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RESEARCH ARTICLE

# Ephemeral stream reaches preserve the evolutionary and distributional history of threespine stickleback in the Santa Clara and Ventura River watersheds of southern California

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**Abstract** Much remains to be understood about the evolutionary history and contemporary landscape genetics of unarmored threespine stickleback in southern California, where populations collectively referred to as *Gasterosteus aculeatus williamsoni* have severely declined over the past 70+ years and are now endangered. We used mitochondrial sequence and microsatellite data to assess the population genetics and phylogeography of unarmored populations sampled immediately downstream from the type locality of *G. a. williamsoni* in the upper Santa Clara River, and assessed their distinctiveness with respect to low-armor populations in the downstream sections of the river and the adjacent Ventura River. We also characterized the geographic limits of different plate morphs and evaluated the congruence of those boundaries with barriers to dispersal in both river systems and to neutral genetic variation. We show substantial population structuring within the upper reach of the Santa Clara River, but little partitioning between the lower Santa Clara and Ventura Rivers—we attribute these patterns to different ancestry between spatially subdivided populations within the same drainage, a predominance of downstream gene flow, and ability for coastal dispersal between the Santa Clara and Ventura Rivers. We also show that alleles from introduced low-plate stock have infiltrated a native population in at least one upper Santa Clara River tributary, causing this formerly unarmored population to become gradually low-plated over a 30 + year time period. Measures of genetic diversity, census surveys, and severe habitat disturbance all indicate that unarmored stickleback near the type locality are currently at high risk of extinction.

**Keywords** Threespine stickleback · Santa Clara River · Conservation genetics · Phylogeography · Natural selection

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## Introduction

Threespine stickleback (*Gasterosteus aculeatus*) are known for their bright male nuptial coloration, unusual pelvis, prominent denticulated spines, and thin bony plates (i.e. skeletal 'armor') covering the myotomes on the sides of the body. These traits provide some of the best-studied examples of adaptive evolution in vertebrates (Bell and Foster 1994; Schluter 2000; Boughman 2001; Colosimo et al. 2005; Chan et al. 2010; Jones et al. 2012). Variation in the amount of body armor has been used to define taxonomic and conservation units within this species 'complex'. These units differ primarily in the number of lateral plates, with complete or fully-plated morphs having up to 36 plates on a side (*G. aculeatus aculeatus*), to partial morphs that have a gap between the abdominal plates and

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those on the caudal peduncle, to low-plate morphs having 3-7 abdominal plates on a side (*G. a. microcephalus*), and finally to un-plated or unarmored morphs which lack plates altogether (*G. aculeatus williamsoni*; Miller and Hubbs 1969; Hagen and Gilbertson 1972; Bell 1976). The extent of plate development is correlated with a suite of environmental variables. Complete armor is associated with the presence of predatory fish and exposure to marine conditions through permanent residence or anadromy, while intermediate or partial armor is often found in freshwater (Bell and Foster 1994; Reimchen 2000). The least common phenotype is the unarmored form, which is exclusive to freshwater habitats.

The unarmored *G. williamsoni* was first described from specimens collected in the upper Santa Clara River in Soledad Canyon, California (Girard 1854), and subsequently accorded subspecific rank *G. a. williamsoni* (as reviewed in Miller 1960; Miller and Hubbs 1969). Later survey work led to the discovery of unarmored stickleback populations in a number of other streams in southern California (Culver and Hubbs 1917; Miller 1961; Bell 1978), including additional locations in the upper Santa Clara River and its tributaries (Baskin and Bell 1976). However, since the early 1940s, most populations have become extirpated, including those that once occurred in all of the major river drainages (Los Angeles, San Gabriel and Santa Ana Rivers) in the Los Angeles Basin (Miller 1961; Moyle 2002). Unarmored fish persist naturally in only three areas today, the Santa Clara river drainage, a landlocked cluster of small populations at high elevation in the San Bernardino Mountains, and in San Antonio Creek north of Point Conception (a fourth site in San Diego County, California contains transplanted fish). Their declines over short time frames, combined with an otherwise long history in the region and former predominance in the freshwater ichthyofauna of southern California (Miller 1960; Swift et al. 1993; Moyle 2002), led to their federal listing as an endangered species in October 1970 (35 Federal Register 16047) and their state listing in California in June 1971.

The taxonomic and evolutionary framework for *G. aculeatus* as described by Miller and Hubbs (1969) has served as a blueprint for the management of *G. a. williamsoni*. They delimited subspecies according to three morphotypes (fully-plated/armored, low-plated, and unplated) and considered the many intermediate populations throughout the species range as introgressed forms. Although numerous studies over the past 40 years have shown this to be an inaccurate view of *G. aculeatus* evolution (Hagen and McPhail 1970; Schluter and McPhail 1992; Bell and Foster 1994; McKinnon and Rundle 2002), management of unarmored stickleback populations is rooted in this nomenclature and concept of evolutionary history for the species. This is likely due to two main factors—first, the listing rule for *G. a. williamsoni* (35 FR 16047) took place immediately following the publication of Miller and Hubbs's landmark paper (1969), which was the most extensive review of the systematics and morphological evolution of *G. aculeatus* at the time; second, recent genetic studies on southern California stickleback are lacking (but see Buth et al. 1984; Buth and Haglund 1994; Orti et al. 1994), forcing managers to rely on outdated concepts and information when attempting to implement conservation plans.

In addition to the type locality occurring in upper Soledad Canyon, unarmored stickleback in the Santa Clara River watershed have drawn much attention from management agencies due to the instability of the current habitat. This instability is caused by many factors, including regulated water discharge, alteration of land use and configuration, streambed channelization, use of septic tanks in some rural areas, livestock grazing, introduction of non-native species, and the effects of run-off and land destruction created by oil extraction and the mining of minerals, sand, and gravel (Stillwater Sciences 2007; USFWS 2009; Beller et al. 2011). The watershed is also located within a tectonically active area in the northern Transverse Ranges of California—the uppermost reaches drain from steep slopes in the northern San Gabriel and Sierra Pelona Mountains, where uplift rates have been rapid since the Pleistocene (Hall 2002). Recent fires and subsequent debris flows during El Niño storm events have had dramatic effects on aquatic vertebrates in this area (Hitchcock et al. 2004; Richmond et al. 2013), including the extirpation of at least one stickleback population from the San Francisquito Creek tributary (T. Hovey, CADFW; pers. comm.). Because stickleback reproduction and subsistence is sensitive to a number of ecological conditions (Moyle 2002), these factors and others suggest that their last remaining occupied reaches within the Santa Clara River are unlikely to persist without adequate management and protection.

In this study, we used microsatellite markers, mitochondrial DNA sequence data and comparative analysis of plate morphology from contemporary and historical samples to characterize the evolution and population genetic structuring of threespine stickleback in the Santa Clara River watershed. Our main objective was to evaluate the degree to which unarmored populations in the headwater regions are genetically distinctive from other stickleback populations in the lower Santa Clara River and the geographically adjacent Ventura River, and to delimit the spatial boundaries of the different plate morphs and genetic groups within and between the two drainages. We also sought to determine the genetic origin of a population in the Bouquet Creek tributary of the upper Santa Clara River,

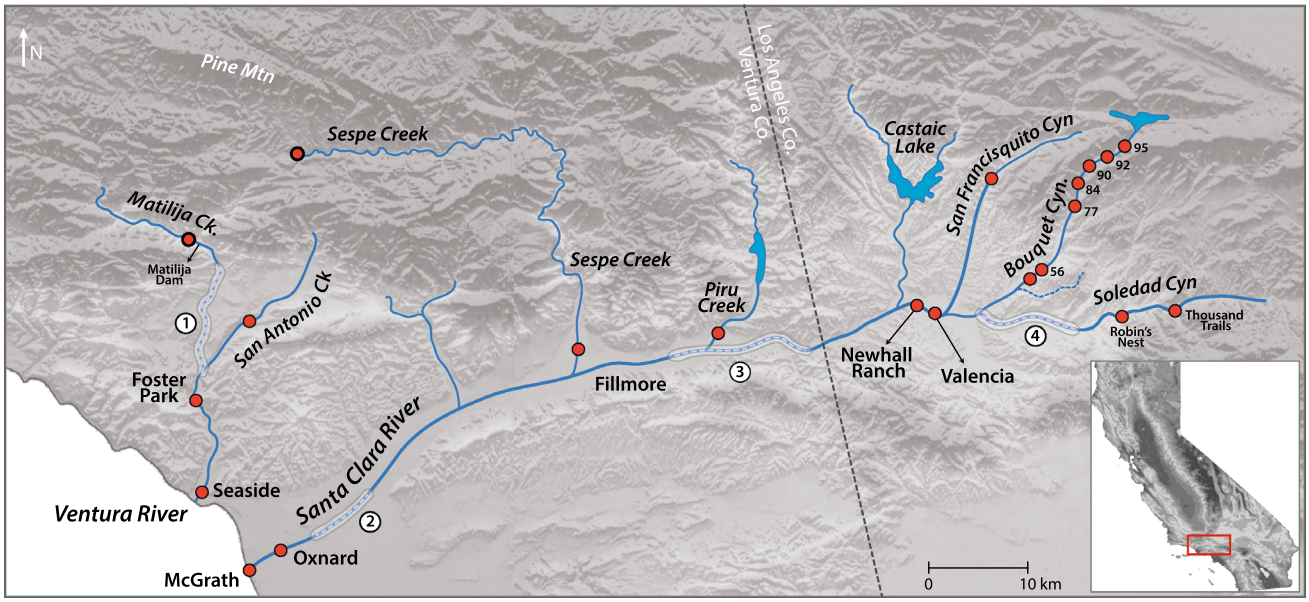


**Fig. 1** Sampling locations for threespine stickleback in the Santa Clara and Ventura Rivers—inset map of California highlights the study region. Lightened, dashed areas show the four main dry gaps: *1* Matilija Gap, *2* Saticoy Gap, *3* Piru Gap, and *4* Santa Clarita Gap. Gap lengths are approximate and vary depending on amounts of rainfall. Numbers adjacent to sampling points in Bouquet Canyon refer sampling reaches in Table S1. Map scale is approximate

where threespine stickleback were first recorded during the 1970s (USFWS 2009).

## Materials and methods

### Field sampling

We obtained tissue samples from U.S. Geological Survey monitoring surveys over the past decade, museum collections, and professional colleagues. All specimens were collected with appropriate U.S. Federal Recovery Permits (TE-045994, TE 793644-6 &7) and or California State Scientific Collecting Permits (SCP-2679, SCP-90)—any direct contributions by professional colleagues were also obtained with appropriate scientific collecting permits from the California Department of Fish and Wildlife and are available on request. Fish were captured using seining and dip nets and tissues were stored in 95 % ethanol.

The Santa Clara River is an interrupted perennial stream that extends from sea level to about 2,700 m across a predominantly east–west axis (where west is downstream toward the Pacific Ocean). The entire watershed drains approximately 4,212 km$^2$. We sampled stickleback across the full length of the river, including its main tributaries and from four locations in the geographically adjacent Ventura River (Fig. 1). Several flood plains with intermittent surface flow divide the perennial reaches of the Santa Clara main channel (Miller 1960; Cox et al. 2003; Stillwater Sciences 2007; Beller et al. 2011); we briefly describe them here, for clarity in the remainder of the paper (see also Fig. 1). The uppermost dry gap (hereafter the 'Santa Clarita Gap') is approximately 16.0 km long and occurs in the Santa Clarita Basin between the towns of Saugus and Lang—the type locality for *G. a. williamsoni*, which no longer supports stickleback, lies to the east of this Basin at Kentucky Springs in Soledad Canyon (Miller 1960). A second, more substantial dry gap extends from approximately 5.6 river-km below the Los Angeles County line west to an area near the mouth of Piru Creek and can reach up to 24.0 km in length (i.e. the Piru Gap). A third intermittent reach exists further downstream in Saticoy and covers approximately 5.3 river-km (i.e. the Saticoy Gap).

We also screened a small sample of unarmored stickleback from San Felipe Creek, a tributary to the Salton Sea in San Diego County—this population was transplanted from Soledad Canyon on separate occasions dating back to 1972 and was intended as a 'genetic reservoir' in the event that the Soledad population became extirpated (Swift et al. 1993).

### Morphological data collection

We conducted plate counts on all genotyped individuals under a Leica M125 microscope at 10X magnification, provided they were larger than 25.0 mm (following Hagen and McPhail 1970). Skeletons were stained with Alizarin Red following the Schluter Lab protocol (www.zoology.

ubc.ca/~schluter). In some cases, counts were made on additional fish collected at the same time and place as those that were genotyped to increase the sample size for statistical analysis.

We used ANOVA and a Tamhane's T2 post hoc to test for significant differences among groups of samples. For the Santa Clara main stem, we compared three groups: sites west of the Piru Gap towards the ocean, sites east of the Piru Gap but west of the Santa Clarita Gap (i.e. Newhall Ranch, Valencia, San Francisquito Canyon, and Bouquet Canyon), and sites east of the Santa Clarita Gap, which includes the two sampling areas just downstream from the type locality for *G. a. williamsoni* (i.e. Thousand Trails and Robin's Nest). All stickleback populations east of the Santa Clarita Gap are recognized as the unplated *G. a. williamsoni*; those between the Piru and Santa Clarita Gaps, including the tributaries that drain into this reach, are predominantly unplated (with the exception of upper Bouquet Creek—see Results); those west of the Piru Gap out to the ocean are exclusively the low-plate morph *G. a. microcephalus*.

We also compared samples among six stream reaches covering approximately 4.7-km within Bouquet Creek, where low-plate stickleback are suspected of being introduced as by-catch with hatchery rainbow trout *Oncorhynchus mykiss* for sport fishing (Swift et al. 1993). Reach numbers and their respective coordinates are listed in Table S1. We also examined a temporal series of specimens collected in 1979, 1982, 2001, and 2011 from lower Bouquet Creek to test for increases in plate number over the past ~35 years. These specimens were collected from an area that extends between the Zuni Campground and the juncture of Bouquet and Texas Canyons (reach 56; Table S1). Specimens from 1979 to 1982 were obtained from the Natural History Museum of Los Angeles County (lot no. LACM44585-1, 44567-1); the 2001 samples are part of an in-house collection at the USGS San Diego Field Station; the 2011 samples were obtained from the Santa Barbara Museum of Natural History (lot no. SBMNH1825). All statistical analyses were conducted in (R_Core_Team 2013).

## Genetics: microsatellite and mtDNA data collection

We extracted genomic DNA from muscle tissue or fin clips using a Qiagen DNeasy Blood & Tissue Kit. These samples were then used to screen 22 microsatellite loci that were largely developed by (Peichel et al. 2001) and known to be polymorphic in other population-level studies of European, Asian, and North American stickleback (Mäkinen et al. 2006; Kitano et al. 2007; Caldera and Bolnick 2008). This initial screening was based on a larger dataset that included representatives of threespine stickleback populations across southern California, the data of which will be published elsewhere.

We amplified the microsatellites in 10 µl multiplex reactions using a Qiagen Multiplex PCR kit and 50-100 ng of DNA template following the manufacturer's protocol. Table S2 provides summary statistics for the microsatellite loci used in our final analyses. Genotyping runs were performed on an ABI 3100S Automated Sequencer (Applied Biosystems, Foster City, CA, USA) at the San Diego State University Microbiology Core Facility using the LIZ600 size standard (Applied Biosystems). We scored the alleles using GeneMarker v1.85 (Softgenetics LLC, State College, PA) and tested for pairwise linkage disequilibrium among loci using Genepop on the web (Raymond and Rousset 1995).

We also sequenced a portion of the mitochondrial control region (CR; ~450-base pairs [bp]) and the cytochrome *b* gene (cyt*b*; ~1,020-bp) for three to five individuals per location, including several representatives of low- and fully-plated *G. aculeatus* populations in northern California and the Pacific Northwest. We selected these markers because they have been widely used in other studies on stickleback phylogeography, allowing us to combine our data with previously published sequences obtained from stickleback around the world. Cycling conditions and primer sets for each marker and are detailed in (Mäkinen and Merilä 2008). DNA sequencing was performed using Sanger methods and Big Dye v3.1 chemistry on an ABI3730XL DNA analyzer at Genewiz (La Jolla, CA, USA). We edited the data using Sequencher v5.1 (Gene Codes Corporation, Ann Arbor MI) and aligned the sequences by eye. The sequence alignment is available as a NEXUS file on the dryad database (to be completed pending acceptance).

## Population structure and phylogeography

We used Structure v2.3.1 (Pritchard et al. 2000) to assign individuals to natural groups and to detect clinal shifts in allele frequencies. We performed two main sets of analyses using the admixture model, one assuming correlated allele frequencies across sampling locations and another assuming uncorrelated frequencies (Pritchard et al. 2000; Falush et al. 2003). For each set, we ran 10 independent simulations for a pre-specified number of clusters $K$ (250,000 steps, discarding the first 100,000 steps) and plotted the average $\ln P(D|K)$ scores against $K$ values ranging from 2 to 10 in Structure Harvester (Earl and vonHoldt 2012). The $K$ value corresponding to the asymptote of the $\ln P(D|K)$ curve and the $\Delta K$ statistic (Evanno et al. 2005) were then used to approximate the number of clusters $K_{max}$ in the dataset. To summarize the results, we generated alignments of the assignment coefficient matrices using the GREEDY

algorithm in ClumpP v1.1.2 (Jakobsson and Rosenberg 2007) and visualized the consensus alignments using Distruct v1.1 (Rosenberg 2004).

To identify the placement of Santa Clara and Ventura River populations within the broader phylogeographic context of *G. aculeatus*, we used the CR and cyt*b* sequences to estimate a phylogenetic tree in MrBayes v3.1.2 (Ronquist et al. 2011). Outgroup samples consisted of published sequences from low- and fully-plated morphs from Japan (Watanabe et al. 2003), the northern Atlantic Ocean (Mäkinen and Merilä 2008), and the sister species *G. wheatlandi* (Kawahara et al. 2009), plus our own sequences for samples from the Pacific northwest. We used Bayesian Information Criterion (BIC) scores generated in jModeltest 2.1.2 (Posada 2008) to identify best-fit substitution models for each gene region and ran two separate, model partitioned Markov Chain Monte Carlo simulations for $2 \times 10^7$ steps, sampling from the posterior every 1,000th step. We assessed convergence and effective sample sizes for each model parameter in Tracer v1.5 (Drummond and Rambaut 2007) and discarded the first 10 % of the posterior samples after verifying stationarity in the parameter estimates.

To estimate the divergence times between major clades recovered in our analysis, we used a relaxed clock approach in BEAST v1.8 (Drummond and Rambaut 2007) and rate calibrations for CR and cyt*b* inferred in Mäkinen and Merilä 2008. Their calibrations were based on the 10 Mya divergence estimate between the common ancestor of *Pungitius pungitius* and *G. aculeatus* as inferred from fossil data (Bell and Foster 1994). We used an uncorrelated lognormal clock model and explored results using three different tree priors (Bayesian Skyline, expansion growth, and constant size) to test for possible effects on branch length and divergence estimates.

### Estimates of genetic diversity and differentiation

We used GenoDive v 2.0b25 (Meirmans and Van Tienderen 2004) to calculate observed ($H_O$) heterozygosity, gene diversity ($H_S$), and the $F_{ST}$ analog $\Theta$ (Wier and Cockerham 1984). Significant differences in pairwise $\Theta$ values were determined using $1 \times 10^4$ permutations of the dataset in GenoDive. We estimated allelic richness ($A_R$) based on a minimum sample of 16 gene copies in HPRare (Kalinowski 2005). We compared $H_O$, $H_S$, $A_R$, $\Theta$ and Queller and Goodnight's (1989) index of relatedness $R$ using permutation tests and different hierarchical configurations of the data in Fstat v2.93 (Goudet 2001) to test for spatial differences in the amount of genetic diversity and differentiation. We measured departures from Hardy–Weinberg equilibrium (HWE) for each locus and population using Genepop v1.2 (Raymond and Rousset 1995).

We also performed an analysis of molecular variance (AMOVA) in Arlequin v3.11 (Excoffier et al. 2005) to examine the proportion of allelic variation explained by different hierarchical groupings of the data (within sampling locations, among sampling locations, among groups of sampling locations as suggested by clustering analyses). We also included comparisons between sites with different plate morph identities to test whether the locus-by-locus variation differed between plate morph groups.

Last, we screened all loci for evidence of natural selection using the $F_{ST}$-outlier approach (Beaumont and Nichols 1996; Beaumont 2005) implemented in Lotisan (Antao et al. 2008). This approach calculates an expected distribution of $F_{ST}$ versus $H_E$ based on an island model of migration (Wright 1931) and assumes that markers are neutrally-evolving—loci having excessively high or low $F_{ST}$ compared to neutral expectations are inferred to be under different forms of natural selection (i.e. directional or balancing).

## Results

### Morphology

Two obvious plate morph groups exist in the Santa Clara River, with the sharpest division in plate counts centered on opposite sides of the Piru Gap (ANOVA, df = 2; $F = 238.829$; $P < 0.001$; Table 1). Unarmored morphs were exclusive to sites east of the gap toward the headwaters, whereas low-plate morphs were largely restricted to sites west of the gap towards the river mouth. Nearly 100 % of the fish collected from Soledad and San Francisquito Canyons lacked body armor entirely, whereas further downstream in Valencia, individuals showed a slight but significant increase in plating (ANOVA, df = 3; $F = 8.586$; $P < 0.001$). Nonetheless, a relatively high proportion of the Valencia fish had either no plates or one plate total (0.63), or no plates on at least one side (0.72).

Although Bouquet Creek lies within an area that is otherwise exclusive to unarmored fish (i.e. above the Piru Gap), plate counts in the creeks' upper reaches were significantly different from the remaining Santa Clara River populations (ANOVA, df = 2; $F = 368.88$; $P < 0.001$: Fig. 2). The highest counts were recovered from samples collected furthest up the canyon (mean plate count for Reach 95 = 6.78 ± 0.98; Fig. 3); plate counts from all upper reaches combined were also significantly different from those in Reach 56 near the Zuni Campground (mean = 5.57 ± 0.30; ANOVA, df = 4; $F = 64.43$; $P < 0.001$). We also found that the mean number of plates per fish increased across sampling years for reach 56 (ANOVA: $F$-ratio = 5.11, df = 3, $P < 0.01$), indicating

**Table 1** Geographic and plate count data. Latitude/longitude data points approximate the middle of a sampling reach

| Name | PopID | Lat | Long | Elev (m) | dist (km) | n | Mean no. plates ± SE |
|---|---|---|---|---|---|---|---|
| Soledad 01 (Thousand Trails) | 1 | 34.44245 | −118.21085 | 772.0 | 120.0 | 24 | 0.0 ± 0.0 |
| Soledad 02 (Robin's Nest) | 2 | 34.43933 | −118.31721 | 610.0 | 108.7 | 24 | 0.2 ± 0.2 |
| San Francisquito Canyon. | 3 | 34.54573 | −118.51623 | 506.0 | 96.9 | 23 | 0.0 ± 0.5 |
| Valencia | 4 | 34.42671 | −118.57650 | 324.0 | 75.1 | 25 | 1.8 ± 0.0 |
| Newhall Ranch | 5 | 34.43471 | −118.60312 | 316.0 | 70.1 | 12 | [a] |
| Bouquet Canyon. MM15.89 | 6 | 34.50920 | −118.45320 | 527.0 | 89.4 | 24 | 4.0 ± 0.5 |
| Bouquet Canyon. Reach 77 | 6 | 34.54850 | −118.43211 | 685.0 | 121.6 | 25 | 5.4 ± 0.5 |
| Bouquet Canyon. Reach 84 | 6 | 34.55401 | −118.41619 | 729.0 | 123.3 | 11 | 5.4 ± 0.8 |
| Bouquet Canyon. Reach 90 | 6 | 34.56041 | −118.40477 | 769.0 | 125.1 | 10 | 5.7 ± 1.2 |
| Bouquet Canyon. Reach 92 | 6 | 34.56283 | −118.40004 | 809.0 | 125.5 | 31 | 5.4 ± 0.5 |
| Bouquet Canyon. Reach 95 | 6 | 34.56741 | −118.39450 | 838.0 | 126.3 | 9 | 6. 8 ± 1.0 |
| Piru Creek | 7 | 34.41729 | −118.78950 | 209.0 | 54.8 | 24 | 9.0 ± 0.2 |
| Lower Sespe Creek | 8 | 34.38435 | −118.95346 | 110.0 | 35.7 | 8 | 8.6 ± 0.4 |
| Upper Sespe Creek | 9 | 34.55774 | −119.25324 | 1010.0 | 101.7 | 11 | 9.5 ± 0.3 |
| Oxnard | 10 | 34.23645 | −119.19606 | 19.0 | 6.3 | 24 | 8.4 ± 0.2 |
| McGrath (Santa Clara mouth) | 11 | 34.23011 | −119.26061 | 1.0 | 0.0 | 8 | 8.5 ± 0.6 |
| Seaside Park (Ventura mouth) | 12 | 34.281755 | −119.30903 | 6.0 | 0.8 | 8 | 8.8 ± 0.4 |
| Foster Park | 13 | 34.35201 | −119.30748 | 68.0 | 9.5 | 31 | 10.0 ± 0.4 |
| San Antonio Creek | 14 | 34.43267 | 119.25093 | 194.0 | 22.2 | 24 | 7.9 ± 0.5 |
| Matilija Creek | 15 | 34.50077 | −119.34370 | 391.0 | 19.7 | 32 | 11.3 ± 0.3 |

Other column headers are as follows: *Elev.* elevation of the sampling location; *dist* distance from the sampling location to the ocean; *n* number of individuals used for plate counts; *no. plates* mean number of total plates ± standard error

[a] Ten of the 12 samples from Newhall Ranch were below the minimum size threshold for obtaining accurate plate counts

that body armor has been steadily increasing in the lower section of Bouquet Creek since at least the 1980s (Fig. 2).

Below the Piru Gap, all fish were categorically low-plate morphs, with mean plate counts ranging from 8.38 in Oxnard to 8.96 in lower Piru Creek (Table 1). Ventura River samples also consisted of low-plate morphs only. Plate counts differed significantly across sampling locations within the Ventura River (ANOVA, df = 3; $F = 14.35$, $P < 0.001$), with the most heavily plated fish occurring in the upper Matilija Creek tributary. In fact, plate counts in Matilija Creek were the highest among all sampling sites in both watersheds.

## Population structuring: microsatellites

A plot of the lnP(D|$K$) scores from the STRUCTURE runs and the $\Delta K$ statistic showed evidence of three main clusters that were non-randomly associated with geography (Fig. 3). Unarmored samples in the upper Santa Clara River formed two distinctive clusters, one restricted to Soledad Canyon east of the Santa Clarita Gap and the other including San Francisquito Canyon, Valencia, Newhall Ranch and lower Bouquet Creek ($K = 3$–6, Fig. 3). The translocated San Felipe Creek population also retained a strong signature of its source population in Soledad Canyon. Valencia samples showed evidence of asymmetric admixture with Soledad Canyon samples, as expected if fish movement is predominantly one-way and downstream.

Although geography was generally a good predictor of clustering patterns, stickleback from upper Bouquet Creek were an exception to this general finding. These fish showed clinal admixture corresponding to different sampling reaches—only a proportion of the samples from the lowest reaches (reaches 77 and mile marker 15.89) clustered with unarmored fish from San Francisquito Canyon, Valencia and Newhall Ranch ($K = 3$–6; Fig. 3), as expected based on geography. In contrast, samples from the uppermost reaches (95 and 92) showed greater affinity to low-plate populations from the lower Santa Clara River below the Piru Gap. The cline was centered on reaches 90, 84, and 77, which occur just below a waterfall that separates this stretch of creek from reaches 95 and 92. Linear regression and standard correlation analysis of assignment coefficients $Q$ against elevation of the sampling location showed that the lower an individual was sampled in the Bouquet Creek, the greater the tendency for that individual

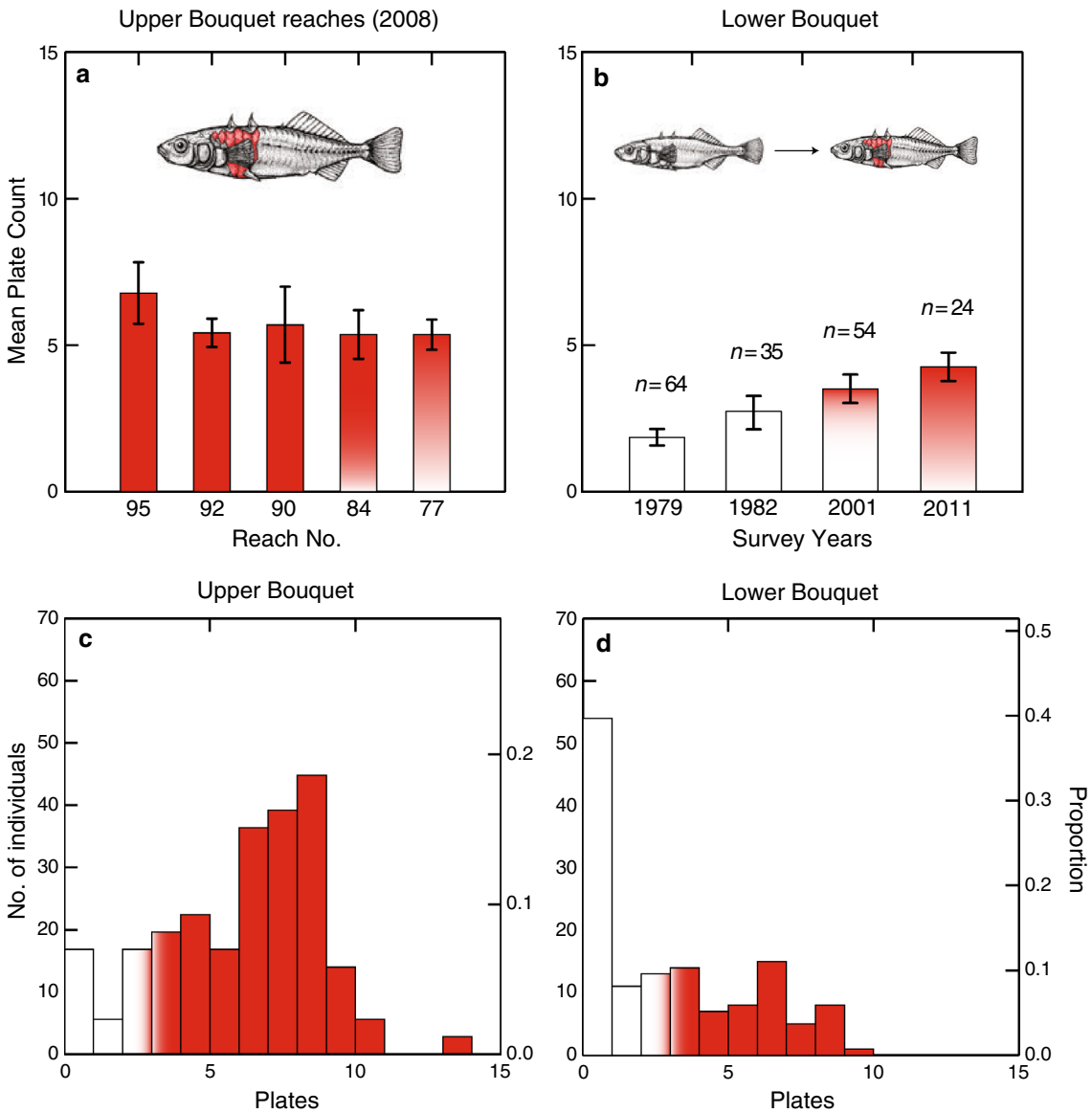


**Fig. 2** Mean plate counts (±SE) across reaches and survey years for Bouquet Creek, with *red* coloration indicating low-plate morphs. Sample sizes and reach locations are provided in Table S1. **a** Mean plate counts for upper Bouquet Creek showed no significant differences across upper reaches in 2008. **b** Plate counts by survey year for reach 56 near the Zuni Campground. **c** Histogram showing the distribution of plate counts for all upper reaches combined in 2008. **d** Distribution of plate counts for reach 56 (*lower*) based on the combined 1979, 1982, and 2001 samples

to cluster with unarmored fish in the main channel and San Francisquito Canyon (Spearman's $\rho = 0.58$, $P = 0.006$; $\rho = 0.70$, $t = 4.26$, $P < 0.001$; Table S3).

In the lower Santa Clara River, low-plate samples were virtually indistinguishable from Ventura River populations at $K = 2$–3, although some admixture with the upper Santa Clara River was apparent (Fig. 3). As we fit the data to $K \geq 4$, Ventura River fish became more distinctive, whereas lower the Santa Clara River populations remained admixed. Upper Sespe Creek samples also showed greater affinity to Ventura River samples at higher $K$ than to individuals from lower reaches of the same tributary or the Santa Clara main stem. At $K = 8$, Matilija Creek samples collected above the Matilija Dam clustered separately from those in the Ventura River below the dam.

When we partitioned the data into the three main clusters, the AMOVA showed that most of the variation was explained by the within-population variance component $V_C$ (63.9 %, $F_{CT} = 0.28$; $P < 0.001$); 28.3 % of the total variation was explained by differences among clusters ($F_{ST} = 0.36$; $P < 0.001$), whereas populations within clusters explained only 7.9 % of the variation ($F_{SC} = 0.11$;

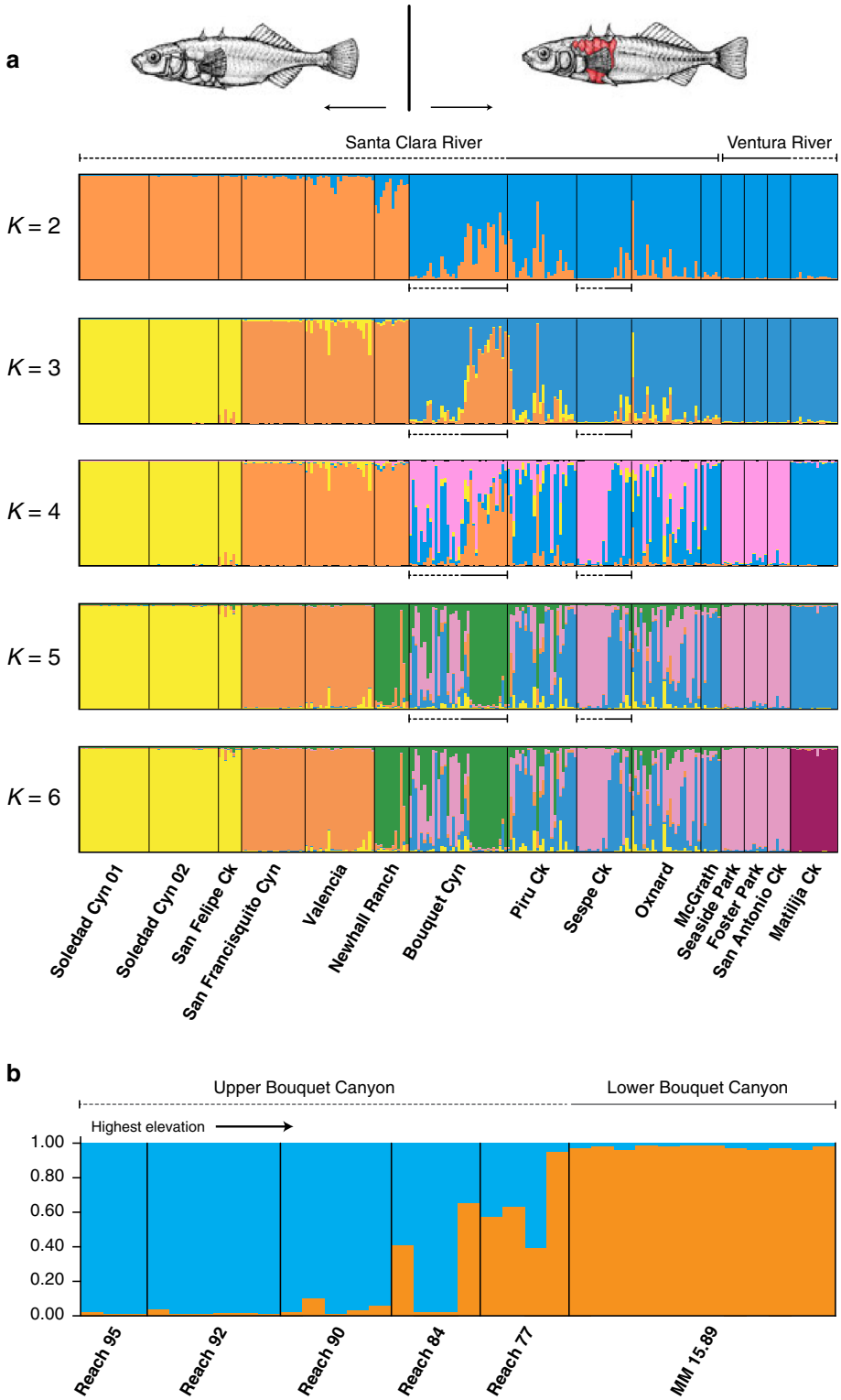


**Fig. 3** Assignment plots based on the microsatellite data. *Cartoons* denote the plate morph identity of the sampling locations, with the *vertical bar* showing the major division between morphs in the Santa Clara River. *Bars* above and below each assignment plot indicate spatial divisions within the sampling areas(*dashed* upstream reaches;*solid* downstream reaches). **a** Assignments for the full Santa Clara and Ventura River dataset for $K = 2$-6. **b** Cluster assignments for Bouquet Creek showing the cline centered on reaches 84 and 77; clusters are separated by survey reach (sampling elevation decreases from left to right). Locations of each reach are provided in Table S1

$P < 0.001$). When grouping populations by plate morph (i.e. $K = 2$), we found that most of the variation was again explained by $V_C$ (60.4 %, $F_{CT} = 0.27$; $P < 0.001$), whereas 26.8 % of the variation was explained by differences between the two phenotypic groups ($F_{ST} = 0.40$; $P < 0.001$). Thus, a similar amount of the total variation (28.3 vs. 26.8 %) was explained by differences among the three main genetic groups as between the two plate morphs. When comparing low-plate populations in the Ventura and lower Santa Clara River, only 2.5 % of the variation

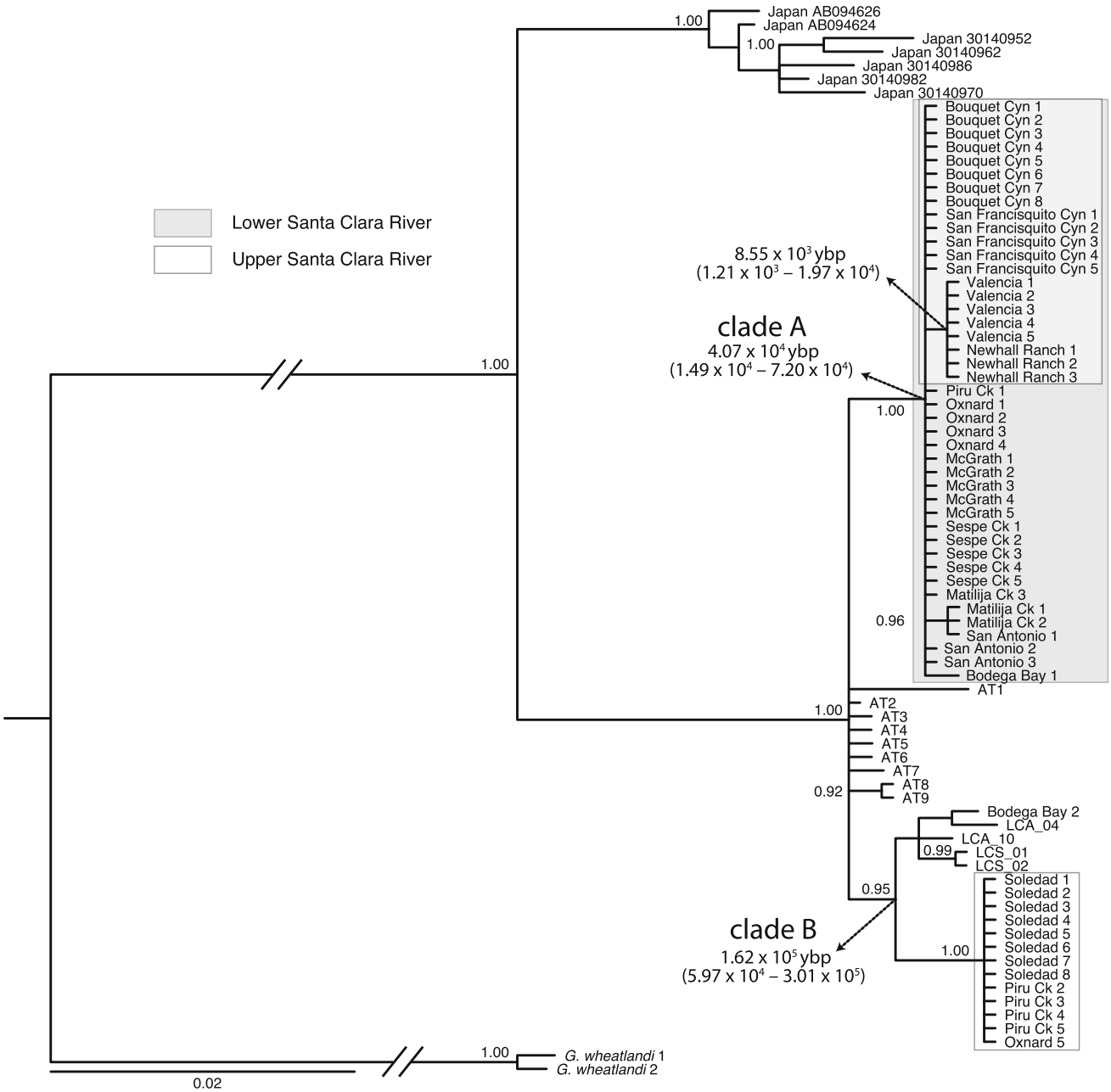


**Fig. 4** 50 % majority consensus tree from the MrBayes analysis. Haplotypes were named according to sampling location. Numbers above the branches are posterior probabilities. *AT* Trans-Atlantic lineage of Mäkinen and Merilä (2008); *LCA* fully-plated, Pacific Northwest; *LCS* low-plate Pacific Northwest

was explained by the among group variance ($V_A = 0.10$; $F_{ST} = 0.12$; $P < 0.001$), indicating little differentiation between the two groups.

## Population structuring and admixture: mtDNA

We recovered four mitochondrial haplotypes (18 parsimony informative sites) that formed two well-supported clades, and neither was exclusive to low- or unplated morphs (Fig. 4). In clade A, a single high frequency haplotype was recovered from nearly all fish collected below the Santa Clarita Gap and throughout the Ventura River ($f = 0.74$; 31/42 sequenced individuals in the Santa Clara River below the Santa Clarita Gap and in the Ventura River). A second clade A haplotype was restricted to the Santa Clara main stem in Valencia and Newhall Ranch ($f = 0.19$), and third low frequency haplotype ($f = 0.07$)

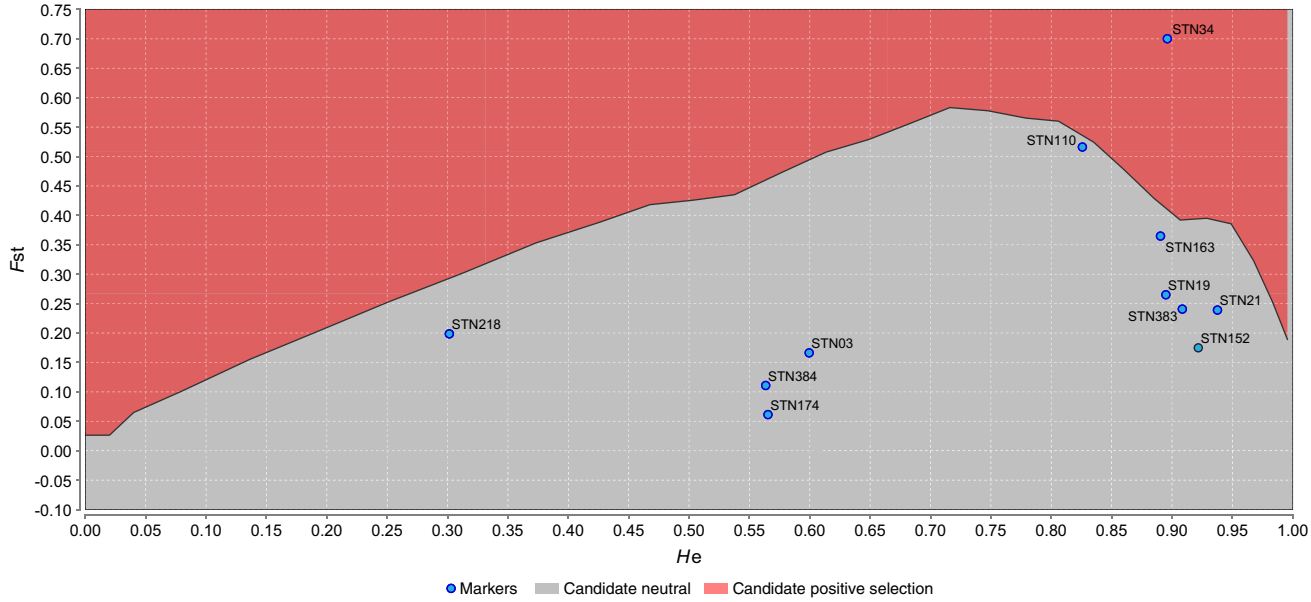


**Fig. 5** Expected distribution of $F_{ST}$ and $H_E$ for selected and neutral markers based on an island model of migration

was recovered in three individuals from the Matilija and San Antonio Creek tributaries in the Ventura River.

Haplotype diversity and differentiation was higher for Clade B, which consisted of broadly distributed samples from the Pacific Northwest, Bodega Bay (northern coastal California), and Soledad Canyon. These samples included the full range of threespine stickleback plate morphs. We recovered a single, divergent haplotype from all fish in Soledad Canyon, with the same haplotype appearing at low frequency in several low-plate individuals well downstream in Piru Creek and Oxnard. The time to most recent common ancestry of haplotypes in clade B was also approximately four times older than for the haplotypes in Clade A (Fig. 5).

A number of different haplotypes from the northern Atlantic Ocean were interspersed between the two major Pacific clades, but no phylogeographic structure was discernable among any of these Atlantic haplotypes, consistent with Mäkinen and Merilä (2008). Haplotypes from threespine stickleback in Japan formed a well-supported, monophyletic sister group to all ingroup samples.

## Genetic diversity and differentiation estimates

Table 2 provides information on sample sizes and diversity estimates for each sampling location. We detected an outlier locus that may be under natural selection in unarmored populations (STN34; see below), so we excluded this locus from all assignment tests to avoid biasing the clustering patterns. Two of the 11 loci (STN21 and STN383) were significantly out of HWE for some locations, but no loci were out of HWE across all populations. To test the extent to which STN21 and STN383 may have affected the cluster assignments, we re-ran the STRUCTURE analyses excluding these loci from the dataset and found that the results were qualitatively consistent; thus we were confident that their inclusion was not misleading our results.

**Table 2** Genetic diversity estimates for each sampling location

| Name | Cluster | $n$ | $n_P$ | $A_R$ | $H_O$ | $H_S$ |
|---|---|---|---|---|---|---|
| Soledad 01 (1000 Trails) | 1 | 24 | 7 | 1.60 | 0.16 | 0.20 |
| Soledad 02 (Robin's Nest) | 1 | 24 | 7 | 1.65 | 0.21 | 0.22 |
| San Felipe Creek | 1 | 8 | 7 | 2.10 | 0.36 | 0.36 |
| San Francisquito Creek | 2 | 23 | 8 | 2.05 | 0.26 | 0.31 |
| Valencia | 2 | 24 | 10 | 2.65 | 0.35 | 0.43 |
| Newhall Ranch | 2 | 12 | 10 | 3.40 | 0.47 | 0.56 |
| Bouquet Creek | 2 | 34 | 11 | 4.72 | 0.64 | 0.72 |
| Piru Creek | 3 | 24 | 11 | 5.06 | 0.64 | 0.66 |
| Sespe Creek | 3 | 19 | 11 | 4.83 | 0.55 | 0.63 |
| Oxnard | 3 | 24 | 11 | 5.51 | 0.65 | 0.68 |
| Santa Clara River mouth | 3 | 8 | 11 | 5.20 | 0.62 | 0.66 |
| Seaside Park | 3 | 8 | 11 | 3.90 | 0.54 | 0.61 |
| Foster Park | 3 | 8 | 11 | 4.60 | 0.51 | 0.61 |
| San Antonio Creek | 3 | 8 | 11 | 4.10 | 0.54 | 0.61 |
| Matilija Creek | 3 | 16 | 11 | 3.50 | 0.48 | 0.54 |

The cluster category refers to the group in which individuals from each sampling location were assigned with the highest probability in the STRUCTURE analyses. $n$, sample size for genotyped individuals; $n_P$, number of polymorphic loci; $A_R$, allelic richness; $H_O$, observed heterozygosity, $H_S$, gene diversity (Nei 1987)

**Table 3** Comparison of genetic diversity estimates among clusters in the Santa Clara River

| | $A_R^*$ | $H_O^*$ | $H_S^*$ | R | $F_{ST}$ |
|---|---|---|---|---|---|
| Cluster 1 | 1.78 | 0.21 | 0.23 | 0.19 | 0.11 |
| Cluster 2 | 2.62 | 0.34 | 0.41 | 0.36 | 0.24 |
| Cluster $3_A$ | 4.14 | 0.58 | 0.63 | 0.17 | 0.72 |
| Cluster $3_B$ | 4.60 | 0.62 | 0.66 | 0.08 | 0.05 |
| Cluster $3_C$ | 3.70 | 0.51 | 0.58 | 0.25 | 0.15 |

The asterisk denotes indices with significant differences across clusters (5000 permutations, $P \leq 0.01$). Cluster assignments were based on the STRUCTURE analyses: cluster *1* Soledad 01-02 and San Felipe Creek; cluster, *2* Valencia, San Francisquito Canyon, and Newhall Ranch; cluster, $3_A$ lower Santa Clara + Ventura River populations (excluding Bouquet Canyon); cluster, $3_B$ lower Santa Clara River only; cluster, $3_C$ Ventura River only, $A_R$ allelic richness, $H_O$ observed heterozygosity, $H_S$ gene diversity, *R* Queller and Goodnight's relatedness index, $F_{ST}$ Wright's fixation index

When comparing groups of populations as defined by the STRUCTURE analysis ($K = 3$), we found significant differences in allelic richness $A_R$ (5000 permutations of the dataset, two-sided; $P = 0.005$), observed heterozygosity $H_O$ ($P = 0.004$), and gene diversity $H_S$ ($P = 0.007$) between the upper Santa Clara unarmored populations and the lower Santa Clara low-plate populations (Table 3). In addition, five loci were invariant in Soledad Canyon, three were invariant in San Francisquito Canyon, and two were invariant in Valencia, whereas all loci were polymorphic at the remaining sampling locations. Linear regression analysis comparing location (measured as the distance of the sampling location to the Santa Clara river mouth at McGrath) with allelic richness $A_R$, observed heterozygosity $H_O$, and gene diversity $H_S$ all showed significant negative relationships ($A_R$, $r^2 = 0.81$, $P = 0.0002$; $H_O$, $r^2 = 0.81$, $P = 0.0002$; $H_S$, $r^2 = 0.79$, $P = 0.0003$), indicating that genetic diversity sharply declines in populations sampled successively further up river. In fact, the lowest diversity estimates were obtained for fish sampled in Soledad Canyon. This same pattern was reflected in mtDNA haplotype diversity, where all Soledad Canyon fish shared a single haplotype that was differentiated from the multiple haplotypes recovered below the Santa Clarita Gap.

Estimates of population differentiation based on Wier and Cockerham's $F_{ST}$ analog $\Phi$ showed that fish from most sampling sites were significantly differentiated. However, there were three exceptions where sampling localities appear to be in regular communication: (1) the two Soledad Canyon sites in the upper Santa Clara, (2) the lagoon and most sites east of the Piru Gap (McGrath, Piru Creek, lower Sespe Creek and Oxnard), and (3) all sites below Matilija Creek in the Ventura River. We treated the two Sespe Creek samples separately as the headwater sample was highly differentiated from the sample collected closer to the mouth of the tributary. When we evaluated pairwise differentiation for populations forming the three main clusters ($K = 3$), we found no significant differences within each cluster (range $\Phi = 0.07$–$0.24$; $P = 0.83$).

## Outlier locus

Allelic variation at STN34 was consistent with a marker that could be linked to a quantitative trait locus under natural selection in unarmored populations. Only three fish from all unarmored sites combined (including San Felipe Creek, 100 of 103 individuals; allele frequency = 0.97) were not homozygous for allele 185, two of which were from Valencia and one from San Francisquito Canyon. Of these three individuals, all were heterozygotes at STN34, and in each case one of the alleles present was 185. The allele was also present at some but not all other sampling locations, with Sespe Creek showing the highest frequency (0.37) among the low-plate populations.

A locus-by-locus AMOVA separating unarmored from low-armor samples showed that the among-group variance component for STN34 explained 66.8 % of the variation between plate morph groups, far more than any of the remaining loci. Similarly, the between-group $F_{ST}$ value ($F_{ST} = 0.71$, $P < 0.001$) greatly exceed that of all other loci (range $F_{ST} = 0.19$–$0.57$). When we performed the AMOVA using unarmored samples only, the among-population variance component was negative ($V_b = -0.00011$, % variation $= -0.75$) and $F_{ST}$ was close to zero ($F_{ST} = 0.01$, $P = 0.26$), as expected given the near fixation of a single allele for these samples. In contrast, when we analyzed only the low-plate fish, STN34 showed significant population structure and accounted for about 9.14 % of the total variation ($V_b = 0.02392$; $F_{ST} = 0.12$, $P < 0.001$). These analyses suggest that the absence of body armor is correlated with the presence of specific variation at STN34; in low-plate populations, STN34 behaved similar to the other presumptively neutral markers, whereas in unplated populations, the predominance of a single allele was consistent with a locus under selection.

Corroborating these results, the expected distribution of $F_{ST}$ versus $H_E$ based on an island model of migration showed that STN34 had a significantly higher $F_{ST}$ than expected under neutrality when populations were grouped by plate morph (Fig. 5). When we analyzed a subset of the data containing only low-plate populations, STN34 no longer showed evidence of being an outlier, but rather conformed to the neutral expectation. However, when we examined only unarmored populations using the same approach, STN34 again showed evidence of selection, only in this case the signature was for balancing selection (i.e. maintenance of the same allele across unarmored populations).

## Discussion

The genetics of threespine stickleback in the Santa Clara and Ventura Rivers reveal a complex population history, with contemporary genetic signatures arising from population-level processes and at least one incidental translocation overlaying older signatures of deeper lineage divergence events. Our findings have a number of important implications for management, particularly for the endangered unarmored populations in the upper Santa Clara watershed, and suggest that plate morph distributions and the effects of geophysical features on gene flow have remained stable in both the Santa Clara and Ventura River watersheds for a substantial amount of time.

### Spatial distribution of plate morphs

The distribution of plate morphs in this study is consistent with previous surveys of the Santa Clara River dating to the 1960's (Miller and Hubbs 1969; Bell 1978), where unplated fish were restricted to upstream reaches east of the Piru Gap, and low-plate fish were restricted to downstream reaches west of the Piru Gap towards the ocean. We found a slight yet significant increase in body armor at the Valencia and Newhall Ranch sampling areas, although plate counts in these fish were still within the limits of a typical unarmored population (Miller and Hubbs 1969; Bell 1976). The biological significance of this increase is not clear, although changes in water temperature, salinity, pH, and ion concentration are all known to affect the development of body armor (Lindsey 1962; Giles 1983; Bell et al. 1993; MacColl et al. 2013). Both sites are juxtaposed between two large wastewater treatment facilities that discharge into the Santa Clara River, and it is possible that some aspects of the treated water (most notably temperature and chloride levels) may be influencing plate development without causal genetic differentiation. Alternatively, these fish may simply harbor some genetic variation that translates to a slight increase in the 'armoredness' of the population, although the source of this variation and whether it actually exists is not known.

Stickleback in upper Bouquet Creek represent the single exception to the upstream–downstream partitioning of plate morphs in the Santa Clara River. The genotype and phenotype of these fish more closely resembles a typical downstream Santa Clara River low-plate population than any of the more geographically proximate, unarmored populations in San Francisquito Canyon, Valencia or Soledad Canyon. Unarmored stickleback were first reported in Bouquet Creek in the 1970s (USFWS 2009) at a time when trout stocking had already commenced for sport fishing. Trout were harvested from the Fillmore State Fish Hatchery on the lower Santa Clara River just east of the confluence with Sespe Creek, where stickleback were established in in-channel hatchery ponds and were known to be inadvertently transported along with trout (Swift et al. 1993; USFWS 2009). Although no official record of inadvertent stickleback movement exists for Bouquet Creek, the fact that plate condition and genotypic affinity clearly ties these fish to lower Santa Clara River populations leaves little doubt that the upper Bouquet Creek population exists as a result of accidental introduction.

While the data supporting a non-native introduction are convincing for upper Bouquet Creek, this same hypothesis fails to explain the historical presence of unarmored stickleback in the lowest reaches of the creek, and why they were unknown prior to the 1970s. Regardless of why the population went undected before the 1970s, our plate counts revealed a temporal trend for increasing plate counts in this reach over several decades, suggesting that the genetic background of introduced, low-plate fish from the upper creek reaches have infiltrated a formerly unarmored, native stock in the lower reaches. Our STRUCTURE analyses confirm this scenario, as individual assignments reveal a cline in cluster membership down the canyon. Only in the lowest reaches of Bouquet Creek do fish cluster with unarmored populations in Valencia, Newhall Ranch and San Francisquito Canyon. Interestingly, the cline is positioned immediately downstream of a waterfall that likely restricts upstream gene flow (but not necessarily prevents it under certain conditions), whereas movement downstream from the upper reaches is unimpeded. Consistent with this prediction, we see admixture of upstream alleles from reaches 95 and 92 into reaches 90, 84, and 77 just below the waterfall, but no admixture in the opposite direction.

Below the Piru Gap, our data show evidence of a continuous low-plate assemblage that extends to the Santa Clara river mouth at McGrath State Beach. The phenotypes of these fish are similar to those in the Ventura River, although we detected some variation in plate counts within the upper Ventura River watershed. This variation mirrors the patterns described in Bell and Richkind (1981), where fish from upper Matilija Creek had significantly higher plate counts compared to those from the lower reaches of the Ventura main stem. They attributed this pattern to the presence of predatory trout above the Matilija Dam and the absence of trout below the dam, and showed that there was a step-cline in plate counts centered on a 10 K dry gap above the mouth of Matilija Creek (stations 10 and 11 *in* Bell and Richkind 1981). Plate counts from this study are consistent with this pattern, suggesting that that the cline has remained stable since at least the late 1970s.

We note that more precise plate count estimates can be obtained only with larger sample sizes of individuals from each sampling location. In this study, sample sizes reflect either low stickleback abundance for a given site and/or

permit limitations on take. Nonetheless, these data sufficiently characterize the distribution of the main plate morph groups and indicate that their locations have remained stable for at least the past ~60 years, and possibly much longer.

## Geographic barriers to gene flow and population structuring

Our results show that the main genetic breaks in the Santa Clara and Ventura River systems are closely linked to stable versus ephemeral reaches in the main river channels, rather than the drainage of origin or positive assortative mating by plate morph. The most extensive dry gap (i.e. Piru Gap) spatially coincides with the largest genetic division of the two river systems (Fig. 3), as well as the step-cline in plate counts. Thus, the Piru Gap serves to reinforce both the morphological and genetic separation of the two plate morph groups. These findings are similar to allozyme studies conducted in the early 1980s in showing that the low- and unplated morphs are genetically differentiated across the Santa Clara River and that little gene flow occurs between them (Buth et al. 1984).

We found further evidence of genetic subdivisioning between unplated populations occurring on either side the Santa Clarita Gap (i.e. Soledad Canyon vs. Newhall Ranch/Valencia/San Francisquito), although gene exchange appears less restricted across this reach compared to the Piru Gap. Admixture patterns revealed in the STRUCTURE analyses again indicate a predominance of one-way gene flow across this area, as do patterns in mtDNA haplotype variation, the latter of which shows that stickleback are at least occasionally transported across both the Santa Clarita and Piru Gaps. This is seen by the fact that several fish from the mouth of Piru Creek and Oxnard carried the unique Soledad Canyon mtDNA haplotype. However, these presumptively older gene exchanges, while preserved in the mitochondria, are clearly overridden by more recent gene flow among the more interconnected populations of the lower Santa Clara main stem.

Lack of genetic subdivision among the lower Santa Clara River populations extends to the Ventura River, as the marine separation between the two rivers has a negligible effect on the genetic composition of low-plate populations found in either system. Lower sea level earlier in the Holocene 8–10,000 years would likely have conjoined these drainages (Sommerfield and Lee 2004), and the earliest historic mapping suggests that mouth migration of the Santa Clara would have allowed it to approach to within 2 km of the Ventura River during the nineteenth century (Jacobs et al. 2011). In addition, mitochondrial and microsatellite data from the closed lagoon specialist tidewater goby (*Eucyclogobius newberryi*) suggest frequent communication between these drainages despite otherwise limited dispersal (Dawson et al. 2001; Barlow 2002).

Contemporary gene flow may also occasionally occur via ocean dispersal during winter storm events. Stickleback tolerate marine conditions (Bell 1979; Guderley 1994; Kassen et al. 1995), and storm discharges commonly create freshwater or brackish plumes that extend from coastal watersheds well out into the Southern California Bight (Noble et al. 2003; Schiff and Bay 2003; Reeves et al. 2004; Nezlin et al. 2005). These plumes reach as far as 10 km offshore during normal storm events (~2 year recurrence) and up to 30 km during exceptional floods (~10 year recurrence), and almost certainly transport stickleback within them (Warrick et al. 2004). In fact, the river mouths are in such close proximity (~5 km, well within the dispersal capabilities of stickleback) that their effluent mixes and cannot be distinguished at 1.1-km resolution satellite images (Nezlin et al. 2005). The recent collection of a fully armored adult stickleback in Malibu Lagoon in March, 2006 (LACM 56405-1, 51 mm SL) documents the potential for non-local genetic sources to invade coastal populations.

We found some evidence of genetic structure within the Ventura River, virtually all of which involved the Matilija Creek population. As is the case in the Santa Clara River, an ephemeral reach extending from approximately 13–23 km above the Ventura river mouth best explains the clustering patterns. Bell and Richkind (1981) report similar results for allozymes, and showed that populations from both above and below the Matilija Dam were genetically differentiated from populations closer to the river mouth—they attributed this partitioning to the dry gap, whereby genes from below the gap were unable to penetrate upriver to the Matilija tributary. Adding to the effect of this dry gap, our samples were collected from the upstream side of the Matilija Reservoir, which serves as yet another, more substantive barrier to upstream gene flow.

## Spatial discordance in microsatellite and mitochondrial variation

Both the microsatellite and mtDNA data indicate substantial mixing between lower Santa Clara and Ventura River stickleback populations, as many individuals shared identical haplotypes between the two drainages (Fig. 4). However, the extent of mitochondrial admixture between the two Rivers showed evidence of further upstream transport in the Santa Clara River than did shared alleles for the microsatellites. In the former, the Santa Clarita Gap appears to be the major upstream barrier to movement of the mitochondria, whereas in the latter, the Piru Dry Gap is clearly the more significant barrier. Both data types unequivocally indicate that unarmored fish in Soledad

Canyon are highly distinctive and have been effectively isolated (with respect to upstream gene flow from below the Santa Clarita Gap) from other populations downstream for a substantial amount of time.

It is likely that the signal of further upstream movement of the mitochondria versus microsatellites (i.e. above the Piru Dry Gap) is a consequence of the inadvertent transplanting of lower Santa Clara River fish into Bouquet Creek. Although fish movement from Bouquet Creek into the main channel is currently unlikely due to stream channel alteration at the base of the canyon, it is entirely plausible that stickleback were swept down from the upper creek reaches during high flow episodes some time after the initial transplantation took place, and before the amount of stream flow alteration reached its current levels. This would explain the presence of a lower Santa Clara River mtDNA haplotype above the Piru Gap, yet is consistent with all other evidence that these fish are incapable of natural upstream movement across the Piru Gap. It also explains the absence of lower Santa Clara River mtDNA haplotypes in Soledad Canyon, given that stickleback would be incapable of transporting those haplotypes upriver across the Santa Clarita Gap.

The regional structuring of differentiated mtDNA haplotypes within the Santa Clara River, in conjunction with the spatial subdivisioning of microsatellite genotypes, clearly shows that two divergent lineages persist within this drainage, and that the more inland, unarmored populations in Soledad Canyon constitute the more divergent of the two lineages (Fig. 4). The 458-bp fragment of the control region is approximately 2.0 % divergent between Soledad Canyon and the lower Santa Clara, and the 1022 bp fragment of the cytochrome *b* gene is about 1.0 % divergent; when the two genes are concatenated (1480 bp), the divergence is approximately 1.2 %. This is quite remarkable given that the same genes sequenced for eight marine and 41 freshwater stickleback populations across Europe showed only 0.57 % divergence for both genes combined (Mäkinen and Merilä 2008). This degree of genetic differentiation among the two main phylogroups within the Santa Clara River, combined with their spatial exclusion on either side of the Piru Gap, suggests that they are remnants of separate dispersal events into the Santa Clara River watershed, with Soledad Canyon unarmored fish representing the earlier of the two invasions.

## A signal of selection in unarmored populations

By partitioning our sampling locations according to morph identity and performing different tests of selection on the STN34 locus, our data displayed patterns expected for a genomic region under natural selection. STN34 is known to be under stabilizing selection in Fennoscanadian freshwater and marine stickleback populations and is physically linked to a ubiquitin-conjugating enzyme (Mäkinen et al. 2006). Although the general function of ubiquitin-conjugating enzymes is known, their significance with regard to the absence of body armor in this system is not known. The obvious implication is that allele polymorphism at STN34 is somehow associated with this condition, but no studies investigating the genetic underpinnings of plate loss to date have shown such an association, nor have any specifically focused on the evolutionary transition between low- and unplated morphs. Alternatively, variation at STN34 could be signaling the influence of selection on some other trait unrelated to armor development.

Although our results are encouraging, additional comparative studies on separate unarmored stickleback populations (i.e. different drainages; see Buth and Haglund 1994) are necessary before any firm conclusions regarding selection at STN34 can be made. We are currently conducting such a study across all extant populations recognized as *G. a. williamsoni* in southern California. If the same allelic variant is detected at high frequency across these geographically isolated populations, support for a link between STN34 polymorphism and the absence of body armor is gained, especially if unarmored populations have evolved independently among the various low-plate lineages in southern California.

## Conservation implications

The restricted distribution of unarmored stickleback in the upper Santa Clara reaches, lack of genetic variation, and ongoing anthropogenic disturbance of their natural evolutionary ecology all indicate that these fish are at high risk of extinction. Since the Soledad Canyon samples were collected for this study in 2008, repeated surveys in 2009, 2010 and early 2011 failed to recover any unarmored stickleback in parts of the canyon where they were abundant in previous years (C. Dellith and T. Hovey, pers. obs.); however, areas with suitable habitat were left unexplored due to land access issues, so it is possible that unarmored fish still persist in the canyon. Neither the Thousand Trails nor Robin's Nest sites have been surveyed since January 14th 2011, and an unusually large amount of rainfall due to an El Niño-Southern Oscillation in the Pacific (14.9-cm in December 2010 for the Santa Clarita area, and approximately 11.1-cm to date for 2011) may have left these populations severely depleted or locally extirpated.

The higher overall genetic diversity of the lower Santa Clara and Ventura River stickleback populations likely reflects a decreased susceptibility to bottlenecks relative to those in the upper Santa Clara watershed because of location—diversity is more easily maintained because of at

least occasional gene exchange between the lower Santa Clara and Ventura River populations, and the greater potential for influx of nonresident anadromous alleles. In contrast, upper Santa Clara River populations are more vulnerable to demographic collapse because of the effects of storm run-off over steeper elevational gradients (Cannon et al. 2011; Parise and Cannon 2012), increased isolation due to dry flood plains and other habitat features, limited ability to disperse upstream, and the fact that populations are not as easily replenished following major storm episodes or fire-flood sequences. In fact, the low genetic diversity at most loci in upper Santa Clara River stickleback is consistent with the signature of a sustained bottleneck. If a large population size cannot be maintained within Soledad Canyon, genetic diversity augmentation and or population re-establishment through translocation (e.g. from San Felipe Creek) may be the only way to prevent the extinction of unarmored stickleback from remaining areas nearest to the type locality.

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