

A few stickleback suffice to transport adaptive alleles to new lakes

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Abstract

Threespine stickleback fish provide a striking example of local adaptation despite recurrent gene flow. The species is distributed around the Northern hemisphere in both marine and freshwater habitats. It is thought that these numerous, smaller freshwater populations have been established “de novo” from marine fish, and that a shared freshwater phenotype is often established using standing genetic variation. Here we use genealogical simulations to determine the levels of gene flow that best matches observed patterns of allele sharing among habitats in stickleback, and more generally to better understand how gene flow and local adaptation in large metapopulations determine speed of adaptation and reuse of standing genetic variation. We find that rapid, repeated adaptation using a shared set of alleles maintained at low frequency by migration-selection balance occurs over a realistic range of intermediate rates of gene flow. Low gene flow leads to slow, independent adaptation of distinct habitats, whereas high gene flow leads to large migration load. We quantify how F_{ST} scans for adaptive alleles are more likely to succeed with higher rates of gene flow. In addition, we find the origin of many freshwater adapted alleles in the introduced lakes to be propagated from the original generation of marine individuals that had inhabited the lake. The results support existing theory of local adaptation, and provide a more concrete look at a particular, empirically motivated example.

Introduction

The canonical model for the genetics of adaptation, first formulated by Fisher in the early part of the 20th century, involves the sequential fixation of new mutations. While it has proven valid in numerous studies in the field as well as in the lab, this model is now rightfully understood as incomplete for many species in nature that have more complicated population structures. What role does genomic variation play in meta-populations with connection to an array of selective pressures? How could migration-selection balance and ecological speciation actually benefit species as a whole? A growing number of studies have identified instances of convergent or shared adaptive evolution in small demes that utilize standing genetic variation (SGV) found in the static population from which they split Nelson and Cresko [2017], Schrider and Kern [2017], Barrett and Schluter [2008]. However, it is still widely unknown which variations of evolutionary processes such as gene flow, recombination, selection and mutation, promote the maintenance and re-use of such genetic polymorphisms. In addition, we would like to know how complex geographic systems, with a variety of selective pressures, interact with these evolutionary forces to help or hinder the use of SGV in newly colonized subpopulations.

Recently, a large flood of data resulting from evolutionary systems of organisms have come from population genomic studies powered by advances in sequencing technologies. One such organism is the threespine stickleback. Many studies of species have exhibited a long standing evolutionary history of migration-selection balance between marine and freshwater populations across the globe. The rigidity of the stickleback's ability to frequently prosper in newly created freshwater environments has made this fish a good model for understanding the genetic basis of adaptive evolution. The ancestral marine form of stickleback has given rise to millions of independently derived populations in recently de-glaciated regions of the Northern Hemisphere. While geographic isolation prevents direct migration between a large majority of these patches of freshwater populations, They appear to exhibit very similar phenotypes, as well as many shared, adaptive alleles often found to be identical by decent (IBD). Impressively, independent local adaptation of marine individuals to freshwater environments has been observed to take place in tens of generations, and the adaptive alleles are found in freshwater populations that have been geographically isolated since the end of the last ice age \approx 13,000 years ago. For example, in 1964 the Great Alaskan Earthquake caused an uplift of Middleton island and in turn, introduced a group of freshwater ponds around the perimeter of the island. Quickly inhabited by the surrounding marine population of stickleback, Lescak et al. [2015] observed significant phenotypic changes in less than 50 years that appear to be parallel to freshwater stickleback that have been separated for over thousands of years. In these freshwater stickleback, the number of lateral plates are reduced and the opercle shapes shows the same expansion of the dorsal region and reduction of the ventral region as observed in the large majority of freshwater demes.

But how can evolution occur at such a rapid pace? Waiting for new mutations to arise in each lake would take much longer, and the genotypes being identical by state is even more improbable. This would seem to suggest that freshwater alleles are maintained in the marine individuals allowing the accelerated selection on SGV found in marine individuals which colonize the lake. The first clear example of the global reuse of SGV was the gene *eda* which has been shown to be an important regulator for the number of lateral plates. While the low lateral plate version of this gene arose millions of years ago, it is found in freshwater ponds which have formed much more recently. Contemporary population genomic studies employing genome-wide haplotype analyses has provided evidence that *most* regions of the genome that distinguish marine-freshwater genetic differences share this pattern [Nelson and Cresko, 2017].

Schluter and Conte [2009] formulated the “transporter” hypothesis to explain these observations, a conceptual model in which alleles beneficial in freshwater populations are maintained at migration-selection balance in the larger oceanic population and thus available for subsequent adaptation into new freshwater habitats. The hypothesis seems fits available data, but a number of quantitative questions remain. Are the actual population sizes, migration rates, and fitness differentials consistent with this hypothesis? How many of these differentially selected alleles are there, and how are they arranged within the genome? Does adaptation to a new lake rely on presence of early-generation hybrids between freshwater and marine fish, i.e., on long haplotypes recently inherited from freshwater-dwelling stickleback? Or, is the process more akin to the atomization and reconstruction effected by the Star Trek technology that motivated the hypothesis' name? More generally, how do the dynamics and eventual genomic organization depend on the level of gene flow and strength of local adaptation, both across the entire stickleback meta-population and separately within each lake?

In this paper, we used forward simulations modeled on the threespine stickleback meta-population to

ask how gene flow affects the genetic and genomic architecture of local adaptation. We know in practice that stickleback populations in new habitats can adapt in only tens of generations [Lescak et al., 2015]; what level of between-habitat gene flow is required for such rapid adaptation? Genome scans for between-habitat differentiation have detected a number of causal QTL; what proportion of the loci actually underlying adaptation do we expect to be detected by such scans? How does our power to detect causal loci depend on the amount of gene flow?

We find that only a few stickleback per generation, per lake, suffices to maintain sufficient genetic variation in the ocean so that new freshwater populations can adapt from standing variation in only tens of generations. We also find that continued gene flow of marine individuals into recently established lakes is not important for adaptation, and so it is only “downstream” gene flow that allows freshwater adaptations to be “transported” between lakes. This rapid, local adaptation can only occur over an intermediate range of levels of gene flow – low gene flow prevents variation from being transported, while high gene flow prevents local adaptation.

Methods

To explore these questions, we used SLiM [Haller and Messer, 2017, 2018] to implement forwards-time simulation of populations of individuals with explicitly represented genomes in which selection acted upon a single continuous quantitative trait. The details of the model were motivated by current understanding of threespine stickleback evolutionary history and demography, and included divergent selection in the two habitats which had substantial spatial structure. Certain aspects of the model remain simplistic due to computational constraints. Possibly the most important caveat is that simulated population sizes are much smaller than the census size of the threespine stickleback populations observed in nature (see the Discussion for more on this).

Habitat and geography Simulations have two habitat types – Marine and Freshwater – defined by their selective pressures, each with 5,000 diploid individuals. The arrangement of these habitats, depicted in Figure 1, roughly models a set of freshwater habitats along a stretch of coastline. The marine habitat is a continuous, one-dimensional range of length 25 units, while the freshwater habitat is divided into 25 discrete subpopulations (which we call “lakes”), each connected to the marine habitat at regularly spaced intervals (positions $i - 1/2$ for $1 \leq i \leq 25$).

Divergent selection is mediated by a single quantitative trait with different optima in marine and freshwater habitats. This situation roughly models the cumulative effect of the various phenotypes thought to be under divergent selection between the habitats, such as armor morphology, body size, craniofacial variation and opercle shape. The optimal trait values in the marine and freshwater habitats are +10 and -10 respectively, and fitness of a fish with trait value x_{ind} in a habitat with optimal value x_{opt} is determined by a Gaussian kernel with standard deviation 15, i.e.,

$$f(x_{\text{ind}}; x_{\text{opt}}) = \exp \left\{ \frac{1}{2} \left(\frac{x_{\text{ind}} - x_{\text{opt}}}{15} \right)^2 \right\}.$$

In short, the difference between an individual’s trait value and the optimum determines that individual’s fitness. We chose the difference between optima and strength of stabilizing selection in each habitat so that

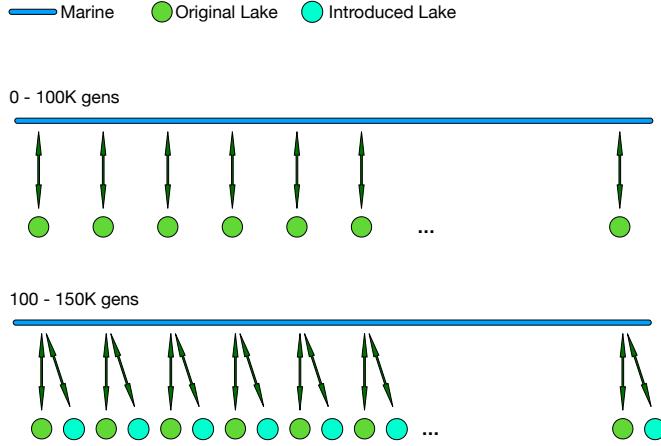


Figure 1: **Diagram of simulated populations:** a single, continuous, one-dimensional marine habitat (blue) is coupled to randomly mating “lakes” at discrete locations with arrows representing migration patterns. After an initial period of 100K generations with 25 lakes, an additional 25 lakes are added (at the same set of locations) and populated with marine individuals to simulate the appearance of newly accessible freshwater habitats colonized by marine stickleback. The marine habitat, and each set of 25 lakes, each contain 5,000 individuals at all times.

- (a) around 10 (diploid, homozygous) mutations were sufficient to move from one optimum to the other, and
- (b) well-adapted fish from one habitat would have low, but nonzero, fitness in the other habitat.

Genetic architecture of the trait Each individual carries two linear chromosomes, each of size 10^8 loci. Mutations that can affect the trait under selection can occur at rate 10^{-10} per locus per generation in ten regions of 10^5 loci each, spread evenly along the chromosome. Each mutation in these regions is either additive, completely recessive, or completely dominant (with equal probability). Effect sizes for these mutations are chosen randomly from an exponential distribution with mean $1/2$, either positive or negative with equal probability. Individual trait values (x_{ind}) are determined additively from the diploid genotypes. Concretely, an individual that is heterozygous and homozygous for mutations at sets of loci H and D respectively has trait value $x_{\text{ind}} = \sum_{i \in H} h_i s_i + \sum_{j \in D} s_j$, where h_i and s_i are the dominance coefficient and the effect size of the mutation at locus i . Subsequent mutations at the same locus replace the previous allele. We also used SLiM’s ability to record *tree sequences* [Haller et al., 2018] to add neutral mutations across the entire genome at a rate of 10^{-7} per locus per generation, as described in Kelleher et al. [2018].

Population dynamics We use SLiM to simulate a Wright–Fisher population with non-overlapping generations and a fixed population size of 5,000 diploid individuals in each habitat. Each generation, the two parents of each new offspring are chosen proportional to their fitness (all individuals are hermaphroditic), and the contributing genomes are produced by Poisson recombination with an average of one crossover per chromosome per generation (10^{-7} per locus per generation). Since the total population across *all* 25 lakes is

fixed at 5000, and the Wright–Fisher model assumes unrealistic global population regulation, we normalize the fitnesses of each individual such that approximately 200 offspring are generated in each lake, each generation. to do this, we divide fitness values of each freshwater individual by the mean fitness in their lake, so that the mean fitnesses of all lakes are equal before selection happens.

As depicted in Figure 1, dispersal occurs both locally along the coastline in the marine habitat as well as between the marine habitat and the lakes, with a lake–ocean migration rate denoted m . All individual dispersal events can be thought of as occurring at the juvenile stage in the life cycle of the simulation. Each new individual in each habitat has parents from the other habitat with probability m (in which case we call it a “migrant”), and parents from the same habitat with probability $1 - m$. The first parent of each nonmigrant individual in the freshwater habitat is chosen from the freshwater habitat proportional to fitness, and a mate is chosen from the same lake as the first, also proportional to fitness. The offspring then lives in the same lake as the parents. Parents for each nonmigrant marine individual are chosen similarly: first, a single parent is chosen proportionally to fitness in the marine habitat, and then a mate is chosen, also proportionally to fitness but re-weighted by a Gaussian function of the distance separating the two, with standard deviation 1/2. Concretely, if the first parent is marine individual i , then marine individual j is chosen as the mate with probability proportional to $f(x_j) \exp(-2d_{ij}^2)$, where $f(x_j)$ is the fitness of individual j and d_{ij} is the distance between the two locations. Finally, each new marine offspring is given a position displaced from the first parent’s position by a random Gaussian distance with mean 0 and standard deviation 0.5, and reflected to stay within the habitat range. Parents for each freshwater migrant are chosen in the same way as for nonmigrant marine individuals, and are assigned to the lake nearest to the position of the first marine parent. Similarly, parents for each marine migrant are both chosen from the same lake as before, and the offspring is given a spatial location in the marine habitat at the location of the parent’s lake.

New lakes To study how marine-derived populations adapt after colonizing newly appearing freshwater habitats, we introduce a new set of 25 lakes after 100,000 generations. These new lakes are populated with marine individuals to emulate a freshwater lake being colonized by oceanic stickleback. This creates two *sets* of lakes, which along with the marine population have a total of 15,000 individuals. Since this introduction of lakes doubles the number of lake-to-marine immigrants, the probability that a new marine individual has freshwater parents is $2m$ instead of m .

Recording genealogical history We used SLiM’s ability to record *tree sequences* [Haller et al., 2018, Kelleher et al., 2016] to output the genealogical history of all individuals at the time of introduction of new lakes, at the time of adaptation, and at the end of the simulation. This allowed us to directly query the true origins of adaptive alleles. In addition, it allowed for much larger simulations by avoiding the computationally expensive task of simulating neutral mutations which were retroactively added to the gene trees at a rate of 10^{-7} per locus per generation, as described in Kelleher et al. [2018].

The output tree sequence from each simulation allows us to explore the origin of the genetic basis of adaptation in the new lakes. To do this, we constructed the genealogical tree relating all extant chromosomes at each locus along the genome. Using these trees we classified each adaptive allele, in each genome in the new lakes at the time of adaptation, into four categories:

1. a “*De novo*” allele: deriving from a new mutation that occurred in a new lake.

2. a “*Migrant*” allele: deriving from a migrant not in the initial generation that colonized the lake
3. a “*Captured*” allele: present in initial colonists of the new lake, and both common (above 50%) in the original lakes, and uncommon (below 50%) in the ocean.
4. a “*Marine*” allele: present in initial colonists of the new lake, but not a “captured” allele.

The proportion of trait-affecting alleles in new lakes that fall in these categories measures the degree to which selection in the new environments made use of (1) new mutation, (2) post-colonization migration, (3) standing variation at migration-selection balance, and (4) standing variation at mutation-selection balance.

We also used neutral mutations to calculate F_{ST} , calculated on a per-locus basis and then averaged in windows as a measure of between-population relative differentiation. Concretely, if p_f and p_m are the frequencies of a given mutant allele in the freshwater and marine habitats, respectively, and $\bar{p} = (p_f + p_m)/2$, then we compute F_{ST} for that mutation as $1 - p_f p_m / (\bar{p}(1 - \bar{p}))$.

Results

To observe the impact of gene flow on selection in the new lakes, we varied the ocean-lake migration rate, m , across separate simulations from 5×10^{-5} to 5×10^{-1} . Below, we often refer to these migration rates in terms of the number of migrants per lake, per generation, which we denote M . Since each lake contains 200 individuals, $M = 200m$. Many aspects of adaptation changed substantially across this range, including the speed of adaptation, degree of sharing of adaptive alleles between lakes, and the population genetic signals left behind. At very low rates of gene flow, each new lake’s population adapted almost completely independently through *de novo* mutation, which took a very long time ($\approx 20,000$ generations). At very high rates of gene flow, local adaptation was constrained by the large influx of locally maladaptive alleles Bolnick and Nosil [2007]. Between these two extremes, genetic variation that allowed adaptation to freshwater habitats could move relatively easily between lakes. Perhaps surprisingly, only a few migrants per generation from the lakes to the ocean were needed to maintain sufficient genetic variation in the ocean to accelerate adaptation in new lakes.

Local Adaptation: differentiation with gene flow

As shown in Figure 2, local adaptation occurred in all simulations with the exception of the highest gene flow at which half of each population was composed of migrants. Figures 3 and S4 show how mean trait values in freshwater and marine populations diverged over time, until the trait means were close to the optimal values in each habitat. Establishment of new alleles in the lakes is visible in the first few thousand generations of Figure 3 as jumps in the mean trait value which move the trait by an amount of order 1 every few hundred generations. At the lowest rate of gene flow, $M = 0.01$, differences at around 20 commonly polymorphic sites (about 10 that shift the trait in each direction) were responsible for most of the adaptive differences between freshwater and marine habitats. As expected, increasing migration rate decreased differentiation between habitats: as seen in Figure 4, F_{ST} between marine and freshwater habitats at neutral sites steadily declines as migration increases.

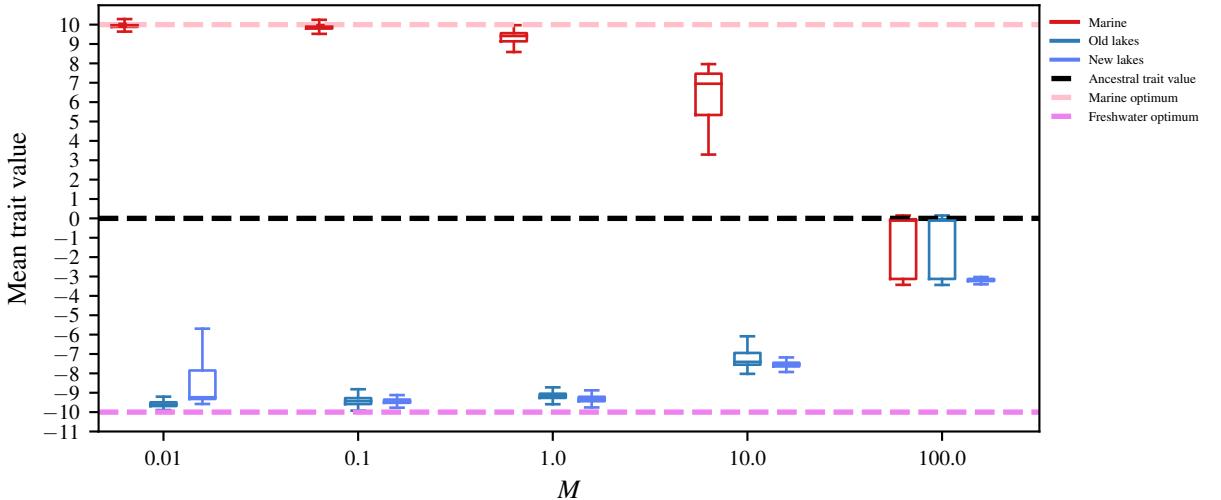


Figure 2: Distribution of mean trait values across generations of the simulation, for different migration rates. The dashed pink and purple lines at ± 10 give the optimum phenotypes in the marine and freshwater environments, respectively.

Adaptation occurred much more quickly at higher migration rates, both in the old and new sets of lakes. We measured this “time to adaptation” as the number of generations until average trait values in old and new lakes were within 0.5 of each other, shown in Figure 5 for different rates of gene flow. Adaptation of new lakes took over 18,000 generations at the lowest rate of $m = 0.01$, while at $m = 1$ migrant per, lake per generation, new lakes managed to adapt in just under 60 generations.

Sharing of freshwater adapted alleles

At low migration rates, the *initial* period of adaptation takes roughly 25 times longer for lakes than it does for the ocean. This difference occurs because at low migration rates, each lake must wait for its own novel mutations to arise in order to adapt. Because the marine habitat is continuous, with 25 times more individuals than any one lake, there is a much larger influx of new mutations to be selected upon. At higher migration rates, greater mixing allows the initial lakes to share alleles instead of developing their own genetic basis for adaptation.

To investigate in more depth how locally adaptive alleles found in the original lakes are shared between lakes, as well as how they spread to the new lakes, we defined and tracked the distribution of “pre-existing freshwater adapted alleles” at the beginning of each generation. To be considered in this category, an allele must participate in the genetic basis of local adaptation for at least one of the original lakes. Concretely, these are any non-neutral mutations whose frequency is above 50% in at least one original lake and below 50% in the marine habitat. Figure 6A shows the distribution of the number of these alleles across generations. At $M = 0.01$, each lake has a private set of about 10 mutations nearly fixed in that lake but not elsewhere: new lakes independently acquire new adaptive alleles rather than pre-existing ones. At $M = 0.1$, we again

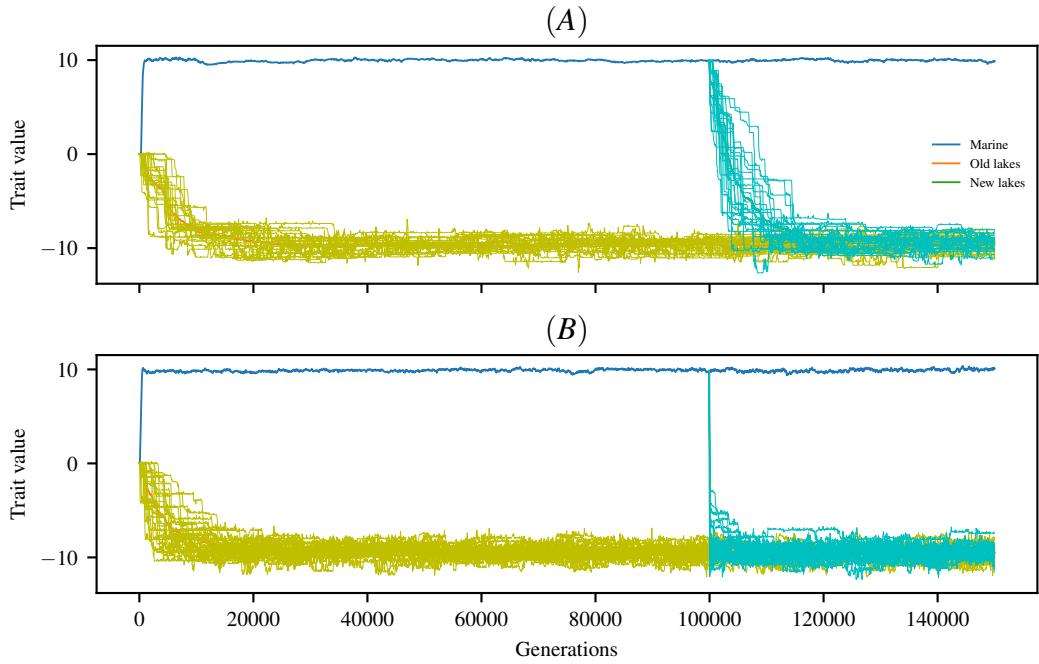


Figure 3: Mean individual trait values in the marine habitat (blue line), the original lakes (yellow lines; average in orange), and the new lakes (light green lines; average in dark green), across the course of two simulations, with migration rates of (**top**) $M = 0.01$ and (**bottom**) $M = 0.1$ migrants per lake per generation, respectively. Optimal trait values in the two habitats are at ± 10 . Analogous plots for other migration rates are shown in Figure S4.

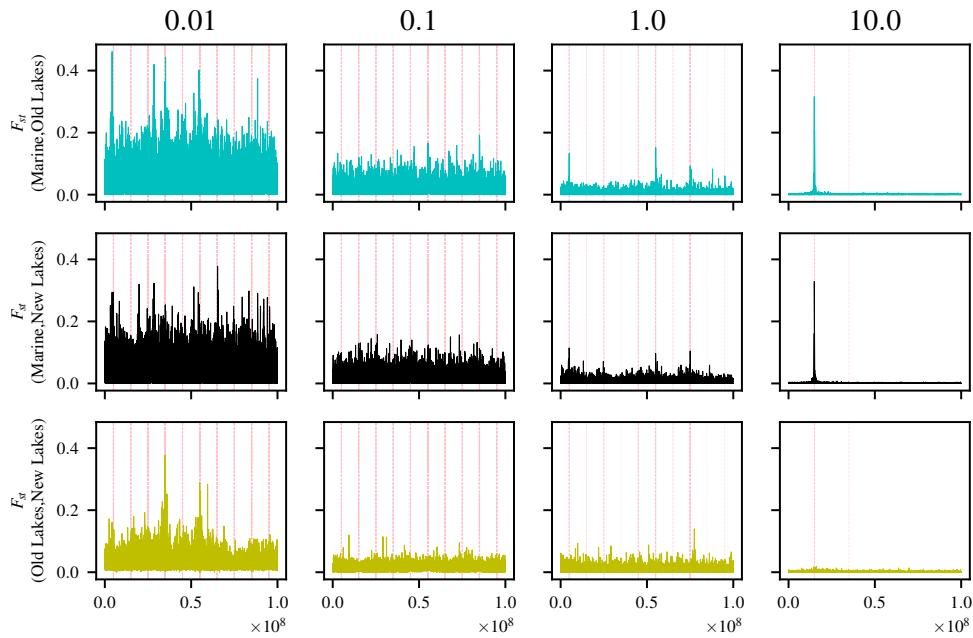


Figure 4: Average F_{ST} in windows of 500Mb between: **(top)** marine habitat and old lakes; **(middle)** marine habitat and new lakes; and **(bottom)** old and new lakes. Each plot shows F_{ST} values for a separate simulation, with columns corresponding to increasing gene flow from left to right. F_{st} is calculated between all marine individuals, and all lakes pooled together. All locations of pre-existing freshwater adapted alleles have been highlighted by pink dashed vertical lines with an alpha value of 0.3, so darker shades of blue imply more freshwater adapted alleles at that location.

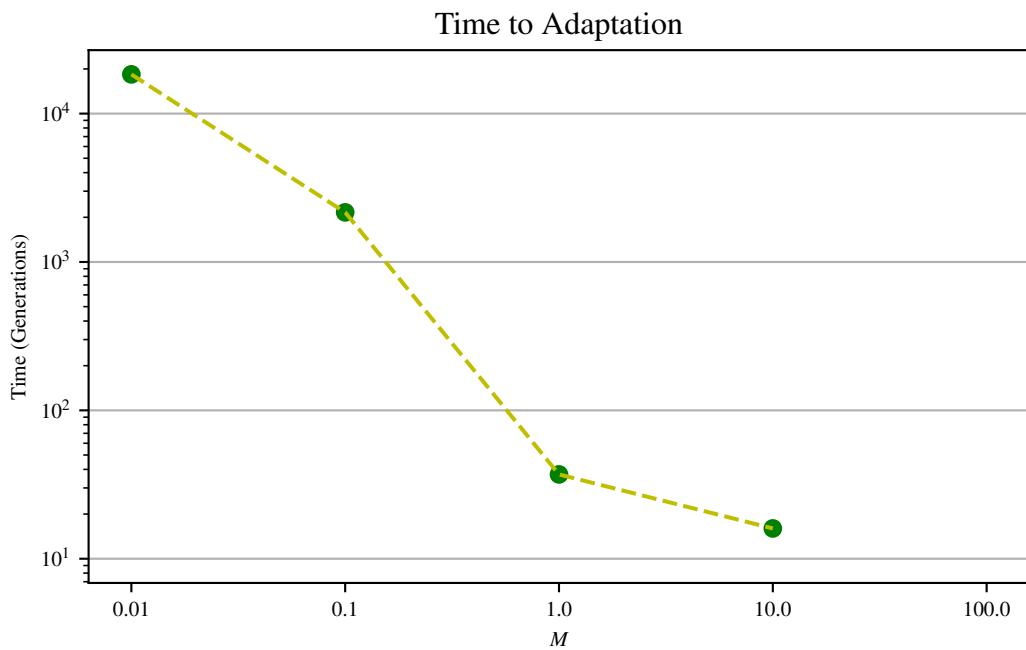


Figure 5: Time to adaptation as a function of migration rate. The time to adaptation is measured as the number of generations until the introduced population's mean phenotype comes within 0.5 of the original lakes average phenotype. Each point represents a single simulation run. (Adaptation did not occur at the highest rate of gene flow.)

observe the original lakes adapting nearly independently from each other, but now the new lakes adapt using pre-existing alleles present in the original set of lakes. Concurrently, the average marine individual carries ≈ 2 pre-existing freshwater adapted alleles, standing variation which was nearly absent at $M = 0.01$. As migration rate increases past this, the total number of pre-existing freshwater adapted alleles declines (Figure 6C). Interestingly, the frequency of these alleles in the ocean stays relatively constant across the reasonable rates of gene flow.

Figure 6B shows the distribution, through time, of the mean *percentage* of currently-defined freshwater adapted alleles that each genome in each of the populations carries. If all individuals across lakes carried the same set of alleles determining their trait value, this would be 100%. At the lowest migration rate $m = 0.01$, each genome in the original lakes have almost exactly 1/25th of the total number of pre-existing freshwater adapted alleles – this is because each one of the 25 lakes has adapted with a unique set of alleles. Since these are *pre-existing* alleles, the value is zero for introduced lakes. Figure 6A shows us that at 0.1 migrants per lake per generation and above, the average individual across the new lakes has nearly the same amount of pre-existing freshwater adapted alleles as individuals across the old lakes. As expected, the genetic basis of the freshwater phenotype seems to simplify as migration increases – higher rates of migration allow adaptive alleles of larger effect to travel more efficiently through the population, even though they are deleterious in the ocean.

The numbers in Figure 6 suggest that the dramatic increase in speed of local adaptation we observed above occurs because higher gene flow between populations allows sharing of freshwater alleles between populations. We confirmed this by using recorded tree sequences to identify the origin of each trait-affecting allele in each individual in the new lakes, as defined in the Methods. Figure 7 shows that at the lowest rate of gene flow the majority of adaptive alleles are derived from “*de novo*” mutation. As gene flow increases, a larger fraction of adaptive alleles derive from pre-existing variation in the marine population at the time of introduction. In other words, greater mixing at higher migration rates allows lakes to share alleles instead of developing their own genetic basis of adaptation.

While increased migration allows sharing of adaptive alleles between lakes, at $m = 10.0$ the constant influx of alleles between the habitats creates substantial migration load. The rate at which migration load becomes substantial, 10 migrants per lake per generation, only replaces 5% of each population each generation with migrants from the other habitat, but this is sufficient to shift the mean trait values to nearly half their optimal values, as seen in Figure 2.

Scans for selection

Here, we take a closer look at the genomic architecture of local adaptation between the two habitats. Can measures of local differentiation such as F_{ST} be used to identify the causal loci? Figure 4 shows plots along the genome of average per-locus F_{ST} values in 500bp windows between the marine habitat and all freshwater habitats pooled together. Higher rates of dispersal showed more distinct F_{ST} peaks over polymorphic loci, while “background” levels of F_{ST} increase as gene flow decreases, swamping out this signal until the regions under selection are indistinguishable. This is likely the contribution of two separate forces of natural selection: first, stronger genetic drift with less migration leads to higher background F_{ST} , and second, greater sharing of adaptive alleles providing a shared signal across populations.

At first glance, this suggests that genome scans for local adaptation based purely on measures of differ-

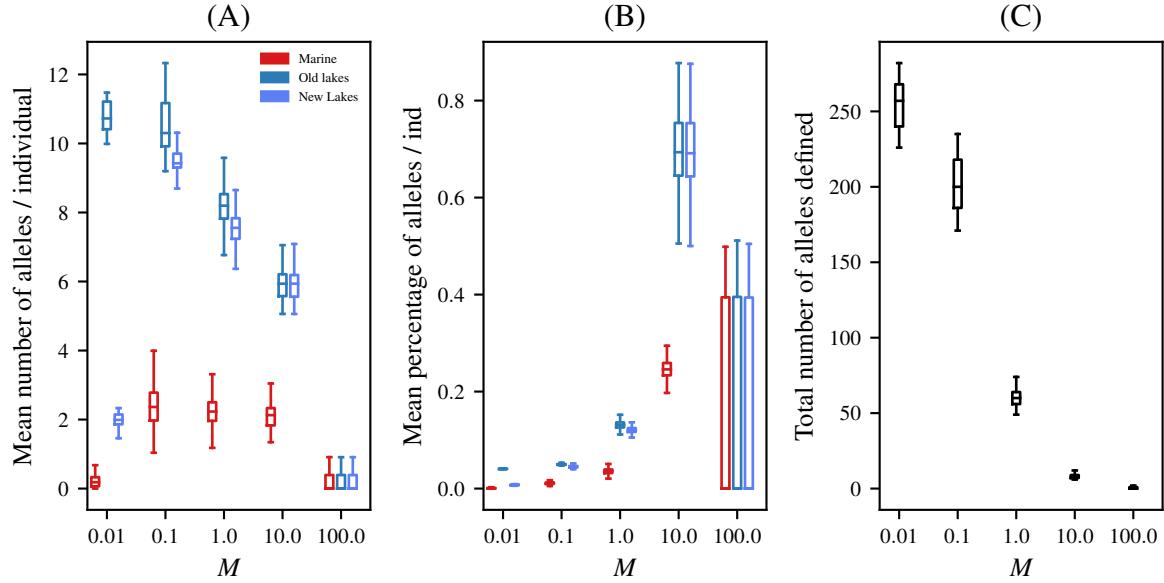


Figure 6: Amount of standing freshwater variation by habitat, across migration rates. Each plot counts “pre-existing freshwater adapted alleles”, that are common in the original lakes but rare in the ocean (see text for definition). **(A)** Mean number of these alleles per individual. **(B)** Mean percentage of these alleles per individual. **(C)** Total number of these alleles (so, $B = A/C$). The number of alleles meeting these conditions changes over the course of the simulation, and each plot shows distributions of these values across generations. The horizontal axis shows M , the mean number of migrants per lake per generation.

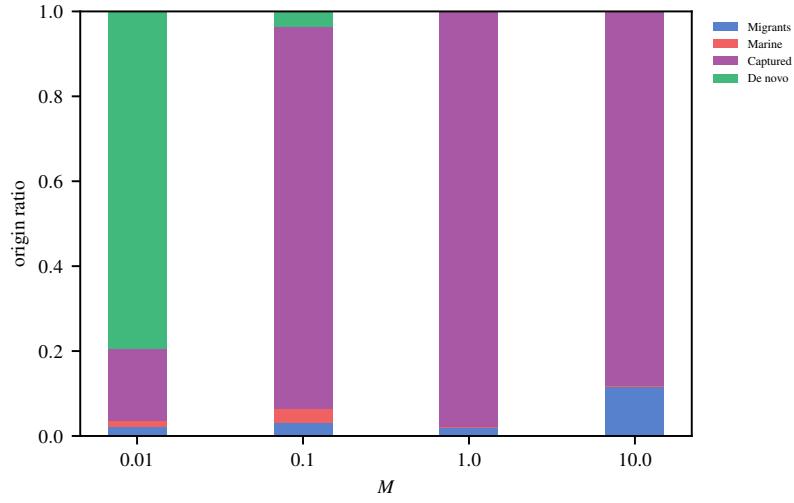


Figure 7: **(Origin of adaptive alleles:)** Each bar plot shows the origins of all trait-affecting alleles above frequency 50% in at least one new lake, classified as **(red)** new mutations, **(blue)** post-colonization migrants, **(green)** “captured” from pre-existing lakes, or **(orange)** standing marine variation. See Methods for precise definitions of these categories.

entiation will only be successful given enough migration between habitats. But how many of these peaks are actually underlying trait differences that form the basis for local adaptation? To quantify this, Figure S1 shows the power and false positive rates that would be obtained by an F_{ST} cutoff that declared everything above a certain value to be a causal locus. What we observe most plainly in this graph is that $M = 1.0$ and $M = 10.0$ migrants per lake, per generation are the only rates of dispersal which provide reliable true negatives when observing peaks. This means that a large percentage of peaks past an F_{ST} cutoff of 0.05 actually lie on top of regions which could impact trait differences. Unfortunately, the low statistical power for all rates of M would seem to suggest that many regions which impact trait differences do not appear as F_{st} peaks. This may imply that genome scans for local adaptation based purely on measures of differentiation may not be as reliable as previously thought. To summarize, our study find that with high rates of dispersal between population, F_{st} peaks may be a good indication of causal loci. However, this is a one way implication, meaning even with high dispersal, not all causal loci will appear as F_{st} peaks.

It's important to note that our study in Figures 4 and S1 pooled together all lakes and all marine individuals for the calculations. For comparison, Figures S5 & S2 shows the same scans but calculated only on between individuals in the marine with spacial location ≤ 5.0 and the individuals in the respective 5 lakes. These graphs show even less power and True Positives only at $M = 10.0$ migrants per lake, per generation

Origin of introduced freshwater adaptive alleles

We have thus far found that the speed of adaptation depends strongly on the degree to which alleles can be shared between populations. However, the large increase in speed of adaptation between dispersal rates of $M = 0.1$ and $M = 1.0$, seen in Figure 5, remains mysterious. Figure 6A shows that with $M = 0.1$ migrants per lake per generation, there is substantial sharing of alleles between populations, and yet at this rate new lakes take around 2,000 generations to adapt. This is surprising because we see a similar quantity of allele sharing at $M = 1.0$, but much more rapid adaptation (≈ 60 generations). A reasonable explanation for this 33-fold discrepancy is that new lakes must wait for an additional influx of freshwater alleles through migration beyond what is present in the initial generation. However, Figure 7 shows this is not the case: at both $m = 0.1$ and $m = 1.0$, the vast majority of alleles responsible for local adaptation came from individuals in the original generation (“captured”).

	$m=0.01$	$m=0.1$	$m=1$	$m=10$
best	2.77	-12.02	-21.02	-7.02

Table 1: Haplotypic variation present in the new lakes at time of colonization, across rates of gene flow, quantified as the most negative trait value achievable with intact haplotypes, averaged across populations (see text for details).

An alternative explanation is that at lower rates of gene flow, freshwater alleles present as standing variation in the marine habitat are more tightly linked to marine alleles. This implies the adaptation in the new lakes must wait for recombination to separate freshwater adapted alleles from nearby marine adapted alleles. This might be expected at low dispersal rates since freshwater alleles may need to be “masked” by nearby compensatory alleles to remain in the ocean for long periods of time. Reversing this “masking” then slows the process of adaptation that uses this genetic variation. In other words, higher dispersal from freshwater to the ocean maintains relatively intact freshwater haplotypes that can be more easily rebuilt

in the marine environments. Recall that trait-affecting mutations only occur in relatively small regions of 10^5 loci in which recombination occurs only once in every one thousand meioses. This implies that even if there is a sufficient amount of variation in the initial population of a lake to shift the trait from the marine optimum (+10) to the freshwater optimum (-10), rebuilding the most beneficial haplotype may prove to be a non-trivial task for selection. For example, suppose there are 10 variants segregating at low frequency with effect size -1 each, but each is paired with a compensatory allele with effect size +1. Each local haplotype is therefore neutral. This might also explain why at $M = 0.1$, marine individuals still hold on average several alleles that shift the trait in the freshwater direction. (Figure 6A).

To quantify the genetic variation available *without* recombination within the ten genomic regions, we first found, within each population, the haplotype with the largest net negative effect at each of the ten genomic regions. Summing these ten numbers, we get the maximum amount that selection could move the population in the freshwater direction without recombining within these regions. The mean of this value across the 25 lake populations is shown in Table 1 – typical populations at $M = 0.1$ migrants per lake per generation could shift to a phenotype of -12.02 (and so have sufficient variation to adapt without recombination), but the “best” haplotypes in the populations at $M = 1.0$ have effect sizes nearly twice as big at a mean total of -21.02. The amount of variation available at $M = 10.0$ is lower (only -7.02), presumably because of migration load, while at $M = 0.01$ almost no alleles with negative effect are present. Note that some of these haplotypes will likely be lost to drift – indeed, if they did all fix, then populations at $M = 0.1$ would adapt much more quickly. However, the comparison still provides a good comparison.

Theoretical expectations

We now revisit our observations above in the light of population genetics theory. The discussion will be roughly based on estimates, with a focus on intuition rather than precise calculations. Because we model stabilizing selection on an additive trait controlled by a moderately large number of loci within each population, more precise expectations might be obtained through quantitative genetics [Svardal et al., 2014] or even Fisher’s geometric model [Barton, 2001, Chevin et al., 2014].

Suppose a new allele enters a lake, either by migration or mutation. If, when it is rare but present in n copies, it has fitness advantage s – i.e., the expected number of copies in the next generation is $(1+s)n$ – then the probability that it escapes demographic stochasticity to become common in the population is approximately $2s$ [Lambert, 2006, Haldane, 1927] (assuming Poisson reproduction, as we roughly have here). If the current population all differ from the optimum trait by z , and the allele has effect size $-u$ in heterozygotes, then the fitness advantage of the allele would be $s(u) = \exp(-\beta((z-u)^2)/\exp(-\beta z^2)) \approx 2\beta zu$, where in our parameterization, $\beta = 1/450$. This tells us two things: (1) the rate of adaptation decreases as the population approaches the optimum, and (2) larger mutations (in the right direction) are more likely to fix.

New mutations The total rate of appearance of new mutations per lake is $\mu_L = 0.04$, which are divided evenly in six categories: additive, dominant, and recessive, in either direction. This implies that a new additive or dominant effect mutation appears once every 75 generations, on average. Effect sizes are randomly drawn from an Exponential distribution with mean 1/2, and so the probability that a dominant mutation manages to establish in a population differing from the optimum by z is roughly $\int_0^\infty 4\beta zu \exp(-2u) du = \beta z$,

and so the rate of establishment of dominant mutations is $\beta z/75$, i.e., about one such mutation every $33750/z$ generations. At the beginning of the simulation where $z = 10$, we would then expect the fixation of alleles to take around 3,000 generations. The distribution of the effect sizes of these successfully established mutations has density proportional to $u \exp(-2u)$, i.e., is Gamma with mean 1 and shape parameter 2. Since additive alleles have half the effect in heterozygotes, they have half the probability of establishment. During the initial phase of adaptation, the populations begin at around distance $z = 10$ from the optimum. Combining these facts, we expect adaptive alleles to appear through mutation within lakes at first on a time scale of 3,000 generations, with the time between local fixation of new alleles increasing as adaptation progresses, and each to move the trait by a distance of order 1. This agrees roughly with what we see in Figures 3 and S4.

Standing variation An allele that moves the trait u units in the freshwater direction, in heterozygotes, has fitness roughly $\exp(-\beta u^2) \approx 1 - \beta u^2$ in the marine environment, i.e., a fitness differential of $s = \beta u^2$. The product of population size and fitness differential in the marine environment for a mutation with $u = 1$ is therefore $2Ns \approx 22$, implying that these alleles are strongly selected against but might drift to moderate frequency if recessive. The average frequency of such an allele in the marine environment at migration-selection equilibrium is equal to the proportion of individuals in the ocean replaced by migrants per generation divided by the selective disadvantage, i.e., around $m/\beta u^2$. Each new lake is likely to contain a few copies of alleles at frequency above 1/400 (since each new lake is initialized with 400 randomly selected genomes). With $u = 1$, this occurs if $m/\beta > 1/400$, and so since $1/\beta = 450$, if $m \geq 5 \times 10^{-6}$ the chances are good that any particular lake-adapted allele that is present in all pre-existing lakes will appear at least once in the fish that colonize a new lake. However, an allele with $u = 1$ only has probability of around 1/20 of establishing locally, so an allele of this size must be present in about 20 copies to ensure adaptation. Putting these together, we expect migration-selection balance to maintain sufficient genetic variation for new lakes to adapt if $m \geq 10^{-4}$, which corresponds to $M \geq 0.4$, agreeing with our results. However, this calculation treats each allele independently; in practice we found that standing freshwater variation in the ocean were masked by linkage to compensatory marine variants.

Migration The key quantity regulating the amount of standing variation in the ocean was the *downstream* migration rate, from lakes to the ocean. How important is the upstream migration rate? If sufficient genetic variation is not present in a new lake initially, it must appear either by new mutation or by migration. Since a proportion m of each lake is composed of migrants each generation, it takes $1/m$ generations until the genetic variation introduced by migrants equals the amount initially present at colonization. This implies a dichotomy: either (a) migration is high, and adaptation is possible using variants present at colonization or arriving shortly thereafter, or (b) migration is low, so adaptation takes many multiples of $1/m$ generations. Since in our model lower migration also reduces the amount of variation available in the ocean, we expect very little contribution of subsequent migration across any value of m , as seen in Figure 7.

Discussion

In this paper, we have described a realistic simulation model of a coastal meta-population to observe the effects of selection of genetic variation (SGV) across a wide range of gene flow rates. We have shown that historical introgression, at our given parameter sets, is able to reproduce rapid and parallel adaptation similar to what we've seen in real populations such as Middleton island. Selection is able to rebuild the freshwater haplotype at a rapid pace (in tens of generations) from marine populations as a medium between all freshwater populations. Almost all rates of migration were helpful in the efficiency of the population to locally adapt except for the highest at which migration load limited the ability of the populations to reach the local optimum.

While our findings prove useful to the understanding of stickleback, they are not autonomous to this species. Now, we will discuss the broader implications of our findings on the more general framework of similar systems, we also explore what other questions remain. Small patches of similar selective pressures can be found in many places such as high altitudes, underwater volcanic vents, or post forest fire environments. Recently, [Ferretti et al., 2018], discovered seven new species of *Hapalotremus* (*Theraphosinae*), living at the extremely high altitudes distributed along the Andes and Yungas in western South America. With the ancestral species at much lower altitudes, one could image high altitude alleles that could potentially be carried to help in the colonization of neighboring mountain environments. Systems of meta-populations like this could potentially arise in many places where the ancestral species carries around with it beneficial alleles for selective pressures it has encountered in the past and remains connected to through hybridization events.

Atmosphere's ability to carry whoever scotty is beaming up ... Perhaps the most notable finding in this study is that large, ancestral populations have the capacity to maintain alleles which are potentially deleterious for an extended amount of time. This principle, deriving solely from the properties of sex and natural selection, could be applicable to almost any species experiencing the influence of divergent selection with introgression. And to explore further how this process is carried out exactly, many of the questions lie in understanding which properties of genomic architecture and demographics allow this transportation to be possible. In our simulations, the continued gene flow from freshwater populations meant that the alleles were maintained at a constant rate with fixed demographics. Two possible questions that might arise from this are: (1) How do population sizes, demographics, and evolutionary histories impact an ancestral population's capacity for carrying maladaptive alleles in its opposing environment, and (2) What role does genomic architecture play in the ability for a haplotype to be rebuilt.

Realized genomic architecture Additionally have also shown introgression is beneficial for inferring causative loci from divergence (F_{st}) along the genome. This is generally because noise of selectively neutral alleles divergence can appear causative when genetic drift causes more differences between populations that have little gene flow between them. It's important to know that in all scenarios, hitchhiking of selectively neutral alleles could also be mistaken for being causative as they often display the same amount of divergence. In general, our study may suggest that diversity scans may not prove as useful in causal loci inference as previously thought. It is important to note that these summary statics could prove to be very useful if used beyond just the peaks seen when looked at as a function of genomic position. Many methods, such as advanced machine learning, have proved to be very good at taking in noisy data and parsing out important

information Schrider and Kern [2018].

Implications for the “Transporter”-hypothesis This study was in large, supportive of the “Transporter”-hypothesis suggested by [Schluter and Conte, 2009]. Provided there is enough dispersal, we found that repeated colonization and adaptation of freshwater stickleback was able to produce beneficial alleles which could then be supported by the marine stickleback population through the offspring of hybridization events. When new freshwater environments were colonized by the marine stickleback, selection was able to rebuild the freshwater haplotype. Shown in Table 1, we found that a populations capacity to carry maladaptive alleles remains pretty constant over realistic ranges of dispersal, but low rates of gene flow may ”mask” the alleles through linkage which counteracts the fitness disadvantage. This linkage prevents the ability for a sub-population to rapidly select on the beneficial SGV as recombination acts as a bottleneck to evolution in this situation. Additionally, In Figure 7 we found that the genetic basis for local adaptation, when selecting upon SGV, is propagated largely from the initial generation of inhabitants. This contrasts the belief that subsequent migration is a necessity in newly established populations ability to rebuild a haplotype. In other words, this finding suggests that with high probability, a randomly chosen subset from ancestral species in the meta-populations like marine stickleback, carry the capacity to rapidly adapt without *any* continued connection to the rest of the species.

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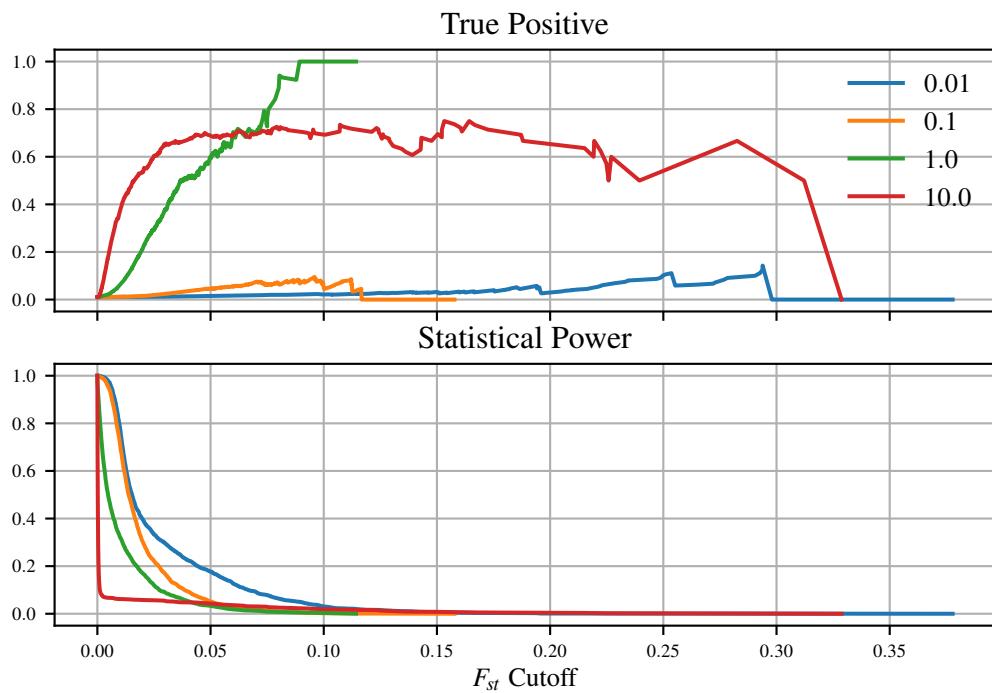


Figure S1: Statistical Power and False Positives as a function of F_{st} threshold. Statistical Power is the likelihood that a SNP will be predicted to have an effect on phenotype when there is an effect to be detected (?). False Positives give us the ratio of SNPs that effect phenotype to total SNPs greater than the F_{st} threshold. **TODO: fix this caption; x-axis label (F_{ST}).**

Supplementary material

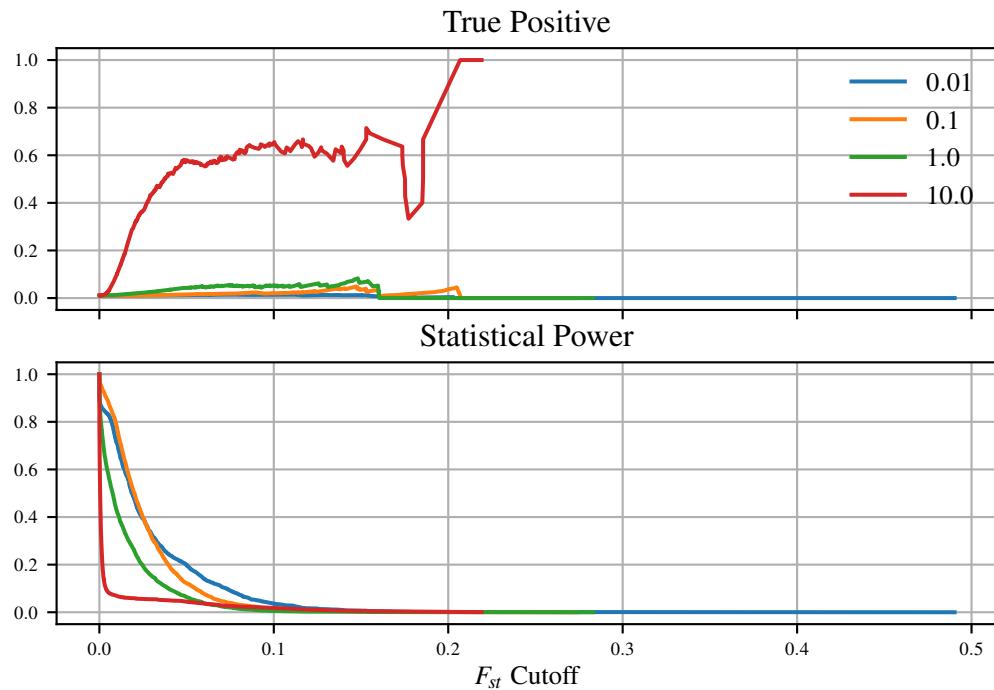


Figure S2: Statistical Power and False Positives as a function of F_{st} threshold. Statistical Power is the likelihood that a SNP will be predicted to have an effect on phenotype when there is an effect to be detected (?). False Positives give us the ratio of SNPs that effect phenotype to total SNPs greater than the F_{st} threshold. **TODO: fix this caption; x-axis label (F_{ST}).**

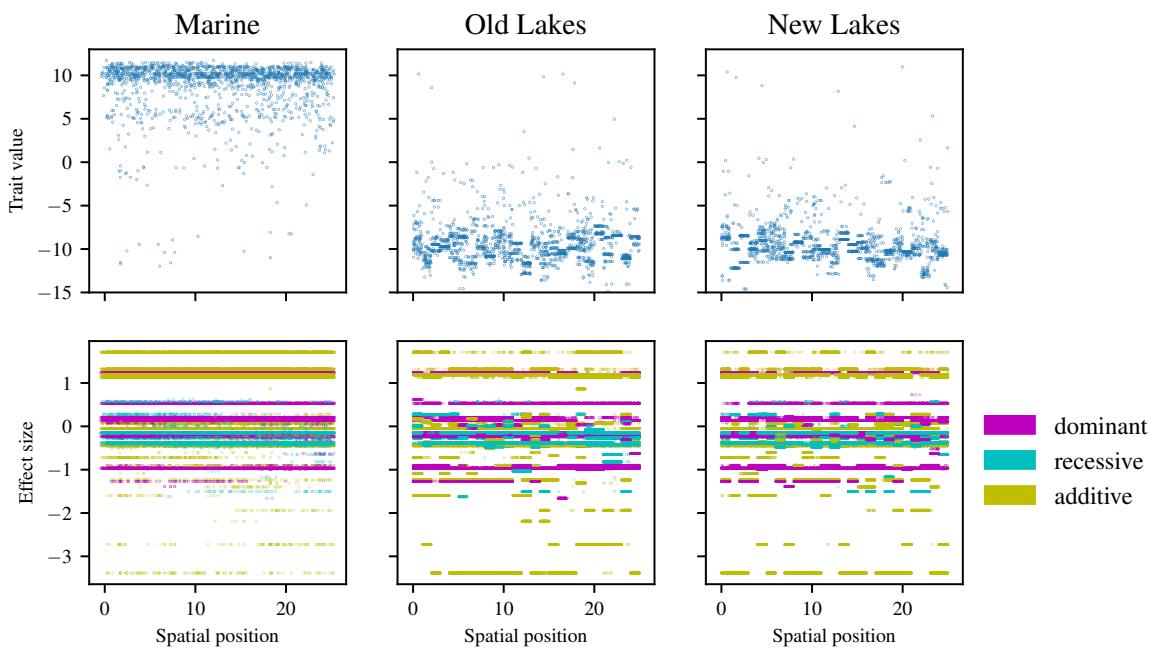


Figure S3: Phenotypes (**Top**) (effect size and mutation type) and haplotypes (**Bottom**) as a function of spatial position of the individual possessing the genome. The phenotype of each haplotype is, by definition, the sum of the effect sizes of each mutation in the genome.

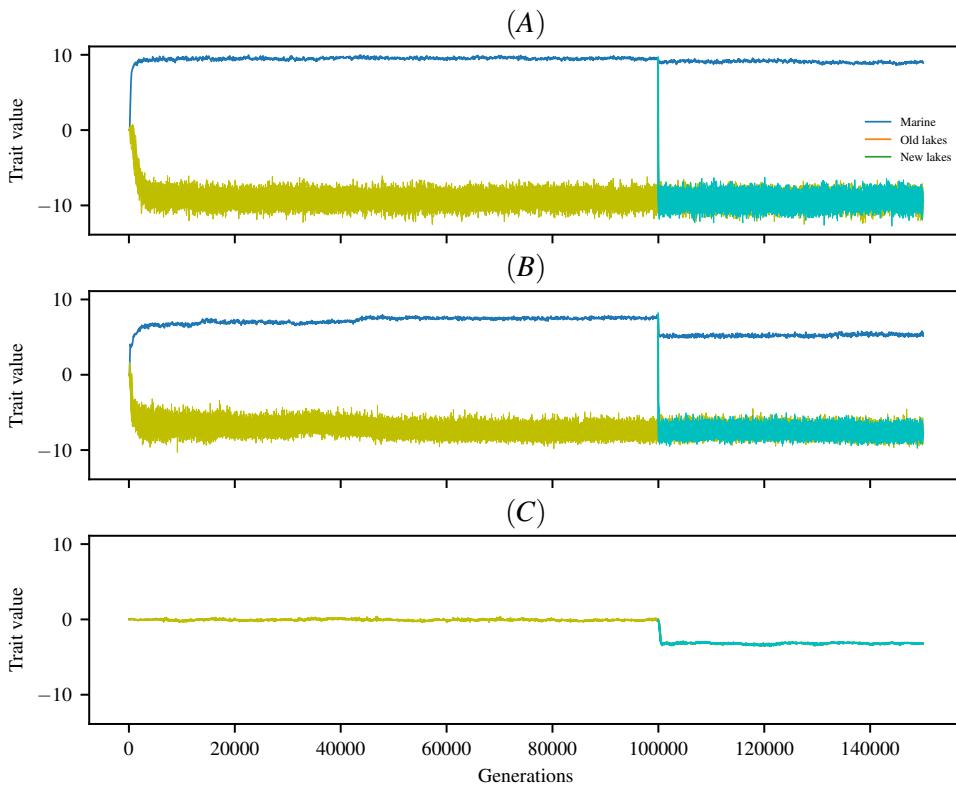


Figure S4: Mean individual trait values in the marine habitat (blue line), the original lakes (yellow lines; average in orange), and the new lakes (light green lines; average in dark green), across the course of two simulations, with migration rates of (A) $m = 5 \times 10^{-3}$, (B) $m = 5 \times 10^{-2}$ and (B) $m = 5 \times 10^{-1}$. (i.e., 1, 10, and 100 migrants per lake per generation, respectively). Optimal trait values in the two habitats are at ± 10 .

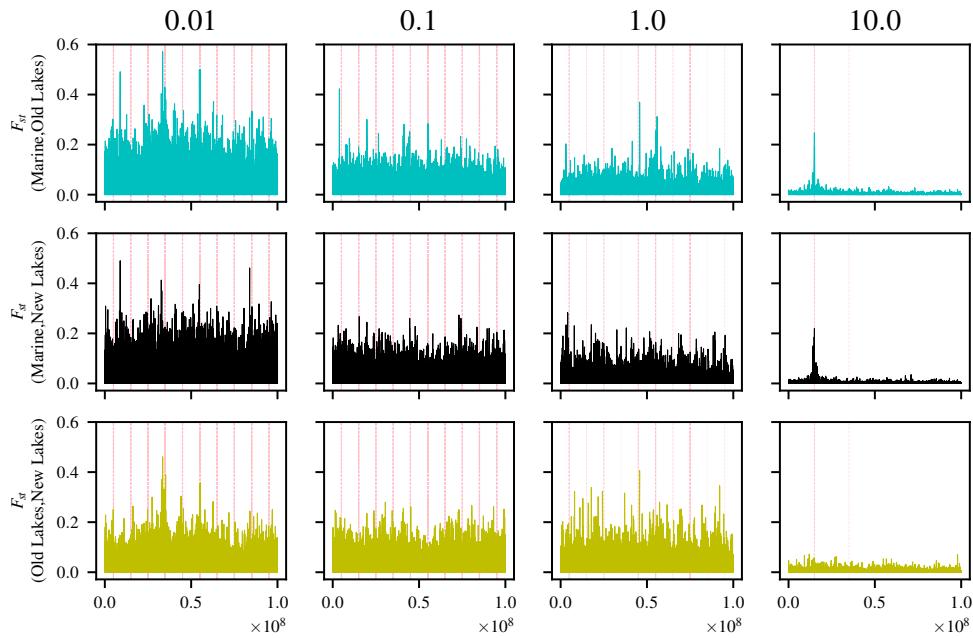


Figure S5: Average F_{ST} in windows of 500Mb between: **(top)** marine habitat and old lakes; **(middle)** marine habitat and new lakes; and **(bottom)** old and new lakes. Each plot shows F_{ST} values for a separate simulation, with columns corresponding to increasing gene flow from left to right. F_{st} is calculated between all marine individuals with spatial location ≤ 5.0 , and the five corresponding lakes. All locations of pre-existing freshwater adapted alleles have been highlighted by pink dashed vertical lines with an alpha value of 0.3, so darker shades of blue imply more freshwater adapted alleles at that location.