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Genomic parallelism and lack thereof in contrasting systems of three-spine sticklebacks

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Abstract

Parallel evolution and the extent to which it involves gene reuse has attracted much interest. Whereas it has theoretically been predicted under which circumstances gene reuse is expected, empirical studies that directly compare systems showing high and low parallelism are rare. Three-spine stickleback (*Gasterosteus aculeatus*), where freshwater populations have been independently founded by ancestral marine populations, represent prime examples of phenotypic and genomic parallelism, but cases exist where parallelism is low. Based on RAD (Restriction site Associated DNA) sequencing we analyzed SNPs and chromosome inversions in populations in Denmark and Greenland showing low and high parallelism, respectively. We identified parallelism across freshwater populations in Greenland at genomic regions previously identified to be associated with marine-freshwater divergence. These same markers also separated Danish marine and freshwater sticklebacks, albeit to a weaker extent. Hence, parallelism was not absent in Denmark but possibly constrained by spatially and temporally varying selection. Divergence time estimates found one Danish freshwater population to be much older than the others. It also deviated strongly with respect to parallelism, and may represent earlier postglacial colonization based on a different pool of standing variation and eliciting different adaptive responses to freshwater conditions. These findings provide empirical support to previous suggestions that the time since replicate populations had access to a common pool of standing variation is a major factor determining gene reuse. Finally, based on the observed parallelism in the Greenlandic system we discuss the predictability of adaptive responses in newly established populations.

Keywords: RAD sequencing, genomic parallelism, chromosome inversion, divergence time

47 Introduction

48 Understanding the processes that underlie adaptation of populations to different environments is a central
49 theme in evolutionary biology (Barrett & Hoekstra 2011; Kawecki & Ebert 2004; Savolainen *et al.* 2013). In
50 many cases, populations have independently adapted to similar environmental conditions by means of similar
51 phenotypic traits, that is parallel evolution (Conte *et al.* 2012; Elmer & Meyer 2011; Losos 2009; Schluter &
52 Nagel 1995). However, parallelism at the phenotypic level can involve different processes, ranging from
53 convergent evolution based on *de novo* mutations arising independently in different populations, to selection
54 based on standing genetic variation leading to both phenotypic and genomic parallelism across populations
55 based on the same genetic polymorphisms. The latter is often referred to as gene reuse (Conte *et al.* 2012;
56 Elmer & Meyer 2011; Schluter & Conte 2009).

57 Several cases of phenotypic parallelism have been described involving different extents of gene reuse. For
58 instance, similar adaptive coloration has been observed in different populations of the beach mouse
59 (*Peromyscus polionotus*), but different genetic architecture underlies the trait in different populations,
60 suggesting convergent evolution without gene reuse (Hoekstra *et al.* 2006). Another example involves the
61 American lake whitefish (*Coregonus clupeaformis*), which in a number of lakes shows co-existing dwarf- and
62 normal-sized morphs that exhibit morphological, physiological and life-history adaptations to limnetic and
63 epibenthic habitats, respectively (Bernatchez *et al.* 2010; Dalziel *et al.* 2015). However, at the genomic level
64 there is relatively limited parallelism, and when it occurs it is for the most part partial, *i.e.* found in some but
65 not all population pairs (Gagnaire *et al.* 2013). Finally, marine and freshwater populations of three-spine
66 stickleback (*Gasterosteus aculeatus*) are generally reported to show differences in lateral armour plating.
67 Marine sticklebacks are covered by armour plates from head to tail (Barrett 2010; Bell 2001), mostly assumed
68 to be a defence against fish predators (Reimchen 2000). Freshwater sticklebacks in most cases show reduced

numbers of lateral plates, with predation by invertebrates and/or abiotic factors suggested as selective agents (Barrett 2010; Leinonen *et al.* 2011; Reimchen 2000). This parallelism across freshwater populations is accompanied by parallelism at the underlying major quantitative trait locus EDA (Colosimo *et al.* 2005; O'Brown *et al.* 2015), representing a classic case of phenotypic parallelism and gene reuse. In fact, parallelism at the trait of lateral plating and the EDA gene is accompanied by a generally high degree of parallelism at the genomic level (Deagle *et al.* 2013; Deagle *et al.* 2012; Hohenlohe *et al.* 2010; Jones *et al.* 2012; Terekhanova *et al.* 2014). Whole genome sequencing has revealed 242 genomic regions that show parallel divergence between marine and freshwater sticklebacks (Jones *et al.* 2012), albeit generally without knowledge of the phenotypic effects of this variation.

In a meta-analysis of the probability of gene reuse in parallel evolution, it was suggested that this was most likely to occur when populations are young and have recently had access to the same pool of standing genetic variation (Conte *et al.* 2012). This condition is fulfilled in three-spine sticklebacks, where freshwater populations are assumed to have been founded independently after the last glaciation (ca. 11 kya) by wide-spread marine ancestral populations harbouring a large pool of standing genetic variation. Alleles that are rare in marine populations but beneficial in freshwater conditions are subsequently selected to high frequencies. Moreover, gene flow from established freshwater populations reintroduces freshwater-adapted alleles into marine gene pools where they are subsequently available for founding of new freshwater populations, the so-called Transporter Hypothesis (Schluter & Conte 2009). In contrast, a system like dwarf-normal sized morphs of lake whitefish in different isolated lakes (Bernatchez *et al.* 2010; Dalziel *et al.* 2015; Gagnaire *et al.* 2013) is not expected to have involved a recent common pool of standing variation, and a complex phylogeographic history is furthermore involved (Gagnaire *et al.* 2013). Given these circumstances, the findings of pronounced parallelism in sticklebacks but limited gene reuse and convergent rather than parallel evolution in lake

91 whitefish fit the predicted patterns, although cases also exist where gene reuse is limited even when replicate
92 populations have access to the same pool of standing variation (Ravinet *et al.* 2016).

93 Although threespine stickleback has emerged as a prime model of parallelism and gene reuse, there are
94 nevertheless important exceptions to these patterns. First, parallelism at the trait of lateral plating is not a
95 species-wide phenomenon. Full-plated morphs dominate freshwater populations far inland in Central and
96 Eastern Europe (Banbura 1994; Munzing 1963; Roesti *et al.* 2015) and parts of Eastern North America (Hagen &
97 Moodie 1982), and mixed morph compositions have been observed at a number of locations (Cano *et al.* 2006;
98 Ferchaud & Hansen 2016; Munzing 1963). Second, phenotypic parallelism without strict gene reuse has also
99 been observed, notably reduction of pelvic armour in replicate freshwater populations (Chan *et al.* 2010). This
100 involves a regulatory region associated with the gene *Pitx1*, where mutations have occurred independently
101 across populations. Third, at the genomic level instances of lack of parallelism across freshwater populations
102 have been observed, such as in the Jutland Peninsula, Denmark (Ferchaud & Hansen 2016). In that study, RAD
103 (Restriction site Associated DNA) sequencing (Baird *et al.* 2008; Hohenlohe *et al.* 2010) using the restriction
104 enzyme *SbfI* identified 11 genomic regions showing some parallelism, defined as being outliers in at least two
105 independent comparisons of marine and freshwater populations. However, no genomic regions were
106 consistently outliers across all freshwater populations, and only three regions overlapped with outlier regions
107 reported in previous studies (Hohenlohe *et al.* 2010; Jones *et al.* 2012; Terekhanova *et al.* 2014). It was
108 suggested that stronger environmental heterogeneity across freshwater habitats in this geographical region as
109 compared to other previously studied regions could explain the results.

110 Analyzing different systems within the same species showing high and low parallelism, respectively, would
111 provide a particularly powerful approach to assess possible factors involved in parallelism and gene reuse, such
112 as demographic history and differences or similarities of selection regimes. We therefore contrasted two

113 systems of threespine sticklebacks: a temperate region showing pronounced environmental heterogeneity,
114 based on reanalysis of RAD sequencing data from the Jutland Peninsula, Denmark (Ferchaud & Hansen 2016);
115 and 2) new RAD data from an Arctic region represented by the Nuuk Fjord region in Greenland, showing
116 environmental similarity across freshwater habitats and overall simple and species-poor ecosystems. The use
117 of RAD sequencing for detecting genome-wide selection has recently been much debated (Catchen *et al.* 2017;
118 Lowry *et al.* 2017; McKinney *et al.* 2017). In the present study, RAD sequencing using the restriction enzyme
119 SbfI was unlikely to detect all selection ongoing in the genome, which was also not the purpose of the study.
120 However, it represents a random sample of the genome (Catchen *et al.* 2017; Hohenlohe *et al.* 2010) and has
121 been shown to detect regions of the genome under parallel selection in threespine stickleback (Bassham *et al.*
122 2017; Hohenlohe *et al.* 2010). We already knew that parallelism was low in the temperate region in Denmark,
123 both at the trait of lateral plating and at the genomic level. The Greenlandic populations had previously been
124 found to show a clear pattern of low-plated freshwater populations and full-plated marine sticklebacks (Liu *et*
125 *al.* 2016), which we expected would also be reflected in genomic parallelism across Greenlandic freshwater
126 populations. Hence, RAD data were used for contrasting parallelism in the two systems and for testing
127 hypotheses about the factors underlying differences in parallelism.

128 We identified genomic outlier regions among all populations from each of the two systems and conducted
129 analyses that allowed for direct comparison of genomic parallelism between the two systems (Roesti *et al.*
130 2014). We furthermore analyzed chromosome inversions based on RAD data. Inversions confer protection
131 against recombinatorial break-down involving inverted and non-inverted copies, and can thereby maintain
132 haplotypes with adaptively important combinations of variation (Kirkpatrick & Barton 2006). They play
133 important roles in adaptation and speciation processes in a range of species (Coluzzi *et al.* 2002; Noor *et al.*
134 2001; Sodeland *et al.* 2016). Inversion haplotypes have also previously been suggested to be under differential
135 selection in freshwater and marine sticklebacks (Jones *et al.* 2012) and parapatric lake and stream populations

(Roesti *et al.* 2015). Finally, we used an allele frequency spectrum based approach (Gutenkunst *et al.* 2009) for analyzing demographic history and estimating the time of founding of freshwater populations. This allowed for assessing to what extent the freshwater populations were young and had recently had access to a common source of standing variation (Conte *et al.* 2012). Using these analyses, we asked which factors, specifically differences in selection, environmental heterogeneity and demographic history could explain the different outcomes of high and low parallelism in the two systems. We specifically focused on the following issues: 1) Does low genomic parallelism in the Danish system reflect heterogeneity of selection regimes across populations, whereas parallelism in other systems reflects similarity of selection regimes? A recent study involving multiple population pairs of lake-stream stickleback showed variation in parallelism ascribed to environmental variation, which lends support to this explanation (Stuart *et al.* 2017). 2) The time since replicate populations had access to a common pool of standing variation has been suggested to be a major determinant of genomic parallelism (Conte *et al.* 2012). Could differences or homogeneity in divergence time between marine and freshwater populations explain the outcomes of low and high genomic parallelism, respectively? 3) Finally, we discussed the biological implications of genomic parallelism, specifically with reference to Greenland where ongoing climate change is expected to result in northern expansion and establishment of new freshwater populations.

Materials and methods

Study populations

We focused on the Nuuk Fjord region in Greenland and the Jutland Peninsula in Denmark, the latter represented by previously published data (Ferchaud & Hansen 2016). We aimed to include populations representing comparable geographical scales (see Fig. 1). Five populations were sampled from the Nuuk Fjord, including one marine population (KobM) and four freshwater populations (KobF, Qar, Kap1, Kap3), and six

populations were included from the Jutland Peninsula, involving one marine population (Nor) and five freshwater populations (Mos, Had, Hal, Kro, Kib; see Table 1 for sample sizes, full names and geographical locations of populations). The samples were collected in 2011-2013 using hand net, cast net, electrofishing and minnow traps.

The main environmental characteristics of habitats are listed in Supporting Information, Table S1. Briefly, all freshwater populations from Greenland were from oligotrophic lakes with Arctic char (*Salvelinus alpinus*) as the main fish predator. Kap1 and Kap3 were from the same system of lakes, with Kap1 being near the outlet in the sea and Kap3 being located further upstream, separated by a lake and stretches of river. Except for Kap1 and Kap3 the freshwater localities were presently inaccessible from the sea due to natural waterfalls. In Denmark, two of the freshwater populations were from relatively large lakes (Mos, Hal), two from small lakes (Had, Kib), and one from a river (Kro), which is a tributary to Hal. Specifically concerning Kib it is known that the lake was partially drained in 2009, likely causing a bottleneck in the stickleback population (Ferchaud & Hansen 2016). All Danish freshwater localities were eutrophicated and a wide range of fish predators were present, but varying considerably among lakes. None of the populations were presently accessible from the sea due to anthropogenic barriers (see Supporting Information, Table S1).

Lateral plate morphs and age determination

We counted the number of lateral plates under a dissection microscope and furthermore recorded presence or absence of a keel; in the latter case the number of keel plates was not included in the total plate count. We defined morphs as low-plated (10 or fewer plates), partially plated (10 - 21 plates) and full-plated (> 21 plates) (Le Rouzic *et al.* 2011).

In order to obtain information about generation length, we determined the age of a subset of individuals from Hal, Mos, Kap1 and KobF using sagittal otoliths. The individuals were chosen to represent sizes at which

sticklebacks are expected to reproduce. Total fish length was measured and the otoliths were dissected under a stereomicroscope, cleaned and subsequently embedded in Crystal Bond 509 thermoplastic glue (Ted Pella Inc., Redding, CA, USA) on a microscope slide. The age was read following the procedures by Moser *et al.* (2012). The number of translucent otolith increments, which are accreted in the late winter – spring (Allen & Wootton 1982; Moser *et al.* 2012), were counted in randomized order (without knowledge of origin and size of fish) under a stereomicroscope using 32-50 times magnification. Based on the results we estimated the mean age of reproducing individuals and used this as an approximation for generation length (Hill 1979). This information was used for translating divergence time estimates (see below) from numbers of generations into years.

RAD sequencing and SNP calling

Genomic DNA was extracted from muscle tissue using standard phenol–chloroform extraction. RAD sequencing (Baird *et al.* 2008; Hohenlohe *et al.* 2010) using the restriction enzyme SbfI was outsourced to Beijing Genomics Institute (BGI, Hong Kong, China). Paired-end sequencing was conducted on the Illumina HiSeq platform, resulting in read lengths of 90 bp. Only the first read (containing the restriction site) was used, as sequencing depths of the second reads (without the restriction site) were expected to be insufficient for reliable SNP calling.

The reads were sorted according to their unique barcodes, trimmed to 75 bp and quality-filtered using programs from the FASTX-Toolkit (<http://hannonlab.cshl.edu/fastx-toolkit>), as described in detail in Pujolar *et al.* (2013). Reads were subsequently mapped to an updated assembly of the three-spine stickleback reference genome (Glazer *et al.* 2015) using Bowtie v1.1.1 (Langmead *et al.* 2009), allowing a maximum of two mismatches. Reads with multiple alignments to the reference genome were discarded in order to avoid

paralogs. An R script `convertCoordinate` (Glazer *et al.* 2015) was used for translating genomic positions between this genome assembly and the assembly by Jones *et al.* (2012).

We used Stacks v1.13 (Catchen *et al.* 2013) to call SNPs from the mapping results. Only RAD loci from the 20 autosomal chromosomes were included, whereas those from the sex chromosome (Chr XIX) and the smaller scaffolds were excluded. We set a minimum depth of 10 reads per individual, and a RAD locus must be present in all the populations and in at least 80% of the individuals in each population. Between individuals the same RAD locus was allowed to differ by no more than two base pairs, and only biallelic SNPs were retained. Furthermore, loci falling within the top 1% of the overall depth distribution were removed in order to avoid paralogs; as sequencing depth will vary among individuals, for each locus an overall depth was calculated by averaging over all individuals after standardizing its depth within each individual. The resulting SNP data set was exported to VCF (Danecek *et al.* 2011) and PLINK (Purcell *et al.* 2007) files for downstream analyses.

Genetic diversity, linkage disequilibrium and population structure analyses

Expected and observed heterozygosity was estimated using VCFtools v0.1.12b (Danecek *et al.* 2011). We calculated individual heterozygosity and population-level observed (H_O) and expected (H_E , in this case equal to nucleotide diversity π) heterozygosity. In all cases, heterozygosity estimates were divided by the number of bases surveyed by the RAD loci, thus providing genome-wide estimates.

We used PLINK 1.07 (Purcell *et al.* 2007) to estimate the decay of linkage disequilibrium (LD), based on the correlation between alleles at pairs of loci (Hill & Weir 1988) along Chromosome IV (34.15 Mb) and using a minor allele frequency threshold of 0.1. The results, including the distance at which LD had decayed by 50%, were plotted using an R script from <https://jujumaan.com/2017/07/15/linkage-disequilibrium-decay-plot/>. These analyses were conducted in order to assess if differences in LD between the Danish and Greenlandic systems could lead to differences in power for detecting genomic parallelism.

223 Φ_{ST} (Excoffier *et al.* 1992), an F_{ST} analog, was estimated between all pairs of populations, and the patterns of
224 differentiation were visualized by constructing a neighbor-joining tree based on Φ_{ST} , using the R package “ape”
225 (Paradis *et al.* 2004).

226 Genetic structure was furthermore analyzed using Principal Component Analysis (PCA) based on individual
227 multilocus genotypes. This analysis cannot tolerate missing values. All missing values in the VCF file were
228 therefore removed in a two-step process. First, individuals with missing calls in more than 1000 SNPs were
229 removed, following which all SNPs containing missing values were removed. PCA was conducted using the R
230 package “ade4” (Dray & Dufour 2007).

231 We also implemented a fastSTRUCTURE analysis (Raj *et al.* 2014), which in addition to detecting population
232 structure can reveal possible migration and admixture. SNPs with global minor allele frequency (by pooling all
233 individuals from all populations) lower than 0.1 were removed. We used a "simple prior" and conducted
234 analyses assuming K (number of groups) ranging from 2 to 15. The results were visualized in R (code provided
235 in <https://github.com/shenglin-liu/plotFastStructure>), and the optimal K, based on highest likelihood score, was
236 estimated with the script “chooseK.py” of the fastSTRUCTURE package.

237 Outlier scans and analysis of parallelism

238 We conducted two sets of outlier scans involving all Danish and Greenlandic populations, respectively, using
239 the method BayeScan 2.0 (Foll & Gaggiotti 2008). For this purpose we only used SNPs with a minor allele
240 frequency ≥ 0.1 in the pooled samples from Denmark and Greenland, respectively. Prior odds were 100, and a
241 False Discovery Rate (FDR) of 0.05 was used. We further defined a window of 100 Kb on each side of outlier
242 SNPs in order to define genomic regions that could be compared to the analyses of parallelism using delta-
243 divergence (see below).

Subsequently, we used the index delta-divergence (Roesti *et al.* 2014) for analyzing parallelism across freshwater populations. It builds on the principle that if several derived populations (in this case freshwater sticklebacks) have been founded from a common original source population (in this case marine sticklebacks), then genomic regions under parallel selection across derived populations should be 1) outliers in multiple pairwise comparisons between the same original source population and different derived populations, but 2) show low divergence in pairs of derived populations. Hence, in practical terms delta-divergence represents the difference between marine-freshwater and freshwater-freshwater population divergence. We calculated delta-divergence, plotted it along the genome for Denmark and Greenland separately, and used 0.2 as a threshold to define significantly elevated peaks following Roesti *et al.* (2014). Moreover, we defined a window of 100 kb in each direction from SNPs under putative parallel selection in order to define genomic regions that could be compared to regions previously found to show divergence between freshwater and marine sticklebacks, using the Stickleback Genome Browser (<http://sticklebrowser.stanford.edu>) (Jones *et al.* 2012). We searched the three-spine stickleback genome for possible candidate loci under selection within the genomic regions of interest using Biomart in <http://ensembl.org>.

Finally, in order to analyze population relationships for loci suggested to be under parallel selection, we constructed PCA plots involving all individuals from all populations. This was based on two sets of SNPs suggested by the delta-divergence index to be under parallel selection in Denmark and Greenland, respectively.

Analysis of chromosome inversions

Previous results based on whole genome sequencing identified three inversions in the threespine stickleback genome that were also associated with freshwater adaptation, that is Chr (Chromosome) I: 21.5-21.9 Mb, ChrXI: 5.4-5.9 Mb, and ChrXXI: 5.8-7.5 Mb (Jones *et al.* 2012). In the Glazer *et al.* (2015) assembly these coordinates correspond to ChrI: 26.1-26.4 Mb, ChrXI: 6.2-6.5 Mb, and ChrXXI: 10.0-11.4 Mb. We devised a method for

detecting candidate inversions, used this to examine whether the same inversions could be detected in our data and recorded their frequencies in marine and freshwater populations.

Inversions suppress recombination between inverted and non-inverted copies. As a result, many SNPs in this region should share the same allele frequency in a population, especially if the two haplotypes (inverted and non-inverted) have diverged for a long time. When comparing the genome-wide distribution of F_{ST} between populations exhibiting different frequencies of the inverted copy, we should consequently see a "rectangular peak" of F_{ST} for neighbouring SNPs in the inverted region (see examples from this study in Supporting Information, Fig. S1). We developed an exploratory algorithm for detecting candidate inversions and their haplotype frequencies within populations, implemented in an R script available in <https://github.com/shenglin-liu/Find-inversions>. The algorithm is based on F_{ST} calculation, but reversing the roles of populations and SNPs. Conventionally, F_{ST} is calculated between populations across many SNPs, but in this algorithm F_{ST} is calculated between SNPs across several populations over a sliding window. Thus, SNPs sharing the same allele frequency, i.e. the rectangular peaks, will have consistently low F_{ST} across populations. For more detailed information we refer to the github page, and a file with the parameters used for the present study is available in DRYAD. We detected candidate inversions using this algorithm and assessed whether these genomic regions are under parallel selection in freshwater populations by plotting frequencies of the inferred haplotypes across all populations.

Demographic history

As selection is most efficient in large populations and as the degree of parallelism and gene reuse is expected to be highest in populations that have recently diverged from a common pool of standing variation (Conte *et al.* 2012), we estimated divergence time and effective population size (N_e). This was conducted using *daadi* v1.6.3 (Gutenkunst *et al.* 2009), which makes use of allele frequency spectra (AFS) of populations. We generated joint

AFS for all 55 pairwise combinations of the 11 populations. The code for the R scripts used for generating joint AFS including correction for missing genotypes is provided in <https://github.com/shenglin-liu/vcf2sfs>. $\partial a \partial i$ is model dependent and can only estimate parameters of a predefined demographic scenario. We used a simple split model as a basis, representing a scenario where two new populations diverged from a common ancestral population at a certain time in the past. Two additional models were subsequently devised: a split-and-bottleneck model, where one of the two new populations went through a drastic population size change in the recent past, and a split-with-migration model, where gene flow was allowed between the two new populations after the split (Supporting Information, Fig. S2).

Each model was fit for 30 rounds with the starting parameters of each round perturbed by a factor of two from those of the first round. The round that gave the highest likelihood was taken as the final result. Results were further verified by comparing the observed joint AFS obtained from the data with the expected (simulated) joint AFS obtained from the model, as high similarity between them indicates successful fitting. All estimates were scaled by ancient population size, N_0 , obtained from the formula: $N_0 = \theta / 4\mu / L$, where θ is estimated by $\partial a \partial i$, μ is mutation rate per nucleotide per generation, and L is the total number of bases surveyed by the RAD loci. For consistency with a previous study (Ferchaud & Hansen 2016), we assumed a standard mutation rate of 1×10^{-8} . Confidence intervals for the estimates were generated through 300 bootstraps. Each bootstrap was run for 30 rounds with perturbed starting points to optimize the estimation.

Results

Lateral plate morphs and age determination

At the phenotypic trait of lateral plating, sticklebacks from Greenland showed near perfect parallelism, with all freshwater sticklebacks being low-plated without keel (except for a single individual from Kap1 exhibiting a keel) and all marine sticklebacks being full-plated (Table 1). In contrast, there was limited parallelism across Danish

freshwater populations, as reported previously (Ferchaud & Hansen 2016). Hence, Mos with a water-way distance to the sea of ca. 100 km (Supporting Information, Table S1) was fixed for the full-plated morph, the majority of Hal and Kro individuals were low-plated with keels, whereas all Had and Kib individuals were low-plated without keel (Table 1).

Age determination of individuals at sizes where reproduction is expected to take place (based on our observations ≥ 4 cm) showed pronounced differences between Greenlandic and Danish sticklebacks (Supporting Information, Fig. S3). Examples of otolith growth rings from individuals from Greenland and Denmark are shown in Supporting Information, Fig. S3.a and b, and Fig. S3.c shows length and ages for all individuals. In Denmark, ages ranged from two to five years, with a mean of 2.5 years (omitting the single five year old individual). In Greenland, ages ranged from three to eight years with a mean of 4.4 years (omitting the single eight year old individual). Although the latter individuals are old, a previous study of Fennoscandian sticklebacks also identified sticklebacks as old as 6 years in Northern Finland (DeFaveri & Merila 2013).

Alignment and SNP calling

Alignment success and sequencing depths are shown in Supporting Information, Table S2. The proportion of aligned reads per individual ranged from 0.72 to 0.76 (mean: 0.74, s.d.: 0.01) and sequencing depth of loci per individual ranged from 19.9 to 596.9 (mean: 70.4, s.d.: 48.6). A total of 238 SNPs were removed after filtering out the top 1% of the overall depth distribution. A total of 32,713 SNPs were obtained from 23,504 RAD loci representing 1,621,776 bp, accounting for 0.39% of the whole genome.

Genetic diversity, linkage disequilibrium and population structure

329 Individual heterozygosity showed higher diversity in marine than freshwater populations and higher diversity in
330 Greenland as opposed to Danish freshwater populations (Supporting Information, Fig. S4). Observed (H_O) and
331 expected (H_E) population-level heterozygosity was highly similar within each population (Table 1).

332 The decay of LD across Chromosome IV, here quantified as the genomic distance at which LD had decayed by
333 50%, ranged between 0.01 and 0.02 Mbp in the Greenlandic freshwater populations and 0.05 and 2.31 Mbp in
334 the Danish freshwater populations, whereas it was virtually zero in the Danish marine population, Nor, and
335 0.01 Mbp in the Greenlandic marine population KobM (Supporting Information, Fig. S5).

336 Pairwise Φ_{ST} was considerably higher between pairs of Danish populations (0.059-0.313) than between pairs of
337 Greenlandic populations (0.022-0.062), whereas Φ_{ST} was relatively low (0.039) between the Danish and
338 Greenlandic marine populations (Supporting Information, Table S3). These patterns were also evident from a
339 neighbor-joining tree based on Φ_{ST} values (Supporting Information, Fig. S6), which furthermore identified
340 Danish and Greenlandic sticklebacks as two distinct groups.

341 PCA was conducted for 184 individuals and encompassed 23,925 SNPs. The 13 individuals that were removed
342 due to missing data showed overall low sequencing depth (Supporting Information, Table S2). PCA could
343 separate all populations except Kap1 and Kap3 (Fig. 2.a), with the first five axes accounting for most variance
344 (Fig. 2.b). Populations from Denmark were distinguished along axis 1 and 2 (Fig. 2.a and 2.c) whereas
345 Greenland populations were separated along axis 6 and 9 (Fig. 2.a). One individual from Kro (a tributary to Hal)
346 grouped with Hal and was likely an immigrant (Fig. 2.c).

347 Analyses using fastSTRUCTURE assuming K from 2 to 15 identified K = 9 as representing the most likely
348 configuration, which separated most populations from each other, except Kap1 from Kap3 and Hal from Kro
349 (Fig. 3). At low K, the two marine populations (KobM and Nor) showed mixed ancestry. For instance, at K=2 to
350 K=5 Nor displayed admixture between the Greenlandic populations and the Danish freshwater populations,

whereas from K=6 to K=8 KobM displayed admixture between Nor and the Greenlandic freshwater populations. This is in accordance with assumptions that marine stickleback populations are widespread and highly mobile and could be more dynamic over time with respect to migration and admixture as compared to freshwater populations.

Outlier scans and parallelism detection

After filtering out SNPs with minor allele frequency < 0.1, 6745 SNPs remained for Greenlandic populations for the BayeScan analysis, whereas 6957 SNPs remained for Danish populations. A total of 140 outlier SNPs were observed among Greenlandic populations, corresponding to 74 genomic regions assuming a 200 Kb window (Fig. 4.a, Supporting Information Table S4). Far fewer outliers were observed among the Danish populations: 21 SNPs corresponding to 15 genomic regions (Fig. 4b, Supporting Information Table S5).

Delta-divergence analyses revealed much stronger genomic parallelism among freshwater populations in Greenland than in Denmark. This corresponded to the levels of phenotypic parallelism observed for lateral plate polymorphism in these two systems. The analyses identified 40 SNPs among Greenlandic populations showing values > 0.2 (Fig. 5.a and Supporting Information, table S6). When using a window size of 200 Kb, this corresponded to 19 genomic regions distributed across 12 chromosomes. Sixteen of the 19 genomic regions (84.2%) were also outliers in the BayeScan analyses, and conversely 16 of all 74 BayeScan outlier regions (21.6%) detected among populations in Greenland were due to parallelism across freshwater populations. In addition, seven of the delta-divergence outlier regions (36.8%) overlapped with genomic regions previously found to show elevated marine-freshwater divergence (Jones *et al.* 2012) and included well-known candidate genes associated with morphology (EDA in Chr IV) (Colosimo *et al.* 2005) and osmoregulation (atp1a1 in Chr I) (Supporting Information, tables S6 and S7).

372 Fewer SNPs (21) of elevated delta-divergence were observed for the Danish populations, most of which just
373 barely exceeded the threshold of 0.2 (Fig. 5.b and Supporting Information, table S8). They corresponded to 19
374 genomic regions distributed across 11 chromosomes. Only two of the 19 regions (10.5%) overlapped with
375 BayeScan outliers among all Danish populations (Supporting Information, Table S5), and conversely two of the
376 15 BayeScan outlier regions (13.3%) coincided with parallelism across freshwater populations. Only two of the
377 19 regions (10.5%) overlapped with high delta-divergence regions in Greenland and only one of the regions
378 (5.3%) overlapped with genomic regions previously found to show elevated divergence between freshwater
379 and marine sticklebacks (Jones *et al.* 2012) (Supporting Information, table S8). There was consequently also
380 limited overlap with respect to genes found in these genomic regions (Supporting Information, table S9).

381 We conducted PCA using the SNPs showing elevated delta-divergence among populations from Greenland and
382 Denmark, respectively. We were particularly interested in the first axis, expected to reflect ecotype-related
383 adaptation. SNPs showing high delta-divergence across freshwater populations in Greenland provided, as
384 expected, clear separation of Greenlandic marine and freshwater populations along axis 1 (Fig. 5.c). The same
385 SNPs also separated the Danish freshwater populations from Had, Kib, Hal and Kro from the marine Nor
386 population along axis 1, but less distinctly so as compared to the Greenlandic populations, and there was
387 considerable variation along axis 2 (Fig. 5.c). Interestingly, the Mos population was close to marine sticklebacks
388 along axis 1, coinciding with the fact that it showed full plating. The SNPs showing elevated delta-divergence
389 among Danish populations provided, as expected, some separation of Danish marine and freshwater
390 populations (Fig. 5.d). However, they did not separate marine and freshwater populations from Greenland,
391 suggesting that these SNPs are not generally associated with adaptation to freshwater environments.

392 In total, the analyses showed high parallelism across freshwater populations in Greenland and lower
393 parallelism in Denmark. This applied to both the phenotypic level (lateral plates) and the genomic level. The

394 results also documented, however, that genomic regions under parallel selection in Greenland showed some,
395 albeit weaker parallelism in most Danish freshwater populations, with Mos being a notable exception.

396 Inversion polymorphism

397 Our algorithm for detecting inversions successfully identified three major inverted regions that have previously
398 been reported (Jones *et al.* 2012), *i.e.* ChrI: 26.1-26.4 Mb, ChrXI: 6.2-6.5 Mb, and ChrXXI: 10.0-11.4 Mb.

399 Moreover, an additional candidate inversion was suggested in ChrIX: 5.7-7.9 Mb, although further validation
400 would be required. The frequencies of inferred haplotypes in Fig. 6 documented strong parallelism across
401 freshwater populations in Greenland for ChrI: 26.1-26.4 Mb and ChrXI: 6.2-6.5 Mb. Hence, KobM was fixed for
402 one haplotype in both genomic regions, whereas Kap1, Kap3, Qar, and KobF were almost fixed for the
403 alternative haplotype. In Denmark, parallelism was less pronounced. Nor showed the same pattern as KobM
404 and was fixed or almost fixed for the same haplotypes, whereas the freshwater populations showed highly
405 varying haplotype frequencies. Had, Kro and Kib were fixed for the freshwater-specific haplotype in Chr I,
406 whereas Mos and Hal showed intermediate frequencies. Mos was fixed for the marine-specific Chr XI
407 haplotype, whereas the freshwater-specific copy was found at low to intermediate frequencies in Had, Hal, Kro
408 and Kib. The inversion in ChrXXI: 10.0-11.4 Mb and the candidate inversion in ChrIX: 5.7-7.9 Mb did not exhibit
409 clear patterns of parallelism in neither Greenland nor Denmark.

410 Demographic history

411 The basic split model showed generally high similarity between observed and expected joint AFS, suggesting
412 that estimation was reliable (Supporting Information, Fig. S7), except for the small populations HAD and KIB,
413 where a split-and-bottleneck model may be more appropriate (see below). Ancestral effective population size
414 estimates were similar across all population pairs (Fig. 7). Contemporary effective population size estimates
415 were consistent for the same populations involved in different pairwise analyses (Fig. 7). Marine populations

416 showed the highest contemporary effective population sizes, higher than the ancestral effective population
417 size, consistent with the expectation of expansion of marine populations after the last glaciation (Liu *et al.*
418 2016). All freshwater populations were smaller compared to the ancestral effective population size, with the
419 populations from Kro, Had and Kib showing the smallest effective population sizes.

420 Estimates of divergence time (Table 2) showed that in Greenland all freshwater populations diverged from
421 each other and from the marine KobM population at about the same time (except between Kap1 and Kap3),
422 approximately 2800-3500 generations ago, assuming a mutation rate of 1×10^{-8} . The more recent divergence
423 time between Kap1 and Kap3 (688 generations ago) was expected given the adjacency of locations (Fig. 1).

424 Divergence time was generally considerably older for the Danish populations as compared to Greenland. Hal
425 and Kro diverged recently (167 generations ago), as expected given that Kro is a tributary to Hal, but most of
426 the freshwater populations diverged ca. 5,000-7,000 generations ago from marine sticklebacks (Nor) and from
427 each other. Mos deviated from the other freshwater populations by showing older divergence time estimates;
428 10,000 generations divergence time from Nor and 6,600-10,000 generations involving all the other Danish
429 freshwater populations.

430 Previous analysis of the Danish RAD data suggested bottlenecks in the Kib and Had populations (Ferchaud &
431 Hansen 2016), and the basic split model might not accurately reflect the real demographic history. Application
432 of the split-and-bottleneck model, allowing one of the two populations to experience a recent bottleneck,
433 yielded generally older divergence time estimates involving these two populations (Supporting Information,
434 Table S10), and the likelihood increased considerably. Application of the split-with-migration model mostly
435 decreased the likelihood except for analyses involving Mos, where divergence time increased considerably
436 (Supporting Information, Table S10).

These estimates assumed a standard mutation rate (1×10^{-8}). Considering the estimated ages of sticklebacks, the generation length is likely on the order of ca. 2 years in Denmark and 4 years in Greenland. Despite the difference in divergence time between marine and freshwater populations in Greenland and Denmark, as measured in generations, divergence time therefore may be quite similar measured in years. Absolute dating of divergence time requires a species-specific mutation rate estimate. If we assume a rate of 3.7×10^{-8} (Liu *et al.* 2016), the results from the split model translates into divergence between freshwater and marine populations ca. 3,000-3,800 years bp in Greenland. Similarly, in Denmark divergence between marine and most freshwater populations should have occurred ca. 2,700-3,800 years bp. Divergence time between Mos and the other Danish marine and freshwater populations would range from 3,300-5,400 years. An estimate of 1.42×10^{-8} derived from transcriptome sequencing data from ninespine (*Pungitius pungitius*) and three-spine stickleback (Guo *et al.* 2013) would suggest divergence time between marine and freshwater populations 7,900-9,900 years bp in Greenland, 7,000-9,900 years bp in Denmark, and 9,300-14,100 years bp between Mos and other Danish marine and freshwater populations (between 14,100 and 17,600 years bp, if a split-and bottleneck model was assumed for Kib, Had and Kro).

Discussion

Most previous studies of phenotypic and genomic parallelism have made deductions based on comparisons involving multiple species (Conte *et al.* 2012; Raeymaekers *et al.* 2017), have focused on detecting if parallelism is a general feature within single species or species complexes (Bradbury *et al.* 2010; Gagnaire *et al.* 2013; Losos 2009; Ravinet *et al.* 2016; Terekhanova *et al.* 2014), or have focused on detecting the genetic architecture and possible genomic parallelism associated with individual traits (Chan *et al.* 2010; Colosimo *et al.* 2005; Cooley *et al.* 2011; Laurent *et al.* 2016). Here we took a different approach and made use of the presence of separate systems within a single species showing contrasting patterns of parallelism. This allowed for adding

different aspects to our understanding of the dynamics of parallelism than could have been obtained from analyzing each system on its own. Hence, the results showed lower parallelism (phenotypic and genomic) across stickleback populations in Denmark as compared to Greenland, but they also demonstrated that parallelism was not altogether absent in the Danish system. SNPs showing high parallelism across Greenland populations also separated Danish marine and freshwater sticklebacks in a PCA, although to a lower degree than in Greenland, and with Mos as a notable exception.

Recent discussions about the utility of RAD sequencing for detecting possible selection have highlighted the importance of considering linkage disequilibrium when assessing results of outlier scans (Catchen *et al.* 2017; Lowry *et al.* 2017; McKinney *et al.* 2017). Importantly, the differences between systems in the present study were not suggested to be a result of different detection power due to different linkage disequilibrium in the two systems. Linkage disequilibrium, and thereby the ability to detect genomic regions showing high divergence, varied across populations but was overall stronger in the Danish relative to the Greenlandic system, and yet it was the system in Greenland that showed pronounced genomic parallelism. There is also emerging evidence that adaptive alleles are not randomly distributed across genomes but tend to accumulate in low-recombining regions (Berner & Roesti 2017; Samuk *et al.* 2017). However, it seems unlikely that differences should exist in low-recombining regions between the Danish and Greenlandic systems that could explain the different outcomes with respect to parallelism. It should nevertheless be noted that with the genomic coverage achieved by RAD sequencing combined with linkage disequilibrium it is in principle not possible to distinguish if the same or different causative mutations underlie genomic regions showing parallel divergence. Even using whole genome sequencing this would still be a challenge and require dedicated molecular biology experiments, such as those conducted by O'Brown *et al.* (2015) for identifying causative mutations in the EDA gene. In the present study, however, the low genomic parallelism observed across Danish freshwater populations does imply that gene reuse is low in this system, whereas the pronounced genomic parallelism across freshwater

population in Greenland implies a high degree of parallel selection based on a shared pool of standing variation, even if this cannot be ascertained at the level of each genomic region.

Another potential problem is reduced effective population size and bottlenecks creating stochastic heterogeneous genomic divergence. This makes it difficult to distinguish between regions of high divergence caused by drift or selection (Poh *et al.* 2014; Storz 2005), and specifically for separating the effects of genetic drift and selection-driven parallel evolution (MacPherson & Nuismer 2017). Analysis of joint AFS suggested that Had, Kib and Kro were the smallest populations. Yet, considering chromosome inversions these three smallest populations exhibited a pattern similar to the Greenlandic freshwater populations for the inversion in Chromosome I with fixation of the same haplotype, whereas Mos and Hal from larger lakes and exhibiting higher effective population sizes showed a mixture of haplotypes. As this inversion is likely to be under selection (Jones *et al.* 2012), this does suggest that selection is detectable in these small populations. This is also in accordance with pairwise sliding windows F_{ST} analyses in Ferchaud & Hansen (2016), which found that Had and Kib were in fact the populations that conformed the most to a pattern of parallelism across freshwater populations.

In the following, we discuss factors that could underlie differences in the degree of parallelism between systems: differences in selection regimes across populations and differences in divergence time between marine and freshwater populations. Based on the parallelism observed in the Greenland system, we furthermore discuss the general biological significance of parallelism and the extent to which it reflects overall patterns of adaptive processes.

Differences in selection regimes

A factor potentially explaining reduced parallelism concerns differences in selection regimes; if selection regimes are heterogeneous across a set of populations, then this would expectedly lead to reduced parallelism

504 as compared to situations where selection regimes are homogeneous. This has indeed been documented in
505 sticklebacks (Stuart *et al.* 2017) and for example in experimental evolution studies with *Pseudomonas*
506 *fluorescens* (Bailey *et al.* 2015). Selection regimes were expected to show high similarity across freshwater
507 populations in Greenland, and in accordance with this a high degree of parallelism was observed. Conversely,
508 there were stronger differences in environmental conditions across the Danish freshwater environments and
509 lower parallelism. Yet, it is a striking feature of the inversion polymorphisms in Chr I and XI that they showed
510 virtual fixation of alternate haplotypes in freshwater and marine populations in Greenland, whereas in the
511 Danish freshwater populations both haplotypes were retained, except for Chr XI, where Mos was fixed for the
512 putatively marine-specific haplotype. Similarly, the PCA in Fig. 5c showed that freshwater and marine
513 sticklebacks in Denmark can be separated based on marine-freshwater outlier SNPs identified in the
514 Greenlandic populations that encompass several genomic regions previously found to be associated with
515 marine-freshwater divergence (Jones *et al.* 2012), but not as distinctly as in Greenland. It suggests that
516 polymorphism is retained in the Danish populations despite selection (see also allelic frequencies of these SNPs
517 in Supporting Information, Table S6). This raises the possibility that spatially and temporally fluctuating
518 selection and/or disruptive selection occurs (Ellner & Hairston 1994; Hedrick 2002; Kingsolver *et al.* 2001),
519 which would maintain polymorphism at loci under selection, causing decreased divergence between marine
520 and freshwater populations and reducing parallelism. Disruptive selection, temporal shifts of selection and
521 negative frequency-dependent selection have been demonstrated at the phenotypic and molecular levels in
522 sticklebacks (Barrett *et al.* 2008; Bolnick & Lau 2008; Bolnick & Stutz 2017; Marchinko *et al.* 2014) and are
523 overall well established phenomena in evolutionary biology (Grant & Grant 2002). However, resolving which
524 dynamics could be involved in the present case (e.g. temporal variation in abundance of fish predators
525 maintaining variation at loci affecting morphology) would require long-term genetic and environmental
526 monitoring of systems showing high and low parallelism, respectively.

527 Differences in marine-freshwater divergence time

528 A second factor represents the time since replicate populations diverged from a common ancestor, suggested
529 to be a critical factor determining the extent of gene reuse in parallel evolution (Conte *et al.* 2012). Specifically,
530 a longer divergence time between freshwater and marine sticklebacks in Denmark could explain the low
531 parallelism. Marine-freshwater divergence time measured in generations was approximately two times higher
532 in Denmark compared to Greenland. However, our finding that sticklebacks from Greenland are much older
533 than individuals from Denmark at comparable sizes suggest that measured in number of years divergence time
534 may be quite similar. Evolution occurs over generations rather than years, and it is at least theoretically
535 possible that the more generations elapsed in the Danish system could result in reduced genomic parallelism in
536 Denmark relative to Greenland. Adaptation to freshwater conditions after colonization by marine sticklebacks
537 has been found to occur rapidly at both phenotypic and genomic levels (Bassham *et al.* 2017; Bell *et al.* 2004;
538 Le Rouzic *et al.* 2011; Lescak *et al.* 2015; Terekhanova *et al.* 2014), so this would imply that the genetic
539 architecture of adaptive traits in replicate populations should initially be similar and then change increasingly
540 over time if different episodes of selection occur in individual populations.

541 The Mos population was an important component in the reduction of genomic parallelism among the Danish
542 populations. Here, divergence time may be a decisive factor as it showed considerably older divergence from
543 other populations. Given the dynamic nature of marine populations it is even possible that its marine ancestral
544 source population was significantly different from present marine sticklebacks in the region. If this is the case,
545 Mos and the other Danish populations may not have had access to the same source of standing variation. In
546 fact, the genetic composition of the Mos population is puzzling, more resembling that of marine populations as
547 based on the loci showing high delta divergence in the Greenlandic system (Fig. 5.c). Here it should be
548 considered, however, that we do not know if these loci mark genomic regions associated with e.g.

morphological traits favoured by selection in Mos and marine populations, or if they mark regions associated with adaptation to marine and freshwater *sensu stricto*, such as physiological traits. Some of the regions are with high certainty associated with genes affecting morphology, such as EDA in Chromosome IV (O'Brien *et al.* 2015), and many of the genomic regions harboured genes potentially affecting morphological traits (Supporting Information, Table S7). On the other hand, Mos showed a frequency of 0.85 of a marine-specific SNP allele close to the *atp1a1* gene in Chromosome I (Supporting Information, Table S6). This gene is strongly associated with osmoregulation in fishes, including sticklebacks (McCairns & Bernatchez 2010; McCormick 2001). At the same time, however, the genomic region in which it resides harbours an inversion reducing recombination (Jones *et al.* 2012), which increases the possibility that it hitch-hikes with a different gene that is the actual target of selection.

We attempted more precise dating of the divergence time between marine and freshwater populations by first assuming a mutation rate of 3.7×10^{-8} , calibrated on the basis of the demographic history of stickleback populations inferred using PSMC analysis (Liu *et al.* 2016). However, this suggested very recent founding that seems incongruent with subfossil data showing the presence of sticklebacks in Greenland and Denmark 9,000 and at least 6-7,000 years bp, respectively (Bennike 1997; Enghoff 1994). Assuming instead a mutation rate of 1.42×10^{-8} (Guo *et al.* 2013) yielded estimates that seem realistic, suggesting founding of freshwater populations in both Denmark and Greenland ca. 7-11,000 years bp and a divergence time of up to 14,600 years bp for Mos compared to other populations. However, applying this same mutation rate for PSMC based on whole genome sequences would suggest that populations in Denmark were founded during the Last Glacial Maximum ca. 20,000 years bp, which is unrealistic (see Fig. S4 in Supporting Information of Liu *et al.* (2016)). Several factors may underlie these seeming incongruences, such as inaccuracies in estimating mutation rates, heterogeneity across marine populations (DeFaveri *et al.* 2013; Guo *et al.* 2015) and historical admixture causing present marine populations to have diverged from the ancestral populations, and failure of the real

572 demographic history of populations to be reflected by the demographic models used in the $\partial\text{a}\partial\text{i}$ analyses.
573 Despite these caveats, however, we find it likely that Mos represents a different and earlier postglacial
574 founding event, possibly from a different source than the other populations included in the study and that this
575 underlies the lack of parallelism associated with this population.

576 Biological significance of parallelism

577 Do studies of parallelism and gene reuse provide information about the predictability of evolution? If so, this
578 would be particularly interesting in the case of Greenland, as the Arctic is one of the regions most strongly
579 affected by ongoing climate change (Hinzman *et al.* 2005), and three-spine sticklebacks have been observed to
580 expand northwards colonizing freshwater bodies where they have not previously been recorded (Svenning *et al.*
581 2015). In Western Greenland sticklebacks are absent in lakes north of ca. 72°N, but subfossil data (6800 ^{14}C
582 years old) show that they have previously been distributed ca. 200 km further North (Bennike 1997) and have
583 since then become extirpated. The current distributional range is therefore expected to be delimited by
584 temperature and not by lack of available habitat, and further northward expansion is anticipated as the climate
585 warms. Given the observed parallelism across extant freshwater populations, involving 21.6% of the total
586 number of outliers observed among populations in the system, it could be expected that new colonization of
587 freshwater habitats would be facilitated by transport of beneficial alleles from other extant freshwater
588 populations via marine sticklebacks, as assumed by the Transporter Hypothesis (Schluter & Conte 2009).

589 Studies of recently colonized stickleback populations provide results supporting this (Bassham *et al.* 2017;
590 Lescak *et al.* 2015; Terekhanova *et al.* 2014). However, as pointed out by e.g. Kaeuffer *et al.* (2012), the degree
591 of non-parallelism (in the present case 79.4% of outliers) is just as important to consider as parallelism, as this
592 could both indicate idiosyncratic responses to similar selection regimes and responses to differences in
593 selection regimes across environments. Also, outlier scans as in the present study will tend to detect loci

underlying traits with a simple genetic architecture, such as morphological traits. Many other phenotypic traits are, however, highly polygenic, in which cases selection is unlikely to leave genomic footprints that can be easily detected using conventional genomic scans (Pritchard & Di Rienzo 2010; Pritchard *et al.* 2010). Moreover, for highly polygenic traits there can be different genetic pathways leading to similar phenotypic adaptations, thereby causing reduced parallelism at the genomic level (Arendt & Reznick 2008).

In total, we therefore expect that newly founded freshwater stickleback populations in Greenland would show similar parallel phenotypic and genomic level responses as reported in other studies (Lescak *et al.* 2015; Terekhanova *et al.* 2014). We would at the same time be hesitant to extrapolate this to a general expectation that adaptation to freshwater conditions involves parallel evolution and gene reuse. Adaptation undoubtedly encompasses many traits that may exhibit a complex polygenic architecture, not necessarily leading to gene reuse and genomic parallelism.

Conclusions

Our analysis of two stickleback systems showing different levels of phenotypic and genomic parallelism allowed for teasing apart some of the factors underlying these differences. First, the results demonstrated that at the genomic level there was not a clear limit between presence and absence of parallelism. Instead, differences were gradual. We suggest that this reflects different modes of selection acting in the different systems; strictly directional versus spatially and temporally varying selection. Second, the population contributing the most to decreased parallelism in the Danish system had diverged earlier from a marine ancestor than the other populations and may represent a separate postglacial recolonization event from a different source. Our results illustrate the complexity of parallelism and point towards inclusion of more factors, such as population history, for improving our general understanding of its dynamics and biological significance.

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622 **Data Accessibility**

623 Sequence reads have been deposited in the NCBI Sequence Read Archive under project numbers
624 SAMN02585793 and SAMN03076274 (samples from Denmark) and PRJNA369168 (samples from Greenland). R
625 scripts for identifying candidate inversions, for plotting results from fastSTRUCTURE, and for creating allele
626 frequency spectra from a VCF file are available at <https://github.com/shenglin-liu/Find-inversions>,
627 <https://github.com/shenglin-liu/plotFastStructure>, and <https://github.com/shenglin-liu/vcf2sfs>, respectively. A
628 VCF file with the SNP data and an example of the Find_inversions script with the parameters used in this study
629 are available on DRYAD (doi:10.5061/dryad.818c434).

630 **Supporting Information**

631 Table S1 and Figs. S1-S7 are included in the file Supporting_Information_TableS1_FigS1-7.pdf. Tables S2-S10
632 are included in the file Supporting_Information_Table_S2-10.xlsx.

633

634 **Authors' contributions**

635 MMH, SL and ALF conceived the project. RN and MMH planned and coordinated sampling of sticklebacks. SL
636 conducted the bioinformatics and statistical analyses with inputs from ALF and MMH. PG analyzed otoliths and

estimated ages of sticklebacks. SL and MMH wrote the paper with input from all other authors. All authors read and approved the final manuscript.

References

- Allen JRM, Wootton RJ (1982) Age, growth and rate of food consumption in an upland population of the 3-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology* **21**, 95-105.
- Arendt J, Reznick D (2008) Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? *Trends in Ecology & Evolution* **23**, 26-32.
- Bailey SF, Rodrigue N, Kassen R (2015) The effect of selection environment on the probability of parallel evolution. *Molecular Biology and Evolution* **32**, 1436-1448.
- Baird NA, Etter PD, Atwood TS, *et al.* (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *Plos One* **3**, e3376.
- Banbura J (1994) Lateral plate morph differentiation of freshwater and marine populations of the 3-spined stickleback, *Gasterosteus aculeatus*, in Poland. *Journal of Fish Biology* **44**, 773-783.
- Barrett RDH (2010) Adaptive evolution of lateral plates in three-spined stickleback *Gasterosteus aculeatus*: a case study in functional analysis of natural variation. *Journal of Fish Biology* **77**, 311-328.
- Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics* **12**, 767-780.
- Barrett RDH, Rogers SM, Schluter D (2008) Natural selection on a major armor gene in threespine stickleback. *Science* **322**, 255-257.
- Bassham S, Catchen J, Lescak E, Von Hippel F, Cresko W (2017) Sweeping genomic remodeling through repeated selection of alternatively adapted haplotypes occurs in the first decades after marine stickleback colonize new freshwater ponds. *BioRxiv*, <https://doi.org/10.1101/191627>.
- Bell MA (2001) Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica* **112**, 445-461.
- Bell MA, Aguirre WE, Buck NJ (2004) Twelve years of contemporary armor evolution in a threespine stickleback population. *Evolution* **58**, 814-824.
- Bennike O (1997) Quaternary vertebrates from Greenland: A review. *Quaternary Science Reviews* **16**, 899-909.
- Bernatchez L, Renaut S, Whiteley AR, *et al.* (2010) On the origin of species: insights from the ecological genomics of lake whitefish. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**, 1783-1800.
- Berner D, Roesti M (2017) Genomics of adaptive divergence with chromosome-scale heterogeneity in crossover rate. *Molecular Ecology* **26**, 6351-6369.
- Bolnick DI, Lau OL (2008) Predictable patterns of disruptive selection in stickleback in postglacial lakes. *American Naturalist* **172**, 1-11.
- Bolnick DI, Stutz WE (2017) Frequency dependence limits divergent evolution by favouring rare immigrants over residents. *Nature* **546**, 285-288.
- Bradbury IR, Hubert S, Higgins B, *et al.* (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **277**, 3725-3734.

- Cano JM, Matsuba C, Makinen H, Merila J (2006) The utility of QTL-Linked markers to detect selective sweeps in natural populations - a case study of the EDA gene and a linked marker in threespine stickleback. *Molecular Ecology* **15**, 4613-4621.
- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology* **22**, 3124-3140.
- Catchen JM, Hohenlohe PA, Bernatchez L, *et al.* (2017) Unbroken: RADseq remains a powerful tool for understanding the genetics of adaptation in natural populations. *Molecular Ecology Resources* **17**, 362-365.
- Chan YF, Marks ME, Jones FC, *et al.* (2010) Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* **327**, 302-305.
- Colosimo PF, Hosemann KE, Balabhadra S, *et al.* (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* **307**, 1928-1933.
- Coluzzi M, Sabatini A, della Torre A, Di Deco MA, Petrarca V (2002) A polytene chromosome analysis of the *Anopheles gambiae* species complex. *Science* **298**, 1415-1418.
- Conte GL, Arnegard ME, Peichel CL, Schluter D (2012) The probability of genetic parallelism and convergence in natural populations. *Proceedings of the Royal Society B-Biological Sciences* **279**, 5039-5047.
- Cooley AM, Modliszewski JL, Rommel ML, Willis JH (2011) Gene duplication in *Mimulus* underlies parallel floral evolution via independent trans-regulatory changes. *Current Biology* **21**, 700-704.
- Dalziel AC, Martin N, Laporte M, Guderley H, Bernatchez L (2015) Adaptation and acclimation of aerobic exercise physiology in Lake Whitefish ecotypes (*Coregonus clupeaformis*). *Evolution* **69**, 2167-2186.
- Danecek P, Auton A, Abecasis G, *et al.* (2011) The variant call format and VCFtools. *Bioinformatics* **27**, 2156-2158.
- Deagle BE, Jones FC, Absher DM, Kingsley DM, Reimchen TE (2013) Phylogeography and adaptation genetics of stickleback from the Haida Gwaii archipelago revealed using genome-wide single nucleotide polymorphism genotyping. *Molecular Ecology* **22**, 1917-1932.
- Deagle BE, Jones FC, Chan YGF, *et al.* (2012) Population genomics of parallel phenotypic evolution in stickleback across stream-lake ecological transitions. *Proceedings of the Royal Society B-Biological Sciences* **279**, 1277-1286.
- DeFaveri J, Merila J (2013) Variation in age and size in Fennoscandian three-spined sticklebacks (*Gasterosteus aculeatus*). *Plos One* **8**, e80866.
- DeFaveri J, Shikano T, Shimada Y, Merila J (2013) High degree of genetic differentiation in marine three-spined sticklebacks (*Gasterosteus aculeatus*). *Molecular Ecology* **22**, 4811-4828.
- Dray S, Dufour AB (2007) The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**, 1-20.
- Ellner S, Hairston NG (1994) Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *American Naturalist* **143**, 403-417.
- Elmer KR, Meyer A (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends in Ecology & Evolution* **26**, 298-306.
- Engelhoff IB (1994) Fishing in Denmark during the Ertebølle Period. *International Journal of Osteoarchaeology* **4**, 65-96.
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.
- Ferchaud AL, Hansen MM (2016) The impact of selection, gene flow and demographic history on heterogeneous genomic divergence: threespine sticklebacks in divergent environments. *Molecular Ecology* **25**, 238-259.

- Foll M, Gaggiotti O (2008) A genome scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**, 977-993.
- Gagnaire PA, Pavey SA, Normandeau E, Bernatchez L (2013) The genetic architecture of reproductive isolation during speciation-with-gene-flow in lake whitefish species pairs assessed by RAD sequencing. *Evolution* **67**, 2483-2497.
- Glazer AM, Killingbeck EE, Mitros T, Rokhsar DS, Miller CT (2015) Genome Assembly Improvement and Mapping Convergent Evolved Skeletal Traits in Sticklebacks with Genotyping-by-Sequencing. *G3-Genes Genomes Genetics* **5**, 1463-1472.
- Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**, 707-711.
- Guo BC, Chain FJJ, Bornberg-Bauer E, Leder EH, Merila J (2013) Genomic divergence between nine- and three-spined sticklebacks. *Bmc Genomics* **14**.
- Guo BC, DeFaveri J, Sotelo G, Nair A, Merila J (2015) Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks. *Bmc Biology* **13**.
- Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD (2009) Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *Plos Genetics* **5**, e1000695.
- Hagen DW, Moodie GEE (1982) Polymorphism for plate morphs in *Gasterosteus aculeatus* on the East Coast of Canada and an hypothesis for their global distribution. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **60**, 1032-1042.
- Hedrick PW (2002) Pathogen resistance and genetic variation at MHC loci. *Evolution* **56**, 1902-1908.
- Hill WG (1979) Note on effective population size with overlapping generations. *Genetics* **92**, 317-322.
- Hill WG, Weir BS (1988) Variances and covariances of squared linkage disequilibria in finite populations. *Theoretical Population Biology* **33**, 54-78.
- Hinzman LD, Bettez ND, Bolton WR, *et al.* (2005) Evidence and implications of recent climate change in northern Alaska and other arctic regions. *Climatic Change* **72**, 251-298.
- Hoekstra HE, Hirschmann RJ, Bunday RA, Insel PA, Crossland JP (2006) A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* **313**, 101-104.
- Hohenlohe PA, Bassham S, Etter PD, *et al.* (2010) Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *Plos Genetics* **6**, 23.
- Jones FC, Grabherr MG, Chan YF, *et al.* (2012) The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**, 55-61.
- Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP (2012) Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution* **66**, 402-418.
- Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225-1241.
- Kingsolver JG, Hoekstra HE, Hoekstra JM, *et al.* (2001) The strength of phenotypic selection in natural populations. *American Naturalist* **157**, 245-261.
- Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation and speciation. *Genetics* **173**, 419-434.
- Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* **10**, R25.
- Laurent S, Pfeifer SP, Settles ML, *et al.* (2016) The population genomics of rapid adaptation: disentangling signatures of selection and demography in white sands lizards. *Molecular Ecology* **25**, 306-323.
- Le Rouzic A, Østbye K, Klepaker TO, *et al.* (2011) Strong and consistent natural selection associated with armour reduction in sticklebacks. *Molecular Ecology* **20**, 2483-2493.
- Leinonen T, Herczeg G, Cano JM, Merila J (2011) Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: a test of the refuge use hypothesis. *Evolution* **65**, 2916-2926.

- Lescak EA, Bassham SL, Catchen J, *et al.* (2015) Evolution of stickleback in 50 years on earthquake-uplifted islands. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E7204-E7212.
- Liu S, Hansen MM, Jacobsen MW (2016) Region-wide and ecotype-specific differences in demographic histories of threespine stickleback populations, estimated from whole genome sequences. *Molecular Ecology* **25**, 5187-5202.
- Losos JB (2009) *Lizards in an evolutionary tree : the ecology of adaptive radiation in anoles* University of California Press, Berkeley.
- Lowry DB, Hoban S, Kelley JL, *et al.* (2017) Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. *Molecular Ecology Resources* **17**, 142-152.
- MacPherson A, Nuismer SL (2017) The probability of parallel genetic evolution from standing genetic variation. *Journal of Evolutionary Biology* **30**, 326-337.
- Marchinko KB, Matthews B, Arnegard ME, Rogers SM, Schluter D (2014) Maintenance of a genetic polymorphism with disruptive natural selection in stickleback. *Current Biology* **24**, 1289-1292.
- McCairns RJS, Bernatchez L (2010) Adaptive divergence between freshwater and marine sticklebacks: Insights into the role of phenotypic plasticity from an integrated analysis of candidate gene expression. *Evolution* **64**, 1029-1047.
- McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. *American Zoologist* **41**, 781-794.
- McKinney GJ, Larson WA, Seeb LW, Seeb JE (2017) RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry *et al.* (2016). *Molecular Ecology Resources* **17**, 356-361.
- Moser D, Roesti M, Berner D (2012) Repeated lake-stream divergence in stickleback life history within a Central European lake basin. *Plos One* **7**, e50620.
- Munzing J (1963) Evolution of variation and distributional patterns in European populations of 3-spined stickleback, *Gasterosteus aculeatus*. *Evolution* **17**, 320-&.
- Noor MAF, Grams KL, Bertucci LA, Reiland J (2001) Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 12084-12088.
- O'Brown NM, Summers BR, Jones FC, Brady SD, Kingsley DM (2015) A recurrent regulatory change underlying altered expression and Wnt response of the stickleback armor plates gene EDA. *Elife* **4**, e05290.
- Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**, 289-290.
- Poh YP, Domingues VS, Hoekstra HE, Jensen JD (2014) On the prospect of identifying adaptive loci in recently bottlenecked populations. *Plos One* **9**, e110579.
- Pritchard JK, Di Rienzo A (2010) Adaptation - not by sweeps alone. *Nature Reviews Genetics* **11**, 665-667.
- Pritchard JK, Pickrell JK, Coop G (2010) The Genetics of Human Adaptation: Hard Sweeps, Soft Sweeps, and Polygenic Adaptation. *Current Biology* **20**, R208-R215.
- Pujolar JM, Jacobsen MW, Frydenberg J, *et al.* (2013) A resource of genome-wide single-nucleotide polymorphisms generated by RAD tag sequencing in the critically endangered European eel. *Molecular Ecology Resources* **13**, 706-716.
- Purcell S, Neale B, Todd-Brown K, *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-575.
- Raeymaekers JAM, Chaturvedi A, Hablutzel PI, *et al.* (2017) Adaptive and non-adaptive divergence in a common landscape. *Nature Communications* **8**.
- Raj A, Stephens M, Pritchard JK (2014) fastSTRUCTURE: Variational inference of population structure in large SNP data sets. *Genetics* **197**, 573-589.

- Ravinet M, Westram A, Johannesson K, *et al.* (2016) Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. *Molecular Ecology* **25**, 287-305.
- Reimchen TE (2000) Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: Functional implications for the ancestral plate condition. *Behaviour* **137**, 1081-1096.
- Roesti M, Gavrillets S, Hendry AP, Salzburger W, Berner D (2014) The genomic signature of parallel adaptation from shared genetic variation. *Molecular Ecology* **23**, 3944-3956
- Roesti M, Kueng B, Moser D, Berner D (2015) The genomics of ecological vicariance in threespine stickleback fish. *Nature Communications* **6**, 8967.
- Samuk K, Owens GL, Delmore KE, *et al.* (2017) Gene flow and selection interact to promote adaptive divergence in regions of low recombination. *Molecular Ecology* **26**, 4378-4390.
- Savolainen O, Lascoux M, Merila J (2013) Ecological genomics of local adaptation. *Nature Reviews Genetics* **14**, 807-820.
- Schluter D, Conte GL (2009) Genetics and ecological speciation. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 9955-9962.
- Schluter D, Nagel LM (1995) Parallel speciation by natural selection. *American Naturalist* **146**, 292-301.
- Sodeland M, Jorde PE, Lien S, *et al.* (2016) "Islands of Divergence" in the Atlantic cod genome represent polymorphic chromosomal rearrangements. *Genome Biology and Evolution* **8**, 1012-1022.
- Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology* **14**, 671-688.
- Stuart YE, Veen T, Weber JN, *et al.* (2017) Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology & Evolution* **1**, 0158.
- Svenning MA, Aas M, Borgstrom R (2015) First records of three-spined stickleback *Gasterosteus aculeatus* in Svalbard freshwaters: An effect of climate change? *Polar Biology* **38**, 1937-1940.
- Terekhanova NV, Logacheva MD, Penin AA, *et al.* (2014) Fast evolution from precast bricks: genomics of young freshwater populations of threespine stickleback *Gasterosteus aculeatus*. *PLoS Genet* **10**, e1004696.

Figure legends

Fig. 1. Maps showing the locations of the sampled three-spine stickleback populations in Denmark, Northern Europe and the Nuuk Fjord region, Western Greenland. The red rectangles represent marine populations and the red triangles freshwater populations.

Fig. 2. Principal Component Analysis showing the genetic relationships among individuals from the 11 studied populations. a. Parallel coordinate plot of individuals along the first 10 axes. b. Eigenvalues of all the 183 axes. c. Two- dimensional plot of individuals positioned along the first two axes. Kap1, Kap3, Qar and KobF are

845 freshwater populations and KobM a marine population from Greenland. Had, Mos, Hal, Kro and Kib are
846 freshwater populations and Nor a marine population from Denmark.

847 Fig. 3. Population structure analysis using fastSTRUCTURE (Raj *et al.* 2014). Individual admixture proportions
848 are shown assuming numbers of groups (K) ranging from 2 to 15, with fastSTRUCTURE indicating K = 9 as the
849 most likely configuration.

850 Fig. 4. Results of outlier scans across all populations from a) Greenland and b) Denmark using BayeScan 2.0 (Foll
851 & Gaggiotti 2008). The dashed grey line denotes the significance threshold at a False Discovery Rate of 0.05.

852 Fig. 5. Analysis of genomic parallelism across freshwater populations of three-spine sticklebacks in Greenland
853 and Denmark. a. Plot of genome-wide delta-divergence (Roesti *et al.* 2014) along 20 chromosomes in
854 freshwater populations in Greenland (the sex chromosome XIX was excluded). Values exceeding 0.2 were
855 deemed significant and are indicated by a horizontal dashed line. b. Plot of genome-wide delta-divergence
856 along 20 chromosomes in freshwater populations in Denmark. c. Genetic relationships among individuals from
857 all populations assessed using Principal Component Analysis (PCA) based on the SNPs showing high (> 0.2)
858 delta-divergence in populations from Greenland. Individuals were plotted along axes 1 and 2. d. Genetic
859 relationships among individuals from all populations assessed using Principal Component Analysis (PCA) based
860 on the SNPs showing high (> 0.2) delta-divergence in populations from Denmark. Individuals were plotted along
861 axes 1 and 2.

862 Fig. 6. Frequency of inverted haplotypes in chromosomal regions showing inversion polymorphisms across the
863 11 studied populations. Inversions in Chr I, IX, XI and XXI were analyzed.

864 Fig. 7. Effective population size estimates for the 11 populations using $\partial a \partial i$ (Gutenkunst *et al.* 2009) under the
865 split model. The estimates were based on joint allele frequency spectra (AFS) between pairs of populations.

866 Each population is therefore represented by 10 estimates, which are presented in each plot. Similarity of
867 estimates for different pairwise AFS indicates high consistency of results. Two estimates of effective population
868 size are provided; contemporary effective population sizes are indicated by red dots, whereas grey dots denote
869 ancient effective population size.

For Review Only

Table 1. Sample codes, locations and habitats of the sampled populations along with sample sizes for RAD sequencing and analyses of lateral plate morphology. Genome-wide expected (H_E) and observed (H_O) heterozygosity is also listed along with the percentages of individuals showing low, partial and full-plated morphology and showing presence or absence of a keel.

Sample code	Geographic coordinates	Location	Region	Habitat	Sample size, RADseq	H_E	H_O	Sample size, plate morphology	Morph			Keel	
									Low	Partial	Full	Yes	No
KobM	64.137 ; -51.385	Kobbefjord	Greenland	Marine	20	0.00190	0.00191	19	0%	0%	100%	100%	0%
Kap1	64.430 ; -50.209	Kapisilit Lake 1	Greenland	Lake	20	0.00183	0.00180	22	100%	0%	0%	5%	95%
Kap3	64.429 ; -50.198	Kapisilit Lake 3	Greenland	Lake	19	0.00184	0.00182	27	100%	0%	0%	0%	100%
Qar	63.991 ; -51.446	Qarajat Lake	Greenland	Lake	16	0.00174	0.00175	30	100%	0%	0%	0%	100%
KobF	64.132 ; -51.377	Kobbefjord Lake	Greenland	Lake	20	0.00176	0.00176	30	100%	0%	0%	0%	100%
Nor	56.023; 10.262	Norsminde Fjord	Denmark	Marine	20	0.00201	0.00198	25	0%	0%	100%	100%	0%
Had	56.320 ; 10.043	Hadsten	Denmark	Lake	19	0.00116	0.00120	25	100%	0%	0%	0%	100%
Mos	56.029 ; 9.797	Lake Mossø	Denmark	Lake	13	0.00117	0.00117	20	0%	0%	100%	100%	0%
Hal	56.374 ; 9.346	Lake Hald	Denmark	Lake	14	0.00165	0.00163	25	92%	8%	0%	88%	12%
Kro	56.396 ; 9.348	Krobæk River	Denmark	Lake	20	0.00142	0.00142	25	92%	8%	0%	80%	20%
Kib	56.037 ; 8.857	Lake Kibæk	Denmark	tributary Lake	16	0.00103	0.00108	17	100%	0%	0%	0%	100%

Table 2. Estimates of divergence time (in numbers of generations) and their bootstrapped 95% confidence intervals between threespine stickleback populations using *daði* and assuming the split model (see Supporting Information, Fig. S2). The analyses assumed a mutation rate of 10^{-8} . Divergence time estimates assuming split-with-migration and split-with-bottleneck models are listed in Supporting Information, Table S10.

[illegible]

- Kap1
- Kap3
- Qar
- KobF
- KobM
- Nor
- Had
- Mos
- Hal
- Kro
- Kib

SNPs showing parallelism in Greenland

SNPs showing parallelism in Denmark

Chr I: 26.1–26.4Mb **Chr IX: 5.7–7.9Mb** **Chr XI: 6.2–6.5Mb** **Chr XXI: 10.0–11.4Mb**

Kap1

Kap3

Qar

KobF

KobM

Nor

Had

Mos

Hal

Kro

Kib