Reverse Evolution of Armor Plates in Threespine Stickleback

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Running title: Reverse Evolution of Armor in Stickleback

Summary

Faced with sudden environmental changes, animals must either adapt to novel environments, or go extinct. Thus, studying the mechanisms underlying rapid adaptation is not only crucial for understanding natural evolutionary processes, but also for understanding human-induced evolutionary change, an increasingly important problem [1-8]. In the present study, we demonstrate that the frequency of completely-plated threespine stickleback fish (*Gasterosteus aculeatus*) has increased in an urban freshwater lake (Lake Washington, Seattle, Washington) within the last 40 years. This is a dramatic example of "reverse evolution" [9] because the general evolutionary trajectory is towards armor plate reduction in freshwater sticklebacks [10]. Based on our genetic studies and simulations, we propose that the most likely cause of reverse evolution is increased selection for the completely-plated morph, which we suggest may result from higher levels of trout predation after a sudden increase in water transparency during the early 1970s. Rapid evolution was facilitated by the existence of standing allelic variation in Ectodysplasin (*Eda*), the gene that underlies the major plate morph locus [11]. The Lake Washington stickleback thus provides a novel example of reverse evolution, likely caused by a change in allele frequency at the major plate locus in response to a changing predation regime.

Results and Discussion

Reverse Evolution of Armor Plates in Lake Washington

The threespine stickleback (*Gasterosteus aculeatus*) is a good model system for elucidating the ecological and genetic mechanisms underlying phenotypic evolution [12-13]. One dramatic and prevalent phenotypic change in these fish is the reduction of armor plates covering the lateral body surface that occurred repeatedly after freshwater colonization 12,000 years ago [10]. While ancestral marine sticklebacks typically have a continuous row of lateral plates (completely-plated morph), freshwater sticklebacks usually have a reduction in lateral plates, resulting in a gap in the middle part of the plate row (partially-plated morph) or loss of both the middle and posterior plates (low-plated morph). The major gene responsible for reduction of the stickleback lateral plates across the world is Ectodysplasin (*Eda*) [11]. There are two major alleles of *Eda* found in stickleback populations, here referred to as the complete allele and the low allele. Most marine sticklebacks are homozygous for the complete allele, although marine sticklebacks that are heterozygous carriers of the low allele are found at a low frequency [11]. It is proposed that when marine sticklebacks colonize freshwater environments, strong selection results in an increase in the frequency of the low *Eda* allele, leading to the prevalence of low-plated fish in freshwater.

In contrast to the prevalence of the low-plated morph in many freshwater environments [10, 11], we found a high frequency of completely-plated stickleback in Lake Washington, an urban freshwater lake in Seattle [14-16]. In 2005, we found all three lateral plate morphs were present, with 49% complete morphs, 35% partial morphs, and 16% low morphs (Figures 1A and 2C). Although a previous study had also shown that all three morphs were present in Lake Washington in 1968/1969, only 6% were classified as complete morphs (Figure 1A; [17]). Instead, the low morph, with a mode of seven plates, was the most common morph until the late 1960s (Figures 1B and C). In 1976, bimodal peaks appeared, one corresponding to fish with seven plates and another corresponding to fish with 32 plates (Figure 1C; [18]). The frequency of fish with 33 plates was even higher in the 2005 sample (Figure 1C). The increase in completely plated fish in the 2005 sample did not reflect bias in the sampling methods ($n = 322$, $\chi^2 = 6.6949$, $df = 4$, $p = 0.1529$) or the seasonal (Figure S1) or geographical (Figure 2C) distribution of differently plated sticklebacks. These data demonstrate that the frequency of plate morph phenotypes has changed dramatically in Lake Washington within the past 40 years, which is equivalent to 40 generations in this stickleback population [18].

Genotyping of the 2005 samples at the *Eda* locus revealed a strong association between plate phenotype and *Eda* genotype in Lake Washington ($n = 196$, $\chi^2 = 227.0$, d.f. = 4, p

$< 10^{-47}$; Figure S2; Table S1). By ANOVA, the *Eda* genotype explains 75.2% of the variance in plate number in the Lake Washington stickleback. This is close to the percent of phenotypic variance in plate number explained by the *Eda* locus in laboratory crosses (76.9 %; [19]). Thus, the increase in the completely plated phenotype in Lake Washington is likely due to an increase in the frequency of the *Eda* complete allele, given the previously established link between plate phenotype and *Eda* genotype in stickleback populations across the world [11].

Gene Flow is Not the Primary Cause of Armor Plate Evolution in Lake Washington

Most marine sticklebacks in Puget Sound are completely plated (Figure 2C), with high frequencies of the complete *Eda* allele (Figure 2D). Because marine sticklebacks can now migrate into the lake through the Lake Washington Ship Canal (Figures 2B and S3), which was built in 1917 [14], an increase in migration may have contributed to the increase of lateral plates in the Lake Washington stickleback. In order to test this hypothesis, we collected sticklebacks in neighboring marine environments (Puget Sound), multiple points in Lake Washington, and in neighboring streams (Figure 2) and genotyped them with 15 microsatellite markers (Table S2). Genetic data were then analyzed with the Bayesian clustering software STRUCTURE [20]. Within the marine, lake, and stream fish that were genotyped, the most probable number of

genetic clusters (*K*) was three (Figure S4). Estimation of ancestry for each individual revealed that the stickleback in Lake Washington have two main genetic sources (Figure 2E). Stickleback sampled from near the ship canal were genetically similar to marine stickleback (indicated by green in Figure 2E), while those sampled close to the streams were more similar to neighboring stream stickleback (indicated by blue in Figure 2E). However, there was no significant correlation between the probability of marine ancestry and plate number (Pearson correlation r = 0.005, p = 0.967; Figure S5). Multidimensional scaling of the genetic distance matrix also confirmed the lack of association between genotypes at neutral loci and plate number (Figure S6). Thus, the increase in armor plates in Lake Washington does not result simply from the presence of marine sticklebacks in the lake.

It may be still possible that an increase in long-term migration from Puget Sound has contributed to the overall increase in the complete morph in the lake. To test this possibility, we first estimated migration rates (*m*; fraction of migrants per generation) from the genetic data and then examined whether the empirically estimated *m* can explain the observed plate evolution. We used both Isolation with Migration (IM) and LAMARC software [21-23] to estimate the *m* between a Lake Washington population and a Puget Sound marine population (Table S3). The *m* from Puget Sound into Lake Washington was estimated as 3.03×10^{-4} (IM) or 1.77×10^{-3}

(LAMARC), while the *m* from Lake Washington into Puget Sound was estimated as 6.43×10^{-4} (IM) or 1.20×10^{-3} (LAMARC). Then, we developed deterministic numerical simulations to calculate the *m* required for the observed change of plate phenotype under different selection regimes (Figure S7). In the absence of selection ($s = 0$), migration would need to be 0.148 to explain the observed change from 1969 to 1976 and 0.035 to explain the change from 1969 to 2005. These values are inconsistent with our low ($m < 10^{-3}$) migration rate estimates, suggesting that there was a period of selection that favored the complete morph in Lake Washington.

Changes in Selection Regime in Lake Washington

By using the empirically estimated values of *m*, we found that a selection coefficient *s* (strength of selection for the complete morph) of 0.58-0.72 (Table 1) can explain the evolutionary shift from 1969 to 1976 (from 6% complete morphs to 40.2% complete morphs; Figure 1A). This suggests that the complete morph had 58-72 % higher fitness than the low morph during this period. To explain the transition between 1976 and 2005 (from 40.2% complete morphs to 49% complete morphs), *s* of 0.01-0.03 is required (Table 1). We thus conclude that there was a period of very intense selection for the complete morph between 1970 and 1976, followed by a persistent low level fitness advantage (1-3 %) of the complete morph over the low morph.

One of the dramatic ecological changes that occurred in Lake Washington during the early 1970s is increased water transparency due to the mitigation of eutrophication in the late 1960s. Water transparency in the lake was 1-2 m Secchi depth (the maximum depth at which a white Secchi disk is visible from the water surface) during 1955-1971, and increased to 3.4 m in 1973 and then to 6-7 m from 1976 to the present [14, 15]. Previous behavioral experiments have demonstrated that an increase in water transparency significantly increases the reaction distance of visual predators to their prey, thus leading to increased predation pressure on prey fish [24]. Cutthroat trout (*Oncorhynchus clarki*) are visual predators, extremely sensitive to subtle changes in water transparency [24], and the primary predator of threespine stickleback in both the littoral and pelagic zones of Lake Washington [16, 25, 26]. Therefore, we used a visual foraging model, which calculates the search volume by cutthroat trout as a function of light intensity and turbidity [27, 28], to investigate a possible change in stickleback predation regime. This analysis demonstrated that the increase in lake transparency created an 8-fold increase in the visual search volume by cutthroat trout and also expanded the depth range over which effective visual foraging could occur (Figure 3). Most of the expanded search volume was achieved during 1972-1975, when the mean Secchi disk transparency increased to 3.4 m. Although the cutthroat trout population in Lake Washington did not increase between 1971 and 2006 (Figure S8), our model

suggests that an increase in lake transparency could have changed the predation regime by increasing encounter rates between stickleback and cutthroat trout.

Predation by toothed predators, such as cutthroat trout, is thought to favor completely-plated stickleback because the posterior lateral plates can protect the stickleback from being injured and swallowed [29, 30]. Reimchen predicted that the complete morph would occur in open water habitats of high clarity where capture rather than pursuit defenses predominate [29]. Consistent with this hypothesis, we have shown that the increase in the frequency of complete morphs occurred during the time when the water clarity increased dramatically in Lake Washington, a relatively deep and large lake (a surface area of 8.76×10^7 m^2 and a maximum depth of 65.2 m). Further supporting the hypothesis that an increase in predation by cutthroat trout has contributed to the rapid evolution of Lake Washington stickleback, recent stickleback samples are larger than historical stickleback samples (Figure 1B; Table S4). Larger body size can protect against predation by gape-limited predators, such as cutthroat trout [31, 32]. Although salinity and water temperature have also been proposed as factors contributing to lateral plate evolution [33, 34], we can exclude a role for these abiotic factors in the evolution of Lake Washington sticklebacks (Supplemental Discussion).

Conclusions

We have reported a dramatic example of "reverse evolution" [9], in which there has been an increase in completely-plated sticklebacks in a freshwater lake. Our data demonstrate that selection for the complete morph was particularly strong during the early 1970s, suggesting that the main increase in the frequency of completely plated fish may have occurred in less than a decade. Armor reduction has also been shown to occur within only a few decades after the introduction of marine sticklebacks into freshwater [35-37]. Thus, sticklebacks can respond to environmental changes by either an increase or a decrease in lateral plates within a few decades. Rapid phenotypic evolution in stickleback provides us a great opportunity to further investigate the mechanisms by which animals can respond to rapidly changing environments [38].

The rapid evolution of armor plates in Lake Washington sticklebacks may have been enabled by the presence of standing genetic variation at the major plate locus [11]. Without standing variation, a sudden increase in predation may have led to population extinction before a new mutation appeared [6, 39]. Although an increase in gene flow was not the primary cause of armor evolution, gene flow from the marine population may have enabled rapid armor evolution by contributing to standing genetic variation within the lake [7, 40]. This work provides an example of a rapid phenotypic change that does not result from phenotypic plasticity, which has

been proposed as a major mechanism of contemporary evolution [4, 8]. Thus, investigating the genetic mechanisms that underlie adaptive phenotypes is essential for a better understanding of rapid evolutionary change [2, 4, 8, 41].

Although we suggest that reverse evolution in the Lake Washington stickleback is likely attributable to the change in water clarity, we still lack direct evidence for this hypothesis and additional factors may have also contributed. As in many other cases of rapid evolution [5], it is often difficult to tease apart all the potential factors that contribute to phenotypic evolution. We demonstrated, however, that changes in water clarity are able to influence predator-prey interactions; further attention should be given to the influence of water clarity on predator-prey interactions and animal evolution. In addition, this work highlights the importance of investigating the relationships between environmental changes, species interactions, and the genetic basis of phenotypic evolution, both to better understand the mechanisms of animal evolution and inform conservation efforts.

Supplemental Data

Detailed experimental procedures, a supplemental discussion, eight figures and five tables are provided in the Supplemental Data.

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Figure Legends

Figure 1. Lateral Plate Evolution in Lake Washington Stickleback

(A) Temporal change in the frequency of the completely (black bar), partially (gray bar), and low (white bar) plated morphs in Lake Washington stickleback. Sample sizes are shown above the graph. Right panels show representative images of the three morphs in stickleback. Skeletal structures are visualized by alizarin red staining. Scale bars = 10 mm.

(B) Representative images of stickleback collected in March 1957 and March 2006 in the northern pelagic zone of Lake Washington by midwater trawling.

(C) Histograms of lateral plate number for stickleback collected in 1957, 1968/1969, 1976, and 2005. Sample sizes are the same as in Figure 1A. The most common plate number is also shown in each panel as a mode. Among stickleback collected in 1968/1969, 22% had more than 12 plates, but the individual plate counts for each fish are not available [17]. Plate number was counted from the left side of the fish except in the 1976 sample, for which only right plate number data were available [18]. For the 1957 data, museum specimens in the University of Washington Fish Collection were analyzed. The frequency of morph was significantly different between successive sampling time points (χ^2- test; $p < 0.05$) except between 1957 and 1968/1969 samples ($\chi^2 = 2.375$, $p = 0.305$)

Figure 2. Genetic and Morphological Variation around Lake Washington

(A) Map of Washington State. Blue dots indicate the collection sites of marine stickleback from Puget Sound. Lake Washington is highlighted by a square and magnified in Figure 2B.

(B) Map of Lake Washington and neighboring streams. Numbers indicate the sampling sites in Lake Washington: Point 1, Union Bay; Point 2, northern pelagic zone (Area 1 in [17]); Point 3, Matthews Beach; Point 4, Tracy Owen Park; Point 5, Juanita Beach; Point 6, Yarrow Bay; Point 7, Mercer Slough; Point 8, east channel; Point 9, northern pelagic zone (Area 2 in [17]).

(C) Variation in plate morph frequencies among populations. Each column indicates the frequency of the complete (black bar), partial (gray bar), and low (white bar) morph stickleback for each population. Numbers in parentheses indicate sample size. The frequency of different morphs was not significantly different among different points within Lake Washington ($n = 322$, $\chi^2 = 17.0$, d.f. = 14, $p = 0.255$).

(D) Variation in the allele frequency of *Eda* among populations. The black bar indicates the frequency of the complete *Eda* allele and the white bar indicates the frequency of the low *Eda* allele at *Stn382*. Numbers in parentheses indicate sample size.

(E) Genetic structure of sticklebacks collected in Lake Washington, Puget Sound and neighboring streams. The three different genetic clusters are shown in different colors. Each

individual is represented by a thin column that is partitioned into colored segments indicating the estimated proportion of ancestry from each cluster. Numbers in parentheses indicate sample size.

Figure 3. Changes in Cutthroat Trout Foraging Volume in Lake Washington.

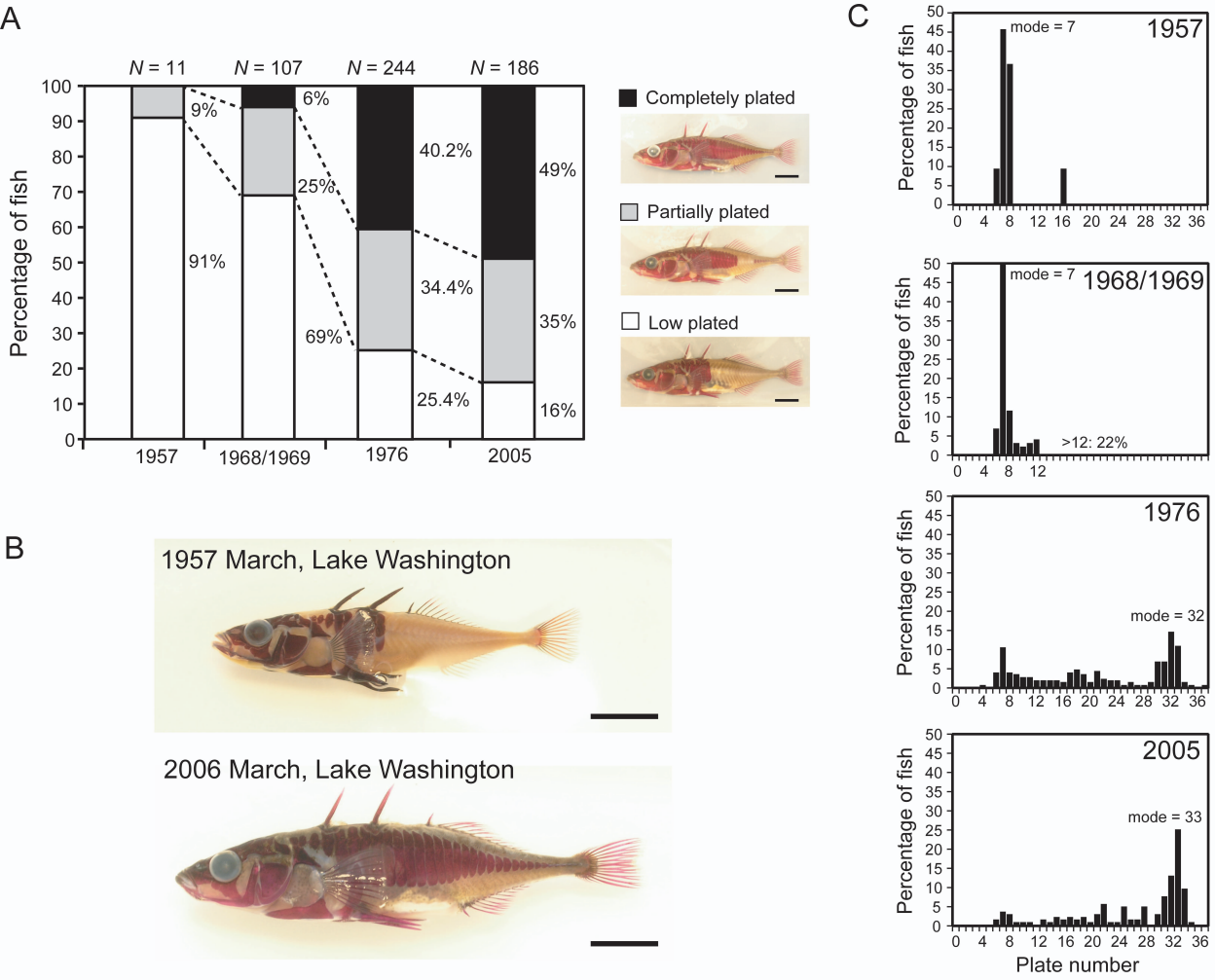
(A-C) Depth-specific search volumes of cutthroat trout are shown during peak eutrophication in 1955-1971 (A), initial recovery during 1972-1975 (B), and the current transparency regime 1976-2006 (C). Different diel periods are indicated with white bars for day, hatched bars for dusk, and black bars for night.

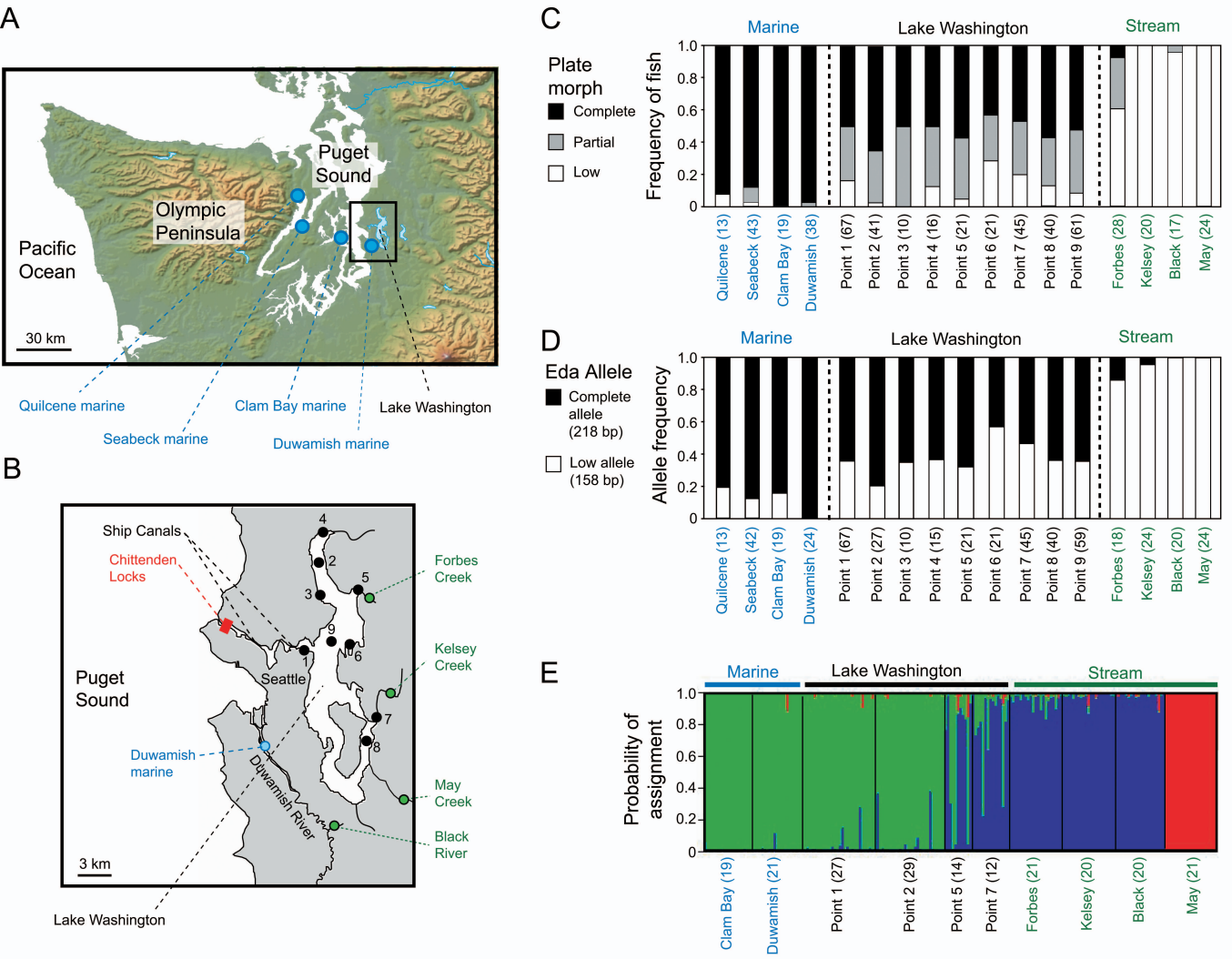
(D) Changes in the search volume by trout are shown as the percentage of the current search volume. Black and white circles indicate the search volumes at the depths of 0-60 m and 10-20 m, respectively.

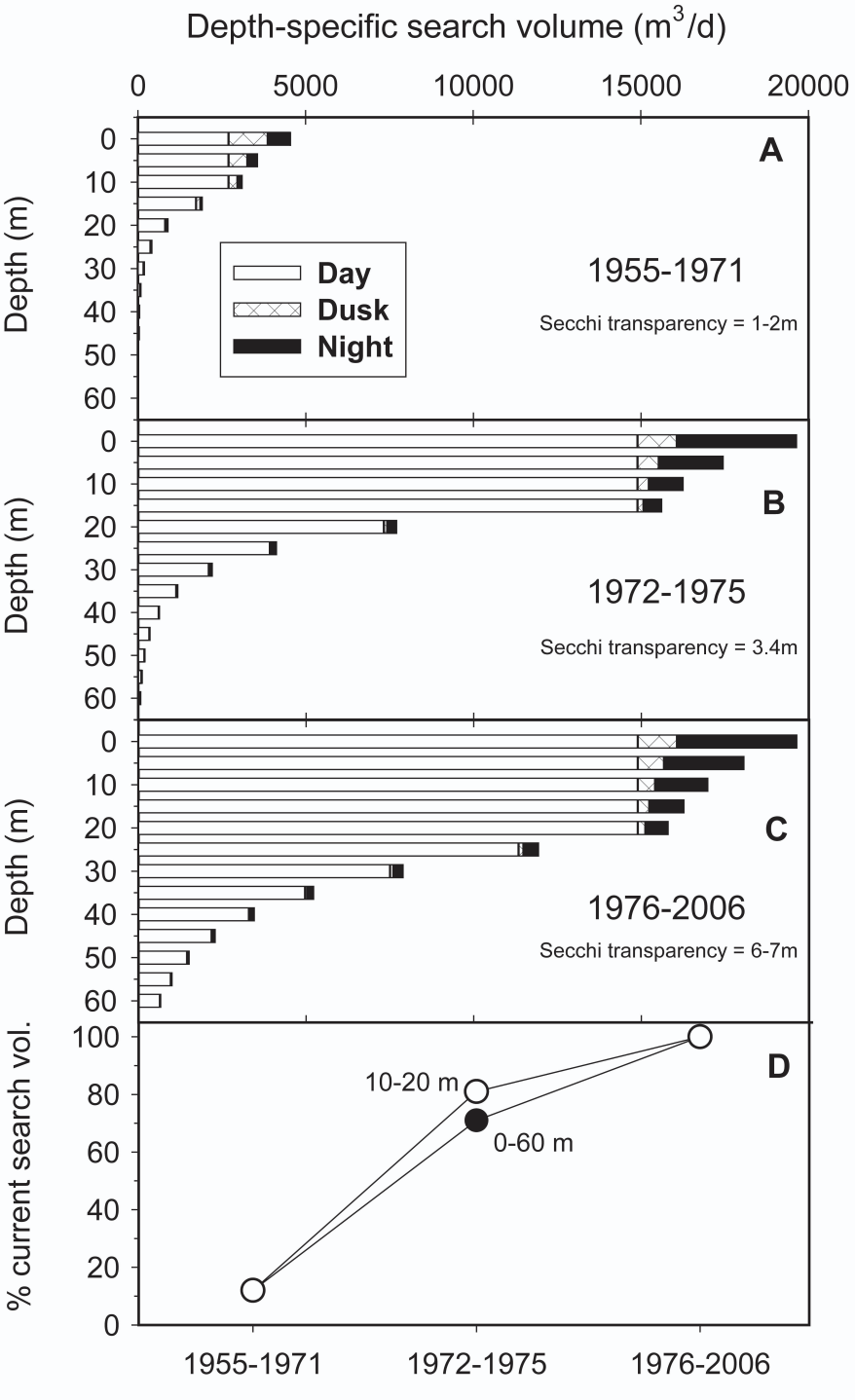
Table 1. Estimation of Strength of Selection (s) for the Complete Plate Morph.

Time period	Dominance of plate morph fitness		
	$h = 0$	$h = 0.5$	$h = 1$
1969-1976	s = 0.708/0.720	s = 0.597/0.606	s = 0.582/0.591
1976-2005	s = 0.013/0.015	s = 0.017/0.020	s = 0.026/0.031

Values of s during different time periods were calculated for different values of the dominance of plate morph fitness (h). Migration rates estimated by LAMARC (left side in each cell) and IM (right side in each cell) were used. Frequencies of 6%, 40.2%, and 49.0% complete plate morphs were used for 1969, 1976, and 2005.


A
N = 11
N = 107
N = 244
N = 186
Percentage of fish
100
90
80
70
60
50
40
30
20
10
0
9%
91%
6%
25%
69%
40.2%
34.4%
25.4%
49%
35%
16%
1957
1968/1969
1976
2005
Completely plated
Partially plated
Low plated
B
1957 March, Lake Washington
2006 March, Lake Washington
C
Percentage of fish
mode = 7
1957
mode = 7
1968/1969
>12: 22%
1976
mode = 32
2005
mode = 33
0 4 8 12 16 20 24 28 32 36
Plate number


A
Puget Sound
Olympic Peninsula
Pacific Ocean
30 km
Quilcene marine
Seabeck marine
Clam Bay marine
Duwamish marine
Lake Washington
B
Ship Canals
Chittenden Locks
Puget Sound
Seattle
Duwamish marine
Duwamish River
Forbes Creek
Kelsey Creek
May Creek
Black River
3 km
Lake Washington
C
Marine
Lake Washington
Stream
Plate morph
Complete
Partial
Low
Frequency of fish
Quilcene (13)
Seabeck (43)
Clam Bay (19)
Duwamish (38)
Point 1 (67)
Point 2 (41)
Point 3 (10)
Point 4 (16)
Point 5 (21)
Point 6 (21)
Point 7 (45)
Point 8 (40)
Point 9 (61)
Forbes (28)
Kelsey (20)
Black (17)
May (24)
D
Eda Allele
Complete allele (218 bp)
Low allele (158 bp)
Allele frequency
Quilcene (13)
Seabeck (42)
Clam Bay (19)
Duwamish (24)
Point 1 (67)
Point 2 (27)
Point 3 (10)
Point 4 (15)
Point 5 (21)
Point 6 (21)
Point 7 (45)
Point 8 (40)
Point 9 (59)
Forbes (18)
Kelsey (24)
Black (20)
May (24)
E
Probability of assignment
Clam Bay (19)
Duwamish (21)
Point 1 (27)
Point 2 (29)
Point 5 (14)
Point 7 (12)
Forbes (21)
Kelsey (20)
Black (20)
May (21)


Depth-specific search volume (m3/d)
0
5000
10000
15000
20000
Depth (m)
A
Day
Dusk
Night
1955-1971
Secchi transparency = 1-2m
B
1972-1975
Secchi transparency = 3.4m
C
1976-2006
Secchi transparency = 6-7m
% current search vol.
D
10-20 m
0-60 m
1955-1971
1972-1975
1976-2006

Supplemental Data

Experimental Procedures

Morphological Analysis

All 2005-2007 samples were collected between March and June by minnow trap and mid-water trawling, except for samples from Points 8 and 9 (Figure 2B), which were collected by purse seining in October 2005 and mid-water trawling in September 2006, respectively. The 1957 sample was collected by Dr. William Aron in the northern pelagic zone of the lake in March by mid-water trawling (University of Washington fish collection No. UW 012691). Plate numbers for the 1968/1969 and 1976 samples were taken from [S1] and [S2], respectively. The 1968/1969 sample was collected at Points 4-6 (Figure 2B) between April and June by beach seining [S1]. The 1976 sample was collected from the southern end of the lake throughout the year by beach seining and dip nets [S2]. For the 1957 museum samples and the 2005-2007 samples, plate number was counted under a dissecting microscope from the left side of the fish stained with alizarin red as previously described [S3]. All fish analyzed were larger than 32mm standard length, at which point plate development is complete [S4]. Fish were assigned to one of three plate morph categories (complete, partial, low) as described previously [S1].

Genetic Analysis

DNA isolation and microsatellite analyses were conducted as previously described [S3], except that the reactions were analyzed on an ABI 3100 (Applied Biosystems, Foster City CA) and visualized with ABI GeneMapper 3.7 (Applied Biosystems, Foster City CA). For genotyping the *Eda* locus, fish were genotyped with the *Stn382* microsatellite marker located in the first intron of the *Eda* gene [S5]: the complete and low alleles gave rise to 218 bp and 158 bp bands, respectively. For population genetic studies, fish were genotyped with 15 microsatellite markers chosen using a random number table. These markers are on 14 different linkage groups (LG) and have allele size distributions consistent with the stepwise mutation model (Table S2). These data were first analyzed with STRUCTURE [S6], which uses Markov chain Monte Carlo simulations to find groupings that minimize Hardy-Weinberg and linkage disequilibrium within cluster groups. Since *Stn46* and *Stn238* are both on LG4, linkage information was included in the input file for STRUCTURE analysis, although no significant linkages were indicated between any markers within each sampling site (exact test [S7], $p > 0.05$, after Bonferroni correction). We estimated the number of clusters in the data by running three simulations for each K value from $K = 1$ through $K = 10$ to calculate the mean log probability of data ($L(K)$), and using the *ad hoc* statistic ΔK, which is based on the rate of change in $L(K)$ between successive K values [S8, S9].

Parameters were estimated after 200,000 iterations, following a burn-in of 25,000 iterations.

For estimation of migration rates, we analyzed the genetic data from the Duwamish marine (n = 21) and Lake Washington (Point 1; n = 27) sticklebacks with both IM and LAMARC software [S10-S12]. Using all Lake Washington stickleback populations for the LAMARC estimation gave rise to similar results (not shown). For calculation of m and N_e, we used a mutation rate of 10^{-4}, as previously described [S13]. We ran LAMARC with 10 short chains of 1,000 samples and 2 long chains of 20,000 samples. IM results were based on recording of 30,000,000 steps after 100,000 of burn-in. In both IM and LAMARC, multiple runs gave rise to similar results.

The pairwise genetic distance (Dps) between individuals from Lake Washington, Puget Sound, and nearby streams was calculated from the proportion of shared alleles using MSA software [S14]. Then, the genetic distance matrix was subject to principal coordinate (PCo) analysis using the R statistical package. The correlations between the first and second PCo and plate number were tested by a Pearson correlation test.

Estimating the Strength of Selection

We first used the simplifying assumption that plate morph was strictly determined by the *Eda*

locus, such that complete morphs had genotype 218 bp/218 bp; partial morphs were 218 bp/158 bp, and low morphs were 158 bp/158 bp. Given the initial frequency of low morphs in 1968/1969, $f(L) = 0.7$, we estimated the initial frequency of allele 158 to be $q = 0.837$. This was used to calculate the frequency of complete and partial morphs assuming Hardy-Weinberg equilibrium. Given initial frequencies of the three morphs, $f(C)$, $f(P)$, and $f(L)$, we next calculated the frequencies of these morphs following selection:

$$f(C)' = \frac{f(C)(1+s)}{\overline{w}}$$
$$f(P)' = \frac{f(P)(1+hs)}{\overline{w}}$$
$$f(L)' = \frac{f(L)}{\overline{w}}$$

where s is the strength of selection for or against complete plate morphs, h determines the dominance of plate morph fitness, and $\overline{w} = 1 + sf(C) + hsf(P)$. Assuming selection acts on stickleback armor phenotypes throughout development, followed by immigration of adults prior to breeding, we then calculated the effects of migration following selection:

$$f(C)'' = (1-m)f(C)' + mf(C_{mar})$$
$$f(P)'' = (1-m)f(P)' + mf(P_{mar})$$
$$f(L)'' = (1-m)f(L)' + mf(L_{mar})$$

where m is the fraction of individuals in Lake Washington who immigrated from marine populations following a round of selection, and $f(C_{mar})$ is the frequency of complete plate morphs in the marine population. We assumed that the marine population is large relative to the Lake

Washington population, so reverse migration does not appreciably affect marine population frequencies and we can adopt a continent-island model. Following a round of selection and migration, we calculated new allele frequencies, and determined the morph frequencies assuming random mating and Hardy-Weinberg equilibrium. Numerical iterations led to estimates of the final morph frequencies after a specified period of time, for a given starting morph frequency. We then iteratively used a large number of parameter combinations (*s*, *h*, and *m*) to determine the *s* necessary for a given observed change assuming a particular heritability and migration rate, or the *m* required for the same change assuming no selection.

Visual Foraging Model

We used a visual foraging model [S15-S17] for predatory cutthroat trout feeding in the pelagic regions of the lake during thermally-stratified periods when most predation on fish was measured [S18]. The model estimated search volumes *SV* (m^3) at any depth during any diel period, based on changes in reaction distance *RD* (m) of predators to forage fishes as functions of light intensity *I* (Lux) and turbidity *T* (NTU), swimming speed *SS* (m/hr), and the duration *D* (hr) of each diel period (Table S5):

$$SV = RD^2 \cdot \pi \cdot SS \cdot D$$

Where for $T \leq 1$ NTU

$$RD = 0.337 \cdot I^{0.118} \ (I < 17 \text{ Lux})$$

or

$$RD = 0.5316 \ (I \geq 17 \text{ Lux}).$$

For $T > 1$ NTU

$$RD = 0.337 \cdot I^{0.118} \cdot T^{0.624} \ (I < 17 \text{ Lux})$$

or

$$RD = 0.5316 \cdot T^{0.624} \ (I \geq 17 \text{ Lux}).$$

Depth-specific search volumes were computed for each diel period at 5-m depth intervals using incident light levels I_0 and light extinction coefficients k and turbidity levels from each of the lake transparency regimes (1955-1971: Secchi depth transparency = 1-2 m; 1972-1975: Secchi depths = 3.4 m; 1976-2006: Secchi depths = 6-7 m; Table S5). Depth-specific light levels I_z were computed for each depth z (m):

$$I_z = I_0 \cdot e^{kz}.$$

Turbidity values were only available during 2002-2003 [S17], so values for earlier transparency regimes were estimated from a regression of Secchi depths (m) versus turbidity ($r^2 = 0.73$; p = 0.03; n = 6):

$T = 4.58 - 0.475 \cdot \text{Secchi}.$

Because light extinction increases with declining transparency, we also attempted to compute an average light extinction coefficient for the 1955-1971 period using a regression of Secchi depth versus k ($r^2 = 0.49$; p = 0.122; n = 6):

$k = -0.708 + 0.051 \cdot \text{Secchi};$

however, the nonsignificant regression created some uncertainty regarding the accuracy of k = -0.63 estimated for the peak eutrophication period. To examine the sensitivity of our analysis to k, we compared the search volume estimates using the regression estimate of k = -0.63 versus k = -0.35, the current high-transparency value. When using the high transparency value for k, the adjusted search volume during the 1955-1971 period only increased from 12% to 20% of the current search volume. This result implies that in the unlikely event that no change in light extinction accompanied the improved water transparency, current predation pressure might have only increased 5-fold instead of 8-fold following peak eutrophication. Thus any error in our light extinction estimate would result in a modest change in the magnitude the predation increase, but would not change the overall conclusion that predation pressure increased markedly between the 1960s and current conditions.

Sr Isotope Analysis

Sr stable isotope analysis of calcified fish tissues can tell us their migratory history, because different water bodies usually have different $^{87}Sr/^{86}Sr$ ratios [S19]. In order to investigate the presence of direct migrants from Puget Sound, threespine sticklebacks were collected in the ship canal above the Chittenden Locks in May 2007 with minnow traps and preserved in ethanol after euthanasia. Sr analysis was conducted as described previously with several modifications [S20]. Seven to 50 mg of fish vertebrae was surgically taken and dissolved in a 15mL Teflon vessel by adding 2mL of 68% ultrapure-grade HNO_3. Upon complete dissolution, the resulting solution was dried and suspended with a few drops of 1N HCl. Ten to 20 mL of water sample were filtered through a cellulose acetate filter (pore size 0.2 μm), transferred to a 30 mL Teflon vessel, and dried on a hot plate. Sr was separated and isolated using classical cation exchange chromatography using AG50WX-12 resin (Muromachi Technos, Tokyo, Japan) with distilled 2N and 6N HCl as elution solution. The concentrations of Sr isotopes were determined using a thermal ionization mass spectrometer (Triton, Thermo Fisher Sci., Yokohama, Japan) at the Research Institute for Humanity and Nature. The $^{87}Sr/^{86}Sr$ ratio of nine standard samples of NBS987 during this study was 0.710259 (2σ mean: ± 0.000005), and all measurements were

normalized to the recommended $^{87}Sr/^{86}Sr$ ratio of 0.710250.

Supplemental Discussion

Because the current salinity (0-0.1 ppt) of Lake Washington has not changed significantly over the last 50 years [S1, S21], the rapid increase in armor plates in Lake Washington stickleback cannot be attributed to changes in salinity [S22, S23]. Although the water temperature of the lake has risen, possibly due to global warming [S24], higher water temperatures are usually associated with the presence of the low plate morph [S22, S23], suggesting that a change in water temperature is not the likely cause of rapid armor evolution.

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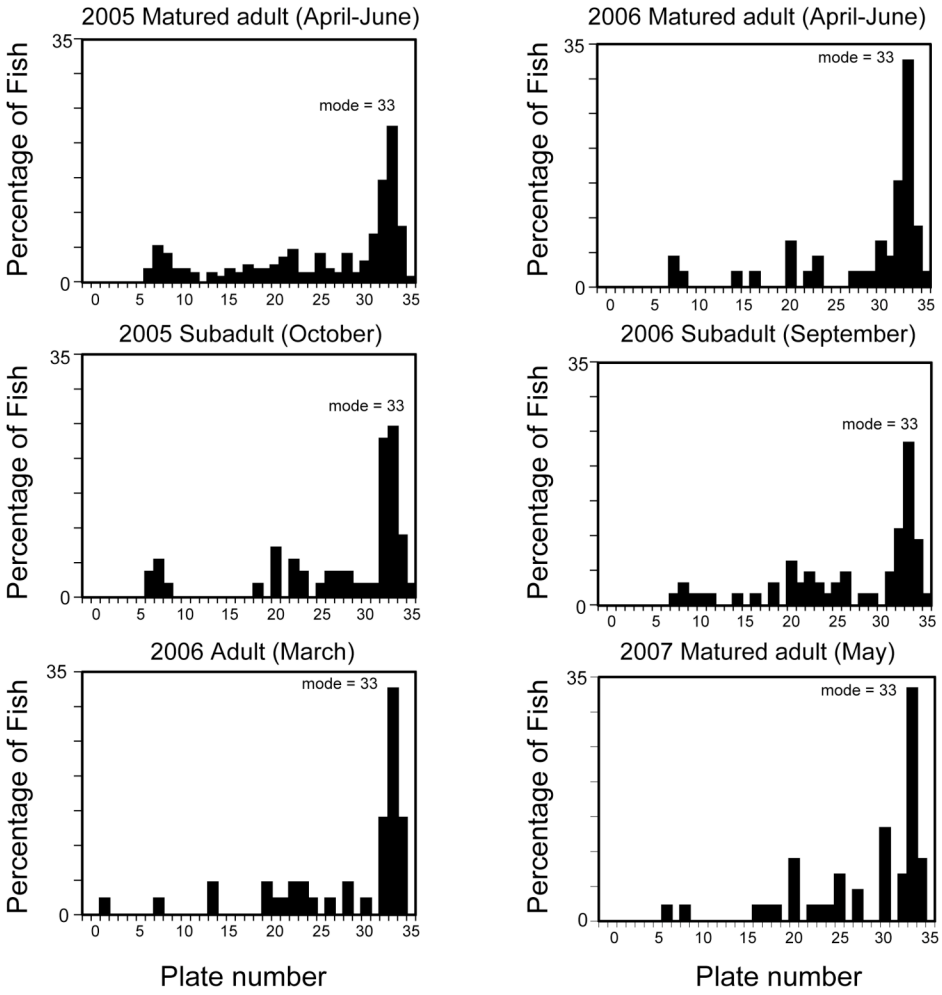

Figure S1. Seasonal Variation in Lateral Plate Phenotype

We analyzed stickleback collected in April-June 2005 from littoral zones (Points 1, 3-7; n = 186), October 2005 from the east channel (Point 8; n = 40), March 2006 from Area 1 of the northern pelagic zone (Point 2; n = 40), April-June 2006 from littoral zones (Points 1 and 5; n = 46), September 2006 from Area 2 of the northern pelagic zone (Point 9; n = 61), and May 2007 from littoral zones (Point 1; n = 45). The frequency of differently plated stickleback was not significantly different among time points (χ^2-test; n = 418, χ^2 = 15.9, d.f. = 10, p = 0.102).

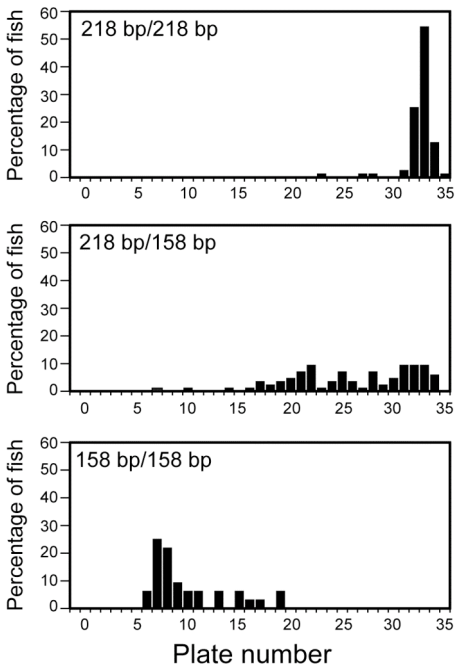

Figure S2. Association between *Eda* Genotype and Lateral Plate Number

Histograms of plate number are shown for the Lake Washington stickleback that are homozygous for the complete allele (218bp allele) (upper panel; n = 79), homozygous for the low allele (158bp allele) (lower panel; n = 32), and heterozygous for the complete allele and low allele (middle panel; n = 85), at the *Stn382* locus. Means (and 95% confidence intervals) of plate number for the 218/218, 218/158, and 158/158 genotypes are 32.58 (31.60-33.56), 26.00 (25.02-26.9), and 10.00 (8.46-11.54). Stickleback collected from Point 1 (n = 67), Point 2 (n = 27), Point 4 (n = 15), Point 5 (n = 21), Point 6 (n = 21), and Point 7 (n = 45) were analyzed.

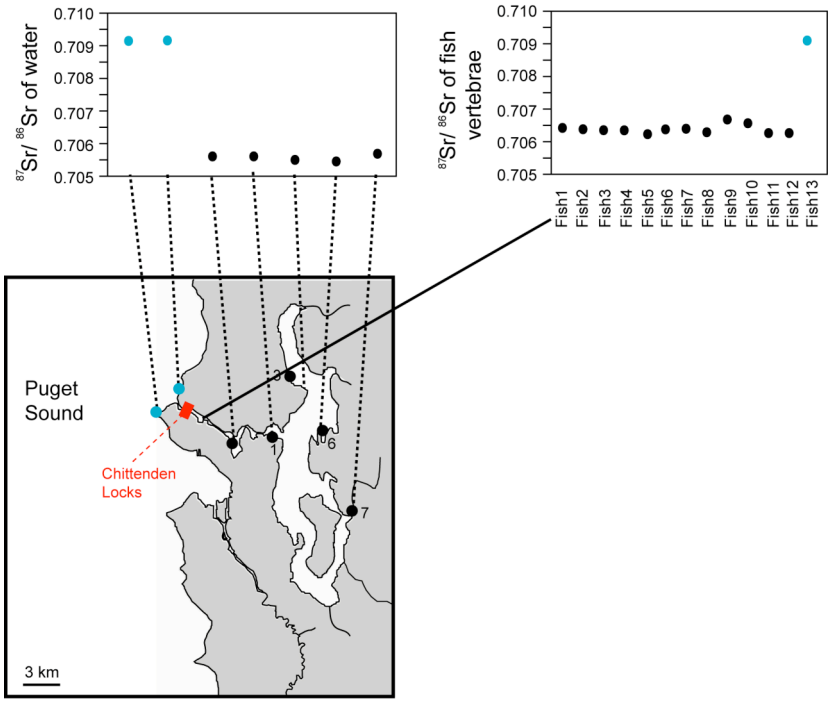

Figure S3. Identification of Migrants by Sr Isotope Analysis

Water was collected from different places and subjected to Sr isotope analysis (left panel), which revealed that the $^{87}Sr/^{86}Sr$ ratio is higher in seawater (blue circles) than lake water (black circles). Sr isotope analysis of the vertebrae of stickleback collected in the ship canal above the Chittenden Locks (right panel) revealed that one fish (blue circle) had a high $^{87}Sr/^{86}Sr$ and is likely to be a migrant from Puget Sound.

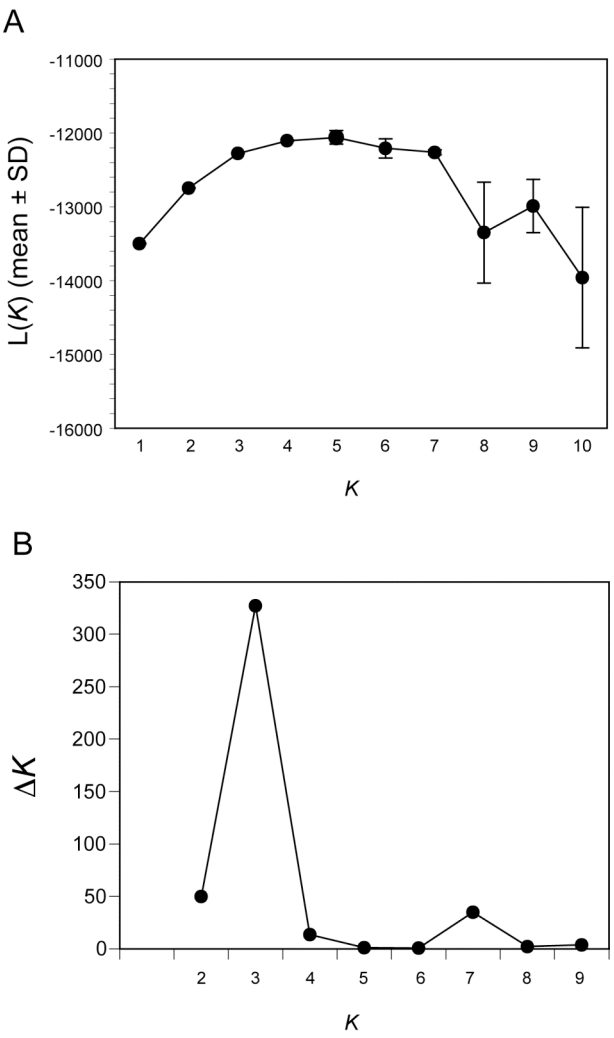

Figure S4. Estimation of Genetic Cluster Number with STRUCTURE

(A) Mean ± S.D. of the log probability of data L(*K*) for three simulations is shown for *K* = 1 through *K* =10. L(*K*) begins to plateau at *K* = 3 or 4.

(B) The *ad hoc* statistic Δ*K* has a unimodal peak at *K* = 3, suggesting that three is the most probable number of genetic clusters (*K*).

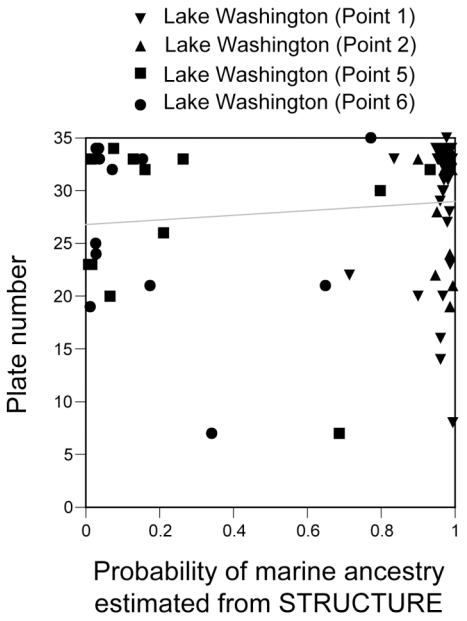

Figure S5. Lack of Correlation between Plate Number and the Probability of Marine Ancestry

The least squares linear regression line is shown.

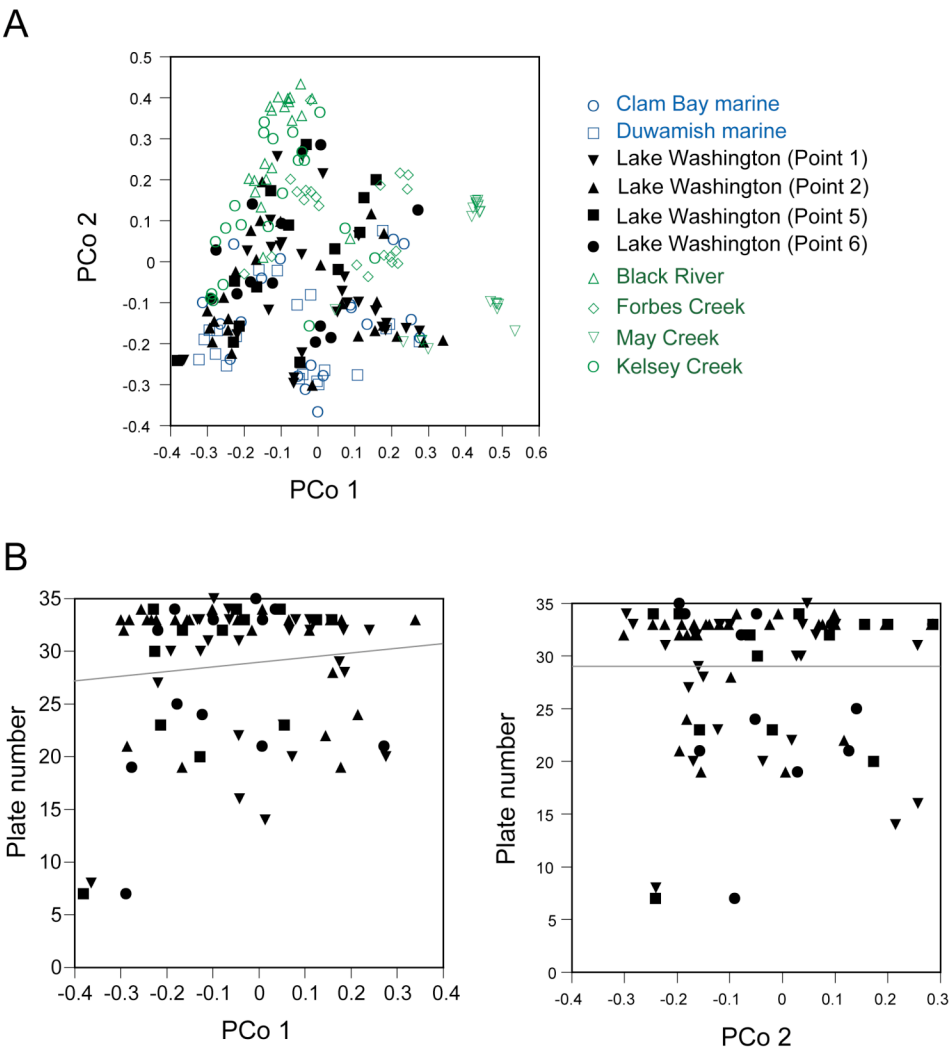

Figure S6. Multidimensional Scaling of Genetic Distance Matrix

(A) Scatterplot between the first (PCo1) and the second principal coordinate scores (PCo2) of the genetic distance matrix based on neutral microsatellite loci for Lake Washington, Puget Sound, and neighboring stream sticklebacks.

(B) Scatterplot between PCo1 and plate number (left panel) and between PCo2 and plate number (right panel) in Lake Washington sticklebacks. The least squares linear regression line is shown.

There is no significant association between plate number and genotypes at neutral microsatellite loci (Pearson correlation test): $r = 0.148$, $p = 0.193$ for PCo1; $r = 0.007$, $p = 0.950$ for PCo2.

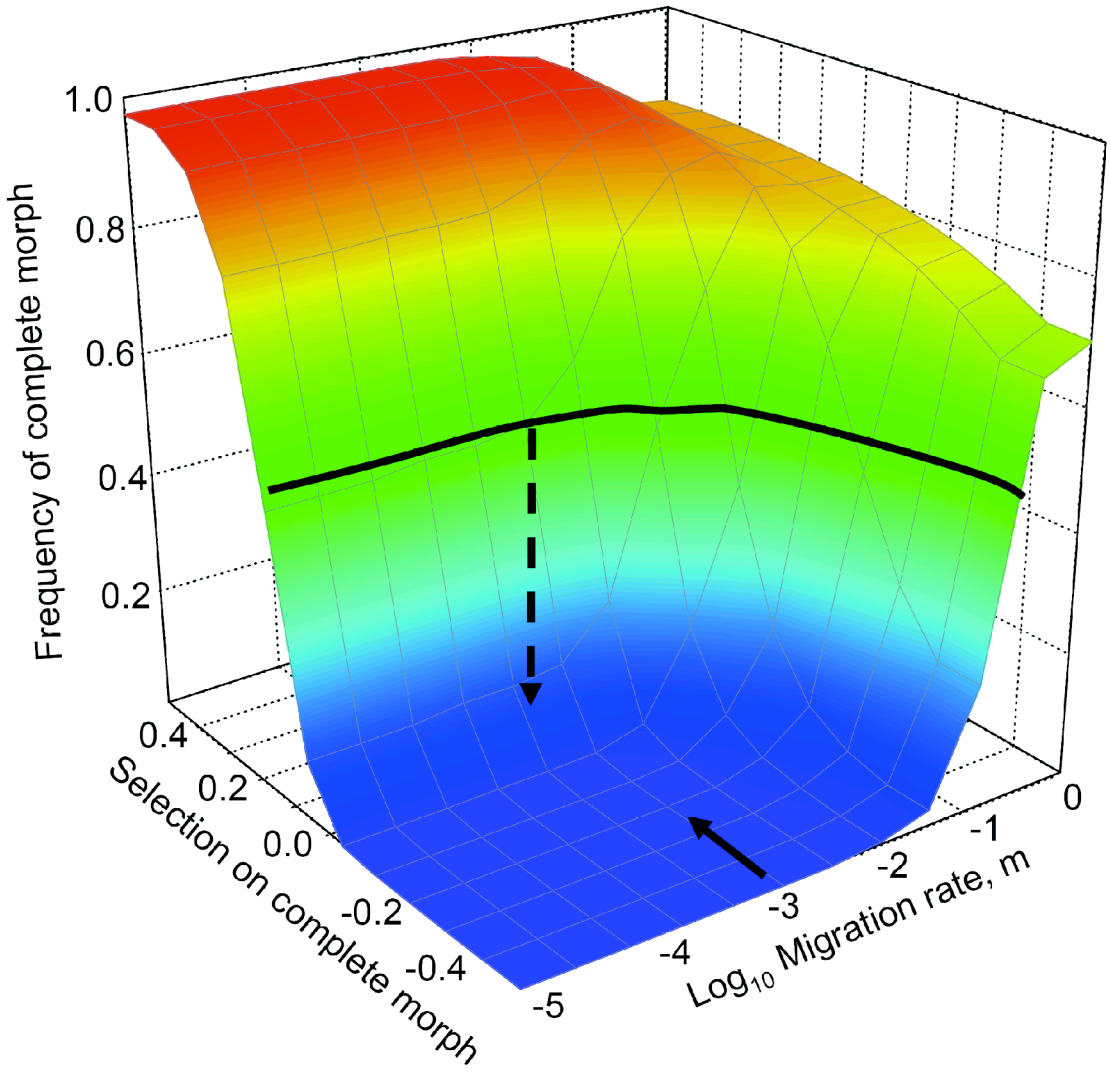

Figure S7. Contribution of Selection and Migration to Plate Evolution.

The expected frequency of completely plated morphs in Lake Washington in 2005 is plotted as a function of selection for or against the completely plated morph, and the rate of immigration from a marine population. The thick dark line represents the combination of migration rates and selection strengths that can yield the observed increase in complete morph frequency between 1970 and 2005 ($h = 0.5$). The shape of the curve changes only slightly for different heritabilities

(h = 0, 0.5, or 1), primarily in elevation of the curve surface (not shown). To illustrate the process of estimating selection strength, we show a solid arrow representing an empirical estimate of the migration rate (the LAMARC estimate of $m = 1.77 \times 10^{-3}$). One then finds the corresponding selection strength that yields the empirically observed evolutionary change (vertical dashed arrow).

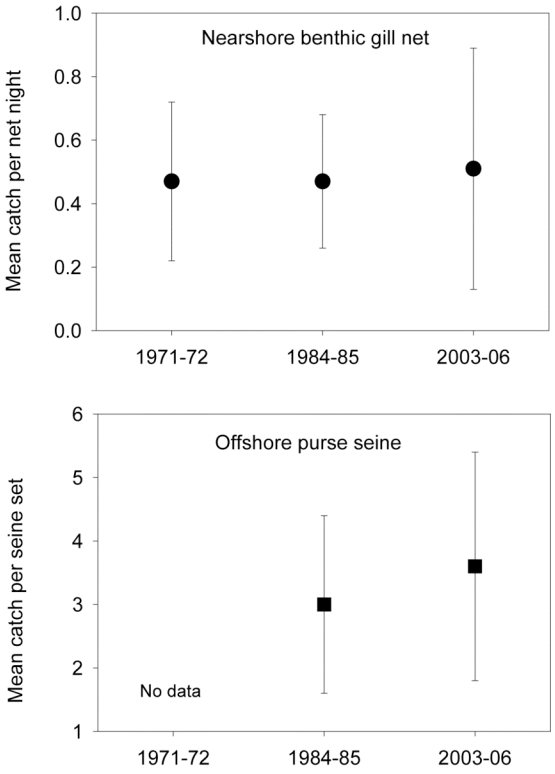

Figure S8. Cutthroat Trout Catch Rates in Lake Washington.

The mean (± 2SE) catch for all sizes of cutthroat trout per 24-hour set in benthic littoral gill nets, and the mean (± 2SE) catch of large (FL ≥ 300 mm) cutthroat trout per purse seine set has not changed in Lake Washington between 1971 and 2006.