Oceans apart: Heterogeneous patterns of parallel evolution in sticklebacks

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Abstract

Reuse of standing genetic variation is thought to be the main mechanism behind the repeated evolution of the same phenotypes in similar environments. An important model system for the study of genomic mechanisms underlying parallel ecological adaptation in the wild is the three-spined stickleback (*Gasterosteus aculeatus*), which has repeatedly colonized and adapted to freshwater from the sea. Previous studies have identified numerous genomic regions showing consistent genetic divergence between freshwater and marine ecotypes, but most of these studies have been based on limited geographic sampling, and with strong biases towards studies conducted in the Eastern Pacific area. We analysed population genomic data from three-spined sticklebacks with comprehensive global sampling using unsupervised methods to detect loci involved in parallel evolution at different geographical areas. In line with previous studies, we find several genomic regions, including two chromosomal inversions, contributing to global differentiation of marine and freshwater ecotypes. However, signatures of parallel evolution were far stronger in the Eastern Pacific region than anywhere else in the world. With simulations, we demonstrate that since freshwater-adapted alleles exist in the marine populations only at low frequencies, they are

easily lost during founder events, thereby reducing the amount of standing genetic variation available for freshwater adaptation outside of the ancestral Eastern Pacific region. Hence, geographic heterogeneity in access to standing genetic variation due to historical demographic factors appears to provide an explanation to marked geographic differences in the pervasiveness of parallel evolution in the three-spined stickleback. Therefore, the degree of genetic parallelism in the three-spined stickleback model system appears not be as pervasive as earlier studies focused on Eastern Pacific populations have led us to believe.

Keywords: *Gasterosteus aculeatus*; genetic differentiation; linkage disequilibrium; local adaptation; parallel evolution

Introduction

The extent to which the evolution of similar phenotypes arises by selection acting on shared ancestral polymorphism (i.e. parallel evolution, Conte and Schluter 2009) or via distinct molecular evolutionary pathways (i.e. convergent evolution; Arendt and Reznick 2008; DeFaveri et al., 2011; Stern 2013) is a major question in evolutionary biology. A powerful approach to disentangle these processes is to study the genomic architecture underlying the repeated evolution of akin phenotypes in similar environments. After the retreat of Pleistocene glaciers, marine three-spined sticklebacks (*Gasterosteus aculeatus*) colonised and adapted to many newly formed freshwater habitats, evolving repeated changes in a number of morphological, physiological, life history and behavioural traits (Bell and Foster, 1994; Gibson, 2005; Östlund-Nilsson et al., 2006; Hendry et al., 2013; Lescak et al., 2015). Thus, this species has become one of the most widely used model systems to study the molecular basis of adaptive evolution in vertebrates in the wild (McKinnon and Rundle, 2002).

Previous studies of the three-spined stickleback model system have quantified the extent of parallel evolution by identifying genomic regions that are consistently differentiated between marine and freshwater ecotypes sampled across different geographic areas (Hohenlohe et al., 2010, 2019; DeFaveri et al., 2011; Jones et al., 2012; Terekhanova et al., 2014, 2019; Ferchaud and Hansen, 2016; Pujolar et al., 2017; Liu et al., 2018). Studies of parallel genomic patterns of ecotype divergence have historically focused on the Eastern Pacific region (Colosimo et al., 2005; Chan et al., 2010; Hohenlohe et al., 2010, 2019; Jones et al., 2012; Nelson and Cresko, 2018), but with several recent studies emerging also from Atlantic populations (Terekhanova et al., 2014, 2019; Ferchaud and Hansen, 2016; Pujolar et al., 2017; Liu et al., 2018). However, only two studies have thus far included samples from a larger (global) geographic range (DeFaveri et al. 2011; Jones et al. 2012). Based on whole genome sequence data from a limited number of individuals (21) that included samples from Eastern Pacific and Atlantic populations, Jones et al. (2012) identified ~200 genomic regions consistently separating marine and freshwater individuals globally, representing roughly 0.5% of the dataset. They also found that 2.83% of the dataset showed signatures of parallel selection in Eastern Pacific freshwater locations, approximately six times more than at the global scale (Supplementary Fig. 3 and Supplementary Table 7 in Jones et al. [2012]),

suggesting that more loci contribute to parallel evolution at smaller geographic (regional) scales. Such global heterogeneous ecotype divergence is consistent with the results of several other studies as well. Focusing on 26 candidate genes in six pairs of marine-freshwater populations across the globe DeFaveri et al. (2011) found that only ~50% of genes under divergent selection were shared across more than three population pairs and none across all. This suggested a limited re-reuse of ancestral polymorphism at the global geographic scale, implicating either an important role of convergent evolution at larger geographic scales (DeFaveri et al., 2011) or geographic heterogeneity in selective pressure among different freshwater ecosystems (DeFaveri et al. 2011; Stern 2013). Based on a subset of the data from Jones et al. (2012) and using a novel method based on linkage disequilibrium for identifying sets of highly correlated loci, Kemppainen et al. (2015) found that approximately 10 times more loci i showed consistent marine-freshwater differentiation in the Eastern Pacific than at the global scale. Furthermore, studies focusing on parallel evolution within oceans, and even smaller geographic regions, show striking differences in the proportion of loci involved in parallel freshwater adaptation between Pacific and Atlantic (Terekhanova et al., 2014, 2019; Hohenlohe et al., 2010; Jones et al., 2012; Ferchaud and Hansen 2016; Pujolar et al., 2017; Nelson and Cresko, 2018; Liu et al., 2018). For instance, Terekhanova et al. (2014) recovered only 19 highly localised genomic regions involved in parallel freshwater divergence in the White Sea in contrast to Hohenlohe et al. (2010) and Nelson and Cresko (2018) who found large genomic regions involved in parallel freshwater divergence across almost all chromosomes in the Eastern Pacific. However, no study has yet addressed these conflicting results, and therefore, the potential mechanisms underlying this apparent large-scale geographic heterogeneity in genome-wide patterns of parallel evolution in three-spined sticklebacks remain unaddressed. To this end, we analysed population genomic data from a comprehensive sampling of all major geographic areas inhabited by the three-spined stickleback, and used supervised and unsupervised methods to detect loci involved in parallel marine-freshwater divergence at different geographical scales. Based on earlier observations (DeFaveri et al., 2011; Terekhanova et al., 2014, 2019; Kemppainen et al., 2015; Ferchaud and Hansen 2016; Pujolar et al., 2017; Liu et al., 2018), we hypothesize that the genetic parallelism in response to colonisation of freshwater environments by marine

sticklebacks is heterogeneous at the global scale, and the degree of genetic parallelism is much stronger in the Eastern Pacific region than elsewhere.

We further seek to understand and discuss the ultimate causes of marked regional differences in genome-wide signatures of parallel genetic differentiation among ecotypes. To explain the mechanism behind the repeated use of the same alleles in independent freshwater populations of sticklebacks, Schluter and Conte (2009) proposed the “transporter hypothesis”. This hypothesis postulates that three-spined sticklebacks have repeatedly colonized and adapted to freshwater environments via selection on standing genetic variation in large marine populations, where recurrent gene flow from previously colonized freshwater habitats maintains freshwater-adapted alleles. Three-spined stickleback populations have persisted in the Eastern Pacific for approximately 26 Mya (Matschiner et al., 2011; Meynard et al., 2012; Betancu-R et al., 2015; Sanciangco et al., 2016), from which the Western Pacific and the Atlantic were colonized much more recently, during late Pleistocene (36.9-346.5 kya, Orti et al., 1994; Fang et al., 2018, 2019). During bottlenecks and founder events, rare alleles are lost at a higher rate than common alleles (Halliburton and Halliburton, 2004; Hyten et al., 2006). Since freshwater-adapted alleles exist in the marine populations only at low frequencies (Schluter and Conte, 2009), it is likely that they were lost to a higher degree than neutral variation during founder events, thereby reducing the amount of standing genetic variation available for freshwater adaptation outside of the Eastern Pacific. However, this hypothesis has not received much attention. To this end, as proof of concept, we used individual-based forward simulations designed to mimic the transporter hypothesis, and the general global ppulation demographic history of three-spined sticklebacks outlined above, to test his hypothesis. We conclude with a discussion on potential biological and demographic explanations for the high degree of geographic heterogeneity in patterns of parallel genomic divergence, and reflect upon the representativeness of the eastern Pacific three-spined stickleback populations as a general model for the study of parallel evolution.

Material and Methods

Sample collection

To study parallel evolution at the global scale, we obtained population genomic data from 166 individuals representing both marine and freshwater ecotypes from the Eastern and Western Pacific, as well as from the Eastern and Western Atlantic Oceans (Fig. 2j, Supplementary Table 1 and Supplementary Fig. 1). Of these, 62 individuals from 17 populations were sampled and sequenced specifically for this study while data from 66 individuals from 38 populations from Fang et al. (2018) and additional 38 individuals (from 24 populations) from other published studies (Jones et al., 2012; Feulner et al., 2013; Yoshida et al., 2014; Supplementary Table 1) were retrieved from GenBank. Fish collected for this study were sampled with seine nets, minnow traps and electrofishing. Specimens were preserved in ethanol after being euthanized with an overdose of Tricaine mesylate (MS222).

To study the extent of genetic parallelism among freshwater sticklebacks with different phylogeographic histories, we classified global samples into seven biogeographic regions based on their phylogenetic affinities: Eastern Pacific, Western Pacific, Western Atlantic, White and Barents Seas, North Sea and British Isles, Baltic Sea and Norwegian Sea (Fang et al., 2018; Fig. 2j). A summary of coordinates, ecotype and population information concerning sampled individuals and re-acquired samples are given in the Supplementary Table 1.

Sequencing and genotype likelihood estimation

Restriction site associated DNA sequencing (RADseq) using the enzyme *PstI* was performed for the 62 individuals sampled in this study using the same protocol as in Fang et al. (2018), where DNA library preparation and sequencing method are described in detail. RADseq data for 66 individuals from Fang et al. (2018) were retrieved from GenBank (Accessions SRR7067148 - SRR7067275), as were the whole genome sequencing (WGS) data of 38 additional samples from published studies (Supplementary Table 1). All RADseq and WGS datasets were mapped using BWA mem v0.7.17 (Li and Durbin, 2010) to the three-spined stickleback reference genome (release-92) retrieved from Ensembl database (Hubbard et al., 2005). Given the heterogeneity in sequencing depth among different datasets, and particularly the very low coverage of the data retrieved from Jones et al. (2012), most of the

analyses were performed directly using genotype likelihoods avoiding variant calling whenever possible. Genotype likelihoods where estimated from the mapped reads using the model of SAMtools (Li, 2011) as implemented in the program suite ANGSD v0.929 (Korneliussen et al., 2014). Full scripts of genotyping and filtering parameters are publically available through DRYAD (doi: XX). Bases with a q-score below 20 (-minQ 20) and reads with mapping quality below 25 (-minMapQ 25), were removed and variants where only retained if they had a p-value smaller than 1e-6 (-SNP_pval $1e^{-6}$ flag in ANGSD). We retained sites with at least 80% effective sample size (-minInd 133) sequenced at a minimum read depth of 2 (-minIndDepth 2). The sex chromosome (Chr. IX; Kitano et al., 2009, Natri et al., 2013) was excluded from downstream analyses due to sex-specific genomic heterogeneity (Schaffner, 2004; Hedrick, 2007). The raw output of genotype likelihoods from 166 individuals comprised 2,511,922 genome-wide loci.

Supervised approaches to determine marine-freshwater divergence

To test to what extent *a priori* population grouping can bias inferences of the pervasiveness of parallel divergence patterns, we estimated allelic differentiation (as estimated by the fixation index, F_{ST}; Weir and Cockerham [1984], as implemented in VCFtools) between the two ecotypes similarly to previously published studies (see Introduction), but using different geographical subsamples from the complete dataset. Using ANGSD, we called the genotype from the raw dataset, keeping only genotypes with a posterior probability over 0.95 and minor allele frequency above 0.1 (-postCutoff 0.95 in ANGSD; --maf 0.1 in VCFtools). Four datasets with different geographic sampling schemes were analysed (Fig. 1 a-d and Supplementary Fig. 2).

In dataset 1 (Fig. 1a), for each SNP we estimated F_{ST} was estimated between all available freshwater individuals from the Eastern Pacific (n=13 and Supplementary Table 3) and a group of marine individuals (n=12) comprising four marine samples from the Eastern Pacific (same samples as in Jones et al., 2012), two marine samples from the Western Pacific and the remaining individuals were randomly sampled from non-Eastern Pacific locations (n=6). This dataset was the one most biased towards freshwater individuals from the Eastern Pacific. The inclusion of two Western Pacific marine individuals was due to the small number

of available Eastern Pacific marine individuals. Dataset 2 (Fig. 1b and Supplementary Table 3) was constructed to simulate the sampling scheme of Jones et al. (2012): it contrasted freshwater individuals (n=26) against marine individuals (n=26), with half of the individuals in each group always coming from the Eastern Pacific (with the exception of the two Western Pacific individuals as above) and the other half from elsewhere. In dataset 3 (Fig. 1c and Supplementary Table 3), a random sample of all available freshwater individuals (n=26) were contrasted against a random sample (n=26) of all available marine individuals (regardless of geographic location). The dataset 4 (Fig. 1d and Supplementary Table 3) was similar to the second dataset, except with the Pacific individuals coming from the Western Pacific instead of from the Eastern Pacific. Thus, the dataset 4 represents a form of the negative control. Random sampling was repeated 100 times under each sampling scheme, F_{ST} was calculated for each locus in each replicate (VCFtools), and the mean F_{ST} values of all replicates were used.

To summarize the pattern of differentiation (genome-wide F_{ST}) for the four datasets, we calculated moving averages of 95% quantiles and medians in windows of 100 SNPs with a step size of 50 SNPs. Since background F_{ST} was likely to be highly dependent on sample sizes and mixtures of individuals from different populations, we focused on the difference between the median and the 95% quantile of genome divergence. A large difference between the two (median and 95% quantile value) indicating a skewed distribution due to an excess of "outlier" loci, i.e. genomic islands of parallel marine-freshwater divergence.

Unsupervised approach to determine marine-freshwater divergence

We conducted Linkage Disequilibrium Network Analysis (LDna) on the whole dataset (2,511,922 SNPs) to identify and extract clusters of highly correlated loci, i.e. sets of loci affected by the same evolutionary processes. LDna starts by producing a single linkage clustering tree (a hierarchical clustering algorithm which combines two clusters connected to each other by at least one edge) based on a pairwise matrix of LD values (as estimated by r^2; Hill 1968) where nodes represent loci and edges represent LD values above given thresholds (Kemppainen et al., 2015). As the LD threshold is sequentially lowered, more and more loci will be connected to each other in a fashion that reflects how similar phylogenetic signals they

contain. For each cluster merger (with decreasing LD threshold) the change in median LD between all pairwise loci in a cluster before and after merger is estimated as λ. When two highly interconnected clusters merge, λ will be large (as compared to when e.g. only a single locus is added to an existing cluster), signifying that these two clusters bear distinct phylogenetic signals. LDna is currently limited to ~20,000 SNPs at a time due to its dependence on LD estimates for all pairwise comparisons between loci in the dataset. To analyse the whole dataset, we applied a three-step LDna approach to reduce complexity of population genomic data in a nested fashion starting from non-overlapping windows within chromosomes (Li et al. 2018), separately for each chromosome and finally for the whole dataset (Supplementary Information 1). In all steps of LDna, we estimated LD between loci from genotype likelihoods using the program ngsLD (Fox et al., 2019), setting the minimum SNP minor allele frequency at 0.05. Full scripts the LDna analyses are provided in the DRYAD (doi: xxx). In the first step, we only kept loci that were in high LD with at least one more locus (r^2>0.8) within a window of 100 SNPs, as most SNPs in the data were not correlated with any other adjacent loci (so called singleton clusters, Li et al. 2018), and thus are unlikely to be informative in our analyses.

The main evolutionary phenomena that cause elevated LD between large sets of loci in population genomic datasets are polymorphic inversions, population structure and local adaptation, all of which are expected to be present in our dataset (Kemppainen et al., 2015). There are specific and distinct predictions about the population genetic signal and the distribution of loci in the genome that arise from these evolutionary phenomena (Kemppainen et al., 2015). First, clusters with LD signals caused by inversions are expected to predominantly map to the specific genomic position where the inversion is situated. In addition, a Principal Component Analysis (PCA) of these loci is expected to separate individuals based on karyotype, with the heterokaryotype being intermediate to the two alternative homokaryotypes (provided that all karyotypes exist in the dataset) and the heterokaryotypic individuals showing higher observed heterozygosities than the homokaryotypes (although this is not always so clear, for instance when the inversion is new and enough mutational differences have not yet accumulated). Second, a PCA based on loci whose frequencies are shaped by genetic drift is expected to separate individuals on the basis of geographic location, with no (or very little) separation between marine and freshwater

ecotypes. Third, clusters with LD signal caused by local adaptation (globally) are expected to cluster individuals based on ecotype, regardless of geographic location, with both the locus distribution and LD patterns being, to some extent, negatively correlated with local recombination rate (Roesti et al., 2013, 2014). The reason for this correlation is that gene flow between ecotypes erodes genetic differentiation on sites linked to locally adapted loci with the exception of regions where recombination is restricted (for instance in inversions, or close to centromeres or telomeres). No such pattern is expected for LD caused by population structuring as the main source of this LD is random genetic drift that, in the absence of gene flow, generates LD in fashion that is independent of genome position (Kemppainen et al. 2015). If a set of loci contributes to local adaptation exclusively in a particular geographic area, PCA based on these loci will only separate individuals based on ecotype in that region. We considered loci to be involved in parallel evolution only if they grouped individuals of the same ecotype from more than one independent location, as otherwise, it is not possible to discern drift from local adaptation, particularly if N_e is small (i.e. genetic drift is strong). To determine if an LD-cluster was likely associated with parallel freshwater divergence we first used expectation maximisation (EM) and hierarchical clustering methods to identify clusters of individuals in PCAs that contained a minimum of seven individuals of which at least 90% of the individuals are freshwater ecotypes (the “in-group”; dotted line; Fig 2a-i, Supplementary Fig. 3). Second, if such in-groups were detected, we further tested whether this cluster contained more freshwater individuals than expected by chance by permutation (Supplementary Information 2). With less than seven in-group individuals, there was no power to detect significant associations, even if all of them were freshwater individuals.

Proof of concept using simulated data

Several potential explanations for geographic heterogeneity in parallel patterns of marine-freshwater divergence in three-spines sticklebacks have been suggested (DeFaveri, et al. 2011). One such explanation that has not received much attention in the context of three-spined sticklebacks is the stochastic loss of freshwater adapted alleles due to founder events when three-spined sticklebacks colonised the rest of the world from the Eastern Pacific in late Pleistocene (see Introduction). Thus, as proof of concept, we here use forward-in-time

simulations to investigate the conditions for such a scenario under which parallel islands of differentiation (i.e. parallel genomic divergence between marine and freshwater ecotypes) can arise.

Our simulations were aimed at recreating the transporter hypothesis model in the Eastern Pacific, and to simulate the colonization of the Atlantic from the Pacific 30-60 Kya during the last known opening of the Bering strait (Hu et al., 2010; Meiri et al., 2014; Fang et al., 2019) and the subsequent colonization of newly available freshwater habitats. Our model starts by allowing local adaptation in the Pacific until mutation/drift/selection equilibrium (Fig. 4a) for 20 Ky after which the Atlantic is colonised from the Pacific between 36 to 40 Kya with no further trans-oceanic gene flow possible beyond 36 Kya. Both oceans are connected to five independent freshwater populations (gene flow between them is only possible via standing genetic variation in the sea) by symmetrical gene flow of N_em=1, where N_em is the number of migrants per generation. While the freshwater populations in the Pacific are populated already from the simulation start, the freshwater populations in the Atlantic can naturally only be colonised following colonisation from the Pacific. To simulate the retreat of the Pleistocene continental ice sheets, and the colonisation of newly formed freshwater habitats, we firstly removed four of the freshwater populations (at 10 Kya) in both oceans. Immediately following this, four new freshwater locations were allowed to be colonised (from the sea) while keeping one of the original freshwater populations as “glacial refugia”, that could continue to feed freshwater adapted alleles to the sea as standing genetic variation. While this process in reality most likely was gradual and not instant, as in our simulations, the end result is the same; most Pleistocene freshwater populations are no longer present today (bar potential glacial refugia), and most present day populations were colonised from the sea (following the retreated of the Pleistocene ice sheets). Thus, post glacial local adaptation is only possible due to the spread of freshwater adapted alleles from the sea in accordance with the transporter hypothesis (Schluter & Conte, 2009; Fig. 4a-e). The carrying capacity (K), that under mutation/drift equilibrium equates to N_e, was kept at 10,000 individuals in the sea and 1,000 individuals in the freshwater populations. This historical global population demographic scenario and the population sizes are in line with findings from Liu, et al. (2016) and Fang et al. (2018, 2019). Generation time was assumed to be two years. We allowed two different levels of trans-oceanic gene flow during the colonisation of the Atlantic (N_em=1 or N_em=5).

From previous studies (Roesti et al. 2013, 2014), we know that recombination rate variation plays an important role in the formation of differentiation islands around locally adaptive loci. Thus, we configured a genetic map where the recombination distance (as measured in centiMorgans, cM; Haldane [1919]) was shorter at the chromosome centres to simulate the presence of centromeres (recombination distance versus physical distance between loci can be seen in Supplementary Fig. 4). We simulated ten equally sized chromosomes of a total length of 100 cM. This relatively short total map length was chosen such that fewer numbers of neutral markers (n=1000; in the interest of computational speed) would be needed to detect differentiation islands. To demonstrate the effect of the number of differentially selected freshwater-adapted traits (each coded by a single quantitative trait locus, QTL), we simulated datasets with 24, 48 and 72 QTL equally distributed among the eight first chromosomes (3, 6 or 9 QTL per chromosome), leaving the remaining two without any QTL. The positions of these QTL within chromosomes were randomly selected (Fig. 5), and then fixed for all simulation replicates (n=20). We also ensured that there was always some recombination distance between a QTL and the nearest neutral locus (but sometimes by necessity very small, e.g. around the simulated centromeres and telomeres, i.e. no QTL position was exactly the same as any of the positions for the neutral markers). The allelic effects of the QTL were either zero (allele 1) or 10 (allele 2), with the selection optima in the marine habitat being zero and the selection optima in all freshwater populations being 20. Thus, a freshwater individual homozygous for allele 2 for a given QTL meant that individual was at its optimal phenotype, and *vice versa* for marine individuals. The selection intensity was set to 100 for the freshwater habitat and 200 for the marine habitat, lower values translating to stronger selection intensity (see quantiNemo manual for details). Smaller selection intensity in the sea allowed higher frequencies of the freshwater allele (allele 2) in the sea as standing genetic variation to facilitate rapid local adaption in the newly formed freshwater populations from the sea. Per-site germ-line mutation rate was set to 1.0×10^{-8} per generation for all loci. Full simulation details can be obtained from DRYAD (doi: xx). Population genomic datasets for both neutral loci and QTL were saved every 50 generations. All allele frequencies started at 0.5 (hence the initial 10,000 generations to allow mutation-drift-selection equilibrium), thus also initially allowing all QTL to be fixed in the Pacific freshwater populations.

The combination of two trans-oceanic gene flow rates (N_em=1 or N_em=5) and three different QTL settings (ca. 3, 6 or 9 QTL per chromosome) resulted in six different parameter settings. Custom R scripts were used to retrieve and parse outputs from the simulations. We monitored the allele frequency of freshwater-adapted alleles for the QTL to assess levels of standing genetic variation at 50-generation intervals in both marine and freshwater habitats. Genome divergence was estimated for the last generation in the simulations; in each replicate simulations, F_{ST} was estimated for neutral loci between the marine and the four freshwater populations that were colonised after the Pleistocene glaciations (pooled) separately for each ocean. The F_{ST} calculations were based only on the neutral loci, thus assuming any genetic signal of selection is due to LD with a QTL. To determine how repeatable evolution was in the simulated data, we obtained the squared correlation coefficient between all pairs of simulation replicates for F_{ST} averaged over 20 bps windows with a step size of 10 bps.

Results

Supervised approaches to determine marine-freshwater divergence

Genome-wide allelic differentiation of four different datasets suggested that parallel marine-freshwater divergence in Eastern Pacific freshwater populations was much stronger and consistent across the genome than in the other geographic regions (Atlantic and Western Pacific). The strongest pattern of differentiation was found when F_{ST} was estimated between Eastern Pacific freshwater individuals and marine individuals randomly sampled from all geographic regions in the dataset (dataset 1, Fig. 1a), with 95%-quantile reaching above 0.5 on most of the 21 chromosomes but with the median F_{ST} remaining close to zero (Fig. 1a, Supplementary Fig. 2). The second dataset (dataset 2, Fig. 1b) mirroring the sampling scheme of Jones et al. (2012) also exhibited strong ecotype differentiation. The overall F_{ST} was lower than in the dataset 1 because of the inclusion of freshwater individuals also from Atlantic, but these two datasets showed similar patterns of marine-freshwater divergence (Fig. 1a-b) as previous studies from the Eastern Pacific (Hohenlohe et al., 2010; Jones et al., 2012; Nelson and Cresko, 2018). When marine and freshwater individuals were randomly sampled

across the data (dataset 3), much less consistent marine-freshwater divergence was found (Fig. 1c, Supplementary Fig. 2) with Chr. I and XX standing out as clear outlier regions. Since our random global sampling is biased towards Atlantic samples (126 individuals *vs*. 40 individuals from the Pacific, Supplementary Table. 1) we also analysed a negative control (dataset 4), focusing on the Western Pacific freshwater ecotypes. In this dataset, no regions showed consistent marine-freshwater divergence with very little variation in the 95%-quantile across the genome (Fig. 1d, Supplementary Fig. 2).

Since F_{ST} is negatively correlated to within-group diversity, the neutral F_{ST} is expected to decrease when pooling many divergent populations (datasets 2,3 and 4; Fig. 1 b-c, Supplementary Fig. 2). However, if allelic differentiation is caused by selection to freshwater-marine environments, F_{ST} is still expected to be high if divergent population are pooled because allele fixation is not random and concordant with how populations were pooled, leading to a strong heterogeneity in the difference between the median and 95% quantile F_{ST} across the genome. Furthermore, the dataset 4 (biased towards sampling of freshwater individuals from the Western Pacific) provided a control test to show that biased geographical sampling alone does not lead to high heterogeneity in 95% quantiles across the genome (Supplementary Fig. 2).

Unsupervised approach to determine marine-freshwater divergence

The first step of LDna on the empirical dataset (2,511,922 SNPs derived from 166 individuals worldwide) identified 214,326 loci that were in high LD with at least one other locus within windows of 100 SNPs (Supplementary Information 1). Performing LDna on each chromosome separately (only using one locus from each LD-cluster from step one; Supplementary information 1) resulted in 81 distinct LD-clusters and from these a final 29 LD-clusters were obtained (pooling within chromosome LD-clusters whenever they were grouped by LDna in the final step; Supplementary information 1), containing in total 71,064 loci (*viz*. Cluster 1-29). Nine of these LD-clusters associated with local adaptation, inversions and divergence between Pacific and Atlantic populations are highlighted in Fig. 2a-i. All 29 clusters are detailed in Supplementary Fig. 3 and Supplementary Table 2.

Cluster 1 (10,184 loci, 0.405% of the dataset) separated all Pacific individuals (East and West) from the Atlantic individuals (Fig. 2a), thus reflecting trans-oceanic geographic structure. Clusters 2 (53,785 loci, 2.141% of the dataset, Fig. 2b) and 21 (2,728 loci, 0.007% of the dataset; correspond to an inversion in Chr. XXI, Fig. 2c) separated most of the Eastern Pacific freshwater individuals from the remaining samples, a pattern that is not expected by chance alone (randomisation test $P < 0.001$, Supplementary Fig. 3, Table 2), reflecting a shared adaptive response amid Eastern Pacific freshwater populations. Two Eastern Pacific freshwater individuals from Kodiak Island, Alaska (ALA population) did not group with the other Eastern Pacific individuals. Therefore, in agreement with earlier phylogenetic analyses (Fang et al., 2018), these two individuals were considered separate from the other Eastern Pacific freshwater individuals for the remaining analyses. Notably, no cluster of similar magnitude separating freshwater individuals from any other large-scale geographic region could be detected, demonstrating that parallel marine-freshwater divergence in the Eastern Pacific is much more prevalent than anywhere else in the world.

Clusters 5, 6, 10, 11, 12, 13, 16, 18, 20, 22, 25, 27 and 29 (in total of 5,232 loci, 0.208% of the dataset; see six representatives in Fig. 2 d-i, and all in Supplementary Fig. 3, Table 2; $P < 0.05$) grouped multiple freshwater individuals from different geographic regions across the Pacific and Atlantic Oceans, reflecting genetic marine-freshwater parallelism on a global (trans-oceanic) scale. LD-clusters 6, 22 and 29 correspond to previously known chromosomal inversions (on chromosomes I, XI and XXI, respectively) associated with marine-freshwater divergence (Jones et al. 2012Fig. 2d, g, i; Supplementary Fig. 3, Table 2). LD-clusters 6, 11, 12 and 27 showed similar marine-freshwater divergence (F_{ST}: Fig. 3a) in datasets biased towards Eastern Pacific freshwater samples (dataset 2) and datasets biased towards Atlantic individuals (dataset 3), suggesting extensive global parallelism. While clusters 5, 10, 13, 22, 25 and 29 also separate freshwater individuals from both Pacific and Atlantic populations from all other individuals, the divergent Atlantic freshwater individuals (in the in-group) were few (Supplementary Fig. 3) and thus these LD-clusters were still biased towards marine-freshwater divergence in the Eastern Pacific (Fig 3 a).

Four LD-clusters (Clusters 4, 8, 9 and 14, in total of 479 loci, 0.019% of the dataset, Supplementary Fig. 3) separated freshwater individuals from only one geographic region

likely reflecting geographic clustering but could also contain some loci involved in non-parallel freshwater adaptation. They are therefore interpreted as inconclusive with respect to their underlying evolutionary phenomena (Supplementary Table 2). Accordingly, loci from these LD-clusters showed little marine-freshwater divergence in the global dataset (Supplementary Fig. 5). Since LD-cluster 24 (47 loci) separated most of the freshwater individuals from the Western Pacific (bar one) from the remaining individuals (Supplementary Fig. 3) it could reflect marine-freshwater divergence exclusively in the Western Pacific. However, the marine and freshwater individuals in this region were sampled far from each other (all freshwater individuals came from Japan and all marine individuals came from south North Eastern Russia; Supplementary Fig. 6 and Supplementary Fig. 1), thus this marine-freshwater divergence could also have been driven by geographic structuring and it was thus also classified as inconclusive. Furthermore, the LD-cluster 19 (241 loci) was identified as a putative chromosomal inversion but it was not associated with marine-freshwater differentiation (Supplementary Fig. 3 and Supplementary Table 2).

Proof of concept using simulated data

In the simulated data, before the Atlantic was colonised from the Pacific, all five freshwater populations in the Pacific were fixed, or nearly fixed for the freshwater-adapted alleles of all locally adapted QTL (Fig. 4f). Following the invasion of the Atlantic (36-40 Kya; Fig. 4f), the increase of freshwater allele frequency in the Atlantic freshwater populations depended on both the number of QTL and level of gene flow from Pacific to Atlantic (Fig. 4f). The highest increase in freshwater-adapted alleles was observed when the number of QTL was small (3 QTL per chromosome) and trans-oceanic gene flow was high ($N_e m$=5, g. 11,000-25,000, Fig. 4f). During post-glacial colonisation of new freshwater habitats from the sea (g. 25,002-30,000), freshwater-adapted alleles (in both Pacific and Atlantic) gradually increased in the newly formed freshwater populations (Fig. 4f), thus reflecting local adaptation. Likewise, this increase was similarly dependent on the number of QTL under selection (both in Pacific and Atlantic) and trans-oceanic gene flow (only affecting the Atlantic, Fig. 4f). These patterns likely reflect the underlying levels of ancestral variation in the sea available for subsequent freshwater adaptation (Fig. 4g). The lowest frequencies of freshwater-adapted alleles in the

sea was always observed in conjunction of the highest number of locally adapted QTL (in both Pacific and Atlantic) and lowest trans-oceanic gene flow (only affecting the Atlantic, Fig. 4g). Furthermore, both the frequency of freshwater adapted alleles in the sea (ancestral variation) and in the post-glacial freshwater populations (local adaptation) depended on whether the QTL were located in low or high recombination regions, with lowest frequencies of freshwater-adapted alleles always observed in low recombination regions (Supplementary Fig. 7a,b). Frequencies of the freshwater-adapted alleles in both Pacific and Atlantic freshwater populations never reached similar frequencies during the post-glacial colonisation (10 Kya; Fig. 4f) as during the last glacial period (10,000-36,000 Kya; Fig. 4f) showing that ancestral variation in the sea in our simulations was not sufficient to allow complete local adaptation (i.e. fixation of all freshwater adapted alleles).

In the simulations, present-day marine-freshwater divergence (mean neutral F_{ST}) was always low for the two chromosomes without QTL and in high recombination regions (Fig. 5). In contrast, F_{ST} for low recombination regions was high for Pacific (for all parameter settings), indicating strong islands of parallel marine-freshwater divergence. This was also true for the Atlantic when the number of QTL was low (3 or 6 QTL per chromosome) and when trans-oceanic gene flow was high ($N_e m$=5). However, when number of QTL was high (9 per chromosome) and trans-oceanic gene flow was low ($N_e m$=1), only one chromosome in the Atlantic (chromosome 6) showed strong marine-freshwater divergence (Fig. 5a). The mean squared correlation coefficient of F_{ST} (measured in sliding windows of 20 SNPs) between replicates was stronger in the Pacific (r^2=0.42-0.57) than in the Atlantic (r^2=0.09-0.42, Supplementary Table 4). This translates in a higher repeatability of genome-wide parallel differentiation island across the replicates in the Pacific than in the Atlantic. The weakest correlation was observed when trans-oceanic gene flow was low and QTL density was high (9 QTL per chromosome; r^2 = 0.09).

Discussion

Using genome-wide SNP data from a comprehensive global sampling of marine and freshwater ecotypes, we demonstrated that only a small proportion of the dataset (13 LD-clusters comprising 0.208% of the dataset) show consistent patterns of parallel ecotype differentiation at the global scale (i.e. in at least two freshwater populations from different biogeographic regions). Two large LD-clusters (comprising 2.149% of the dataset) separated freshwater individuals from Eastern Pacific from all other individuals, while no comparable cluster could be detected for any other geographic region. This shows that parallel evolution in the three-spined stickleback is much more pervasive in the Eastern Pacific than anywhere else in the world. Indeed, marine-freshwater divergence in the Eastern Pacific is even stronger than geographic structuring between the Pacific and Atlantic Oceans (LD-clusters separating freshwater individuals from the Eastern Pacific comprised five times as many loci than the LD-cluster reflecting geographic structuring between Pacific and Atlantic Oceans). With simulations, we demonstrate that this pattern could be explained by the stochastic loss of low frequency freshwater-adapted alleles (in the sea) during range expansion from the Eastern Pacific. This loss was the strongest when trans-oceanic gene flow was low and when the number locally adapted QTL in the Eastern Pacific was high, particularly in low recombination regions where genomic islands of parallel ecotype divergence are expected to occur (Roesti et al. 2014). Supervised F_{ST} genome scans using different sampling strategies demonstrated that a sampling scheme with a large proportion of Eastern Pacific populations could overestimate the number of loci involved in parallel evolution. In the following, we discuss the biological factors potentially contributing to the differences in the degree of marine-freshwater parallelism between Eastern Pacific and non-Eastern Pacific populations of the three-spined stickleback.

Geographic heterogeneity in standing genetic variation

The “transporter hypothesis” (Schluter & Conte, 2009) postulates that three-spined sticklebacks have repeatedly colonized and adapted to freshwater environments via selection on standing genetic variation derived from large marine populations, where recurrent gene

flow from previously colonised freshwater habitats maintains freshwater-adapted alleles. This implicitly assumes that a large pool of locally adapted alleles has accumulated during a long period of time, as gene-flow is expected to spread potentially beneficial mutations across demographically independent populations (Johannesson et al., 2010; Kemppainen et al., 2011). In support of this hypothesis, it has been shown that haplotypes repeatedly used in freshwater adaptation are identical by descent (Colosimo et al., 2005; Roesti et al., 2014) and old, averaging six million years (My) with some as old as 15 My (Colosimo et al., 2005, Roesti et al., 2004, Nelson and Cresko, 2018). These studies analysed populations from the Eastern Pacific region, which represents the oldest and most ancestral marine population (Fang et al., 2018; 2019) where three-spined sticklebacks are thought to have persisted since the split from their close relative, the nine-spined stickleback (*Pungitus pungitus*), approximately 26 Mya (Matschiner et al., 2011; Meynard et al., 2012; Betancu-R et al., 2015; Sanciangco et al., 2016; Varadharajan et al., 2019). However, populations in the Western Pacific and the Atlantic are much younger, as they were colonized from the Eastern Pacific during the late Pleistocene (36.9-346.5 kya; Fang et al., 2018, 2019). Furthermore, there is no evidence for trans-oceanic admixture in previous phylogenetic studies (Fang et al. 2018; 2019) following the split of Pacific and Atlantic clades, and there are no extant populations of three-spined sticklebacks in the arctic Russia between the Kara Sea and the Eastern Siberian Sea. Thus, the spread of freshwater-adapted alleles from the Eastern Pacific to elsewhere via migration through the Bering Strait is unlikely, and has probably not occurred in recent times. Our simulations show that following colonisation of freshwater populations from the sea, the accessibility of freshwater-adapted alleles, which is a function of colonisation history, the number of QTL under selection and recombination rate variation, largely determine the number of loci that show consistent marine-freshwater divergence. Thus, the lower levels of parallelism at the genetic level in European populations could at least in part be explained by access to a smaller pool of standing genetic variation relative to Eastern Pacific populations as a result of a range expansion.

Under the hypothesis that the colonisation of the Atlantic from the Pacific involved a limited number of founder individuals, genetic diversity (expected heterozygosity, H_E, and nucleotide diversity, π) is expected decrease with distance from the source population from which the range expansion started (Ramachandran et al., 2005). We found no evidence to support this

hypothesis (Supplementary Fig. 8 a,b). However, since freshwater-adapted alleles occur in the sea only at low frequencies (and are selected against in the sea), they are less likely to spread to new geographic regions than neutral alleles (Halliburton and Halliburton, 2004; Hyten et al., 2006), so the loss of genetic diversity is expected to be less severe for neutral than freshwater adapted alleles..

Another possible explanation for the lack of parallel islands of ecotype divergence in the Atlantic could be stronger spatial genetic structure in marine populations outside of the Eastern Pacific causing heterogeneity in standing genetic variation available for freshwater adaptation, which was not tested in our simulations. Limited gene flow among marine populations may increase the chance that freshwater-adapted alleles are stochastically lost in some of the sub-populations, thus resulting in smaller and more heterogeneous pools of freshwater-adapted alleles. While there is indeed a significant IBD in our Atlantic marine populations (Supplementary Fig. 8e), spatial structure in the Eastern Pacific seems to be even stronger (Supplementary Fig. 8; data from Morris et al., 2018). Thus, we find no support for the hypothesis that differences in the degree of spatial genetic structuring among marine populations within regions explain the observed patterns.

Based on results of this and earlier studies, there is some evidence for parallel evolution in Europe (Ferchaud & Hansen 2016, Liu et al. 2018, Terekhanova et al., 2014, 2019), though such patterns are locally restricted and involve fewer genomic regions than in the Eastern Pacific. Ferchaud and Hansen (2016) and Liu et al. (2018), reported little parallelism in genomic regions involved in local adaptation, most notably in Denmark and Greenland, compared to the Eastern Pacific. Similarly, Terekhanova et al. (2014) identified a total of 19 distinct genomic regions showing consistent marine-freshwater divergence in the White Sea (later refined to 21, Terekhanova et al. 2019), all of which were also identified as differentiation islands between marine and freshwater ecotypes in the Eastern Pacific (Jones et al., 2012, Hohenlohe et al., 2010). Thus, while local adaptation in all geographic regions indeed likely stem from standing genetic variation originating from the Eastern Pacific (sensu the transporter hypothesis), only Eastern Pacific populations show extensive parallel evolution over a larger geographic area. These results are entirely consistent with our analyses, where

no LD-cluster showed parallel marine-freswater divergence exclusively among non-Eastern Pacific samples.

Another likely explanation for the lack of globally shared genetic parallelism is heterogeneity in selective regimes among freshwater habitats, both between Atlantic and Eastern Pacific Oceans and between different geographic areas in the Atlantic (DeFaveri et al. 2011). However, there is currently no data to assess to what extent differences in selection regimes could contribute to the observed heterogeneity in parallel patterns of marine-freshwater divergence at the trans-oceanic scale. A recent simulation study showed that the probability of genetic parallelism from standing genetic variation rapidly declines as selection starts to change from fully parallel (optima angle of 0°) to divergent (optima angle of 180°), especially when a large number of traits affect fitness (Thompson et al., 2018). For example, populations adapting to optima separated by an angle of just 33° might have only 50% of shared beneficial alleles, even if they have access to the same pool of genetic variation. However, in this model adaptation proceeded via the sorting of naive alleles and not via alleles that are "pretested" by selection (as under the transporter hypothesis) when parallel evolution is particularly likely (Thompson et al., 2018).

Accessibility to ancestral variation and recombination rate variation could explain "differentiation islands"

In the simulated data, correlations of genome-wide patterns of genetic differentiation between independent replicate simulations were strong for Pacific populations (Supplementary Table 4). This demonstrates that – given that the same set of locally adapted alleles are available from standing genetic variation, the underlying recombination rate variation and the population demographic history and selection regimes are the same – evolution of local adaptation can be highly repeatable as also demonstrated by earlier empirical work (e.g. Bassham et al. 2018). According to the divergence hitchhiking model (Feder and Nosil, 2010) clustering of several QTL in low recombination regions should reduce gene flow between ecotypes and thus extend LD across larger genomic regions. This is consistent with our simulations where we were only able to detect genomic islands when several of these QTL were clustered in the same low recombination region. Under such conditions, clear

differentiation islands between ecotypes were detected, in agreement with empirical data (Roesti et al., 2014) showing that differentiation islands between freshwater and marine sticklebacks tend to be located in genomic regions of low recombination (for example close to centromeres). Our results contrast with the results of Flaxman et al. (2013) who, using simulations based on simple genetic maps without recombination modifiers, concluded that under most conditions, divergence hitchhiking is unlikely to significantly contribute to the generation of differentiation islands.

Somewhat surprisingly, we also noted that, while genomic islands of parallel ecotype divergence was most likely when several QTL clustered in the same low recombination region, these were also the QTL where the frequency of the freshwater-adapted allele showed the lowest frequencies in the sea (and thus were least likely to spread to Atlantic during colonisation from Pacific). Since QTL in the low recombination region are less likely to be separated by recombination when freshwater-adapted individuals migrate to the sea, it is reasonable to assume that the selection pressure against these “haplotypes” in the sea is stronger. However, this is not consistent with the empirical data showing that the genomic regions most likely to show global parallel ecotype divergence are inversions, where recombination in heterokaryotypes is particularly restricted. Our simulations assume that freshwater-adapted alleles are selected against in the sea (and is equal for all QTL) while in reality, selection against some of the "freshwater haplotypes/karyotypes” in the sea may be weak or even absent allowing them to easily spread during range expansions. Consistent with this hypothesis, in PCAs based on loci from LD-clusters corresponding to inversions (LD-clusters 6, 22 and 29) several marine individuals also cluster with the freshwater individuals (Fig. 2 d,h), indicating frequent occurrence of the “freshwater karyotypes” in the sea. Indeed, Terekhanova *et al*. (2019) found that the genomic regions most commonly involved in local adaptation in multiple independent freshwater populations were also those with the highest frequencies of these haplotypes in the sea. This is in agreement with our simulation study that predicts that the strongest limiting factor for the transporter hypothesis in the Atlantic is the frequency of the freshwater adapted alleles in the sea.

A difference between LDna and self-organizing map-based iterative Hidden Markov Model (SOM/HMM) used by Jones et al. (2012) to detect genomic islands of marine-freshwater

divergence is that SOM/HMM finds regions of the genome that support a given set of commonly found tree topologies in the data, where the loci may or may not be in high LD with each other, whereas LDna exclusively depends on LD and treats each locus individually. Since our three-step LDna approach firstly only keeps correlated loci (above a given threshold, Supplementary Information 1) within non-overlapping windows within chromosomes, LDna is biased to detect LD-clusters where loci are in LD with at least one physically adjacent locus within a window (of 100 SNPs). Thus, LDna is particularly sensitive to detecting inversions, and may underestimate the number genomic regions involved in parallel genomic divergence globally, if LD is not sufficiently high, or single loci are in LD across regions spanning more than 100 SNPs in the data (on average corresponding to ~ 15kb). Nevertheless, next to the largest LD-clusters that reflected ecotype differentiation exclusively in the Eastern Pacific and the inversions, we identified most of the F_{ST} peaks in the global dataset (Supplementary Fig. 3), thus demonstrating that our method (and parameter settings used for the LDna) is sensitive to the main LD-signals in the data. Most notably, we also found several genomic regions involved in parallel marine-freshwater divergence globally that were not apparent in the F_{ST} analyses (e.g. the marine-freshwater divergence only involved a small proportion of the samples in the total data set), demonstrating the main strength of LDna. This cannot thus explain the complete lack of LD-clusters that differentiate between ecotypes outside of the Eastern Pacific. Furthermore our results are entirely consistent with the lack of large regions of high F_{ST} in the global dataset (with a bias towards non-Eastern populations; Fig. 3 b), the numerous regional studies that show much less parallel evolution outside of the Eastern Pacific and the fact that also in Jones et al. (2012) much stronger marine-freshwater divergence was found within the Eastern Pacific than at the global scale (see Introduction). Therefore, the evidence for a strong discrepancy of marine-freshwater divergence between the Eastern and non-Eastern Pacific populations is indeed substantial.

Jones et al. (2012) described ~200 genomic regions involved in marine-freshwater divergence globally, and we know that this number could much higher in the Eastern Pacific where ecotype divergence is the strongest. Furthermore, islands do not occur on all chromosomes (presumably because these chromosomes do not contain loci for freshwater adaptation), thus the number of QTL responsible for marine-freshwater divergence in the

Eastern Pacific could be substantial in the most QTL-dense chromosomes (e.g. Chromosome IV, where global marine-freshwater divergence is the strongest). In the simulations, we included up to nine QTL per chromosome, with up to six QTL within a single low recombination region within a chromosome (Chr. 3 in the simulations, Fig. 5 a, b). While this might seem like an exceptionally high number, this is nevertheless consistent with empirical data.

Are three-spined sticklebacks a representative model to study parallel evolution?

Since the pattern of parallel genetic differentiation between marine and freshwater stickleback ecotypes in the Eastern Pacific are in stark contrast to what is seen across other parts of the species distribution range, one is posed to ask how representative are the results from the Eastern Pacific stickleback studies with respect to parallel evolution more generally. A recent review of parallel evolution suggests that even dramatic phenotypic parallelism can be generated by a continuum of parallelism at the genetic level (Bolnick et al., 2018). For instance, the coastal ecotypes of *Senecio lautus* exhibit only partial reuse of particular QTL among replicate populations (Roda et al., 2017), and genetic redundancy frequently underlies polygenic adaptation in *Drosophila* (Barghi et al., 2019). Similarly, Conte et al. (2015) studied the extent of QTL reuse in parallel phenotypic divergence of limnetic and benthic three-spined sticklebacks (*Gasterosteus aculeatus*) in Paxton and Priest lakes, and found that 76% of 42 phenotypic traits diverged in the same direction, whereas only 49% of underlying QTL evolved in parallel in both lakes. For highly parallel traits in two other pairs of benthic-limnetic sticklebacks, only 32% of the underlying QTL were reported to be shared (Conte et al., 2012). Finally, using F_{ST} outliers to detect putative genomic targets of selection Kautt et al. (2012, cichlid fishes), Le Moan et al. (2016, anchovy) and Westram et al. (2014, periwinkles), showed that phenotypically very similar populations often share only a small proportion of their F_{ST} outliers. Thus, these studies are also in stark contrast to the original conclusions of widespread genetic parallelism in three-spined sticklebacks.

One exception that seems more general across taxa is the repeated involvement of chromosomal inversions in parallel evolution. Chromosomal inversions could store standing variation as balanced polymorphism and distribute it to fuel parallel adaptation (Morales et al.,

2019). For instance, the same Chr. I inversion involved in global marine-freshwater divergence in three-spined sticklebacks (Jones et al., 2012, Terekhanova et al., 2014, 2019, Liu et al., 2018, this study) also differentiates stream and lake ecotypes in the lake Constance basin in Central Europe (Roesti et al., 2015). Two other clear examples where most genetic differentiation between ecotypes at larger geographic scales is partitioned into inversions come from monkey flowers (*Mimulus guttatus*; Twyford and Friedman 2015) and marine periwinkles (*Littorina saxatilis*; Faria et al., 2018; Westram et al., 2018).

Conclusions

Our results demonstrate that genetic parallelism in the three-spined stickleback model system is in fact not as pervasive as some earlier papers focusing on Eastern Pacific populations have led us to believe. Our analysis of geographically more comprehensive data, with similar and less assumption-burden methods as used in earlier studies, show that the extraordinary genetic parallelism observed in the Eastern Pacific Ocean is not detectable elsewhere in the world (e.g. Atlantic Ocean, Western Pacific Ocean). Hence, the focus on Eastern Pacific has generated a perception bias – what goes on there, does not actually apply to the rest of the world. Furthermore, our simulations show that the spread of freshwater adapted alleles can easily be hampered if the colonisation of the Atlantic (from the Pacific) was limited, in particular for QTL clustered in low recombination regions; i.e. the ones that are most likely to result in parallel islands of ecotype differentiation. Therefore, geographic differences in incidence and pervasiveness of parallel evolution in three-spined sticklebacks likely stem from geographic heterogeneity in access to, and amount of, standing genetic variation, which in turn has been influenced by historical population demographic factors. Hence, while striking genome-wide patterns of genetic parallelism exist (e.g. in Eastern Pacific sticklebacks), the conditions under which such pattern can occur may be far from common, perhaps even exceptional.

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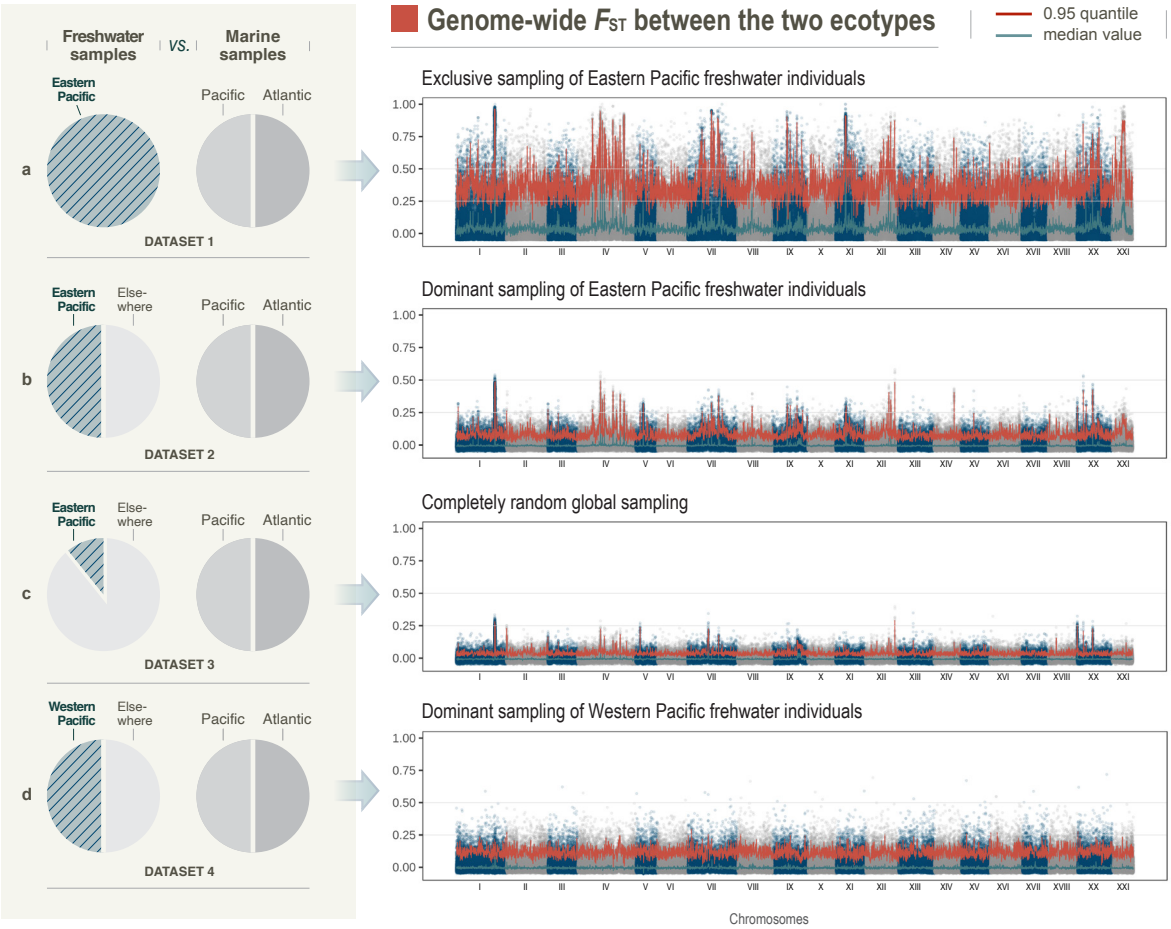
FIGURES

Figure 1 | Marine-freshwater divergence in datasets with different geographic sampling. The four sampling schemes contrasting freshwater and marine individuals with equal sample sizes are illustrated as follows (a) Dataset 1: Eastern Pacific freshwater individuals against global sampling of marine individuals. (b) Dataset 2: half of freshwater individuals are from Eastern Pacific with the remaining individuals randomly sampled from individuals elsewhere. (c) Dataset 3: random sampling of global freshwater individuals against random global sample of marine individuals. (d) A negative control (Dataset 4) where half of the freshwater individuals are from the Western Pacific with the remaining individuals randomly sampled from individuals elsewhere. Genome-wide F_{ST} was calculated 100 times from random sampling under the three sampling schemes, and the mean F_{ST} values are presented on the right side. Red and green lines represent moving averages of 95%-quantiles and medians in windows of 100 SNPs with a step size of 50 SNPs, thus "islands of differentiation" are indicated by a large difference between the 95%-quantile and the median indicating excess of highly differentiated loci.



958 **Figure 2 | Linkage Disequilibrium network analysis (LDna).** (a-i) Nine main LD-clusters of
959 loci identified by LDna. In each plot, the upper depicts the position of the clustered loci across
960 the genome (x-axis) and for each locus, mean LD (r^2) for each locus (relative to all other loci
961 in its cluster; high values indicates strongly connected loci) on the y-axis. The lower presents
962 visualization of principal component analysis (PCA) showing population divergence based on
963 loci from the LD-clusters. The seven different colours represent the geographic origin of
964 populations. Solid and open circles respectively refer to freshwater and marine ecotypes. All
965 identified 29 LD-clusters and corresponding information are listed in Supplementary Fig. 3
966 and Supplementary Table 2. (j) Map of sampled populations with colours matching the PCA
967 results. A flat sampling map is given in the Supplementary Fig. 1.

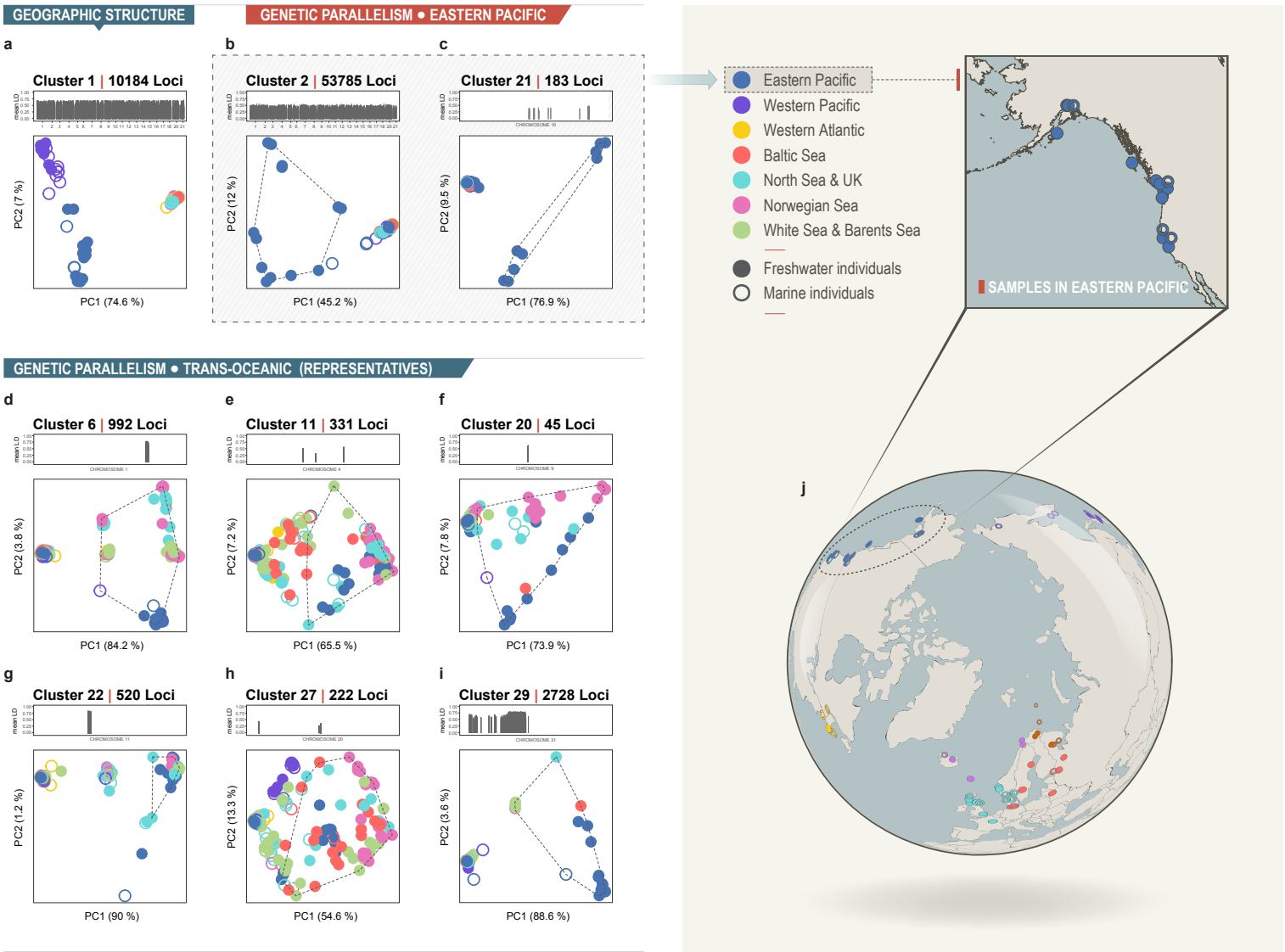


Figure 3 | Genetic parallelism identified by the supervised and unsupervised method. (a) The figure displays loci from the 15 LD-clusters identified by LDna, representing the parallel genomic loci of marine-freshwater divergence across either the Eastern Pacific or the global freshwater populations. The x-axis refers to the genome-wide F_{ST} based on the random sampling of freshwater individuals globally (*viz.* [b] and Fig. 1c). The y-axis represents the genome-wide F_{ST} derived when half of the individuals are from the Eastern Pacific (Fig. 1b). A figure displaying the remaining LDna loci as well as all non-LDna loci across the genome can be found in Supplementary Fig. 2. (b) The genomic position of the parallel genetic variants across the global (trans-oceanic) freshwater populations (13 LD-clusters of lower panel of [a]). The genome-wide F_{ST} (dots in grey colour) is based on the random sampling of freshwater individuals globally (*viz.* Fig. 1c). The LD-clusters are distinguished by corresponding colour at (a).

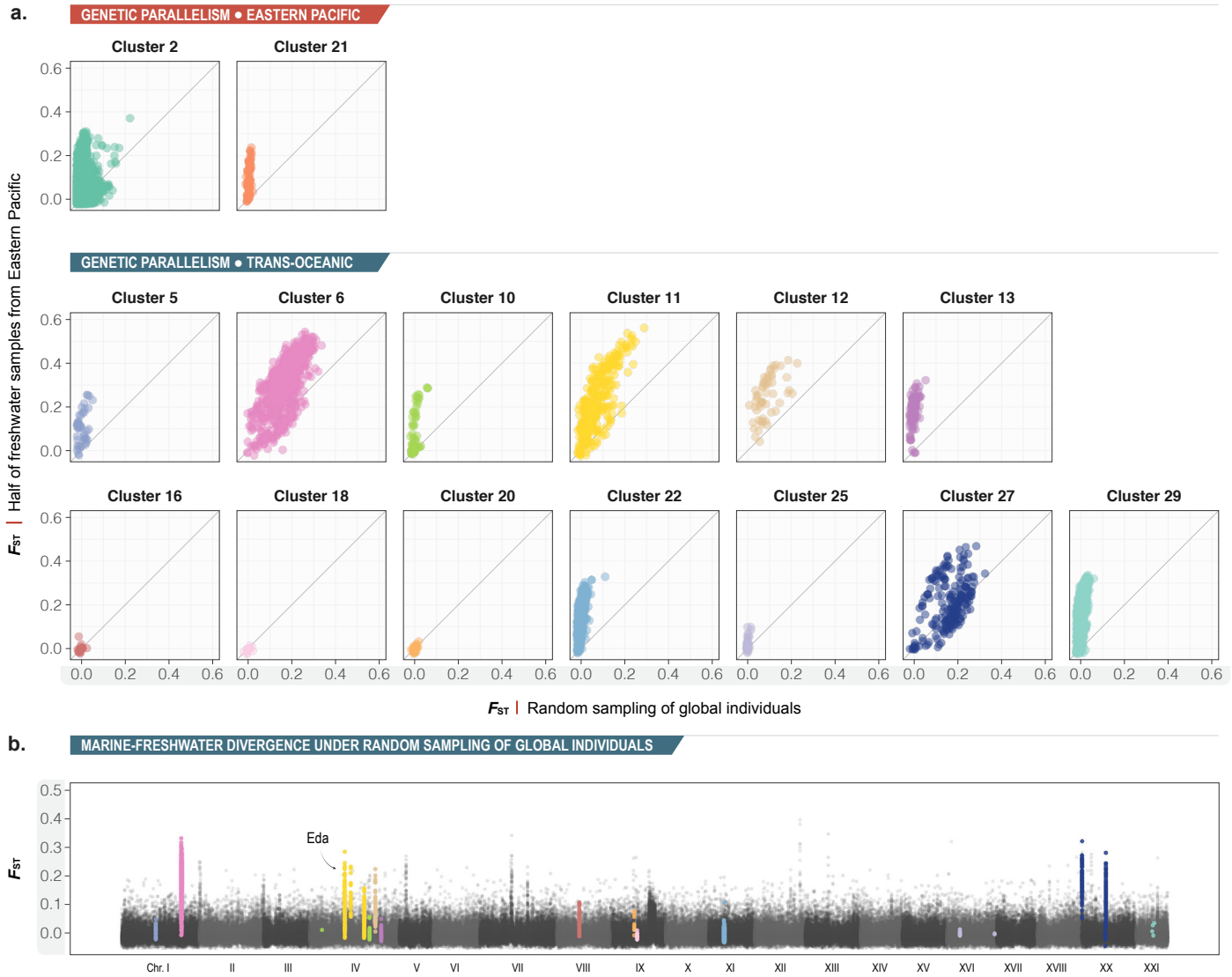
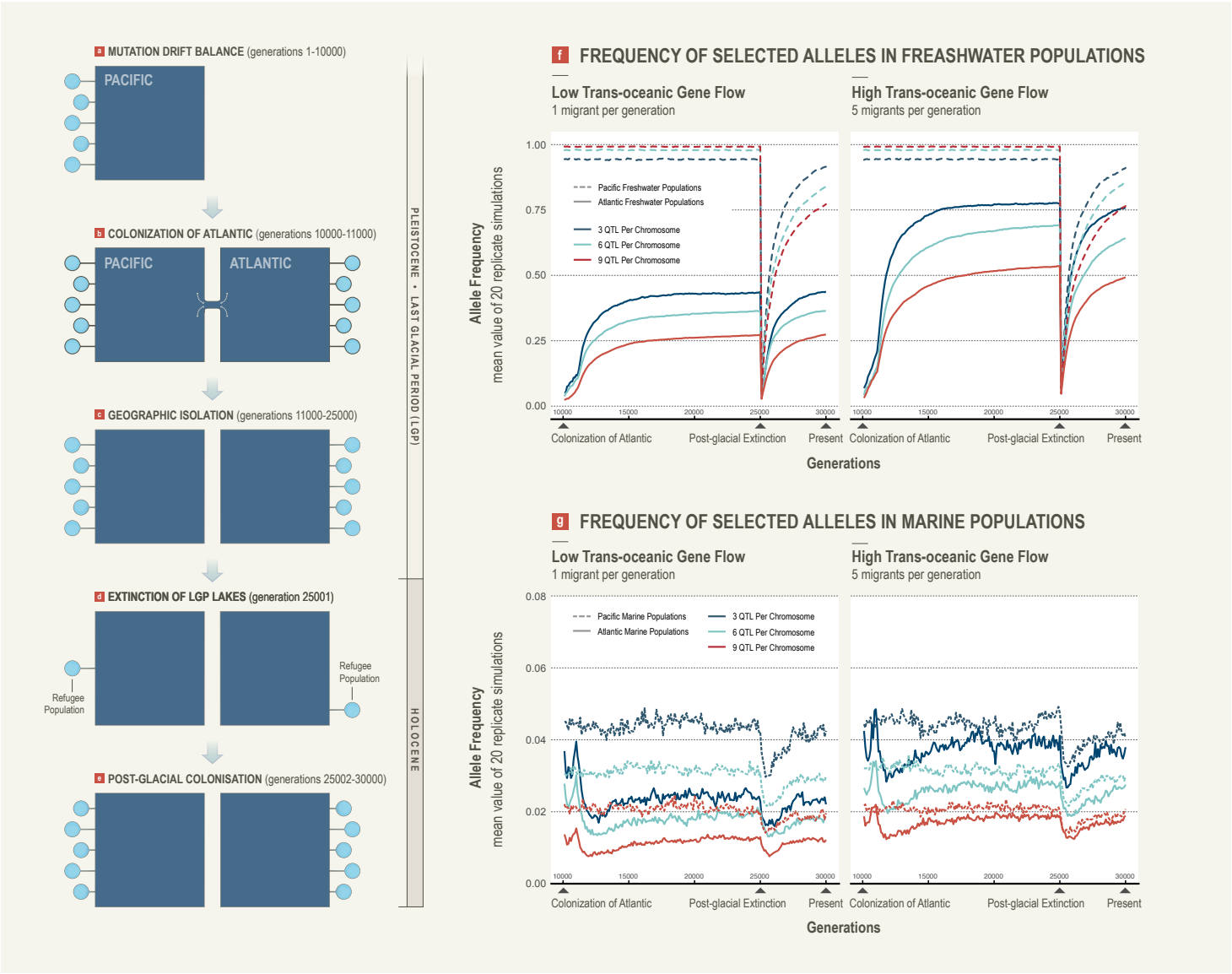
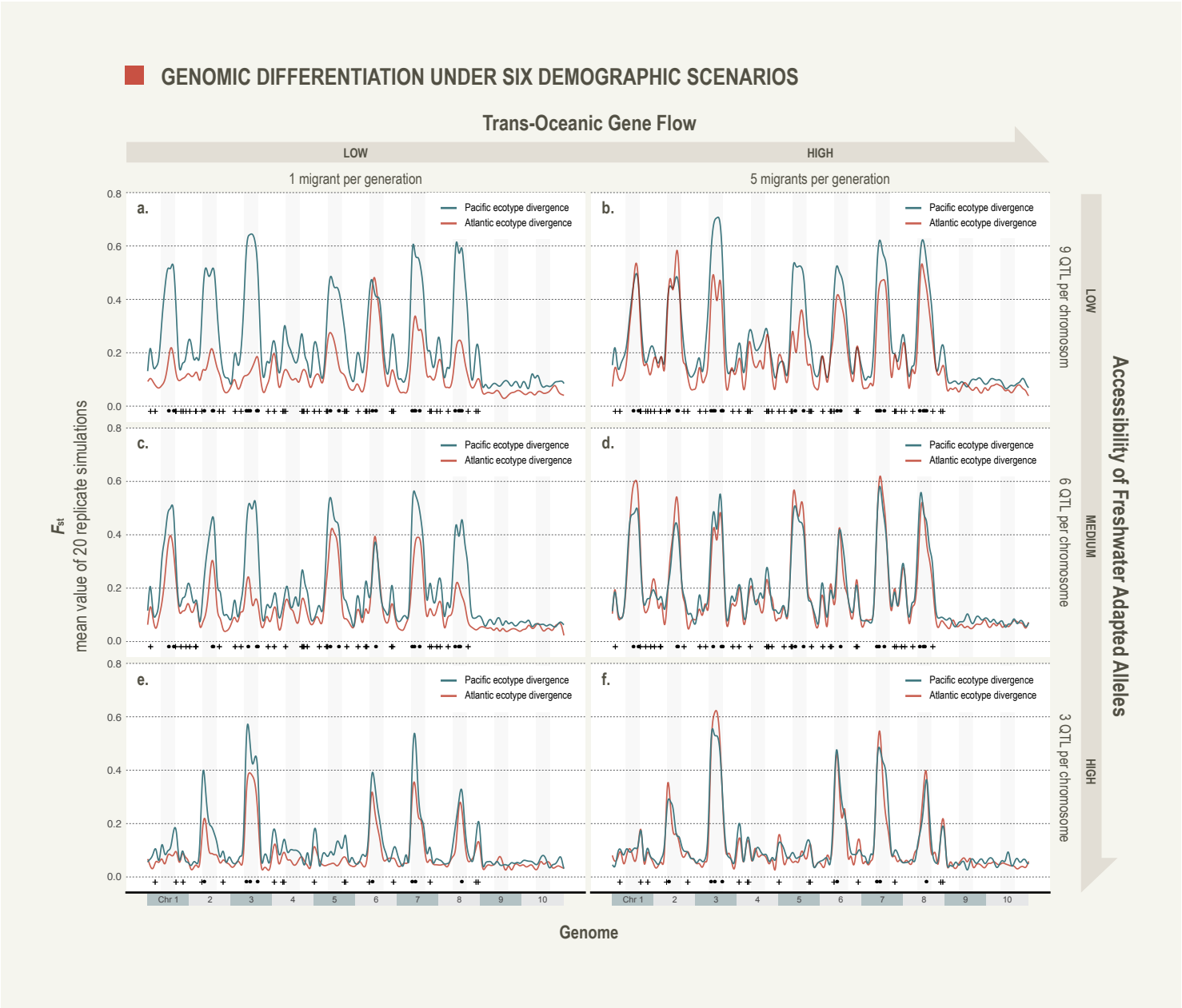


Figure 4 | Ecological genetics in simulated data. (a-e) A schematic of the demographic scenario used for the simulations that is consistent with the "transporter hypothesis" of Conte & Schluter (2009) in the Eastern Pacific. (a) Initial local adaption of the freshwater populations in the Pacific. (b) The colonisation of stickleback populations from the Pacific to the Atlantic. (c) Geographic isolation between the two oceans. (d) Extinction of lakes during last glacial period (LGP) with the survival of refugee populations. (e) The post-glacial colonisation of the new freshwater populations. (f) Frequency of selected (freshwater-adapted) alleles in the new established freshwater populations through generations at high and low levels of trans-oceanic gene flow and three levels of number of locally adapted traits. (g) Frequency of selected alleles in the marine populations through generations at high and low levels of trans-oceanic gene flow and three different numbers of QTL per chromosome.



1001 **Figure 5 | Genomic differentiation in simulated data.** Six plots demonstrate the results of
1002 genome-wide marine-freshwater divergence (F_{ST}) under six demographic scenarios at the
1003 end of simulations (generation 30,000). The F_{ST} is summarized by the mean value from 20
1004 replicate simulations. The white and shaded background represents "High" or "Low"
1005 recombining regions, corresponding to chromosome arms or centromeres, respectively. The
1006 positions of QTL (3, 6 or 9 QTL per Chromosome 1-8) are fixed across simulation replicates.
1007 The crosses (+) and black dots (•), at the base in each plot, represent the QTL in high and low
1008 recombination genomic regions respectively.