	0.065	0.234	0.359	0.391	0.047	0.049	0.290	0.063	0.117	0.109	0.078	0.276	0.362	0.339	0.148	0.304	0.367	0.274	0.310	0.230
111	0.017	0.019	----	0.016	0.031	----	----	0.012	----	----	----	----	----	----	0.034	----	----	----	----	0.129	0.121	0.108
113	0.117	0.222	0.500	0.266	0.047	0.219	0.031	0.049	0.016	0.141	0.483	0.078	0.094	0.121	0.121	0.089	0.016	0.054	0.033	0.226	0.069	0.149
115	0.017	0.056	----	----	----	----	0.031	0.085	0.081	0.109	----	----	0.063	0.034	0.034	0.054	0.148	0.125	0.100	----	0.103	0.041
117	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	0.017	0.018	----	----	----	----	0.069	0.095
119	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	0.041
121	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	0.014
123	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.014
131	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.034	0.014
133	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.014
135	----	----	----	----	----	----	0.016	----	0.032	0.031	----	----	----	0.017	0.069	----	0.033	0.036	0.050	0.016	----	----
143	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----	0.017	----	----	----	----	----	----	----
145	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	0.014
147	0.017	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.034	0.027
149	0.167	0.019	----	----	----	----	0.078	0.012	----	0.016	----	----	0.016	0.172	0.017	0.071	----	----	----	0.097	0.052	0.014
151	0.017	----	0.016	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	0.027
153	0.067	0.389	0.194	0.250	0.531	0.328	0.156	0.476	----	0.484	0.350	0.688	0.484	0.034	0.052	0.143	----	----	----	0.145	0.017	0.014
155	----	----	0.032	0.016	----	----	----	0.012	----	0.016	----	----	----	----	0.017	----	----	----	----	----	----	0.068
157	0.050	----	----	0.031	----	----	0.016	----	----	----	----	----	----	0.017	----	----	----	----	----	----	0.017	0.014
159	----	----	----	----	----	----	----	----	----	----	0.050	0.016	----	----	----	----	----	----	----	----	----	0.014
161	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
187	0.017	----	----	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----
191	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	----	----	----	----	----	----	----
197	0.167	0.074	0.161	0.156	----	0.063	0.328	0.305	0.548	0.141	----	0.047	0.234	0.241	0.190	0.232	0.557	0.411	0.400	0.065	0.052	0.054
199	0.017	----	0.032	0.016	----	----	0.094	----	0.032	----	----	----	----	0.034	0.017	0.036	0.098	0.071	0.050	----	----	----
201	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	----	----	----	----	----	----	----
203	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
205	----	----	----	----	----	----	----	----	----	----	----	0.063	----	----	----	----	----	----	----	----	----	----
	12	7	7	9	6	4	13	8	6	8	4	6	8	10	15	9	6	6	6	8	16	21

Gac7033PBBE

Bin	Big	Long	Step	BBeav	Bev	Seym	Corc	Cloud	LucL	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2	MudAn	RS
180	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.032	----
184	----	----	0.016	----	----	----	----	----	----	0.016	----	----	0.017	----	----	----	----	----	----	----	0.226	0.350
190	----	----	----	----	----	----	0.016	----	0.097	0.047	----	----	0.017	----	----	----	0.125	0.050	0.048	----	0.016	----
192	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018	----	----	----	----	0.048	----	0.065	0.050

194	0.016	0.017	0.047	0.016	0.078	0.109	0.031	0.281	0.097	0.078	0.078	0.177	0.190	0.089	0.033	0.037	0.071	0.050	0.081	----	0.274	0.250
196	0.078	----	0.063	0.097	0.141	0.594	0.078	----	----	0.016	0.109	0.516	0.017	0.018	----	0.037	----	----	0.016	----	0.032	0.100
198	0.188	0.017	0.219	0.177	----	----	0.109	0.031	0.194	0.094	0.016	0.016	0.034	0.161	0.083	0.093	0.161	0.283	0.177	0.207	0.065	0.067
200	0.016	----	0.016	0.032	----	----	0.031	0.047	----	0.047	0.016	----	0.069	0.018	0.033	0.056	----	----	----	----	0.048	0.033
202	0.031	----	----	----	0.031	----	----	----	----	0.031	----	----	----	0.018	0.033	0.019	----	----	----	----	----	----
204	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	0.033	0.019	----	----	----	0.017	----	----
206	----	----	----	----	----	----	----	0.016	0.048	----	----	----	----	----	----	----	0.089	0.067	0.048	----	0.016	----
208	0.016	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	0.017	0.019	----	----	----	----	----
210	0.016	0.017	----	0.016	----	----	0.016	----	----	----	----	----	----	----	----	----	0.019	----	----	----	----	----
212	0.516	0.783	0.563	0.548	0.719	0.281	0.594	0.609	0.452	0.625	0.719	0.210	0.655	0.536	0.683	0.574	0.536	0.550	0.500	0.741	0.032	----
214	0.031	0.033	0.031	0.065	0.031	----	0.063	----	0.032	----	0.063	----	----	0.125	0.067	0.111	----	----	----	0.017	----	----
216	0.078	0.117	0.047	0.016	----	0.016	----	----	----	0.016	----	0.081	----	----	0.017	----	----	----	----	----	----	----
220	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018	----	0.019	----	----	----	----	----	----
222	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017
224	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
226	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.065	----
228	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	0.033
230	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.032	----
232	----	0.017	----	----	----	----	0.016	0.016	0.081	0.016	----	----	----	----	----	----	0.018	----	0.081	0.017	0.016	0.017
234	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
236	----	----	----	----	----	----	0.031	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
238	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017
240	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
242	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017
244	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
246	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	0.017
248	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.033
n=	11	7	8	10	5	4	11	6	7	11	6	5	7	9	9	11	6	5	8	5	18	13

Cier62

Bin	Big	Long	Step	BBeav	Bev	Seym	Corc	Cloud	LucL	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2	MudAn	RS
94	0.017	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018	0.037	----	----	----	----	----	----
98	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
100	----	----	----	----	----	----	----	----	----	----	----	----	----	0.035	0.018	0.019	----	----	----	----	0.016	0.018
102	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018	----	----	----	----	0.036	----	----
104	0.155	0.236	0.246	0.300	----	0.067	0.155	0.065	0.102	0.109	0.078	0.129	0.047	0.053	0.161	0.130	0.121	0.059	0.089	0.107	0.063	----
106	----	----	0.016	----	----	----	----	----	0.068	0.031	----	0.032	0.016	0.035	0.054	0.037	0.052	0.020	----	0.071	0.016	----
108	----	----	----	----	0.031	----	----	0.016	----	----	0.031	----	----	----	----	----	----	----	----	0.071	----	----
110	----	----	----	0.017	----	----	0.034	----	----	----	----	----	----	0.018	----	0.019	----	----	----	----	0.016	0.018
112	----	----	0.016	----	----	----	----	----	----	----	----	----	----	0.053	----	0.019	----	----	----	0.036	0.048	0.018
114	----	----	----	----	----	----	0.052	----	0.017	----	----	----	----	----	0.018	0.019	----	----	----	0.018	0.127	0.161
116	0.086	0.073	0.016	0.067	----	----	0.017	0.048	0.017	0.016	----	----	0.078	0.018	0.018	0.148	0.052	0.078	0.036	0.143	0.127	0.161
118	----	0.036	----	----	----	----	0.017	----	----	----	0.031	----	----	----	----	----	----	----	----	----	0.032	----
120	0.034	0.055	0.066	0.033	----	0.133	0.103	0.048	0.220	0.063	0.016	0.048	0.047	0.105	0.054	0.019	0.259	0.235	0.304	0.054	0.063	0.054
122	0.034	0.055	0.033	0.033	----	----	0.017	----	----	----	----	0.016	----	0.018	----	0.056	----	----	----	0.018	0.048	0.036
124	0.069	0.055	----	0.017	0.125	----	----	----	0.017	----	0.078	0.016	----	0.053	0.071	0.019	0.017	0.020	0.036	0.071	0.048	0.018
126	----	----	0.049	0.017	----	----	----	----	----	----	----	0.032	----	----	0.018	----	----	----	----	0.018	0.016	----
128	0.241	0.091	0.230	0.233	0.609	----	0.155	0.097	0.051	0.281	0.031	0.065	0.219	0.228	0.196	0.148	0.138	0.137	0.125	0.107	0.016	0.054
130	0.034	0.036	0.098	0.100	0.078	0.133	0.138	0.210	0.051	0.125	----	0.016	0.156	0.070	0.107	0.111	0.086	0.039	0.036	0.089	0.032	0.071
132	0.069	0.091	0.049	0.050	0.016	----	0.069	0.016	0.102	0.047	0.281	0.065	----	0.035	0.071	0.037	0.052	0.118	0.054	0.054	0.048	0.036

134	0.103	0.036	0.098	0.017	----	0.167	0.034	0.113	0.068	0.094	0.172	0.113	0.094	0.053	0.036	0.037	0.086	0.059	----	0.018	0.048	0.036
136	0.017	0.036	0.016	0.033	----	----	----	0.145	----	0.063	0.078	0.145	0.109	0.035	0.036	0.019	----	----	----	0.071	0.048	0.018
138	0.017	0.091	0.016	0.033	0.094	----	0.069	0.016	0.136	0.016	0.047	0.065	0.094	0.035	0.036	0.019	0.034	0.020	----	----	0.016	0.089
140	0.034	0.018	0.016	0.017	0.047	0.017	0.017	0.065	0.017	0.063	0.016	0.194	0.047	----	0.018	0.037	----	0.020	0.018	----	0.032	0.036
142	----	----	----	----	----	0.167	----	0.081	----	0.047	0.063	0.048	0.031	0.018	0.036	0.019	----	----	----	0.018	0.063	0.036
144	0.034	----	----	0.017	----	0.317	0.034	0.032	0.102	0.016	0.063	----	0.047	0.070	----	0.037	0.086	0.118	0.196	----	0.032	0.018
145	----	----	----	----	----	----	----	----	----	----	----	----	----	0.053	----	----	----	----	----	----	----	----
146	0.034	0.018	0.033	0.017	----	----	0.069	0.048	----	0.031	----	0.016	0.016	----	0.018	----	----	0.020	----	----	----	----
148	0.017	0.018	----	----	----	----	----	----	----	----	----	----	----	----	----	0.019	----	----	----	----	----	----
150	----	0.036	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	0.036
152	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.032	0.071
154	----	----	----	----	----	----	0.017	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018
156	----	0.018	----	----	----	----	----	----	0.034	----	----	----	----	0.018	----	----	0.017	0.059	0.107	----	----	----
162	----	0.018	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
n=	16	18	15	16	7	7	16	14	14	14	14	15	13	19	19	21	12	14	10	17	23	20

Gacμ10

Bin	Big	Long	Step	BBeav	Bev	Seym	Corc	Cloud	LucL	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2	MudAn	RS
176	----	----	----	----	----	----	----	----	----	----	----	----	----	0.019	0.086	0.036	----	----	----	----	----	0.010
182	0.063	----	0.048	----	----	----	0.141	----	----	----	----	----	----	0.019	----	0.071	----	----	----	0.050	----	0.010
184	0.016	0.050	----	0.032	----	----	0.031	----	----	----	----	----	----	0.019	0.017	0.018	----	----	----	----	0.032	0.010
186	0.047	----	0.016	0.016	0.266	----	----	----	----	0.032	----	----	----	----	----	----	----	----	----	0.017	0.145	0.190
188	----	0.017	----	----	0.063	----	----	0.048	----	0.048	----	----	0.063	----	----	----	----	----	----	----	0.048	0.060
190	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.054	----	----	----	----	0.016	0.030
192	0.016	0.033	0.032	0.048	----	----	0.016	----	----	0.032	----	----	0.016	0.038	0.017	0.036	----	----	----	----	0.048	0.090
194	----	----	----	0.016	----	----	----	----	0.016	0.032	----	----	----	0.019	0.017	----	----	0.065	----	----	0.016	0.040
196	0.188	0.267	0.645	0.710	----	0.063	0.047	0.048	0.203	----	----	0.391	0.047	0.115	0.172	0.125	0.172	0.161	0.250	0.167	0.032	0.040
198	0.031	0.017	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	0.017	0.016	0.070
200	0.047	0.017	----	----	0.047	0.125	0.078	0.032	0.047	0.016	----	0.344	0.031	0.019	0.052	0.054	0.094	0.081	0.063	----	0.065	0.030
202	0.016	0.167	0.065	0.081	----	0.031	0.031	0.065	----	0.016	----	0.094	0.047	0.019	----	----	----	----	----	0.283	0.032	0.030
204	0.016	0.100	0.065	0.065	0.266	----	0.156	0.097	----	0.081	----	0.016	0.094	0.019	----	----	----	----	----	0.117	0.097	0.090
206	0.047	0.017	----	----	0.219	----	0.047	0.016	0.047	0.065	----	0.016	0.047	0.077	----	----	0.031	0.065	0.063	----	0.065	0.050
208	0.016	----	----	----	0.016	----	0.047	----	----	0.048	0.081	0.047	0.031	0.019	0.069	0.036	----	----	----	----	0.032	0.090
210	0.047	0.017	----	----	----	----	----	----	0.016	----	0.048	----	----	----	----	----	0.047	----	0.016	0.033	0.048	0.050
212	0.016	0.033	0.032	----	----	----	0.016	----	0.016	----	----	----	----	----	----	0.018	----	----	----	0.017	0.081	0.030
214	0.031	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	0.030
216	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.018	----	----	----	----	0.048	----
218	----	----	----	----	0.016	----	----	----	----	----	----	----	0.031	----	----	----	----	----	----	----	----	0.010
220	----	0.017	----	----	----	----	0.109	0.048	----	0.081	----	0.063	0.016	0.019	----	0.018	----	----	----	----	0.016	0.010
222	0.031	----	0.016	----	----	----	----	----	----	----	----	----	0.047	----	----	----	----	----	----	----	0.032	0.020
224	0.016	----	----	----	----	----	----	0.016	----	0.048	----	----	0.016	0.019	0.034	----	----	----	----	----	----	----
226	0.016	----	----	----	----	0.188	----	----	----	----	----	0.016	----	----	----	----	----	----	----	0.050	0.048	----
228	0.031	----	----	----	0.016	----	0.016	0.065	----	0.016	----	----	0.047	0.019	0.017	0.036	----	----	----	----	----	----
230	----	0.017	----	----	----	----	----	0.032	----	0.048	----	----	0.047	0.019	0.052	----	----	----	----	----	0.016	----
232	0.016	----	----	----	----	0.109	0.016	0.097	0.016	0.097	----	----	0.141	----	----	----	----	----	----	0.100	----	----
234	----	----	----	----	0.047	----	----	0.081	----	0.048	0.016	----	0.031	0.019	0.034	0.018	0.016	----	----	0.017	----	----
236	----	0.017	----	----	0.031	----	0.047	----	----	0.016	0.016	----	0.016	----	0.017	----	0.016	0.016	0.016	0.017	0.016	----
238	0.031	----	----	0.016	----	0.484	0.063	0.048	0.109	0.032	0.016	----	0.016	0.096	0.121	0.071	0.203	0.048	0.047	----	----	----
240	0.016	----	----	----	----	----	0.016	0.113	0.109	0.032	----	----	----	0.019	0.052	0.018	0.047	0.161	0.109	0.067	----	----
242	0.094	----	0.016	0.016	0.016	----	0.031	----	0.297	0.032	0.113	----	0.031	0.096	0.052	0.196	0.250	0.323	0.281	0.017	----	0.010

244	0.016	0.033	0.016	----	----	----	0.031	0.065	0.063	0.081	0.081	----	0.125	0.096	0.017	0.054	0.047	0.065	0.094	0.033	----	----
246	0.078	----	----	----	----	----	0.016	0.081	0.016	----	0.048	----	0.031	0.019	0.052	0.054	0.016	----	----	----	----	----
248	----	0.050	0.016	----	----	----	0.016	----	----	0.048	0.145	----	----	0.096	0.034	0.036	----	----	----	----	----	----
250	0.031	----	----	----	----	----	----	0.016	----	----	0.097	----	----	0.038	0.017	0.018	----	----	----	----	----	----
252	0.016	----	----	----	----	----	----	----	----	0.016	0.032	----	----	----	0.017	----	----	----	----	----	----	----
254	----	----	----	----	----	----	0.016	----	----	0.016	0.065	----	----	0.019	0.017	----	0.016	----	----	----	----	----
256	0.016	----	----	----	----	----	0.016	----	----	----	0.113	----	----	----	----	----	----	----	----	----	----	----
258	----	0.017	----	----	----	----	----	----	----	----	0.032	----	----	----	0.017	----	----	----	----	----	----	----
260	----	----	----	----	----	----	----	----	0.047	----	0.048	----	----	----	----	0.018	0.047	0.016	0.063	----	0.016	----
262	----	0.017	0.016	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
264	----	0.050	----	----	----	----	----	----	----	----	0.016	----	----	0.019	0.017	----	----	----	----	----	----	----
266	----	0.017	0.016	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----
268	----	0.017	----	----	----	----	----	----	----	0.016	----	----	0.016	0.019	----	----	----	----	----	----	0.016	----
270	----	0.017	----	----	----	----	----	0.032	----	----	----	----	----	----	----	----	----	----	----	----	----	----
272	----	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----
276	----	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----
	27	22	13	9	11	6	22	18	13	24	18	9	23	26	23	21	13	10	10	15	24	22

Gacμ9

Bin	Big	Long	Step	BBeav	Bev	Seym	Corc	Cloud	LucL	Rainb	Frog	Visn	LMC3	LMC2	MC1	LMC1	LC2	LC1	LC3	MC2	MudAn	RS
146	----	----	----	----	----	----	----	0.013	----	----	----	----	----	----	----	----	----	----	----	----	----	----
152	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
156	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
158	----	----	----	----	----	----	----	----	----	----	0.016	----	----	----	----	----	----	----	----	----	----	----
160	----	0.048	----	0.016	0.016	----	----	0.026	----	0.129	0.016	----	0.094	----	----	----	----	----	----	----	0.016	0.078	0.069
162	0.250	0.177	0.094	0.094	----	0.323	0.089	0.171	0.065	0.016	----	0.032	0.109	0.074	0.250	0.127	----	0.032	0.032	0.129	0.063	0.103
164	0.016	0.032	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	0.016	0.034
166	0.016	0.032	0.031	----	----	----	0.018	----	----	----	----	----	----	----	----	----	----	----	----	----	0.031	0.103
168	0.547	0.597	0.625	0.531	0.266	0.355	0.696	0.487	0.774	0.484	0.875	0.726	0.625	0.704	0.517	0.745	0.734	0.694	0.581	0.726	0.422	0.397
170	0.078	0.065	0.063	0.031	0.016	----	0.089	0.079	0.097	0.032	0.031	0.145	0.094	0.130	0.167	----	0.031	0.129	0.177	0.065	0.047	0.034
172	0.016	0.032	0.125	0.156	0.547	0.323	0.018	0.158	0.016	0.177	0.063	0.097	0.047	0.056	0.017	0.055	0.094	0.032	0.145	0.016	0.094	0.052
174	----	0.016	----	0.094	0.156	----	----	----	----	0.032	----	----	----	----	----	----	----	----	----	----	0.094	0.034
176	0.031	----	0.063	----	----	----	0.071	----	----	----	----	----	----	0.019	0.033	----	----	----	----	----	0.016	0.052
177	----	----	----	----	----	----	0.018	----	0.016	0.016	----	----	----	----	----	----	0.063	0.016	----	----	----	----
178	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.017	----	----	----	----	----	----	0.034
180	----	----	----	0.078	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.031	----
182	----	----	----	----	----	----	----	----	0.032	0.016	----	----	----	----	----	----	0.078	0.097	0.065	----	0.016	----
184	0.016	----	----	----	----	----	----	0.066	----	0.097	----	----	0.031	----	----	0.036	----	----	----	0.032	0.016	0.086
188	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.036	----	----	----	----	----	----
190	0.031	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
192	----	----	----	----	----	----	----	----	----	----	----	----	----	0.019	----	----	----	----	----	----	----	----
194	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.031	----
208	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	0.016	----
n=	9	8	6	7	5	3	7	7	6	9	5	4	6	6	6	5	5	6	5	7	16	11