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Contemporary evolution, allelic recycling, and adaptive radiation of the threespine stickleback

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ABSTRACT

Background: Adaptive radiation in the threespine stickleback (*Gasterosteus aculeatus*) is dramatic, highly replicated, and predictable.

Goals: Test hypotheses that divergent phenotypes of freshwater threespine stickleback evolve at the same rate as in other species and that derived freshwater and ancestral oceanic (i.e. marine or anadromous) phenotypes are genetically additive. Update a model for contemporary evolution and adaptive radiation based on new genetic and genomic findings.

Methods: Summarize published information on contemporary evolution of threespine stickleback and compare published stickleback rates of evolution to those of other species. Analyse F1 hybrid and pure crosses to estimate the coefficient of genetic dominance of phenotypes that differ between oceanic and freshwater populations. Review published information on the genetics and genomics of stickleback phenotypes.

Results: Threespine stickleback populations that experience large environmental changes may evolve measurably for multiple traits within ten generations. Rare freshwater-adapted alleles have been recycled from freshwater to oceanic populations by introgression and increase to high frequencies when oceanic stickleback colonize fresh water. These freshwater-adapted alleles tend to be partially recessive, to produce adaptive phenotypic plasticity, and to be linked within several genomic regions, which facilitates their retention in oceanic populations and the evolutionary response to directional selection after invasion of fresh water.

Conclusion: The metapopulation structure and great age of the threespine stickleback have produced a genomic architecture and abundant allelic variation that are conducive to predictable contemporary adaptive radiation involving numerous genes and phenotypic traits.

Keywords: evolutionary genomics, *Gasterosteus aculeatus*, introgression, invasive species, parallelism, recessive allele, threespine stickleback.

INTRODUCTION

Charles Darwin (1859: 84) claimed that 'We see nothing in these slow changes in progress, until the hand of time has marked the long lapse of ages.' Despite Wallace's (1891: 125) opinion that

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'Mr. Darwin was rather inclined to exaggerate the necessary slowness of the action of natural selection,' and subsequent empirical evidence to the contrary notwithstanding, Darwin's view prevailed for almost 150 years. Darwin (1861/1994) also recognized that theory shapes empirical research: 'How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service!' Accordingly, Darwin's view that natural populations evolve very slowly led him to study artificial selection, natural selection's component mechanisms (e.g. exponential population growth, heritability), and evidence for evolution as an historical fact. He did not attempt to study natural selection and evolution in contemporary natural populations. Darwin's successors largely accepted his view and empirical approach. In fact, evidence for contemporary evolution of grasses on toxic mine tailings in the UK (e.g. Gregory and Bradshaw, 1965; McNeilly, 1968) was literally right under his nose, but the 'cloven hoofprint of theory' (Hansen, 1969) – his own theory that evolution is slow – blinded him to this and other similar opportunities.

Darwin's (1859) view that evolution is slow has had two adverse consequences for the development of evolutionary biology. First, it created the expectation that gradual transitions between species would be observable in the fossil record, but this is rarely true (e.g. Eldredge and Gould, 1972; Erwin and Anstey, 1995). In fact, natural selection is so powerful that transitions between fossil species ('punctuations') are usually too fast to be resolved in the stratigraphic record (Schindel, 1980; Gould, 2002; Bell, 2009; Kirkpatrick, 2010). Darwin's view also discouraged investigation of evolution in contemporary populations. Although evolution of industrial melanism (Kettlewell, 1973; Majerus, 1998, 2009) and antibiotic (e.g. Baquero and Blázquez, 1997) and pesticide resistance (Gould, 2010) were recognized, they could be dismissed as the product of unnaturally powerful anthropogenic selection, and they did not lead to the recognition of the importance of contemporary evolution. Hendry and Kinnison's (1999: 1638) definition of contemporary evolution as 'changes that take place within species or populations . . .' and 'microevolution occurring in recent times and on short time scales (less than a few centuries)', and their review of cases and methods to quantify contemporary evolution stimulated interest in this phenomenon. Now, the ubiquity and consequences of contemporary evolution are recognized (Hendry *et al*., 2008).

Among the examples of contemporary evolution that quietly accumulated prior to the focus created by Hendry and Kinnison (1999) were a handful of cases that occurred under relatively natural conditions in the threespine stickleback fish, *Gasterosteus aculeatus*. We review these and subsequent cases (Table 1), discuss the role played by ancestral adaptive variation in contemporary evolution of this species, and update a model for adaptive radiation in *G. aculeatus*.

OVERVIEW OF CONTEMPORARY EVOLUTION IN THREESPINE STICKLEBACK

Studies of stickleback contemporary evolution have included different traits and different methods to compare samples from descendants and their actual ancestors or a surrogate. We focus on Hagen's early contribution to this field, evolutionary rates observed in published studies, and the Loberg Lake population, which we have studied continuously for more than two decades.

Donald W. Hagen's groundbreaking contributions to stickleback evolutionary ecology

The earliest study of contemporary evolution in the threespine stickleback culminated Hagen's refutation of Miller and Hubbs' (1969) claim that variation among western North American threespine stickleback populations is due to gene flow between three nominal subspecies, the marine and anadromous (collectively 'oceanic') *Gasterosteus aculeatus aculeatus* (Fig. 1A), the widespread, freshwater *G. a. microcephalus* (Fig. 1B), and the endemic freshwater *G. a. williamsoni*. These three nominal subspecies are marked by lateral plate counts of about 33, 5–7, and 0 plates per side, respectively. Miller and Hubbs (1969) had implicitly assumed that plate number is non-adaptive. Hagen and McPhail (1970) replied with evidence for adaptive variation of lateral plate number and several other stickleback traits, and Hagen followed up with a series of empirical studies that focused on lateral plate variation. Hagen and Gilbertson (1972, 1973a) defined three lateral plate morphs to avoid use of subspecific names for plate phenotypes and older ambiguous terms. The low morph has fewer than 11 plates per side, restricted to the anterior part of the body (Fig. 2A); the partial morph has more than 11 plates per side in anterior and posterior rows that are separated by an unplated gap (Fig. 2B); and the complete morph has an uninterrupted row of about 33 plates per side (Fig. 2C). Hagen and Gilbertson (1972) showed that high lateral plate number (i.e. 7 plates per side) in low morphs is associated with the presence of predatory fishes in freshwater populations, but not with their distance from the ocean, where gene flow from complete morph, oceanic stickleback would cause plate number to increase in low

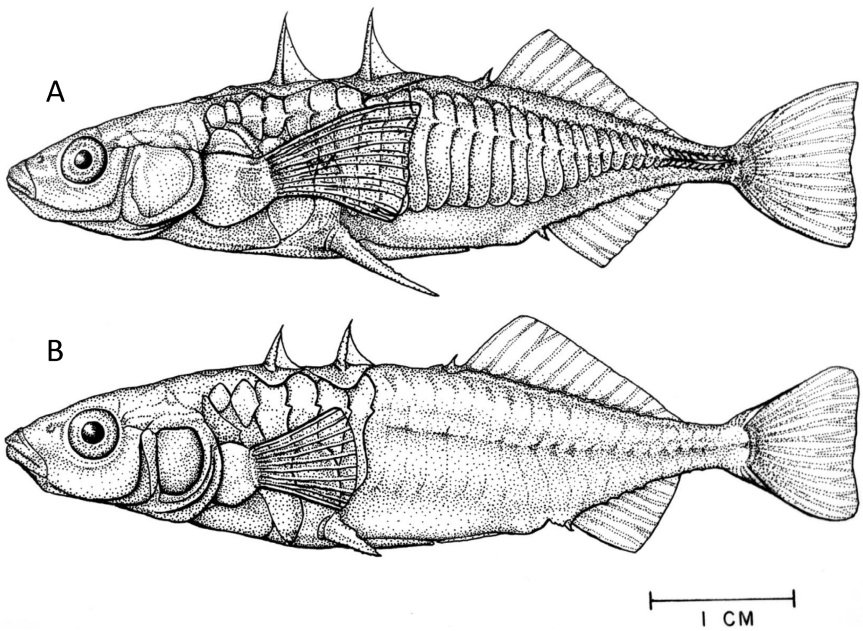

Fig. 1. (A) Anadromous and (B) typical freshwater threespine stickleback. Although anadromous and marine (collectively oceanic) stickleback exhibit limited phenotypic variation, freshwater populations are diverse (Bell and Foster, 1994). Note differences in body shape, lateral plate coverage, and size, shape, and positions of fins. Modified from Bell (1976).

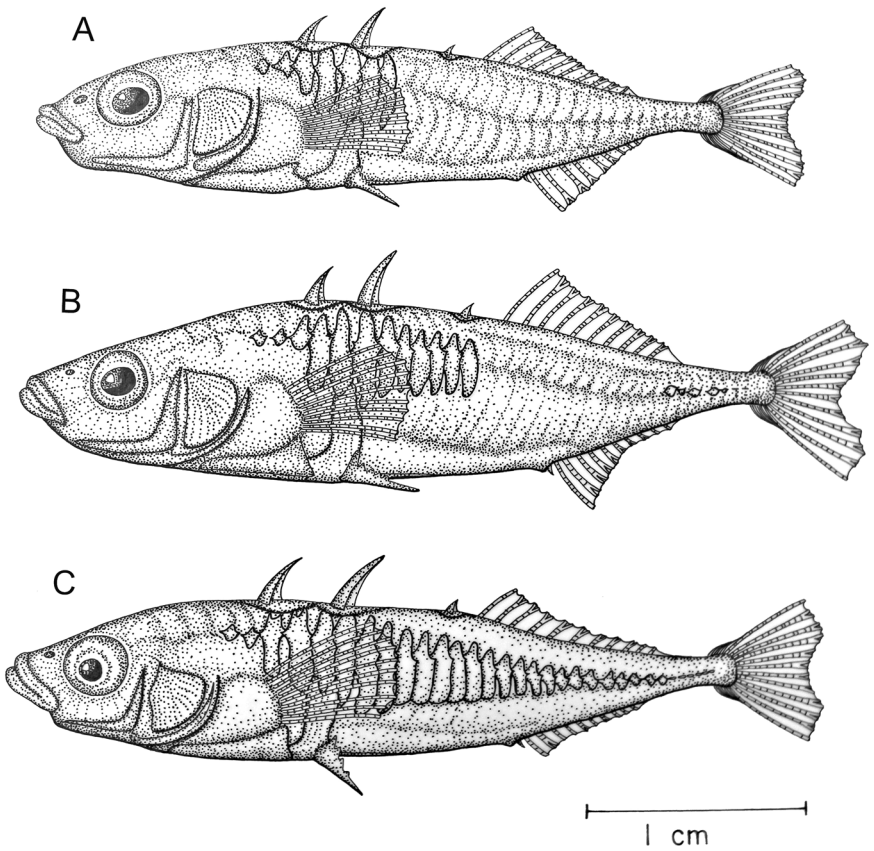

Fig. 2. Lateral plate morphs of *Gasterosteus aculeatus*: (A) low; (B) partial; (C) complete. From Bell and Foster (1994).

morph, freshwater populations. They also showed that plate number variation and morph are genetically determined (Hagen, 1973; Hagen and Gilbertson, 1973a). Hagen and Gilbertson's (1973b) analysis of directional selection and contemporary evolution of lateral plate number in an introduced lake population of mostly low-plated stickleback administered the final blow to Miller and Hubbs' (1969) introgression model for stickleback variation. This study showed that predation by rainbow trout (*Oncorhynchus mykiss*) is selective and favoured higher plate number phenotypes (i.e. 7 and 8 per side) over lower ones (i.e. 5 or 6 per side) in low morph stickleback within two consecutive cohorts and corresponding evolution between two consecutive generations. Hagen's research programme demonstrated that lateral plate number phenotypes in low morphs are adaptive and heritable, and that it can evolve rapidly in response to local natural selection without the need for gene flow (Bell, 2001; Barrett, 2010).

Other cases of contemporary evolution in threespine stickleback

We were already familiar with several studies of contemporary evolution in *G. aculeatus*, and we asked colleagues and did a Google Scholar search to find additional cases. We identified 28 studies published between 1973 and 2013 (Table 1). Fifteen involved oceanic

Table 1. Published studies on contemporary change in threespine stickleback (the environmental change that probably altered selection, resulting in detectable contemporary evolution, is indicated)

Study	Trait	Environmental change	Rate (|haldanes|)	Period (years/gen.)
Hagen and Gilbertson (1973b)	Lateral plate number in low morphs	Introduction (l-l)	—	2/2
	Gill raker number		—	
Francis *et al*. (1985)	Lateral plate morphs	Colonization (a-l)	—	25/~12.5
Klepaker (1993)	Lateral plate morphs	Habitat change (m-l)	—	31/?
	Dorsal spine number		—	
	Lateral plate number		—	
	Multiple (22) morphometric		—	
Reimchen (1995)	Lateral plate number in low morphs	Natural seasonal change (l)	—	3/<1
Ziuganov (1995)	Assortative mating	Introduction (a-l)	—	8/8
	Lateral plate morphs		—	
Bell (2001)	Lateral plate morphs	Colonization (a-l)	—	10/~5
Kraak *et al*. (2001)	Assortative mating	Habitat change (l)	—	7/<7
	Loss of divergence between species		—	
Reimchen and Nosil (2002)	Dorsal spine number	Natural seasonal change (l)	—	17/~7.5
	Pelvic spine number		—	
	Anal spine number		—	
Kristjánsson *et al*. (2002)[a]	Gill raker number	Habitat change (m-l)	—	13/?
	Lateral plate number (unkeeled)		0.03	
	Lateral plate number (keeled)		0.06	
	Dorsal spine number		—	
	Dorsal fin ray number		—	
	Anal fin ray number		—	
	Caudal fin ray number		—	
	Multiple (18) morphometric, including:			
	dorsal spine length		0.18	
	pelvic spine length		0.10	
	anal fin length		0.01	

(*continued*)

Table 1.—(*continued*)

Study	Trait	Environmental change	Rate (|haldanes|)	Period (years/gen.)
Kristjánsson *et al.* (2004)	Pectoral fin ray number	Habitat change (m-l)	—	13/?
Bell *et al.* (2004)	Lateral plate morph	Colonization (a-l)	—	12/~6
	Low lateral plate number (even years)		0.137	
	Low lateral plate number (odd years)		0.104	
	Complete lateral plate number (even years)		0.237	
	Complete lateral plate number (odd years)		0.142	
Olafsdóttir (2004)	Spine length	Introduction (m-l)	—	13/?
Kristjánsson (2005)	Fork length	Introduction (m-l)	—	1/1
	Lateral plate number		—	
	Body shape		—	
Vamosi (2006)	Lateral plate number	Introduction (s & a?-l)	0.029	23/23
Barrett *et al.* (2008)	Lateral plate morphs	Mesocosm (m-l)	—	≤1/≤1
	Ectodysplasin		—	
Kitano *et al.* (2008)	Lateral plate morphs	Habitat change (l)	—	36/36
Gelmond *et al.* (2009)	Lateral plate morphs	Colonization (m-s & l)	—	41/?
	Lateral plate number		—	
	Gill raker number		—	
	Morphometric traits (11)		—	
Arif *et al.* (2009)	Operculum shape	Colonization (a-l)	0.122	15/~7.5
Baker *et al.* (2011)	Egg dry mass	Habitat change? (l)	0.304	10/7.5
	Clutch size		0.207	
	Female breeding standard length		0.234	
	Female breeding age		0.129	
	Male breeding standard length		0.365	
	Male breeding age		0.473	
Le Rouzic *et al.* (2011)	Lateral plate morph	Introduction (l-l)	—	20/20?, 12/~6
	Ectodysplasin		—	
Lind and Grahn (2011)	Multiple (21) AFLP loci	Habitat change (m)	—	>40/>20[b]

Tuomainen *et al*. (2011)	Male courtship behaviour	Habitat change (m)	—	>40/>20
Barrett *et al*. (2011)	Cold tolerance	Introduction (m-l)	0.63	2/2
Aguirre and Bell (2012)	Body shape	Colonization (a-l)	—	20/~10
Furin *et al*. (2012)	Assortative mating	Colonization (a-l)	—	≤22/≤11
Leaver and Reimchen (2012)	Pelvic spine length	Introduction (l-l)	0.19/0.07	16/8–12
	Dorsal spine length		0.25/0.20	
	Gill raker length		0.22/0.17	
	Eye diameter		0.23/0.21	
	Lateral plate 1–3 present (%)		0.12/0.06	
	Lateral plate 4–8 present (%)		0.00/0.03	
Adachi *et al*. (2012)	Standard length	Colonization (l-l)	—	25/?
Hendry *et al*. (2013a)	Lateral plate number	Colonization (s-l)	—	~40/≤35
	Pelvic spine length		—	
	Dorsal spine length		—	
	Pelvic girdle width		—	
	Body shape		—	

Note: Parenthetic abbreviations in the Environmental Change column refer to the source of the original population and the habitat into which it was introduced (first and second letter) or the habitat in which the population evolved (single letter): anadromous (a), lake or pond (l), marine (m), stream (s). Rates are expressed as the absolute value in haldanes measured over the Period in years and generations (gen.). Leaver and Reimchen (2012) presented separate rates for males/females.

[a] Corrected rates (Kristjánsson, 2005).

[b] Based on generation time given for Gulf of Finland stickleback by Tuomainen *et al*. (2011).

populations that had colonized fresh water, four resulted from lake-to-lake (or pond) introduction, seven were caused by seasonal or anthropogenic habitat perturbation, one involved a stream-to-pond introduction, and one was based on a lake population derived from a stream or a mixture of stream and anadromous stickleback. Five of 14 studies of freshwater colonization by oceanic stickleback were based on one population in Loberg Lake, Alaska (see below), and one involved several freshwater populations that had been founded naturally by oceanic stickleback on part of an island that had emerged from the Pacific Ocean during a 1964 earthquake (Gelmond *et al*., 2009). All but three studies (i.e. Reimchen, 1995; Reimchen and Nosil, 2002; Gelmond *et al*., 2009) involved populations that evolved in response to human disturbance, but subsequent contemporary evolution in all 28 cases took place under natural conditions. Thus, they generally simulate the process by which stickleback adaptive radiation occurs and indicate how fast it has taken place.

Rates of change in contemporary threespine stickleback populations

Rates of change in contemporary threespine stickleback populations can be compared with those of other species using haldanes. The haldane (h) was proposed by Haldane (1949) to quantify evolutionary rates and named and popularized by Gingerich (1993). It is calculated as

$$h = (x_2 - x_1)/s_p/g,$$

where x_1 and x_2 are the mean values of the natural logarithm (ln) of traits in an earlier and later sample, respectively, s_p is the pooled mean standard deviation of the natural logarithm of the samples, and g is the number of generations between samples. Haldanes represent change in standard deviation units of the natural logarithm of a trait per generation. The sign of the rate depends on whether its mean value increased or decreased, so we use absolute values. Evolutionary rates tend to vary inversely with g, so it is essential to consider the number of generations over which rates are estimated (Gingerich, 1983, 2001; Stockwell *et al*., 2003).

Rates in haldanes were calculated in only 7 of 28 studies of contemporary change in threespine stickleback, and they produced a total of 24 rates (Table 1). Diverse traits, including lateral plate numbers, spine lengths, operculum shape, and several male and female life-history traits have been studied. Pairs of measurements for different traits from the same study may not be biologically independent, and nine rates are for lateral plate evolution. Leaver and Reimchen (2012) reported separate rates for males and females and used both the source population and the first generation of the introduced population for base values. We averaged the mean of rates for the sexes for each trait and used their comparison to the source population for comparability with other studies. We used the rates from Kristjánsson *et al*. (2002), as corrected by Kristjánsson (2005).

Rates for all traits and studies ranged from 0.00 to 0.63 and averaged 0.18 haldanes. Four sets of rates in haldanes were based on oceanic populations that had colonized or been introduced to fresh water, one was from a lake population that experienced environmental change, another was from a population founded using stream and possibly anadromous stickleback, and the last was a transplant from a large lake to a small pond. Eleven rates for oceanic stickleback in fresh water ranged from 0.01 to 0.63 and averaged 0.16 haldanes. Thirteen rates in stickleback adapting to altered freshwater conditions ranged from 0.00 to 0.47 and averaged 0.20. Unfortunately, six of the rates from freshwater populations but

none from oceanic ones were for life-history traits. Given the differences in traits studied and the low numbers of studies and rates, statistical analysis is not warranted, but these results provide no evidence that rates after colonization of fresh water by oceanic stickleback differ from rates in freshwater residents. Many more estimates will be needed before one can discern variation in rates associated with different traits or types of ecological changes.

Hendry *et al.* (2008) reported 2357 rates of contemporary change in haldanes for diverse species and traits. As in the stickleback data set, rates within studies may not be independent of each other and different studies may include rates for the same trait in different populations. The rates for stickleback must be compared to those from other species with these limitations in mind. In the full data set, rates ranged from 0 to 9.6 and averaged 0.939 haldanes, but these estimates were based on differences measured up to 308 generations apart. Since computed evolutionary rates tend to vary inversely with g (Gingerich, 1983, 2001), we compared the stickleback rates, which were measured over 2 to 23 generations apart (Table 1), to the 342 rates from Hendry *et al.* (2008) that used samples taken less than 25 years apart. Rates in the reduced data set from Hendry *et al.* (2008) ranged from 0 (11 rates) to 1.14 and averaged 0.105 haldanes, so rate estimates in this case did not increase using shorter time intervals.

Contemporary change in threespine stickleback does not appear to be unusually rapid. This result is surprising because phenotypic differences between oceanic and freshwater stickleback populations derived from them can be visually impressive within a few decades (Fig. 1). This misimpression may occur because a large number of traits can change simultaneously within a population.

Contributions of phenotypic plasticity

Much of the phenotypic change observed in contemporary populations of diverse species represents phenotypic plasticity, not evolution (Gienapp *et al.*, 2008; Hendry *et al.*, 2008). Laboratory studies using threespine stickleback have detected phenotypic plasticity of lateral plate number (Lindsey, 1962), body size (McGuigan *et al.*, 2010), vertebral number (Swain, 1992a, 1992b), trophic morphology (Day *et al.*, 1994; Wund *et al.*, 2008), body shape (Sharpe *et al.*, 2008), life-history traits (Baker *et al.*, 2013), and male courtship behaviour (Candolin, 2009). Thus, phenotypic plasticity may make a substantial contribution to change in contemporary populations after they experience a major environmental change.

Leaver and Reimchen (2012) addressed this problem directly using two types of comparisons between a source lake population and an introduced pond population. Comparing the latest sample from the pond population to the first pond sample and to the lake sample, change within the pond population (11 generations) for continuous variables was only 27% for males and 37.5% for females of the change between the latest pond sample and the source sample (12 generations). The directions of change using the lake or first generation in the pond as starting points were generally concordant, but up to three-quarters of the change in the pond occurred during the first generation. Univariate comparisons between laboratory-reared fish from the source and introduced populations were usually non-significant, but principal components analysis indicated a separation of about 28%. Although selection may have been exceptionally strong during the first generation in the pond, it appears that phenotypic plasticity caused up to three-quarters of the change observed over 12 generations.

Some studies of contemporary evolution in threespine stickleback include samples from several generations (Reimchen and Nosil, 2002; Bell *et al*., 2004; Arif *et al*., 2009; Baker *et al*., 2011; Aguirre and Bell, 2012; Leaver and Reimchen, 2012; Le Rouzic *et al*., 2011) (Table 1), and divergence since the first generation is very likely to result from genetic change. Similarly, changes within individual cohorts of lateral plate phenotypes from a single habitat after the phenotypes had become ontogenetically stable (see Bell, 1981) must represent genetic change (Hagen and Gilbertson, 1973b; Reimchen, 1995). Le Rouzic *et al*. (2011) observed contemporary evolution of allele frequencies of the *ectodysplasin* (*EDA*) gene, which strongly influences lateral plate phenotypes (Colosimo *et al*., 2005), and this change could not possibly reflect phenotypic plasticity. Similarly, Lind and Grahn (2011) observed consistent, significant differences between populations from four pairs of habitats with and without local pulp mill effluent pollution for 21 amplified fragment length polymorphisms (AFLP), which also cannot be due to phenotypic plasticity. Despite the substantial contribution of phenotypic plasticity to contemporary change in threespine stickleback populations, there is abundant evidence for contemporary evolution.

There is also good evidence that phenotypic plasticity in threespine stickleback can be adaptive (Swain, 1992b; Day *et al*., 1994; Wund *et al*., 2008) and that the degree of plasticity is evolvable (Day *et al*., 1994). Swain's (1992b) experimental study of phenotypic plasticity and predator avoidance was particularly important. He showed that vertebral phenotypes induced by higher or lower temperatures during development improved predator avoidance at each temperature. Adaptive phenotypic plasticity may constitute a major portion of the phenotypic difference between ancestors and descendants during the first few generations after environmental change (Leaver and Reimchen, 2012), and it can reveal latent variation on which directional selection acts after threespine stickleback are exposed to a new or changing environment (West Eberhard, 2003; Wund *et al*., 2008).

The oceanic-to-freshwater transition

Thirteen studies of contemporary evolution in threespine stickleback concern freshwater populations derived recently from oceanic ancestors (Table 1). These studies are particularly interesting because they simulate the process by which diverse freshwater stickleback populations were founded and diverged from their oceanic ancestors after deglaciation of boreal regions of Eurasia and North America (e.g. Bell, 1976; Bell and Foster, 1994; Bell *et al*., 2004). While the extraordinary diversity of post-glacial freshwater threespine stickleback populations (e.g. Hagen and McPhail, 1970; Moodie and Reimchen, 1976; Lavin and McPhail, 1985; Walker, 1997; Spoljaric and Reimchen, 2007; Ravinet *et al*., 2013; Reimchen *et al*., 2013) demonstrates extensive adaptive radiation within thousands of generations, analyses of contemporary evolution show that oceanic stickleback colonize new freshwater habitats within a few years after they form (Bell, 2001; Bell *et al*., 2004; Gelmond *et al*., 2009) and that much of their existing phenotypic diversity could have evolved within decades (Table 1).

Lateral plate phenotypes and effects of the ectodysplasin gene

The most consistent, conspicuous, and understood difference between oceanic and freshwater stickleback in western North America is lateral plate morphology (Fig. 2). Oceanic stickleback from this region are usually monomorphic or nearly monomorphic for the complete lateral plate morph (Hagen, 1967; Aguirre *et al*., 2008), and populations from lakes and sluggish streams are usually monomorphic or nearly monomorphic for the low lateral plate morph (Hagen and Moodie, 1982; Baumgartner and Bell, 1984; Bell, 1984). Oceanic and freshwater

populations in Western Europe are more polymorphic for lateral plate phenotypes, but complete morphs predominate in oceanic populations and low morphs in fresh water (Heuts, 1947a; Münzing, 1963; Klepaker, 1996). Divergence between oceanic and freshwater threespine stickleback populations is thus similar in both regions, but oceanic populations in Western Europe may carry higher frequencies of freshwater-adapted alleles.

Several studies indicate that consistent divergence of freshwater populations from their oceanic ancestors in western North America results from both the fitness advantage of the low plate morph in fresh water and selection on other phenotypic traits, including growth rate, which are associated with the *ectodysplasin* (*EDA*) locus (Le Rouzic *et al*., 2011; reviewed by Barrett, 2010; Hendry *et al*., 2013b). However, Albert *et al*. (2007) showed that *EDA* or a linked gene (see Hohenlohe *et al*., 2012) also influences body shape, creating the possibility that selection for body shape, which also appears to differ consistently between oceanic and freshwater populations (Walker and Bell, 2000; Leinonen *et al*., 2006; Aguirre and Bell, 2012), will produce a correlated evolutionary response of lateral plate phenotypes after colonization of fresh water by oceanic stickleback. Furthermore, Barrett *et al*. (2008) observed dramatic but inconsistent changes in the frequencies of *EDA* genotypes over short intervals within a generation before plates developed. Barrett *et al*. (2009) observed a significant growth advantage of low-plated stickleback, especially in fresh water. Finally, selection on *EDA* predicted the temporal pattern of lateral plate evolution better than selection on lateral plate phenotypes alone in an introduced population (Le Rouzic *et al*., 2011). Thus, several lines of evidence indicate that evolution of lateral plate phenotypes in fresh water after colonization by oceanic stickleback results both from direct selection on lateral plate phenotypes and indirect selection based on fitness differences of pleiotropic phenotypes (Barrett *et al*., 2009).

However, *EDA* is not the only gene that influences lateral plate number. Although *EDA* explained 76.9% of the plate number variance in the F2 cross that Colosimo *et al*. (2004) studied, three other loci contributed to plate number variance in EDA^C/EDA^L (i.e. complete/low allele) heterozygotes and EDA^L/EDA^L (i.e. low allele) homozygotes. Although the effect of *EDA* is geographically widespread (Colosimo *et al*., 2005), the relationship between *EDA* genotypes and plate phenotypes in a series of Icelandic populations was weaker, explaining only 41.5% and 51.4% of the variation in two populations (Lucek *et al*., 2012). Although the *EDA* gene is likely to be a major factor in lateral plate evolution, it appears that other genes can also have larger effects than suggested by the results of Colosimo *et al*. (2004).

Contemporary evolution of the Loberg Lake population

Five studies used the Loberg Lake stickleback population to examine contemporary evolution, and every trait studied has evolved. This population was established after 1982 (Bell, 2001; Bell *et al*., 2004), and its relatively high genetic diversity indicates that it was founded by numerous individuals (Aguirre, 2007). The size–frequency distribution and patterns of low morph lateral plate number in this population suggest a generation time of about 2 years (Bell *et al*., 2004). When first sampled in 1990, this population resembled anadromous stickleback (Aguirre *et al*., 2008) for lateral plate number and morph frequencies (Bell *et al*., 2004) (Figs. 3, 4), body shape (Aguirre and Bell, 2012) (Fig. 5), and gill-raker number (Bell *et al*., 2004). However, operculum shape may already have diverged substantially; only one of six (17%) specimens had the anadromous operculum shape in 1990, and this frequency declined to 4% by 2004 [$n = 42$ (Arif *et al*., 2009)]. Almost all (i.e. 96%) specimens were completely plated (modally with 33 plates per side) in the 1990 sample, and their frequency then declined dramatically until 1994, after which the rate of decline slowed progressively, plateauing at 3–5% since 2005.

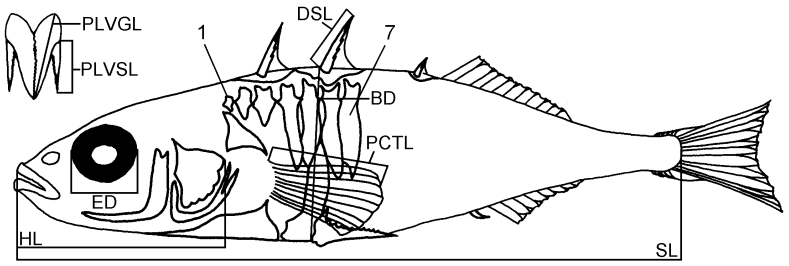

Fig. 3. Traits shown in a lateral view of the body and a ventral view of the pelvis. Length of second dorsal spine (DSL), length of pelvic spine (PLVSL), length of pelvic girdle (PLVGL), standard length (SL), head length (HL), eye diameter (ED), body depth (BD), and pectoral fin length (PCTL). The first (1) and last (7) lateral plates are labelled. Gill rakers are internal structures and are not shown. Modified from Francis *et al.* (1985).

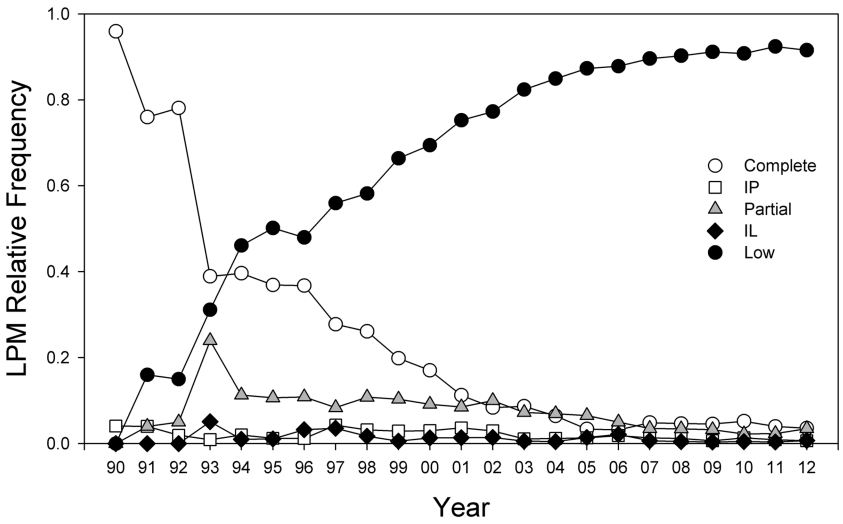

Fig. 4. Temporal variation of the relative frequencies of five lateral plate morphs (LPM) in threespine stickleback from Loberg Lake. See Fig. 2 for lateral plate morphs. Intermediate low phenotypes (IL) have more than 11 plates restricted to the anterior part of the body, and intermediate partial phenotypes (IP) have multiple unplated gaps in the plate row.

Low morphs (modally with 7 plates per side) were absent in 1990, represented 16% in 1991, and have experienced a roughly reciprocal pattern of change to that of complete morphs, increasing slowly from about 87% in 2005 to 92% in 2011 (Fig. 4). Similarly, body shape resembled that of anadromous stickleback originally, converged rapidly with other lake populations by 1992, and has moved further towards them in three apparent small jumps since then (Fig. 4). After 22 years, a population that originally resembled anadromous threespine stickleback has become almost indistinguishable from other lake populations.

By 2005, body size of Loberg Lake stickleback had declined considerably compared with that of local anadromous stickleback (Furin *et al.*, 2012). Mate choice experiments suggested that if Loberg Lake stickleback became sympatric with anadromous stickleback, they would be partially reproductively isolated by size-assortative mating (Furin *et al.*, 2012), which

is an important isolating mechanism between established stickleback species pairs (Hay and McPhail, 1975; Nagel and Schluter, 1998; Ishikawa and Mori, 2000; McKinnon *et al*., 2004). Thus, within about ten generations, partial reproductive isolation existed between the Loberg Lake population and its presumptive anadromous ancestor.

RECESSIVENESS OF FRESHWATER-ADAPTED ALLELES

Hypothesis for retention of freshwater alleles in oceanic populations

Any model for multi-trait contemporary evolution of oceanic threespine stickleback in fresh water must take into account several of the stickleback's properties. Here, we focus on one such property, retention of freshwater-adapted alleles by oceanic populations that rarely express them and in which they are apparently maladaptive. The inheritance of lateral plate morphs (i.e. complete vs. low) provided the preliminary observation to hypothesize that freshwater phenotypes are recessive to oceanic ones, and thus could be retained at a higher equilibrium frequency than if they were additive or dominant. F1 intra-population crosses between complete and low morphs produce inconsistent results (Bańbura and Bakker, 1995), but crosses between completely plated anadromous and low-plated freshwater individuals usually produce completely plated F1 progeny. In these crosses, the allele for the complete morph, EDA^C, is usually dominant to EDA^L for the low morph (Colosimo *et al*., 2004; Cresko *et al*., 2004). Thus, natural hybrids and backcrosses would usually be completely plated and probably would not suffer reduced fitness based on plate morphology in marine habitats. We hypothesize that other freshwater traits are also recessive to their oceanic counterparts. If this hypothesis is true, they should also experience weak selection at low frequencies in the ocean and be carried as recessive standing genetic variation. We present new data from intra-population (i.e. pure) and inter-population (i.e. hybrid) crosses to test this hypothesis.

New crosses

Adults for crosses came from Bear Paw (61.614N, 149.756W) and Boot lakes (61.717N, 150.117W), Matanuska-Susitna Borough, and from an anadromous population in an unnamed marsh along Glenn Highway, north of Anchorage, Alaska (referred to hereafter for convenience as 'Glenn slough', 61.468N, 149.297W). The Bear Paw and Boot lake populations exhibit extreme armour reduction (Bell and Ortí, 1994; Cresko *et al*., 2004). The Glenn slough population exhibits the typical robust armour and other ancestral traits of oceanic populations (e.g. Heuts, 1947b; Hagen, 1967; McPhail, 1994; Walker and Bell, 2000; Cresko *et al*., 2004; Schluter *et al*., 2004; Aguirre *et al*., 2008).

Parents were collected with minnow traps and used in crosses within 24 h of capture (see, for example, Bell *et al*., 1993; Aguirre *et al*., 2004; Cresko *et al*., 2004 for methods). Thirty-six genetic crosses were performed, and 25 produced offspring for analysis (Table 2). Some F1 hybrids could not be used in this study because they were used by Cresko *et al*. (2004) or were infected with *Glugea anomala* (Microsporidia). Anadromous Glenn slough parents for pure and hybrid crosses and lake parents for hybrid crosses were collected on 20 June 1999, late in the breeding season, when freshwater females were rare. Thus, most hybrid crosses used Glenn slough females and lake males. Individuals for the eight freshwater intra-population control crosses were collected on 14 June 2000. Crosses were performed by *in vitro* fertilization, as

Table 2. Crosses, and pelvic score (PS) and lateral plate morph (LPM) frequencies (%) from crosses

	Parent			Family PS									Family LPM		
Cross	Male	Female	*n*	0	1	2	3	4	5	6	7	8	L	P	C
1	Glenn	Glenn	39	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
2	Glenn	Glenn	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
3	Glenn	Glenn	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
4	Bear Paw	Bear Paw	26	19.2	30.8	15.4	7.7	15.4	3.8	3.8	3.8	0.0	100.0	0.0	0.0
5	Bear Paw	Bear Paw	16	56.3	25.0	18.8	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
6	Bear Paw	Bear Paw	20	55.0	15.0	25.0	5.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
7	Bear Paw	Bear Paw	9	66.7	22.2	11.1	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
8	Bear Paw	Bear Paw	3	0.0	33.3	33.3	33.3	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
9	Boot	Boot	14	64.3	21.4	14.3	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
10	Boot	Boot	15	0.0	0.0	66.7	0.0	13.3	6.7	0.0	6.7	6.7	100.0	0.0	0.0
11	Boot	Boot	4	0.0	0.0	25.0	50.0	0.0	25.0	0.0	0.0	0.0	100.0	0.0	0.0
12	Glenn	Bear Paw	25	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	96.0	0.0	0.0	100.0
13	Glenn	Bear Paw	9	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	88.9	0.0	0.0	100.0
14	Glenn	Bear Paw	14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	28.6	71.4
15	Glenn	Bear Paw	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
16	Glenn	Bear Paw	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	8.3	91.7
17	Glenn	Bear Paw	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	11.1	88.9
18	Glenn	Bear Paw	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	20.0	80.0
19	Glenn	Boot	7	0.0	0.0	0.0	0.0	0.0	14.3	14.3	14.3	57.1	14.3	71.4	14.3
20	Glenn	Boot	29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	96.6	0.0	0.0	100.0
21	Glenn	Boot	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
22	Glenn	Boot	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	33.3	66.7
23	Glenn	Boot	5	0.0	0.0	0.0	0.0	0.0	0.0	20.0	20.0	60.0	0.0	0.0	100.0
24	Boot	Glenn	68	0.0	0.0	0.0	0.0	0.0	0.0	2.9	1.5	95.6	0.0	0.0	100.0
25	Boot	Glenn	69	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0

Note: Cross is the cross number, Parent refers to the populations from which Male and Female parents came, and *n* is the number of progeny per cross. See Methods for definitions of phenotypes.

described in Aguirre *et al*. (2004). Offspring were reared in aquaria in one room and experienced similar conditions. They were fed live brine shrimp nauplii until they were large enough to eat frozen adult brine shrimp. Progeny were anaesthetized with MS-222 and sacrificed about 15 months after the crosses were performed, when they had reached adult size. The parents and progeny were fixed in 10% buffered formalin, transferred to 50% isopropyl alcohol, stained with Alazarin Red S to make superficial bones visible, and individually tagged.

Six armour traits were scored. Pelvic score was classified into categories ranging from 0 (absent) to 4 (all four pelvic elements present) per side, and the scores from both sides were summed for each specimen (Bell *et al*., 1993; Bell and Ortí, 1994; Klepaker *et al*., 2013). Lateral plate morph (LPM) was classified as low, partial or complete (see above) on the left side of the body using Hagen and Gilbertson's (1972, 1973b) criteria. The frequencies of intermediate partials and partials, and of intermediate lows and lows (*sensu* Bell *et al*., 2004) were pooled. In addition, the number of lateral plates (LP) on the left side of the body was counted under a dissecting microscope (Fig. 3). LPM and LP are not independent because the range of plate counts usually differs among morphs (i.e. low < partial < complete) (Bell, 1981). Three other armour traits were measured: length of the second dorsal spine (DSL), length of the left pelvic spine (PLVSL), and length of the pelvic girdle along the left side (PLVGL) (Fig. 3).

Gill raker number (GR) is associated with diet, and populations with more gill rakers generally eat smaller prey (e.g. Gross and Anderson, 1984; Lavin and McPhail, 1985; McPhail, 1994). The number of gill rakers on the first right gill arch was counted under a dissecting microscope.

Body shape traits are associated with differences in both diet and predation regime (e.g. McPhail, 1994; Walker, 1997; Aguirre, 2009). Four body-shape traits (Fig. 3) were measured: head length (HL), from the tip of the snout to the posterior edge of the operculum; eye diameter (ED), parallel to the longitudinal axis of the body; body depth (BD), from the origin of the second dorsal spine to the ventral origin of the pelvic spine; and pectoral fin length (PCTL), measured from the origin of the fin to the distal tip of the second pectoral fin ray from the top of the fin (Fig. 3).

Morphometric variables were measured on the left side of the body with digital calipers and log transformed for statistical analysis. Size-corrected family means for each measure were calculated using regression analysis. Briefly, the morphometric variables were regressed on standard length (SL, distance from the tip of the snout to the posterior end of the last caudal vertebra) by cross type (i.e. anadromous controls, lake controls, hybrids) and individual residuals from the regression were averaged by family. The average deviation of each family from the regression line was added or subtracted from the predicted value (by cross type) at the grand mean SL for all specimens included in the study (i.e. 41.925 mm). We used this common slopes approach instead of calculating slopes individually for each family because the small sizes of some families made it impossible to obtain reliable slope estimates. Pelvic spine length could not be size corrected for the lake control crosses because it was 0 for most specimens, so the uncorrected data were used. Unweighted family means were used to compare hybrid crosses to those of anadromous and lake control crosses.

We performed the same analyses with the data uncorrected for size variation to ensure that the results of the statistical analyses were not an artifact of the size-correction method. Family mean SL did not differ between hybrid families from the two lakes ($F_{1,12} = 0.854$, $P = 0.374$; Glenn × Bear Paw, 48.56 ± 2.66 mm; Glenn × Boot, 45.20 ± 2.78 mm), between lake control families ($F_{1,6} = 0.506$, $P = 0.504$; Bear Paw, 45.12 ± 2.32 mm; Boot, 42.34 ± 4.11 mm) or among all cross types ($F_{2,22} = 0.757$, $P = 0.481$; anadromous,

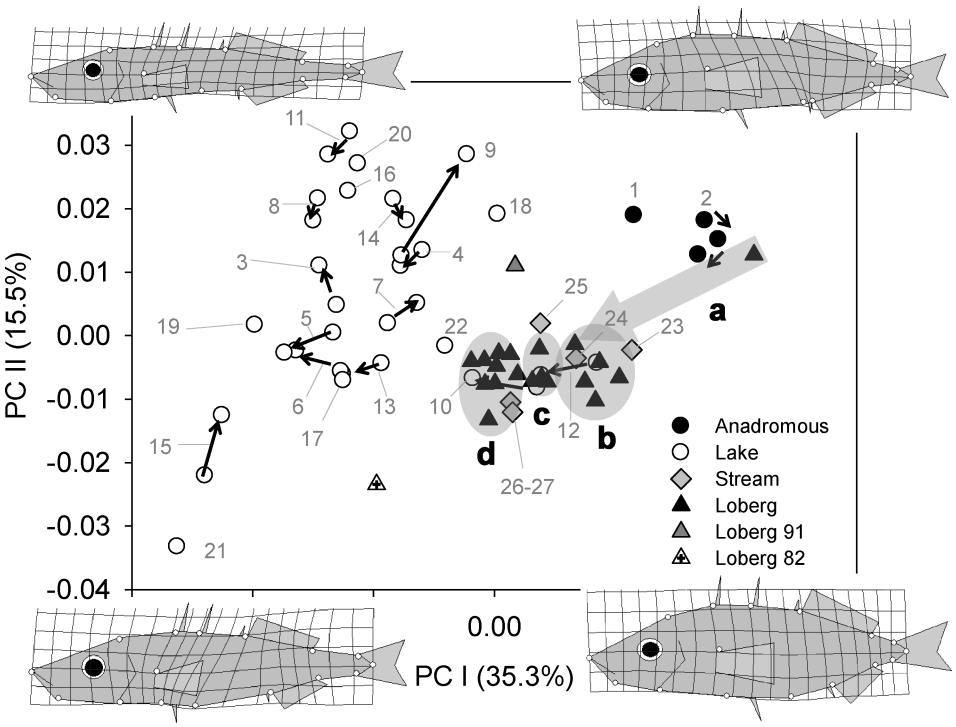

Fig. 5. Principal component (PC) analysis plot of temporal variation of body shape in the Loberg Lake and other Cook Inlet populations of threespine stickleback. The 1982 sample represents the native Loberg Lake population that was exterminated that year. Black arrows connect samples from the same populations from early (arrow tail, usually 1990) and later (arrow head, usually 2004) years (except for the Loberg Lake population). The net shape change between the 1990 and 2009 Loberg Lake samples is much greater than that in any other population. The shaded arrow (a) indicates change in the Loberg Lake population between 1990 and 1992 (the 1991 sample comprised juveniles and is an outlier). Shaded ovals indicate apparent clusters of variation among consecutive years in the Loberg Lake population through time: (b) 1993–1996; (c) 1997–2000; and (d) 2001–2009. Change was dramatic between 1990 and 1992 (a) but irregular within each cluster of consecutive annual samples (b–d). See Aguirre and Bell (2012) for details.

42.93 ± 2.10 mm; hybrids, 46.88 ± 1.91 mm; lake, 44.08 ± 1.99 mm), allowing us to conduct such analyses. With the exception of body depth and pectoral fin length, for which some comparisons became significant in the corrected data, the analysis of the size-corrected and uncorrected data yielded the same results. For simplicity, we present the size-corrected data only.

Morphometric measurements were tested for significant differences using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) *post hoc* test, and LP and GR were assessed with Mann-Whitney *U*-tests (Sokal and Rohlf, 2012). Statistical analyses were performed using SPSS 9.0 (SPSS, Inc., 1998) and BIOMstat 3.301 (Applied Biostatistics, Inc., 2002). None of the family mean morphometric measurements or counts differed between controls from the two lakes, so family means in both populations were treated as a single group. Most phenotypic data did not differ significantly between hybrids from the two lakes either,

so the family means of hybrid crosses from both lake populations were usually pooled, as well. However, hybrid GR depended significantly on the source of the lake parents ($U = 8.0$, $P = 0.038$), and GR in hybrid families involving the Bear Paw Lake (GR_{BP}) and Boot Lake (GR_B) populations were tested separately against anadromous and freshwater control crosses.

The coefficient of dominance (D) describes the degree of genetic dominance (Conner and Hartl, 2004). It represents the fractional difference of the heterozygous phenotype from the mean of the two homozygotes (i.e. additive inheritance). D is calculated as the difference between the heterozygote mean and the mean of the two homozygote means and divided by one-half of the difference between the mean value of the two homozygotes. $D = 0$ indicates that the alleles for the phenotype are additive, $0 < D < 1$ indicates partial dominance of one allele, $D = 1$ is perfect dominance, and $D > 1$ represents overdominance. Negative values of D in our analysis indicate dominance of the freshwater phenotype.

Cross results

Seven morphometric, two meristic (i.e. LP, GR), and two qualitative traits (i.e. LPM, PS) were compared among cross types. The qualitative traits were largely redundant with other meristic or quantitative traits, but they represent biologically important differences. The size-adjusted trait means of hybrids usually differed significantly from those of pure freshwater families but not anadromous ones (Table 3). D was calculated for nine traits (twice for GR), of which the anadromous phenotype was nominally dominant in six, and the freshwater phenotype was nominally dominant in two (Table 3). However, one trait, GR, was almost perfectly additive in hybrid crosses using a Bear Paw Lake parent, but the anadromous phenotype was partially dominant in hybrid crosses using a Boot Lake parent.

Table 3. Unweighted cross means (± standard error) and results of statistical comparisons of size-corrected data between pure and hybrid crosses

Trait	An × An	Hyb	FW × FW	An – FW	An – Hyb	FW – Hyb	D
PLVSL	6.86 ± 0.23	4.83 ± 0.29	0.03 ± 0.02	**6.83*****	**2.03****	**–4.80*****	0.41
PLVGL	8.87 ± 0.17	7.40 ± 0.19	2.06 ± 0.29	**6.81****	1.47^{NS}	**–5.34*****	0.57
LP[a]	32.06 ± 0.60	30.93 ± 0.32	3.86 ± 0.48	**28.2***	1.13^{NS}	**–27.07*****	0.92
DSL	4.89 ± 0.12	4.13 ± 0.17	2.88 ± 0.13	**2.01****	0.76^{NS}	**–1.25*****	0.24
HL	13.46 ± 0.69	13.45 ± 0.10	13.86 ± 0.14	-0.39^{NS}	0.01^{NS}	0.40^{NS}	1.05
ED	4.75 ± 0.38	4.44 ± 0.05	4.33 ± 0.05	0.43^{NS}	0.31^{NS}	-0.12^{NS}	–0.48
BD	9.71 ± 0.40	8.98 ± 0.10	8.50 ± 0.17	**1.21****	0.73^{NS}	**–0.48***	–0.21
PCTL	7.39 ± 0.15	7.06 ± 0.08	6.30 ± 0.13	**1.10*****	0.33^{NS}	**–0.76*****	0.39
GR_{BP}	23.18 ± 0.82	21.29 ± 0.30	19.63 ± 0.32	3.55^{NS}	1.89^{NS}	**–1.66***	–0.06
GR_B	23.18 ± 0.82	22.38 ± 0.26	19.49 ± 0.31	3.69^{NS}	0.38^{NS}	**2.89****	0.57

Note: Trait acronyms are defined in the Methods. An × An, Hyb, and FW × FW are the size-corrected means (mm, except for LP and GR, which are counts) for pure anadromous control, anadromous × lake hybrid, and pure lake control crosses, respectively, at 41.93 mm SL, the grand mean SL of all specimens measured. An – FW, An – Hyb, and FW – Hyb are differences between means for the first cross type minus the second. Significant differences are in bold and significance levels are indicated (F-tests, except LP and GR, for which Mann-Whitney U-tests were used) by superscripts: $^{NS}P > 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Degrees of freedom are 2, 25. D is the coefficient of dominance (see Methods); negative values of D indicate dominance of the freshwater phenotype.
[a] LP number results for hybrid crosses do not include results from family 19, which was an outlier.

Hybrid armour phenotypes always differed significantly from those of the freshwater controls, and anadromous phenotypes were always dominant or partially dominant. Lateral plate and pelvic phenotypes were scored both qualitatively (Table 2) and by number or size. Anadromous crosses produced only complete LPM (Fig. 2C) and fully expressed pelvic structures (PS = 8). Lake crosses produced only low LPM (Fig. 1A), and all but one specimen had pelvic reduction (PS < 8). Hybrid crosses between anadromous and lake stickleback almost always produced complete morphs with PS = 8. Thus, using these qualitative categories, anadromous phenotypes are almost always dominant for plate and pelvic phenotypes.

Using quantitative measures for armour traits (Fig. 3), hybrid means always differed significantly from those of freshwater families, indicating that observed coefficients of dominance are meaningful. Anadromous phenotypes were almost completely dominant for LP (Fig. 6A) and partially dominant for the other three armour traits (i.e. PLVSL, PLVGL, DSL). Thus, F1 hybrids and back cross progeny in nature would resemble their anadromous ancestors for armour.

Turning to body shape traits, mean HL did not differ significantly among the three cross types, so slight overdominance of the anadromous phenotype may be an artifact of measurement error. Mean PCTL in the anadromous and hybrid families both differed significantly from those of the freshwater families, so partial dominance of the anadromous phenotype appears to be significant for this trait. Differences in ED were not significant among cross types, so moderate dominance of the freshwater phenotype also may be an artifact. Even if real, it would not have a large effect on F1 progeny. The mean phenotype for BD in hybrids differed significantly from those of both controls, but dominance of the freshwater phenotype was weak.

Dominance for GR depended on which freshwater population was used (Fig. 6B). Neither hybrid mean differed from that of anadromous families but they both differed from those of the lake controls. GR was almost additive in hybrids using Bear Paw Lake parents, and the anadromous phenotype was partially dominant in the cross with Boot Lake parents.

Discussion of cross results

There is ample evidence that the phenotypic differences between the anadromous and freshwater stickleback populations studied have a genetic basis. Substantial differences between our pure anadromous and lake crosses were consistent among crosses and often statistically significant. The hybrids were usually intermediate to the pure crosses. All of our families were grown under similar conditions, so the effects of phenotypic plasticity should have been limited. We cannot rule out maternal effects because the sire in most hybrid crosses was anadromous, but Berner *et al.* (2011) did not detect maternal effects in their stickleback crosses. Our results are also consistent with previous genetic crosses using oceanic and freshwater threespine stickleback to study the genetics of lateral plate morphs (e.g. Hagen, 1967, 1973; McPhail, 1994; Bańbura and Bakker, 1995; Colosimo *et al.*, 2004; Cresko *et al.*, 2004), pelvic skeleton reduction (Shapiro *et al.*, 2004), including analyses of the Bear Paw and Boot Lake populations we used (Cresko *et al.*, 2004; Chan *et al.*, 2010), gill-raker number (McPhail, 1994), and body shape (McPhail, 1994; Albert *et al.*, 2007). Thus, we are confident that the results of our crosses represent genetic differences between the lake and anadromous populations.

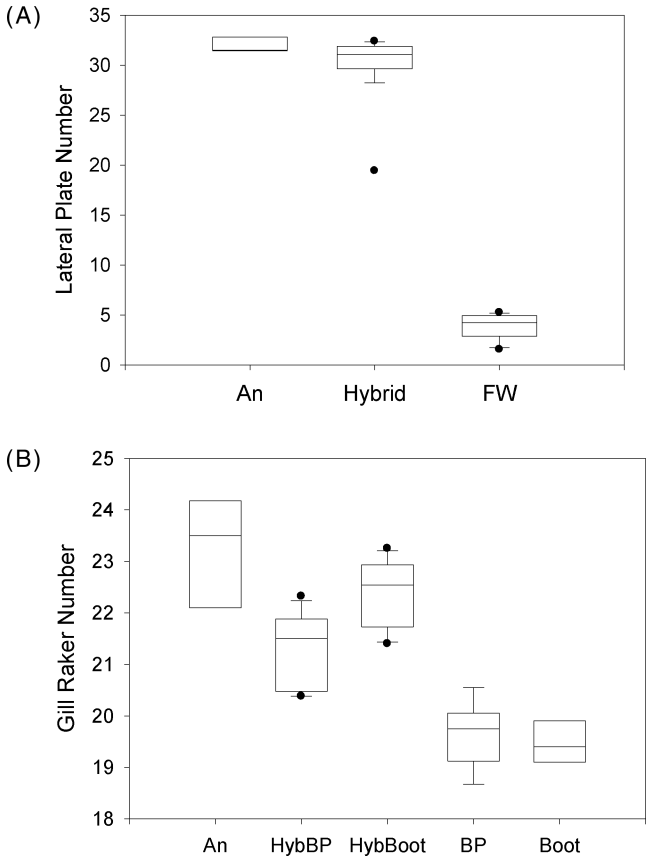

Fig. 6. Results from pure F1 crosses within anadromous and lake populations and hybrid crosses between lake and anadromous parents. (A) Lateral plate number; (B) gill raker number. An, pure anadromous and FW, pure lake crosses. HybBP and HybBoot, hybrid crosses of anadromous to Bear Paw and Boot lake parents, respectively. Boxes are inter-quartile ranges, horizontal lines within boxes are the median, vertical bars above and below boxes are ranges, and solid circles beyond bars are outliers. See Table 2 for the sources of parents for each cross type and Table 3 for cross results for other phenotypes.

Our most important result is that alleles for anadromous phenotypes are partially dominant over the homologous alleles in the lake populations for armour traits, PCTL, and sometimes GR. Freshwater alleles are less likely to be dominant, and their dominance is either weak or phenotypic differences between them and anadromous phenotypes are small. Population processes during millions of generations apparently have produced recessiveness of freshwater-adapted alleles for armour and possibly other traits. Interestingly, however, different null *Pitx1* alleles, which lack the pelvis-specific enhancer (i.e. *Pel*) and cause pelvic reduction, probably arose within freshwater populations after they formed (Chan *et al.*, 2010), and they are also recessive. Dominance may be an incidental consequence of the molecular biology of genes for freshwater phenotypes, but fairly consistent dominance of anadromous alleles for traits that contrast strongly between oceanic and freshwater stickleback suggest that their dominance does not occur by chance.

Although GR is indistinguishable in pure families from Bear Paw and Boot lakes, they appear to have different coefficients of dominance in F1 hybrids. Gill raker number is heritable and presumably polygenic (Hagen, 1973; Hermida *et al*., 2002; Aguirre *et al*., 2004). Perhaps functionally equivalent additive alleles at different loci in the polygenic system for GR were present by chance in the founding populations or arose by mutation since the populations formed and underlie similar GR phenotypes but interact differently with GR alleles from oceanic stickleback. Complementation crosses between these populations would shed light on this possibility.

GENETICS OF CONTEMPORARY EVOLUTION AND ADAPTIVE RADIATION IN THREESPINE STICKLEBACK

Gasterosteus aculeatus is an ancient, widespread monophyletic group of sympatric or parapatric biological species and countless, phenotypically divergent, allopatric, freshwater populations (Bell, 1976, 1995, 2009; Bell and Foster, 1994; McPhail, 1994). Anadromous populations are phenotypically conservative and apparently exhibit limited geographical variation (Walker and Bell, 2000; Aguirrre *et al*., 2008; Bell *et al*., 2009). Freshwater isolates may be young (i.e. post-glacial) and diverge predictably, depending on diet, predation, water chemistry, and hydrodynamics, even within very limited areas (e.g. Lavin and McPhail, 1985; Reimchen *et al*., 1985, 2013; Bell *et al*., 1993; Aguirre, 2009; Ravinet *et al*., 2013). Below we describe the intraspecific phylogeny of the threespine stickleback and discuss the genetic basis for its rapid and predictable adaptive radiation.

Geographical distribution and phylogeny of freshwater isolates

Phenotypically diverse freshwater stickleback isolates are ubiquitous in recently deglaciated coastal lowlands, including islands and isolated fjords that must have been colonized independently from the ocean (e.g. Lindsey, 1962; McPhail and Lindsey, 1970; Bell, 1976, 1995; Bell and Foster, 1994; Gelmond *et al*., 2009), and repeated post-glacial colonization is supported by phylogeographic evidence (Withler and McPhail, 1985; Taylor and McPhail, 1999, 2000; Mäkinen *et al*., 2006; Hohenlohe *et al*., 2010; Deagle *et al*., 2013). Oceanic threespine stickleback represent a phenotypically conservative 'supertramp' (Diamond, 1974) that has frequently colonized new freshwater habitats, and their predictable ecological and phenotypic divergence after freshwater colonization represents a taxon cycle (Wilson, 1961; Ricklefs and Bermingham, 2002). Extant threespine stickleback populations form a star phylogeny, with phenotypically conservative oceanic populations at the centre and freshwater isolates at the tips of the rays of the star. Projecting this phylogeny through time stacks the stars on top of each other going back through time, forming a phylogenetic raceme (Bell, 1987; Williams, 1992; Bell and Foster, 1994). Divergence of freshwater threespine stickleback populations is rapid and produces substantial divergence, and it has been occurring for at least 10 million years. However, this process has not produced a phylogenetic tree with progressively spreading branches that span large phenotypic differences. The most highly divergent populations are young and occur in ephemeral, post-glacial, freshwater habitats, which limits their persistence (Bell, 1987, 1988, 2001; Williams, 1992; Bell and Foster, 1994).

The source of allelic variation for adaptation to fresh water

Anadromous and freshwater stickleback breed in sympatry, enabling reciprocal introgression (Hagen, 1967; McPhail, 1994; Jones *et al*., 2006; Karve *et al*., 2007). Lateral plate morph variation clearly reflects this process. In western North America, oceanic populations are usually monomorphically completely plated, and freshwater populations from lakes and slowly flowing streams are usually monomorphically low plated (Baumgartner and Bell, 1984). *Ectodysplasin* (*EDA*) has the greatest influence on plate morph and number in crosses between anadromous and freshwater populations, and complete morph alleles (EDA^C) from anadromous parents are dominant to low morph alleles (EDA^L) of freshwater parents (Colosimo *et al*., 2004; Cresko *et al*., 2004). Colosimo *et al*. (2005) first showed that EDA^L occurs at low frequencies in oceanic stickleback, and this has been observed in many other oceanic populations (Bell *et al*., 2010; Leinonen *et al*., 2012). EDA^L alleles in most populations are descended from a single allele that has spread around the Holarctic, although a second low *EDA* allele in Japan arose independently from EDA^C (Colosimo *et al*., 2005). In addition, EDA^L alleles of stickleback in adjacent freshwater drainages are more similar to each other than to EDA^L alleles from more distant ones (Schluter and Conte, 2009). The EDA^L alleles must have been present as rare variants in the oceanic populations when they colonized fresh water, risen to high frequencies or become fixed in fresh water, been recycled back into anadromous populations through introgression, and become the basis for evolution of low plate morphs after subsequent freshwater colonizations.

Many other freshwater-adapted alleles appear to have had the same phylogenetic history as EDA^L. The same set of genomic regions in multiple, isolated freshwater populations have been selected after independent colonization of fresh water by oceanic stickleback (Hohenlohe *et al*., 2010; DeFaveri *et al*., 2011; Shimada *et al*., 2011; Jones *et al*., 2012a, 2012b; Deagle *et al*., 2013). Jones *et al*. (2012b) compared genome sequences from 10 pairs of adjacent anadromous and freshwater populations from a wide geographic range. They identified 90 to 174 loci (depending on methods) at which isolated freshwater populations had DNA sequences that are more similar to each other than to their homologues in geographically adjacent oceanic populations. Jones *et al*. (2012b) noted that several genes with recurrent contrasting alleles in oceanic and freshwater pairs have functions that are likely to differ between marine and freshwater environments. Similarly, using Baltic (brackish) and adjacent freshwater populations, Shimada *et al*. (2011) targeted 157 loci with physiological functions and found that 16.6% of them exhibit evidence of directional selection. Seven of these loci appear to be related to osmoregulation. Thus, it appears that many freshwater-adapted alleles may be carried as rare standing genetic variation in oceanic stickleback populations and form the basis for adaptation to fresh water when oceanic stickleback colonize fresh water (Schluter and Conte, 2009; Deagle *et al*., 2013; Feulner *et al*., 2013).

Retention of freshwater-adapted alleles in oceanic stickleback populations

Environment–phenotype associations provide the first line of evidence for divergent natural selection (Endler, 1986). If freshwater-adapted alleles were expressed strongly in F1 anadromous × freshwater hybrids or their backcrosses to anadromous stickleback, they would be exposed to purifying selection in the ocean every generation and be quickly eliminated. However, if they were completely recessive, they would be expressed only in homozygotes. Our results for several traits (see above) suggest that freshwater phenotypes

that contrast strongly between anadromous and freshwater populations, especially armour traits, are often partially recessive to their oceanic-adapted homologues. At low frequencies, q, homozygotes for disadvantageous but recessive freshwater-adapted alleles will rarely be expressed and experience selection. For example, with random mating, a fully recessive allele with a frequency of 1% will be expressed in only one homozygote out of 10,000, or $1/q^2$ individuals; selection against rare recessives will be exponentially weaker than against additive or dominant alleles. Selection against heterozygotes will be weaker when the difference between freshwater and anadromous phenotypes is smaller or the anadromous allele exhibits stronger dominance.

For example, the frequency of EDA^L in oceanic populations is typically about 1% (Colosimo *et al*., 2005; Bell *et al*., 2010; Leinonen *et al*., 2012). The equilibrium frequency of freshwater-adapted alleles in oceanic populations will depend on the magnitudes of purifying selection against them in the ocean and the rate of gene flow from low-plated freshwater populations. If mate choice depends on traits that are dominant in oceanic stickleback, F1 hybrids will tend to backcross to oceanic stickleback, retaining recessive, freshwater alleles in oceanic populations. The more recessive a freshwater-adapted EDA^L allele is on the genetic background of an oceanic stickleback and the greater the rate of gene flow, the higher equilibrium frequency of EDA^L will be in oceanic populations. The same argument would apply to any freshwater-adapted allele that introgresses anadromous stickleback populations. Recessiveness will reduce selection against freshwater-adapted alleles in oceanic populations.

Selection of freshwater-adapted alleles in oceanic stickleback populations after they colonize fresh water

When oceanic stickleback colonize fresh water, natural selection should increase the frequency of freshwater-adapted alleles present in the colonizing population and not lost by the founder effect or subsequent genetic drift (Otto and Whitlock, 1997). Although there is evidence that rare alleles can be lost when freshwater populations are founded (Leinonen *et al*., 2012; unpublished data), the moderate genetic diversity of freshwater stickleback populations suggests that they are not severely bottlenecked at founding (Withler and McPhail, 1985; Taylor and McPhail, 1999, 2000; Hohenlohe *et al*., 2010). Their populations grow rapidly the first year after founding (unpublished data), limiting the period after colonization during which genetic drift is likely to eliminate rare alleles. If recycled freshwater-adapted alleles are absent in a founding population, new mutations in freshwater populations may form the basis for adaptation (Chan *et al*., 2010; Leinonen *et al*., 2012), but that would generally delay adaptation (Barrett and Schluter, 2007; Hunt *et al*., 2008). Contemporary evolution of multiple traits in oceanic (mostly anadromous) populations after they colonize fresh water (Table 1) suggests that standing genetic variation is the foundation for adaptive divergence in fresh water (Schluter and Conte, 2009; Feulner *et al*., 2013).

Since freshwater-adapted alleles are rare and often partially recessive in founding oceanic populations, positive selection will be strongest only in rare (i.e. $1/q^2$) homozygotes. Increases in their frequencies will initially depend on genetic drift, relatively weak selection on heterozygotes, or stronger selection on rare homozygotes, and it should be slow at first (Roughgarden, 1996; Connor and Hartl, 2004; Barrett and Schluter, 2007). Traits with strong, heritable, adaptive phenotypic plasticity should exhibit rapid, non-heritable change in a new environment, respond to positive selection more rapidly, and be more likely to be selected than they would be without this effect (West-Eberhard, 2003). Regardless of phenotypic plasticity, the response

to natural selection will increase as the frequencies of partially recessive alleles rise to moderate levels. It is not clear whether evolution in the Loberg Lake population of low lateral plate morphs, which are encoded by the recessive EDA^L allele, conforms to this expectation because we did not sample the first few generations of this population after it was founded (Bell *et al*., 2004).

Genetic load and genetic linkage

Contemporary evolution of numerous traits simultaneously after oceanic stickleback colonize fresh water raises the potential for genetic load to impact population viability. Freshwater and oceanic stickleback differ at numerous loci (Jones *et al*., 2012b). If they were unlinked, selection against the common, dominant, ancestral alleles at multiple loci after freshwater colonization could result in substantial genetic load (Haldane, 1957). However, if many freshwater-adapted alleles were assembled into a few groups of linked genes, they would usually co-segregate and be selected as a unit, reducing genetic load (Heuts, 1947b). Indeed, many (though not all) genes that differ consistently between members of adjacent pairs of freshwater and anadromous populations and have a recent history of positive selection in freshwater populations are clustered into several genomic regions, sometimes contained within inversions (Hohenlohe *et al*., 2010, 2012; Jones *et al*., 2012b; Hendry *et al*., 2013b). Similarly, loci with divergent alleles between parapatric lake and stream populations tend to be located near the centromere, where recombination is suppressed (Roesti *et al*., 2012, 2013). Regardless of the cause, linkage disequilibrium will facilitate the formation of clusters of adaptive alleles that can increase in frequency as a unit in response to selection (Dobzhansky, 1970).

Linkage of adaptive alleles has at least three important evolutionary consequences: (1) Directional selection on numerous phenotypic traits will cause an evolutionary response in fewer genomic regions with linked genes, reducing genetic load and the likelihood of population decline or extinction. (2) The fitness effects of multiple, linked, adaptive alleles will produce large fitness differentials, higher evolutionary rates, more consistent patterns of phenotypic divergence in freshwater populations, and lower probabilities that rare alleles will be lost by drift in small populations. (3) Groups of alleles that produce integrated phenotypes (Pigliucci and Preston, 2004) that are adapted to either freshwater or marine habitats but not a mixture of the two will co-segregate in F1 hybrids and backcross individuals so that some individuals will have multiple adaptive traits.

Sets of linked alternative alleles in related species have recently been recognized in other taxa and referred to as genomic islands, archipelagos, or continents of divergence (Michel *et al*., 2010; Hohenlohe *et al*., 2012; Nosil and Feder, 2012; Hendry *et al*., 2013b). Thus, selection on traits encoded by blocks of linked alleles that have been recycled from freshwater stickleback into anadromous populations by gene flow helps explain predictable adaptive radiation of freshwater threespine stickleback (Teotónio *et al*., 2009).

Allelic recycling in the threespine stickleback

Schluter and Conte (2009) proposed the 'transporter hypothesis' by analogy with a device in a space drama, *Star Trek*. The transporter room in *Star Trek* disaggregated the space travellers' molecules, transported them through space, and reassembled them at a distant location. Schluter and Conte (2009) proposed that oceanic threespine stickleback populations contain numerous freshwater-adapted alleles that have been acquired by introgressive

hybridization with freshwater stickleback, disaggregated by backcrossing to oceanic stickleback, and reassembled after colonization of fresh water. 'Multiple generations of recombination cause the disintegration of the freshwater-adapted genotype, such that each member of the marine population carries 0, 1, or only a small number of freshwater-adapted alleles' (Schluter and Conte, 2009: 9959). They envisioned that rare, disaggregated freshwater-adapted alleles would have little effect on fitness in oceanic stickleback, but when they founded a new freshwater population, they would be favoured by directional selection, increase in frequency individually, and be reassembled by sexual recombination into the genotype of freshwater populations from which they previously came, just as the space travellers' molecules in *Star Trek* were reassembled at their destination.

Schluter and Conte's (2009) transporter hypothesis requires some modification in light of recent genetic and genomic evidence. First, many loci with contrasting alleles in freshwater and oceanic populations are linked and will be selected as a unit. For example, Linkage Group IV, which includes *EDA*, the major locus for lateral plate variation, contains an extended region of linkage disequilibrium and the entire chromosome may be selected as a unit after freshwater colonization (Hohenlohe *et al*., 2012). Thus, the stickleback transporter does not completely disaggregate the stickleback genotype like the transporter in *Star Trek*. Many freshwater-adapted alleles enter new freshwater populations as standing ancestral variation within blocks of multiple linked genes.

Second, phenotypic plasticity of several stickleback phenotypes appears to be adaptive. Phenotypic expression of alleles that was limited by partial dominance in oceanic populations will increase in fresh water. Adaptive phenotypic plasticity based on these alleles may increase the evolutionary response to selection by revealing recessive genetic variation and immediately increase survival and reproduction before there is an evolutionary response to selection in fresh water (Wund *et al*., 2008; McGuigan *et al*., 2010).

Because many freshwater-adapted alleles are partially recessive, they can be carried in oceanic populations at higher frequencies and persist longer than if they were more strongly expressed in heterozygotes. Consequently, oceanic populations should carry greater standing genetic variation for recessive freshwater-adapted alleles than if these alleles were more strongly expressed (Barrett and Schluter, 2007). Conte *et al*. (2012) found that the probability that the same gene will be used for the same phenotype declines with phylogenetic distance. However, even most geographically and phylogenetically distant, low-plated, freshwater populations of threespine stickleback use EDA^{L} for plate reduction (Colosimo *et al*., 2005; but see Leinonen *et al*., 2012 for an exception). *Gasterosteus aculeatus* is at least 13 million years old (Bell *et al*., 2009), and the same clade of alleles for plate reduction continues to be recycled in distantly related populations. Elevated frequencies of recycled freshwater-adapted alleles in oceanic populations will increase the rate of adaptation after colonization of fresh water because the adaptive alleles start at a higher frequency, they are adaptive for fresh water (i.e. not random mutants), and selection will not be delayed by the time required for new adaptive mutations to occur (Barrett and Schluter, 2007; Hunt *et al*., 2008).

The origin of allelic recycling in threespine stickleback

The metapopulation structure, patterns of gene expression, and genomic architecture of the threespine stickleback form a well-oiled machine to quickly reassemble freshwater phenotypes whenever vacant freshwater habitats are colonized by oceanic stickleback. How was this machine built?

The threespine stickleback is probably primitively marine. Its close relatives, including most other species of the Gasterosteidae (Kawahara *et al*., 2009), Gasterosteales, Cottoidei, and successively larger monophyletic groups to which it belongs (Betancur-R *et al*., 2013) include only or mostly marine species. Accordingly, marine threespine stickleback must originally have lacked freshwater-adapted alleles. The earliest freshwater *G. aculeatus* are at least 10 million years old (Bell, 2009; Bell and Reynolds, 2010), and oceanic fossils are even older (Bell *et al*., 2009); the genetic architecture of the *G. aculeatus* clade has been evolving for a long time. Oceanic stickleback do not need freshwater-adapted alleles to colonize fresh water. For example, a population in Ida Lake, Alaska (61.76217N, 149.58367W) has extremely low microsatellite and mtDNA diversity and has resembled anadromous stickleback for more than 20 years (unpublished data). Lack of evolution in this population for so long suggests that early oceanic populations that lacked recycled freshwater-adapted alleles could have persisted in fresh water long enough for new freshwater-adapted alleles to arise by mutation and increase in frequency. Subsequent hybridization between the early freshwater isolates and their anadromous ancestors would initiate introgression of freshwater-adapted alleles into anadromous populations.

However, any (partially) dominant freshwater alleles that introgressed oceanic populations would rapidly be purged by purifying selection in the ocean, leaving only (partially) recessives. Gene flow from freshwater to anadromous populations would continuously replenish (partially) recessive freshwater-adapted alleles that would be carried as cryptic genetic variation in oceanic populations. The number of loci with recessive freshwater-adapted alleles that can flow from freshwater to oceanic populations without being removed by purifying selection could have increased progressively over at least 10 million years.

Once the stickleback freshwater–oceanic metapopulation system had evolved, any new recessive, freshwater-adapted allele that tends to be revealed by phenotypic plasticity in fresh water would be favoured by selection after invasion. The fitness advantage of increased expression of recessive freshwater-adapted alleles would be most important during the colonization process, when these alleles would be in the heterozygous condition at low frequency. Once they rose to high frequency and homozygotes became common, selection for increased expression in fresh water of recessive alleles in the heterozygous condition would be relaxed. Thus, selection for phenotypic plasticity would be favoured only during brief episodes following freshwater colonization. However, numerous repeated episodes of selection on freshwater-adapted alleles during multiple colonization events over millions of years could cause evolution of adaptive phenotypic plasticity.

Similarly, selection favouring the assembly of loci with freshwater-adapted alleles into groups of linked genes would depend on selection only during adaptation to fresh water. Immediately after colonization, rare freshwater-adapted alleles that were isolated in the genome would be expressed in phenotypes that were adaptive only for the trait that they produce. Positive selection might be weak, and drift would be more likely to eliminate them. In contrast, alleles physically linked to other freshwater-adapted alleles would covary among individuals and increase in frequency due both to direct selection and hitch-hiking on linked alleles that were favoured by selection (Slatkin, 2008; Roesti *et al*., 2013). Like selection for adaptive phenotypic plasticity, selection for linkage among freshwater-adapted alleles would occur episodically after freshwater colonization. Hohenlohe *et al*. (2012) proposed that selection against introgressed freshwater-adapted alleles in oceanic populations would also contribute to evolution of linkage, but this would appear to depend on very low levels of gene flow and selection. However, many successive colonization events during millions of

years could have a major effect on the evolution of linkage between freshwater-adapted alleles.

In summary, alleles that individually increase Darwinian fitness in fresh water might appear rapidly by mutation, but only the (partially) recessive alleles could spread from the freshwater population of origin through oceanic populations as cryptic genetic variation. Recessive freshwater-adapted alleles with a greater phenotypic effect in fresh water than in the ocean (i.e. adaptive phenotypic plasticity) and those in linkage disequilibrium with other freshwater-adapted alleles would be selected more effectively in fresh water than alleles without phenotypic plasticity at unlinked loci. However, selection for adaptive phenotypic plasticity and linkage should occur for only a few generations after colonization of fresh water. Thus, accumulation of recessive freshwater-adapted alleles should have been rapid compared with evolution of increased adaptive phenotypic plasticity and linkage of freshwater-adapted alleles, which would be selected only episodically during new colonization events.

Generality of allelic recycling

There is growing evidence that gene flow between divergent populations and related species can be an important source of genetic variation for the evolution of novel phenotypes (Seehausen, 2004; Conte *et al*., 2012). Furthermore, the interplay of gene flow and divergent selection can favour the genomic architecture that seems to play an important role in contemporary evolution and adaptive radiation of threespine stickleback (Slatkin, 2008; Michel *et al*., 2010; Hohenlohe *et al*., 2012; Nosil and Feder, 2012; Roesti *et al*., 2012). Allelic recycling is clearly important to produce freshwater phenotypes influenced by *ectodysplasin* (Colosimo *et al*., 2004), *Kit Ligand* (Miller *et al*., 2007), *thyroid stimulating hormone-β2* (Kitano *et al*., 2010), and probably many other threespine stickleback genes (Jones *et al*., 2012b; Deagle *et al*., 2013). However, not all divergent phenotypes of freshwater threespine stickleback result from allelic recycling. Pelvic reduction often depends on separate deletion mutations in *Pitx1* from different populations with pelvic reduction (Chan *et al*., 2010). Genomic scans using SNP arrays with three phenotypically similar lake–stream pairs of threespine stickleback populations indicate that many of the alleles for phenotypic convergence among corresponding ecotypes in different drainages were unique to each pair (Deagle *et al*., 2012). Thus, similar phenotypes can also evolve independently in separate stickleback populations without allelic recycling. Convergent phenotypes in related species of other groups may also depend on multiple independent mutations at the same or different loci (Conte *et al*., 2012). The evolutionary genetics of adaptive radiation in the threespine stickleback may depend on a combination of new mutants and old recycled alleles, but recycling of recessive, freshwater-adapted alleles back and forth between oceanic and freshwater populations clearly is an important phenomenon and may not be atypical (Conte *et al*., 2012).

CONCLUSION

Eldredge and Gould (1972) argued that ignoring stasis in the fossil record creates the illusion that it is inconsequential. Similarly, we cannot assess how often stasis has been ignored in perturbed contemporary stickleback populations. While Hagen and Gilbertson (1973b) and Reimchen (1995), for example, recorded significant evolution within one generation, Hendry *et al*. (2013a) failed to observe it 35 generations after the introduction of stream stickleback to

a pond habitat. Similarly, the Ida Lake population we are studying has not evolved freshwater phenotypes during more than 20 years of observation (unpublished data). Hendry *et al.* (2013a) noted that lack of evolution could reflect weak selection, gene flow, and limited time. Their observation is consistent with the slow evolution of armour traits in fossil stickleback for thousands of years, despite directional selection on them (Hunt *et al.*, 2008). Thus, while contemporary evolution has occurred frequently in threespine stickleback, it is not inevitable and may often be too slow to observe in the present.

Much of the phenotypic change exhibited by threespine stickleback when they experience environmental change could be due to phenotypic plasticity. However, initial non-genetic change has been followed by sustained phenotypic evolution in several populations, and contemporary evolution of the frequencies of freshwater-adapted alleles has also been observed. Impressive differences can evolve between ancestral oceanic populations and their freshwater descendants for numerous traits within ten generations (Table 1). Although threespine stickleback do not appear to evolve unusually fast, many traits may evolve simultaneously after they experience habitat changes, giving the impression of extensive, rapid divergence from the ancestor.

The most impressive contemporary evolution of threespine stickleback occurs after oceanic populations colonize fresh water. This transition has been occurring for at least 10 million years. Recycling of linked, partially recessive alleles with adaptive phenotypic plasticity may play an important role in the rate and predictability of divergence. Millions of generations of allelic recycling has assembled the allelic variation, genetic architecture, and expression patterns that facilitate adaptation to fresh water when oceanic populations colonize it.

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