

A few stickleback suffice for the transport of alleles to new lakes

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Abstract

Threespine stickleback populations provide a striking example of local adaptation to divergent habitats in populations that are connected by recurrent gene flow. These small fish occur in marine and freshwater habitats throughout the Northern Hemisphere, and in numerous cases the smaller freshwater populations have been established “de novo” from marine colonists. Independently evolved freshwater populations exhibit similar phenotypes that have been shown to derive largely from the same standing genetic variants. Geographic isolation prevents direct migration between the freshwater populations, strongly suggesting that these shared locally adaptive alleles are transported through the marine population. However it is still largely unknown how gene flow, recombination, and selection jointly impact the standing variation that might fuel this adaptation. Here we use individual-based, spatially explicit simulations to determine the levels of gene flow that best match observed patterns of allele sharing among habitats in stickleback. We aim to better understand how gene flow and local adaptation in large metapopulations determine the speed of adaptation and re-use of standing genetic variation. In our simulations we find that repeated adaptation uses a shared set of alleles that are maintained at low frequency by migration-selection balance in oceanic populations. This process occurs over a realistic range of intermediate levels of gene flow that match previous empirical population genomic studies in stickleback. Examining these simulations more deeply reveals how lower levels of gene flow leads to slow, independent adaptation to different habitats, whereas higher levels of gene flow leads to significant mutation load – but an increased probability of successful population genomic scans for locally adapted alleles. Surprisingly, we find that the genealogical origins of most freshwater adapted alleles can be traced back to the original generation of marine individuals that colonized the lakes, as opposed to subsequent migrants. These simulations provide deeper context for existing studies of stickleback evolutionary genomics, and guidance for future empirical studies in this model. More broadly, our results support existing theory of local adaptation but extend it by more completely documenting the genealogical history of adaptive alleles in a metapopulation.

Introduction

The canonical model for the genetics of adaptation has long been the sequential fixation of new mutations [Maynard Smith and Haigh, 1974, Endler, 1977, Orr, 2005]. While it has proved to be a useful baseline for understanding the genetic variation we see in species today, this model is now rightfully understood as

incomplete for many species in nature that have more complicated population structures [Lai et al., 2019, Schrider and Kern, 2017]. In particular, empirical studies have increasingly identified the need to more deeply incorporate standing genetic variation into adaptation dynamics for metapopulations inhabiting an array of habitats [Hermisson and Pennings, 2005, Barrett and Schluter, 2008]. Populations experiencing diverse selective pressures while still exhibiting significant gene flow often result in more complex genomic signals that are still not fully understood [Charlesworth, 1997, Charlesworth et al., 2003, Nosil et al., 2009, Flaxman, 2013, Samuk et al., 2017]. Concurrently, a growing number of empirical studies have identified instances of convergent evolution using standing genetic variation [Jones et al., 2012, Roesti et al., 2012, Feulner et al., 2015, Schrider and Kern, 2017, Barrett and Schluter, 2008, Nelson and Cresko, 2018, Nelson et al., 2019, Bassham et al., 2018]. However, it is still not clear how variation in evolutionary processes – such as gene flow, recombination, selection and mutation – can promote the maintenance and re-use of standing genetic variation, particularly during colonization and adaptation to new environments [Nelson and Cresko, 2018, Pritchard, 2010, Yeaman and Whitlock, 2011, Schrider and Kern, 2017]. It is similarly unclear whether variation in these evolutionary processes can determine the genetic architecture of evolving traits via standing genetic variation. Theoretical work is mostly limited to one or two loci, or relies on approximations of uncertain validity [e.g., Slatkin, 1975, Petry, 1983, Barton and Bengtsson, 1986, Yeaman and Whitlock, 2011, Ralph and Coop, 2015]. Therefore, detailed simulation models of specific systems can provide an important complement to empirical studies in the lab and field, by helping us understand precisely how standing genetic variation might fuel local adaptation, and what genomic signals we can expect to see.

The ancestral marine form of threespine stickleback fish (*Gasterosteus aculeatus*) has given rise to hundreds of thousands to millions of independently derived freshwater populations in recently de-glaciated regions around the Northern Hemisphere [Bell and Foster, 1994, Thompson, 1997, Cresko et al., 2007, Hunt et al., 2008]. This model organism has provided some of the earliest data showing the heterogeneous nature of divergence across genomes and the much more extensive use of standing genetic variation than once thought [Schluter and Conte, 2009, DeFaveri et al., 2011, 2013, Roesti et al., 2014, Nelson and Cresko, 2018, Bassham et al., 2018, Nelson et al., 2019, Hohenlohe et al., 2010, Terekhanova et al., 2014, Marques et al., 2016]. While geographic isolation often prevents direct migration between freshwater populations, threespine stickleback in them frequently evolve similar phenotypes [Cresko et al., 2004, Colosimo et al., 2004, Stuart et al., 2017, Hanson et al., 2016, 2017, Hirase et al., 2014]. The most recent evolutionary genomic studies on stickleback document that while the overall dynamic of local adaptation to marine and freshwater habitats has been occurring for millions of years [Nelson and Cresko, 2018, Nelson et al., 2019], independent local adaptation of marine individuals to freshwater environments has been observed to take place in just tens of generations [Terekhanova et al., 2014, Lescak et al., 2015, Bassham et al., 2018]. For example, in 1964 the Great Alaskan Earthquake caused an uplift of many islands and coastal regions throughout the Gulf of Alaska. Studies of stickleback populations on uplifted Middleton Island showed that newly created freshwater ponds were invaded by the surrounding marine population of stickleback which evolved the freshwater syndrome of phenotypes in less than 50 years [Lescak et al., 2015, Bassham et al., 2018]. Amazingly, the portions of the genomes of these populations that showed increased divergence from the oceanic population mirrored those previously found to differ between ocean and freshwater populations that have been geographically separated for thousands of years [Hohenlohe et al., 2010, Bassham et al., 2018].

But how can evolution occur at such a rapid pace? Waiting for new mutations to arise in each lake or pond

would take much longer than the decades since the Alaskan earthquake with any plausible target size. Even more improbable is having divergence cluster in such similar genomic regions across independent populations. An alternative hypothesis is that the majority of alleles important for freshwater adaptation are present in the marine individuals due to recurrent gene flow from freshwater back in to marine populations. Schluter and Conte [2009] proposed a conceptual model they termed the “transporter” hypothesis to describe the process by which alleles beneficial in freshwater environments are maintained at migration-selection balance in the larger oceanic population and therefore available to be utilized during subsequent adaptation to new freshwater habitats. (The alleles conferring adaptation to freshwater environments are thereby “transported” to and reassembled in new lakes.) The first clear example of the global reuse of such alleles in stickleback was the gene *eda* which has been shown to be an important regulator for the number of bony lateral plates [Colosimo et al., 2004, Cresko et al., 2004, Colosimo et al., 2005]. While the low lateral plate version of this gene arose millions of years ago, it is found in much younger freshwater ponds around the Northern Hemisphere [O’Brien et al., 2015]. More recently, genome-wide haplotype analyses have provided evidence that *most* regions of the genome that distinguish marine-freshwater genetic differences share this pattern [Nelson and Cresko, 2017].

While the growing body of population genomic data on stickleback evolution supports the transporter hypothesis, a number of questions remain. What are the actual population sizes, migration rates, and fitness differentials consistent with this hypothesis? How many differentially selected alleles exist, how many are used at any one time, and how are they arranged within the genome? A curious natural history observation underlying many of these questions is the fact that some newly formed freshwater habitats, such as the ponds on Middleton Island, are quite small and presumably the number of initial marine migrants is few. The variation carried by these few initial migrants might therefore be a small subset of the total variation, and thus be insufficient to fuel adaptation without subsequent influx of alleles from additional generations of marine migrants carrying the remaining freshwater adaptive alleles.

Here, we use individual-based forward simulations implemented in SLiM that incorporates selection on a quantitative trait explicitly determined additively from a realistically long genome to model the stickleback metapopulation and address these questions [Haller and Messer, 2017, 2018]. We ask how variation in amount of gene flow affects the genetic architecture of local adaptation to newly created freshwater ponds. Because we record the entire genealogy of all alleles [Kelleher et al., 2018], we can determine the distribution and abundance of haplotypes across all marine and freshwater populations to know the timing and proportion of potentially adaptive alleles that are actually utilized in each population. In addition, we can document how these adaptive alleles are distributed across the genomes, and as a consequence determine how this may affect the efficacy of genome scans for between-habitat differentiation.

Methods

To explore these questions, we used SLiM [Haller and Messer, 2017, 2018] to implement forward-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 three-spine stickleback evolutionary, history and demography. The following model included divergent selection in the two habitats which had substantial spatial structure. Certain aspects of the model remain simplistic due

to computational constraints; in particular, total population sizes are much smaller than in reality, although we may capture a good picture of local dynamics.

Habitat and geography Our simulations include two habitat types – marine and freshwater – defined by the nature of 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.,

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

Thus, 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 (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 Individuals are diploid, with a linear chromosome 10^8 bp long, with recombination events occurring at a rate of 10^{-8} per bp per generation. Mutations that can affect the trait under selection occur at rate 10^{-10} per locus per generation in ten regions of 10^5 loci each, spread evenly along the chromosome. This translates to a total adaptive target size of 10^{-4} per individual per generation, or ≈ 1 trait-affecting mutation, per population of 5,000 diploids, per generation. This architecture was chosen to model a moderate number of clusters of trait-affecting genes, each of which having about 1Kb of potentially adaptive target (since mutation rates are closer to 10^{-8} , a mutation rate of 10^{-10} is roughly equivalent to having potentially adaptive sites at one of every 100 bases). 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.

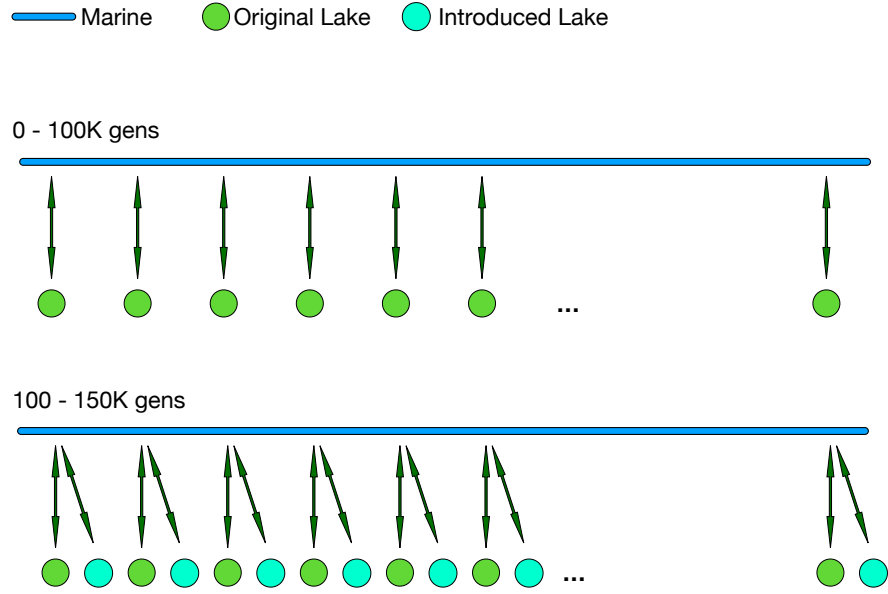


Figure 1: **Diagram of simulated populations:** a single, continuous, one-dimensional marine habitat (blue) is coupled to “lakes”, in which stickleback randomly mate, at discrete locations with arrows representing migration patterns. After an initial period of 100K generations with 25 lakes, an additional 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.

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 (unlike actual stickleback, all individuals are hermaphroditic), and the contributing genomes are produced by Poisson recombination with an average of one crossover per chromosome per generation (10^{-8} 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 so that approximately 200 offspring are generated in each lake, each generation. (A simpler implementation would use SLiM’s “non-Wright-Fisher” model type, which was not available when we wrote the simulations; but we do not expect the difference to affect results.) 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 the marine population is continuous (one-dimensional) while lakes are discrete. Dispersal occurs both locally along the coastline within the marine habitat, as well as between the marine habitat and the lakes, with a lake–ocean migration rate denoted m . No migration occurs directly between the freshwater populations. 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 non-migrant 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 non-migrant 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 non-migrant 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.

Colonization of newly formed 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 that had previously been exchanging alleles with older freshwater populations. 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^{-8} per locus per generation, as described in Kelleher et al. [2018]. The tree sequence output by 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 used neutral mutations to calculate F_{ST} for each locus, which we 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 $1 - (p_f(1 - p_f) + p_m(1 - p_m))/(2\bar{p}(1 - \bar{p}))$ [Gillespie, 1998].

Data availability

The source code used to run and analyze the simulations in this paper are available at <https://github.com/jgallowa07/SticklebackPaper>.

Results & Discussion

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 among 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. Between these two extremes, genetic variation that allowed adaptation to freshwater habitats could move relatively easily

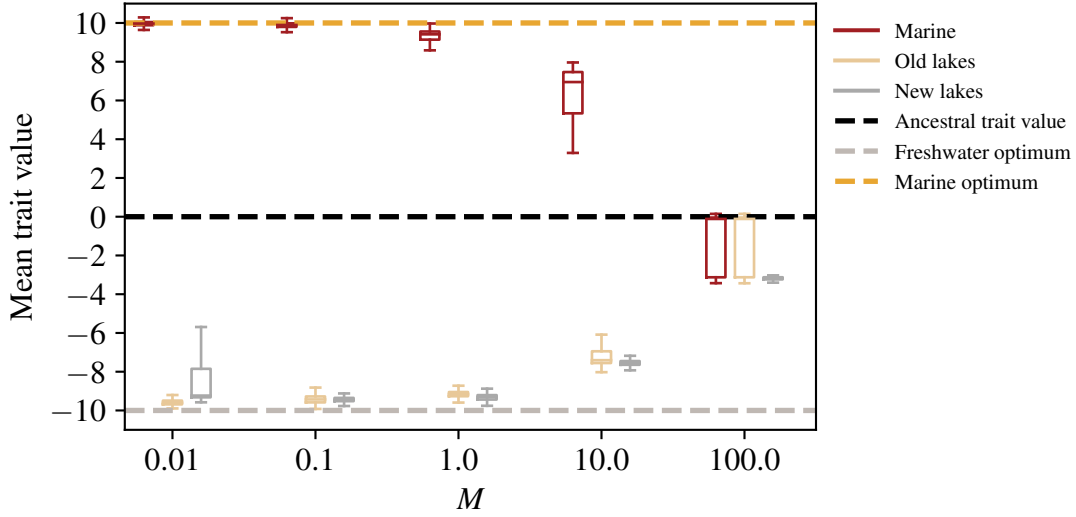


Figure 2: Distribution of mean individual trait values across generations of the simulation (recording started after introduction of the new lakes at 100,000 generations), for different migration rates. The dashed yellow and grey lines at ± 10 give the optimum phenotypes in the marine and freshwater environments, respectively.

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 dramatically accelerate adaptation in new lakes.

Rapid local adaptation at intermediate gene flow rates

Local adaptation occurred in all simulations with the exception of the highest gene flow at which half of each population was composed of migrants, as can be seen by the mean trait values of Figure 2. Figures 3 and S3 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 adaptive alleles in the old lakes during the first few thousand generations manifests as jumps in the mean trait value – which move the trait by an amount of order 1 every few hundred generations (Figure 3). 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.

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 adapted in just under 60 generations.

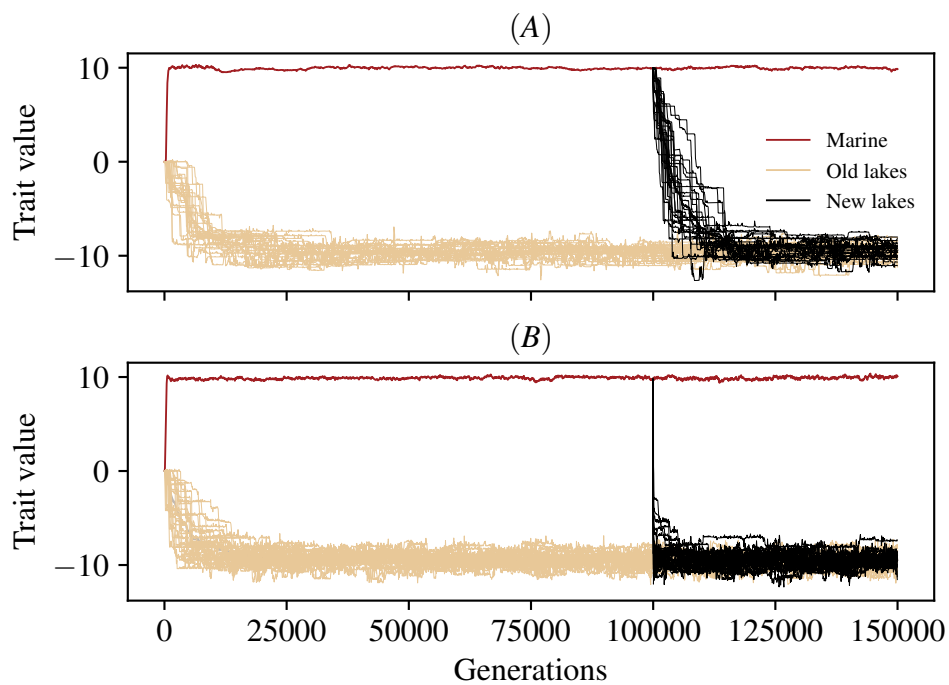


Figure 3: Mean individual trait values in the marine habitat (red line), the original lakes (tan lines; average thicker), and the new lakes (black lines; average thicker), across the course of two simulations, with migration rates of **(A)** $M = 0.01$ and **(B)** $M = 0.1$ migrants per lake per generation, respectively. Optimal trait values in the two habitats are at ± 10 . Analogous plots for other migration rates are shown in Figure S3.

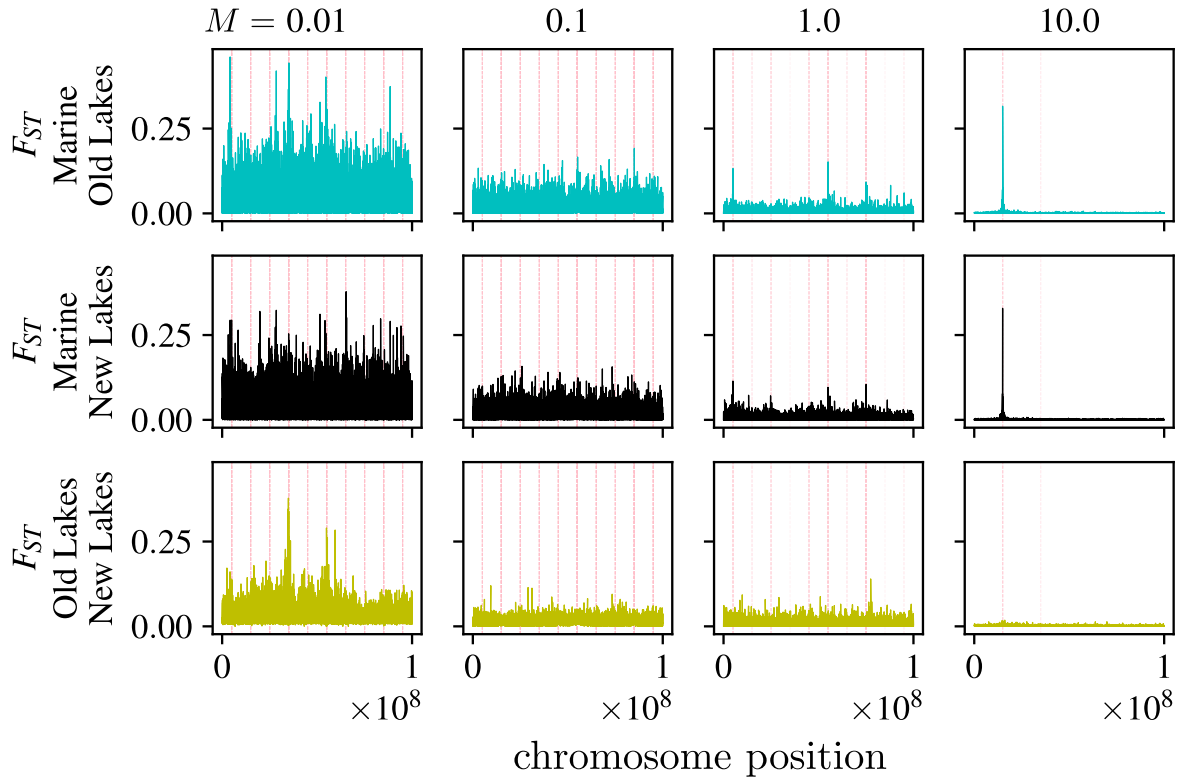


Figure 4: Average F_{ST} in windows of 500Mb between: **(top)** marine habitat and old lakes; **(middle)** marine habitat and new lakes; and **(bottom)** old and new lakes. The columns are ordered by M (the number of migrants per lake, per generation) and show F_{ST} values for a separate simulation, 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 partially transparent pink dashed vertical lines, so darker shades of pink show more freshwater adapted alleles at that location.

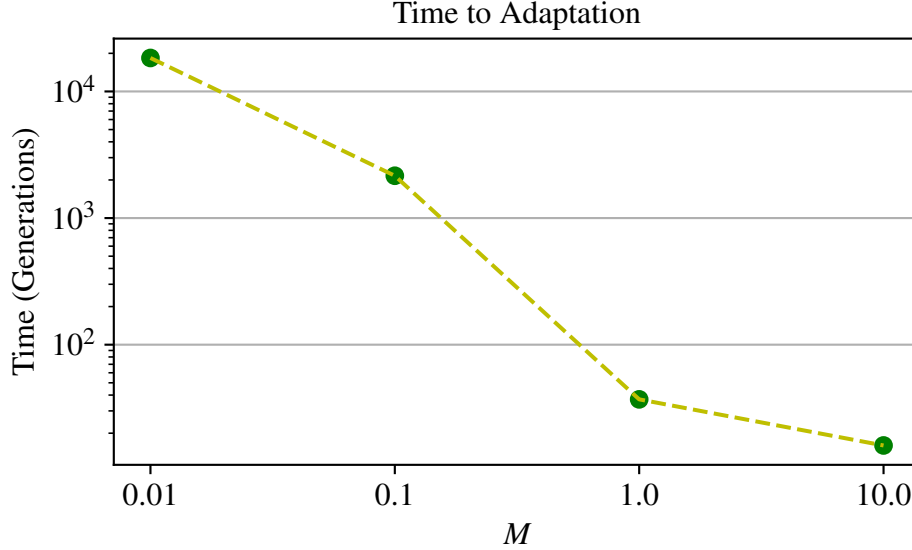


Figure 5: Time to adaptation as a function of migration rate. The time to adaptation is measured as the number of generations until the introduced population’s mean phenotype comes within 0.5 of the original lakes average phenotype. Each point represents a single simulation run. (Adaptation did not occur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

Widespread allele sharing among lakes

Adaptive alleles were shared between lakes at many rates of gene flow, but not at the lowest. 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 in the absence of gene flow, 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 among newly derived 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. (So, “captured” alleles as defined above are pre-existing, but not all pre-existing alleles are captured.) 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 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 $1/25^{th}$ 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 strongly suggest that the dramatic increase in speed of local adaptation we observed above occurs because higher gene flow allows sharing of freshwater alleles among populations. We confirmed this finding 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 among lakes, at $M = 10.0$ migrants per lake per generation the constant influx of alleles between the habitats creates substantial migration load. This level of gene flow only replaces 5% of each population each generation with migrants from the other habitat, but is sufficient to shift the mean trait values to nearly half their optimal values, as seen in Figure 2.

The efficacy of genomic scans of selection depends on gene flow

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? This question has been extensively studied, both with simulation [e.g., Southcott and Kronforst, 2017, Lotterhos and Whitlock, 2015] and in the wild [reviewed in Harrison and Larson, 2016, Hoban et al., 2016]; our simulations provide a detailed look where the truth is known. 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. These F_{ST} values are calculated using all polymorphic loci, including both those that affect phenotype as well as neutral variants. 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 differentiation will only be successful given enough migration between habitats. But how many of these peaks are

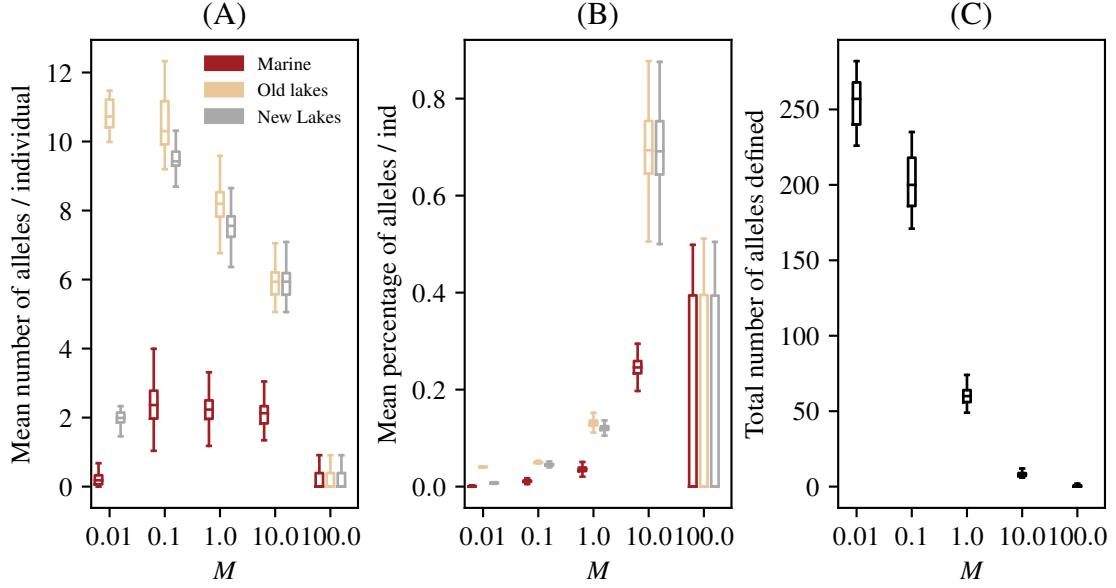


Figure 6: Amount of standing freshwater variation by habitat, across migration rates. Each plot counts “pre-existing freshwater adapted alleles”, that are common in the original lakes but rare in the ocean (see text for definition). **(A)** Mean number of these alleles per individual. **(B)** Mean percentage 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 throughout each simulations. The horizontal axis shows M , the mean number of migrants per lake per generation.

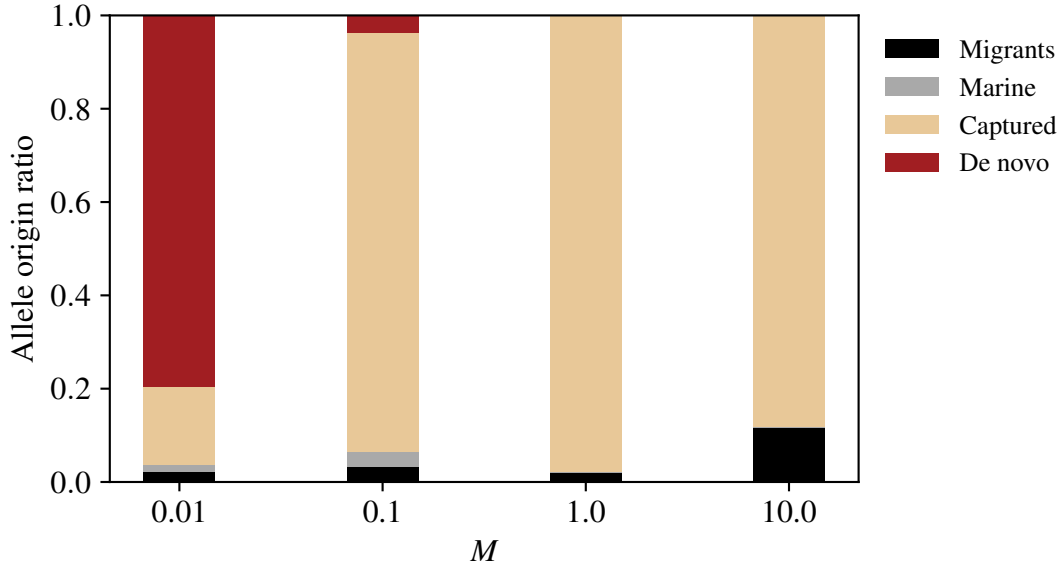


Figure 7: **(Origin of adaptive alleles:)** Each bar plot shows the origins of all trait-affecting alleles above frequency 50% in at least one new lake, classified as **(red)** new mutations, **(blue)** post-colonization migrants, **(green)** “captured” from pre-existing lakes, or **(orange)** standing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

actually underlying trait differences that form the basis for local adaptation? To quantify this, Figure S1 shows the power and true 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 at which a large percentage of peaks (above $F_{ST} = 0.05$) actually lie on top of regions which 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. In other words, with high rates of dispersal between population, F_{ST} peaks may reliably identify causal loci, but not all causal loci will appear as F_{ST} peaks. It’s important to note that F_{ST} in Figures 4 and S1 was calculated after pooling together all lakes and all marine individuals. For comparison, Figures S4 & S2 shows F_{ST} calculated with individuals only in the first fifth of the range (5 lakes and corresponding marine habitat). These show a substantially lower true positive rate, as population-specific noise swamps any signal between the lakes.

As has been suggested elsewhere, introgression aids identification of locally adaptive loci from scans for differentiation. This is generally because shared adaptive alleles provide something akin to independent replicates, allowing signal to emerge above the noise created by genetic drift. However, the required sample sizes and conditions on levels of admixture may be restrictive in practice. More generally, the difficulty of correctly identifying causal loci in even our most ideal situation calls for caution (and proof-of-concept simulations) in designing and carrying out scans for alleles underlying locally adaptive, polygenic traits.

Alleles for freshwater adaptation are mostly present in the initial generation

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 *origin* of the alleles underlying the phenotype is still unknown. In our simulations migration from marine individuals into the new lakes continues throughout introduction and adaptation: but is consistent influx of standing genetic variation a necessity for rapid adaptation of the population? To answer this, we traced the genealogy of *all* adaptive alleles in *all* individuals from the introduced lakes, after local adaptation. Surprisingly, for all cases with the exception of $M = 0.01$, we found that the majority of adaptive allele origins traced back to the original generation of inhabitants in the lake (Figure 7). In our simulations, any reasonable subset of the ancestral population has the potential for rapid adaptation without the need post-colonization hybridization events.

If effectively capturing standing genetic variants is the key to rapid adaptation, as we have presented thus far, why is there a large difference in speed of adaptation between dispersal rates of $M = 0.1$ and $M = 1.0$ seen in Figure 5? This is surprising because we see a similar quantity of allele sharing at $M = 1.0$, but much more rapid adaptation (≈ 60 versus 2000 generations).

| | 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, “Best” quantified as the most negative trait value achievable with intact haplotypes, averaged across populations (see text for details).

A possible 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 that adaptation in the new lakes must wait for recombination to separate freshwater and marine adapted alleles. A freshwater and a marine allele (of roughly equal and opposite effects) in close proximity could form an essentially neutral locus, which would persist in the marine population much longer than a freshwater allele not “masked” in this way. This suggests that a reasonable portion of freshwater alleles in the marine population would be linked to marine alleles. This genetic variation would be more difficult to access, however, so if there is sufficient additive genetic variation available to a new population in unlinked alleles, this will be used first. However, if the genetic variation initially available is not sufficient for a new population to adapt, then adaptation might need to wait for recombination to separate these haplotypes. Similar effects have been noted before in simulation [e.g., Yeaman and Whitlock, 2011].

In other words, perhaps 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, this calculation supports our explanation above: it appears that adaptation at $M = 0.1$ is slower because it must wait for intralocus recombination to free up genetic variation present but masked in the marine population.

Simulation results align with theoretical expectations

How do our results compare to what is expected from population genetics theory? Our simulations included many loci under selection and in linkage to many other selected alleles, which makes precise calculation impossible. Nonetheless, rough calculations based on simple population genetics theory – with the benefit of hindsight – turn out to describe qualitatively most of the aspects of adaptation we observed above. 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], but doing so is beyond the scope of this paper. The arguments we use here are standard in population genetics [see e.g., Gillespie, 1998].

One of the most useful things that theory tells us is about the fate of a new allele in a lake, that has appeared by either migration or mutation. If the allele has fitness advantage s – i.e., when it is rare but present in n copies, 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. Since we are studying a quantitative trait, the fitness effect of each allele depends on the population context: if the individuals in the population all have trait values z units above the optimum, and the allele has effect size $-u$ in heterozygotes, then the fitness advantage of the allele is the ratio of fitnesses with and without the allele. As we calculate fitness here, this is $s(u) = \exp(-\beta((z - u)^2) / \exp(-\beta z^2)) \approx 2\beta z u$, where $\beta = 1/450$. 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 $\mu_L = 0.04$ per generation, and these are divided evenly in six categories: additive, dominant, and recessive, in either direction. Therefore, a new additive or dominant effect mutation appears once every 75 generations, on average. The effect size of each new mutation is randomly drawn from an Exponential distribution with mean $1/2$, and so averaging the probability of establishment over this distribution, we get that the probability that a dominant mutation manages to establish in a population differing from the optimum by z is roughly $\int_0^\infty 4\beta z u \exp(-2u) du = \beta z$. Multiplying the rate of appearance of these mutations with the probability they establish predicts that the rate of establishment of dominant mutations is $\beta z / 75$, i.e., about one such mutation every $33750/z$ generations. During the initial phase of adaptation, the populations begin at around distance $z = 10$ from the optimum, and z decreases as adaptation progresses. Mutations that successfully established are more likely to be strong: the distribution of the effect sizes of these successfully established mutations has density proportional to $u \exp(-2u)$, which is a Gamma distribution with mean 1 and shape parameter 2. There are also additive alleles: these have half the effect in heterozygotes, and so roughly half the probability of establishment. 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 S3.

Standing variation How are freshwater alleles maintained in the ocean, where they are deleterious? Assuming that fish in the marine environment are close to their phenotypic optimum, an allele that when heterozygous moves the trait u units in the freshwater direction, has fitness roughly $\exp(-\beta u^2) \approx 1 - \beta u^2$, 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). For a typical allele with effect size $u = 1$, the equilibrium migration-selection frequency is greater than this threshold if $m/\beta > 1/400$. Since $1/\beta = 450$, if $m \geq 5 \times 10^{-6}$ this suggests that there is a good chance 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 effect size $u = 1$ only has probability of around $1/20$ of establishing locally, and so must be present in about 20 copies to ensure establishment. Putting these calculations together, we expect migration-selection balance to maintain sufficient genetic variation for new lakes to adapt if $m \geq 10^{-4}$, which corresponds to $M \geq 0.4$. This is in good agreement with our observations. However, this calculation treats each allele independently; in practice we found that standing freshwater variation in the ocean might be masked by linkage to compensatory marine variants.

Migration The key quantity regulating the amount of standing variation in the ocean is 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.

We expect at least two other aspects of colonization and migration to be relevant. First, the size of the initial population of marine individuals colonizing each new lake affects how much of the marine genetic diversity is present. In our simulations, new lakes began with a full set of individuals, as may happen in uplifted tidal habitats. If instead each new lake began with only a few individuals (inducing an initial bottleneck), subsequent migration of alleles would likely become more relevant. If in addition, there was no subsequent “upstream” migration, adaptation might need to rely on new mutations, as at our lowest migration rates. However, exactly where these boundaries lie will require additional theoretical or simulation work.

Population size In our simulations, the marine and freshwater populations are of equal size. More generally, we expect the downstream migration rate that is required to maintain standing variation there to scale with the size of the marine population, since the same number of migrants make up a smaller proportion of a larger population. So, if the marine population was well-mixed and tenfold larger than all freshwater populations combined, the number of migrants per lake per generation would need to be ten times larger than in our simulations to maintain sufficient standing genetic variation. If the marine population was a thousandfold larger, adaptation from standing genetic variation that is deleterious in the ocean might be impossible. The fact that repeated adaptation from standing genetic variation has been shown to occur in real world threespine stickleback populations argues that the population sizes of marine and combined freshwater stickleback are not that different. While this might at first seem implausible given the ocean is large and lakes are small, there are hundreds of thousands to millions of lakes inhabited by threespine stickleback in coastal regions of the Northern Hemisphere, and the limiting habitat in the ocean constraining

marine stickleback population sizes is likely to be coastal nesting areas. Together, these facts make the equivalency of marine and freshwater stickleback population sizes much more plausible.

Conclusion

In this paper, we have analyzed a relatively realistic simulation study of a coastal meta-population to understand how repeated local adaptation operates on complex standing variation across a wide range of gene flow rates. We have shown that rapid and parallel adaptation similar to that documented in natural stickleback populations occurs over a realistic range of parameter values. These simulations therefore provide an in-depth look at (and quantitative proof-of-concept of) the “transporter” hypothesis suggested by Schluter and Conte [2009]. Selection is able to rebuild the freshwater haplotype at a rapid pace (in tens of generations) from variation present at migration-selection balance in marine populations. A finding of practical consequence is that the efficacy of F_{ST} -based genomic scans varies significantly with migration rate. Perhaps surprisingly, we found that the majority of adaptive variant can be traced back to the original generation of new habitats, with little role for post-colonization migrant alleles. In other words, a randomly chosen subset of marine stickleback carry the capacity to rapidly adapt in freshwater habitats without *any* continued connection to the rest of the species. Thus, the large marine population was able to harbor and distribute alleles that were deleterious in the ocean habitat, but adaptive in freshwater habitats, to surrounding freshwater populations with only a few migrants per population, per generation. A few good stickleback suffice.

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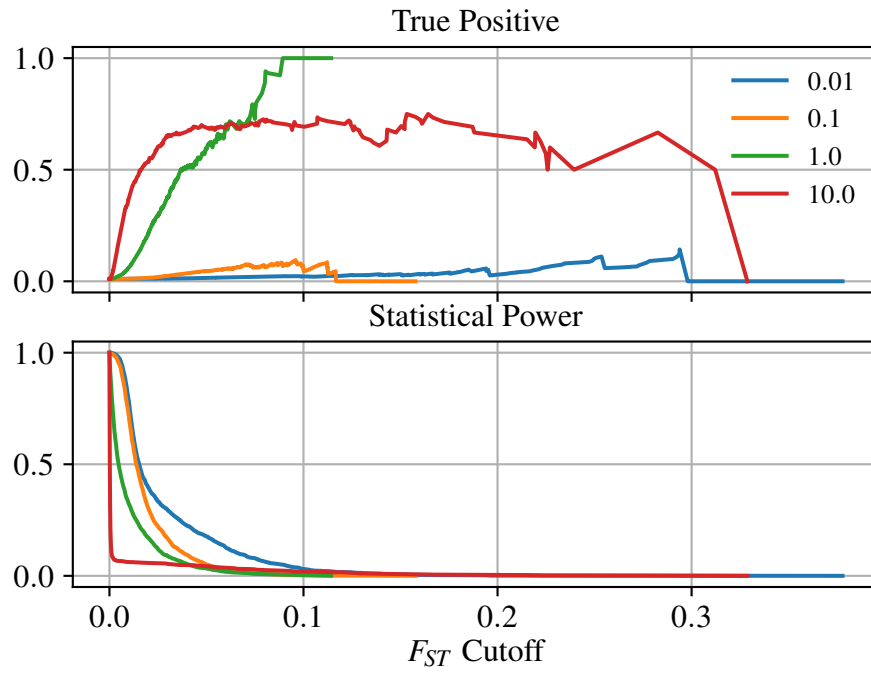


Figure S1: Statistical power and true positive rate as a function of F_{ST} threshold. Statistical power is the likelihood that a SNP will be predicted to have an effect on phenotype when there is an effect to be detected. True positive rate is the proportion of SNPs above the F_{ST} threshold that affect phenotype. Line labels give the respective values of M .

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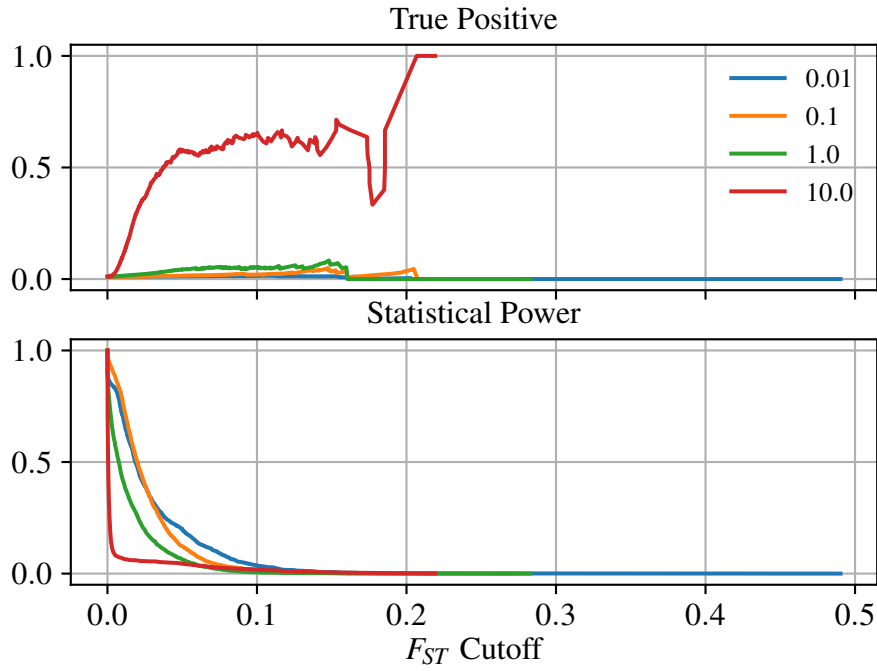


Figure S2: Statistical power and true positive rate as a function of F_{ST} threshold. As in Figure S1 but computed only using the first one-fifth of the total range – i.e., fish in the first five lakes and those in the ocean with coordinate less than 5.0. Line labels give the respective values of M .

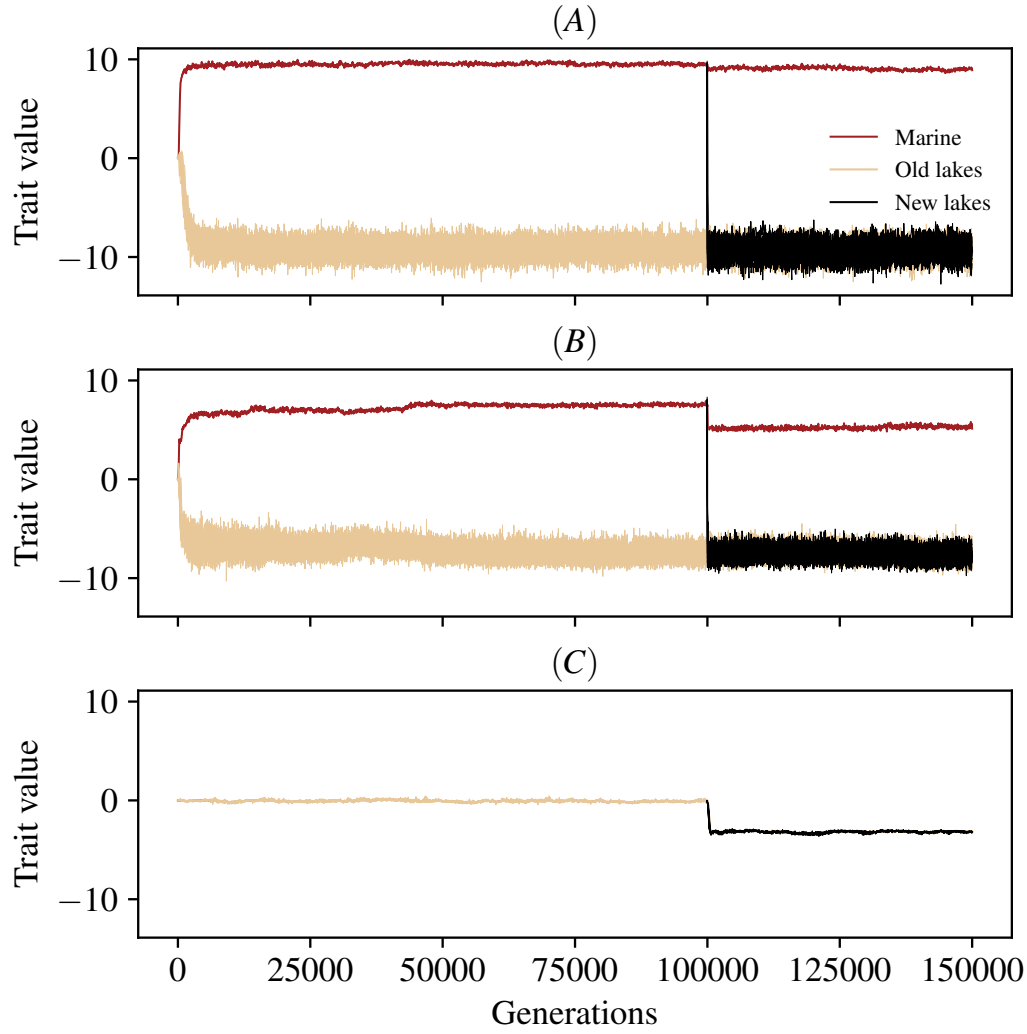


Figure S3: Mean individual trait values in the marine habitat (red line), the original lakes (tan lines; average thicker), and the new lakes (black lines; average thicker), across the course of two simulations, with migration rates of **(A)** $m = 5 \times 10^{-3}$, **(B)** $m = 5 \times 10^{-2}$ and **(C)** $m = 5 \times 10^{-1}$. (i.e., $M = 1, 10$, and 100 migrants per lake per generation, respectively). Optimal trait values in the two habitats are at ± 10 .

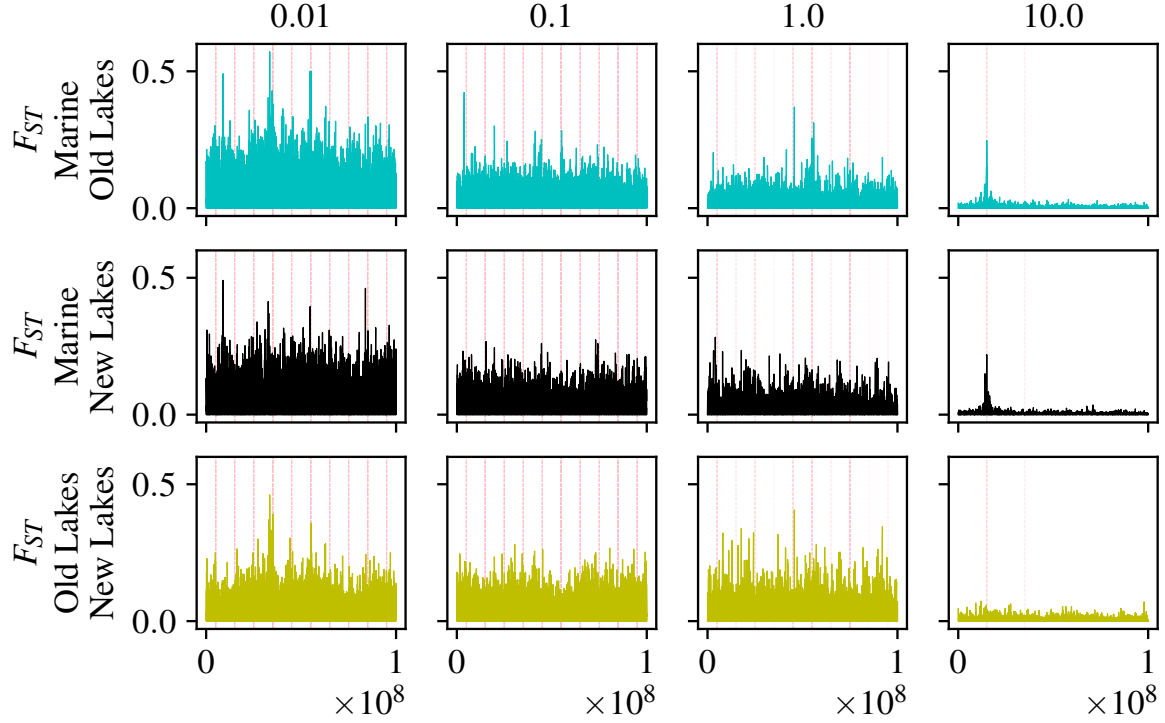


Figure S4: Average F_{ST} in windows of 500Mb between: **(top)** marine habitat and old lakes; **(middle)** marine habitat and new lakes; and **(bottom)** old and new lakes. Each plot shows F_{ST} values for a separate simulation, with columns corresponding to increasing gene flow from left to right. 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 partially transparent pink dashed vertical lines, so darker shades of blue imply more freshwater adapted alleles at that location.

Resubmission Cover Letter
G3

Jared Galloway,
William Cresko,
and Peter Ralph
September 26, 2019

To the Editor(s) –

We are writing to submit a revised version of our manuscript, “A few stickleback suffice for the transport of alleles to new lakes”. Many thanks to the reviewers for thoughtful and helpful comments, to which we have responded in detail. We sympathize that additional simulations would be informative and interesting, but the amount of work that these would entail is beyond our capability at the moment. We appreciate being explicitly given the option to omit them. Otherwise, we think that the revisions have helped to clarify and generally improve the manuscript. We hope you agree.

Sincerely,

**Jared Galloway, Bill Cresko, and Peter
Ralph.**

Reviewer AE:

I agree particularly with questions from two of the reviewers asking for explanation or additional simulations on the founder population size, with reviewer 3's point about clarifying old vs new, and reviewer 1's request for more simulations. I do understand that simulations can be time consuming, however, and want to make clear additional simulations are not a requirement for acceptance (but would be rad if feasible).

Additional simulations exploring more of parameter space would be totally awesome, we agree. However, besides the constraints on our time noted above, we also note that it's not clear how much additional value it would have to delimit the region of parameter space on which our results hold: it would be more satisfying to develop theory that would apply to larger populations of varying sizes with fluctuating migration rates, for instance.

(AE.1) (p. 14, l. 330) *My lab and I also spent some time debating what was meant by the explanation at the bottom of page 13 – I think more effort to clarify here would be helpful.*

Reply: Thanks! We have rewritten this to hopefully be more precise. (p. 14, l. 330)

(AE.2) *Finally, we also wondered why the simulations started at a trait value of 0 instead of 10 – it makes comparison of old vs new a bit harder than seems necessary.*

Reply: We don't have a good reason. Apologies.

Reviewer 1:

This is a very well done work addressing the fundamental problem of local adaptation in metapopulations; stickleback are a nice study system but the findings are relevant for a wide variety of systems. I enjoyed it very much, and my only wish is that the simulation has been run more than once. This is not necessary for speed of adaptation - within-simulation replication of lakes is totally sufficient for this - but for aspects related to genome scanning, numbers of adaptive alleles in a metapopulation, and haplotype-wise effect sizes (table 1), since (especially at high M) the number of adaptive alleles is very few across the whole metapopulation. That said, I do not insist on rerunning the simulation, since it must be very computationally intensive.

Thanks for the nice words! We sympathize, but think that the number of simulations is sufficient for at least estimating the rough size of these effects – these effects are mostly monotonic in migration rate, so that for instance we have an estimate of the number of adaptive alleles at $M = 1.0$ but also know it is bracketed by the values at $M = 0.1$ and $M = 10$. Further simulations would only give us more precise estimates of the mean and an idea of the standard deviation.

(1.1) (p. 12, l. 269) *“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.”* Very cool. It gives a very intuitive guideline

IMPORTANT:
don't re-order or delete “points” below - it messes up the automatic numbering!

as to which genetic architecture of local adaptation to expect given a certain migration rate. There is one neat aspect of this result that is perhaps worth better emphasizing: detectable F_{st} peaks at truly adaptive alleles can only be very few. If we had replicate runs we could say how many true F_{st} peaks, on average, we see at $M=1$ and $M=10$. Many published studies (particularly in marine animals) report dozens if not hundreds of F_{st} outliers throughout the genome; your simulation here suggests that most of them have to be false positives of some kind, especially since migration in the sea is likely to be high.

Reply: We agree with the reviewer that this is an important point, and that our results are consistent with the above hypothesis. However, to quantify and assert that previous empirical studies are reporting false-positives would require many more simulations and analysis in many more situations, which we believe is outside the realm of this paper.

(1.2) Also, about Fig. S1: I am confused what is “statistical power” here : “likelihood that a SNP will be predicted to have an effect on phenotype when there is an effect to be detected”. In this model every SNP has at least *some* effect on phenotype, so what defines “effect to be detected” then?

Reply: Actually, there are many SNPs that do not affect the phenotype, but these were added as a secondary step after simulation, so this is an easy mistake to make. We’ve added a reminder about this. (p. 12, l. 289)

(1.3) (p. 14, l. 323) “Surprisingly, for all cases with the exception of $M = 0.01$, we found that the majority of adaptive allele origins traced back the original generation of inhabitants in the lake (Figure 7)” This is really cool and counter-intuitive, but maybe it is not too surprising since first-arriving (“captured”) alleles are simply much more numerous relative to the alleles that trickle in later, *before the adaptation is complete*? Indeed, initial colonization involves arrival of 200 individuals in a single generation, which is equivalent to $200/M$ generations of subsequent immigration. Considering that at any appreciable M (≥ 0.1) adaptation happens very rapidly (fig 3 and S3), there might simply be not enough additional immigrant alleles to make a contribution. I wonder how this would work if colonization of a lake was a more gradual process, say 2 individuals per generation (not all 200 at once)? Would the early-arriving alleles still have advantage? (I would bet yes)

Reply: We agree that this is an important point to explore, and a good observation. We have appended to the section on migration to more clearly address this (p. 17, l. 430). The available code could certainly be used in further studies to investigate this question more in-depth, but again feel that is outside the scope of this paper and have decided not to conduct more simulation and analysis.

Reviewer 2:

This is an interesting, and as far as I can judge, a sound simulation study focused on population genetics of adaptation in the threespine stickleback. While the problem(s) approached in the manuscript are interesting and relevant, the way the ms has been pitched makes is mostly interesting for community of researchers interested on sticklebacks. By angling the story in more general terms and bringing stickleback in as model system, broader general appeal might have been achieved.

(2.1) *Latin binomial of the focal model species appears only in the references. It would make sense to incorporate it in the abstract and introduction given that the entire ms is focused on this species.*

Reply: Good point; we’ve added it to the first use of the name after the abstract (p. 2, l. 51).

(2.2) Title: *I would make it clear that this is about the “threespine stickleback” - there are many different stickleback species.*

Reply: We believe the first sentence in the abstract makes this clear to the reader.

(2.3) (p. 2, l. 52) *“to millions of independently derived”. Do the references you cite say so? Can you really back up your claim of millions of freshwater populations?*

Reply: This order of magnitude estimate is roughly based on the length of the coastline (roughly 10,000 miles) and the high density of freshwater lakes in many flat, coastal areas. We have reworded this to be more conservative, however. (p. 2, l. 52)

(2.4) (p. 2, l. 55) *“heterogenous nature of divergence”. I think you should cite here the first (paper using global sampling) showing evidence for heterogenous nature of this genetic divergence: DeFaveri et al. (2011). Global analysis of genes involved in freshwater adaptation in threespine sticklebacks (*Gasterosteus aculeatus*). Evolution, 65(6), 1800-1807.*

Reply: Thank you for pointing this out. The citations has been added.

(2.5) (p. 2, l. 59) P2. *“stickleback in them” - threespine stickleback in them*

Reply: Fixed.

(2.6) p.3 Methods *From here on, the ms is generally very thinly referenced. In particular, it remains unclear to reader where the many equations come from: are they something authors have derived themselves (I guess not?), or who did that?*

Reply: We’re not sure what is meant here – the equations in the Methods are almost entirely choices we made in setting up the model: for instance, the choice of a Gaussian fitness function is almost ubiquitous, and the equation for the phenotype in terms of the selection and dominance coefficients is just writing down the definition. Most of these are options available in SLiM, which we cite. We have included a reference for the (simple) definition of F_{ST} that we use (p. 7, l. 204), and added a clarifying comment to the section of the Results and Discussion that is most equation-heavy (p. 16, l. 371).

(2.7) p.5 *One is left to wonder if it realistic to think that the marine population is as big as 25 freshwater populations. Admittedly, this is discussed to some extent in the discussion, but given the abundance of the threespine stickleback in many seas (there is actually quantitative estimates of this in literature), it seems plausible that the marine population(s) is orders of magnitudes larger than that of typical freshwater population, even if combined. I guess a more quantitative view on this in light of literature would be something worth attempting.*

Reply: We agree, this would be very interesting, and an informative exercise. However, we think it's outside the scope of this paper, and hope the reviewer agrees with our discussion of this point at (p. 17, l. 446).

(2.8) (p. 4, l. 134) *Where does the mutation rate 10^{-10} come from? Why this particular value, and how are the results influenced by choice of this?*

Reply: We chose this to reflect our guess at what might be a general consensus for the mutational target size of a polygenic trait (although we recognize that opinions differ). We've added some more discussion to contextualize our choice to the paper (p. 4, l. 134). Some more on this: since per-bp mutation rates are likely closer to 10^{-8} , a rate of 10^{-10} represents roughly one adaptive target per 100 bases across the 10^6 bases where mutations that affect the phenotype might occur. This translates to a total *potential* target size of 10^4 bp, which seems generous but not impossible for a polygenic trait.

(2.9) P4. *It is bit unclear here how LD as was dealt with in the simulations. Are all the loci assumed to be unlinked?*

Reply: All chromosomes are linear with a per-nucleotide recombination rate of 10^{-8} . This has been clarified here (p. 4, l. 131).

(2.10) (p. 6, l. 146) *What happens if generations are overlapping, as is the case in sticklebacks?*

Reply: We know of no reason why in this case overlapping generation would affect the results.

(2.11) (p. 6, l. 153) *Here the authors say that a different model in SLiM would have been more appropriate to be used, but that they “do not expect this to influence the results”. This does not sound very good - why not to check this? Why not to rerun the analyses if a more appropriate model is available?*

Reply: In a perfect world, we would re-run and compare. However, to re-write this model as non-WF would require a relatively significant amount of change to the code as well as much more computational power to re-run the analysis. We know of no reason to expect differences between the two implementations – the main difference being in elegance – and so have opted to stick with the current model. We have altered the wording a bit to better convey this (p. 6, l. 154).

(2.12) Fig 1 *The modeled scenario is probably correct over shorter geographic distances. But there is plenty of empirical evidence to show that marine threespine stickleback populations over larger geographic distances are not genetically homogenous. I think it would be appropriate to acknowledge this somewhere in manuscript.*

Reply: We agree, it should be made clear that the oceanic population is not panmictic but rather has one-dimensional continuous spatial structure. We have clarified this here (p. 6, l. 157).

(2.13) (p. 12, l. 283) *“The efficacy of genomic scans of selection depends on gene flow”. I found this section rather uninformative: all this has been studied in quite detail already in many theoretical (and empirical) papers. This could be shortened. And references to earlier findings would be in place here too - there is no single reference to earlier works here at all.*

Reply: We believe this finding supports existing studies, specifically, how stronger genetic drift with less migration leads to higher background F_{ST} . However, for our study, showing how greater sharing of adaptive alleles provides a shared signal across populations seemed necessary for a study of selection on standing variants. Thus, we have added some more citations to previous work, but otherwise have left this section as is.

(2.14) (p. 15, l. 364) *“Simulation results align with..” It is nice that the results are referenced back to theory. I was left to think that it might have been better to do this by combining this section with the actual results. Now the structure of the ms appears to be: 1) Results & Discussion, 2) Discussion. Furthermore, references to theory are given in the first paragraph, but not after this. Readers do not know where all the equations are derived from.*

Reply: We considered this sort of re-ordering when writing the paper, but decided that it would be more confusing to jump back and forth to the population genetics heuristics. The equations are standard, and we’ve tried better to flag this at the start of the section. (p. 16, l. 371)

(2.15) (p. 17, l. 418) *“we found that standing..” Is “found” right word here? You made an inference, even a guess, that this was so. But you did not prove this?*

Reply: It is a good point that although we found linkage between opposite-effect alleles, this masking might not have a substantial effect. We’ve hedged a bit more in this statement.

(2.16) (p. 17, l. 436) *“Population size”. Here it is argued that population sizes of marine and freshwater populations are not so different. I found it hard to believe this in the light of quantitative data there is. Could it simply be so that marine population is actually very much larger, but structured (as there is genetic evidence for this in many papers) so that the fraction feeding the freshwater colonizations is to some degree isolated from ones feeding the freshwaters in a particular area? This would in effect make relevant portion of the marine population smaller, without need to postulate that the total marine population equals in size to freshwater populations, and that there are millions of lakes inhabited by three spine sticklebacks. Note also that in introduction it was claimed millions of freshwater populations, here you say “if not millions” - inconsistent.*

Reply: We’ve made the two sections consistent, and still heartily agree that quantifying this would be an interesting and useful endeavor, but beyond the scope of the current paper. The proposed substructure in the ocean doesn’t seem very plausible to us, although there is little enough data on oceanic demography that this may well be correct. Still, is our argument that they face similar constraints on breeding ground not convincing?

Reviewer 3:

... Overall, the paper finds clear support for the transporter hypothesis, and uncovers some key conditions under which the transporter mechanism is most likely to occur. An interesting insight is that little gene flow can suffice to provide enough standing variation in the marine gene pool for parallel freshwater adaptation to mostly involve pre-existing (instead of de novo). I also found

interesting that freshwater-adaptive alleles can be maintained for a relatively long time in the marine population, thus indicating that it's not a necessity for gene flow to occur at all times between freshwater and the sea for genetic variation to be shared by descent among freshwater populations. I also liked the paper's insights on the importance of considering entire adaptive haplotypes - and not only the number of adaptive alleles - for repeated adaptation.

The paper is well written and I like this work and don't see any fundamental flaws in the way the simulations were set up or in how the results are interpreted. Of course, one always needs to make certain assumptions when setting up simulations (such as on effect sizes of loci etc.), but I think that the authors explore the parameter space appropriately.

Thanks! We're glad you liked it.

In what follows, I provide suggestions on how to further improve the work. Most of these points should be understood as recommendations. A side-note: Please make sure to add line numbers to your next submission. It is difficult for reviewers to refer to specific text passages without line numbers being present.

Sorry about that; we're submitting with line numbers this time.

(3.1) *Founder effects followed by population growth: I wonder how founder effects during the colonization of the 'new lakes', followed by population growth, would influence the results? It seems that this was never explored, as each new lake had a constant population size of 200 individuals (rather low). I don't expect there to be a qualitative change in the overall results, but the relative contribution of de novo vs. standing variants to adaptation might well be influenced by a more colonization scenario. If the authors decided not to run additional simulations on this issue, it might at least be worth speculating on how founder effects followed by population growth might influence some of their results (such as the results shown in Fig. 7).*

Reply: Good point: we agree this would be one of the first additional parameters to vary. We have discussed this further (p. 17, l. 430), but as we explained above, haven't done more simulations to expand coverage of parameter space.

(3.2) *If possible, it would be interesting to check what type of mutations - in terms of effect size and in terms of dominance - fall under the 'captured', 'marine' and 'de novo' categories. I could well imagine, for example, that mutations that can stay in the marine populations relatively long are more likely to be recessive. Is this true?*

Reply: We thought about this also, and did this comparison in some earlier simulations, but any differential representation was not obvious - there was no large effect - and that we'd need many more simulations to detect anything. So: this seems plausible, but speculatively, it doesn't seem to be a major driver of genetic architecture, but rather a minor adjustment.

(3.3) *I'm a bit confused by how the adaptive loci along the chromosome were modeled. I don't fully understand what you mean by mutations occurring at a rate 10^{-10} per locus per generations in ten regions of 10^5 loci each (Page 4, 'Genetic architecture of the trait'). Why are the adaptive loci themselves organized*

in distinct regions? Why isn't there simply a given number of adaptive loci that are evenly spread across the chromosome?

Reply: We agree this was confusing and have edited this section to hopefully make it more clear (p. 4, l. 134). The motivation for 10 regions of effect was to roughly model the distribution of QTL seen in experiments, and to allow us to contrast closely linked variants (within the same region) with more distant ones.

(3.4) Results & Discussion section *Sometimes, it is not fully clear whether you talk about the 'old' lakes or the 'new' lakes. This is often unclear, for example, in the section "Rapid local adaptation at intermediate gene flow rates". Could you make this clear throughout the manuscript?*

Reply: Good point, we have clarified a few ambiguous spots, e.g., (p. 8, l. 227).

(3.5) Figure 2 *Are these the results after 50k generations?*

Reply: Actually, at the time of introduction (so 100,000 generations), thank you for the clarification, this has been noted in the figure caption.

(3.6) *In the legend of Figure 4, it might be good to re-iterate what the number on top of the panels means exactly. Say that these are actual numbers of migrants per population and what the population size is. What is meant by 'M' is said more clearly in the legend to Figure 6, yet, it would be good to here also state (again) how large the populations are. M can only be understood in context of the actual population size. Otherwise, you would need to provide 'm'.*

Reply: We have revised the figure caption and added the label M to the header.

(3.7) *Figure 5: delete '100' along the x-axis.*

Reply: Good point: Fixed.

(3.8) *Typo on page 14: two '-' instead of only one.*

Reply: Whoops! Fixed; thanks. (p. 16, l. 373)

(3.9) *Page 2, 2nd paragraph (beginning): When you talk about the evolutionary history of stickleback, an appropriate citation would also be Bell & Foster 1994.*

Reply: Good catch - added. (p. 2, l. 54)

(3.10) *Page 2, 2nd paragraph: You provide several empirical papers that demonstrate the re-use of standing variation in stickleback. Your list of papers is fair, although I don't think you chose the best papers of all the ones available. There are papers that are not cited that directly show evidence of standing variation in the sea that is being re-used for freshwater adaptation, such as: Jones et al. 2012 (Curr Biol); Haanel et al. 2019 (EvolLett), Feulner et al. 2014 (MolEcol) etc. Evidence for the reuse of the exact same genetic variation at different locations on the northern hemisphere also comes from inversions in the stickleback genome (Jones et al. 2012 (Nature); Roesti et al. 2015 (NatCom)).*

Reply: Great catch, citations added. (p. 2, l. 41)

(3.11) (p. 2, l. 59) *Page 2, 2nd paragraph: you here talk about 'similar phenotypes' that have evolved repeatedly in freshwater, but you provide only two examples. The cited papers are mostly concerned with the genetic basis of a specific phenotype that has repeatedly evolved (plate loss). However, at the phenotypic level, there are many more examples for marine-freshwater adaptations in stickleback.*

Reply: Good point; we have added citations to more examples. (p. 2, l. 59)

(3.12) *Page 3, 2nd sentence ('An alternative hypothesis ... maintained in the marine due to recurrent gene flow'). I would replace 'maintain' with 'present' because it is unclear at this point whether alleles are actually maintained in the marine population (i.e., stay there for many generations), or whether they are just 'washed into' the marine population over and over again from freshwater without staying long in the marine population at all.*

Reply: Good point. Changed. (p. 3, l. 76)

(3.13) *It might be good to cite Hohenlohe et al. 2010 and/or Roesti et al. 2014 to justify that the marine population is modeled to be panmictic (page 4, top). Both these studies have shown very little genome-wide differentiation between samples taken at different marine locations.*

Reply: This was clarified (p. 6, l. 157). The marine population was not modeled as panmictic but rather a unidimensional continuous space with dispersal.