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Spatio-temporal patterns in pelvic reduction in threespine stickleback (*Gasterosteus aculeatus* L.) in Lake Storvatnet

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ABSTRACT

Questions: The pelvic girdle with associated spines is an integrated anti-predator defence apparatus, and is assumed to protect against piscivores in the threespine stickleback. On the other hand, it might be costly to produce the pelvic apparatus in ion-poor and mineral-challenging freshwater.

Hypothesis: Stickleback with a reduced pelvic apparatus should use more shelter and be more nocturnal, avoiding predation risk. In contrast, stickleback with a well-developed pelvic apparatus should have reduced mortality during ontogeny in encounters with piscivores and thus have a longer expected lifespan. Given these two life-history strategies, we expect assortative mating as a result of divergent selection.

Organism: Marine and freshwater threespine stickleback (*Gasterosteus aculeatus* L.).

Places and times: Two representative ancestral marine populations and 36 freshwater populations in northwestern Norway (Lake Storvatnet, the main focus of the study, and three lakes downstream of it). Material was collected from 2006 to 2009.

Analytical methods: We categorized nominal pelvic apparatus development (CPS morphs) in all fish, and measured metrics associated with these categories in a subsample. We also studied temporal, spatial, and habitat variation in the distribution of pelvic morphs in Lake Storvatnet. In this population, and downstream populations, we contrasted the detailed pelvic morphology with the measured genetic diversity (microsatellites), also estimating gene flow. In Lake Storvatnet, we tested for genetic divergence and signs of potential build-up of reproductive isolation via assortative mating among the observed nominal categories of pelvic reduction (CPS).

Results: Pelvic reduction was seen only in Lake Storvatnet, where more than 50% of fish had a reduced pelvis. The distribution of pelvic morphs was stable over time and did not differ between habitats. The proportion of fish with pelvic reduction decreased with age. Freshwater

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stickleback tended to have a smaller pelvis than marine fish. The Lake Storvatnet stickleback were genetically differentiated from the downstream Lake Gjerhaugsvatn population, and both of these were different from the marine populations, with little gene flow among populations. No apparent genetic structure was found between CPS morphs within Lake Storvatnet. However, genetic factorial correspondence axes were significantly correlated with pelvic principal component axes in Lake Storvatnet, suggesting some phenotype × genetic association.

Conclusion: The weak association between phenotypes and genetic structure observed in this study may reflect the build-up of early steps of reproductive isolation. Given time, such mechanisms may lead to the evolution of assortative mating, which may drive adaptive pelvic morphs (niche peaks), further resulting in genetically divergent populations and pelvic morphs.

Keywords: assortative mating, natural selection, ontogeny, pelvic reduction, regressive evolution, structural mutation dynamics.

INTRODUCTION

Understanding the mechanisms that generate and maintain biological diversity is a major goal in evolutionary biology. When a novel trait is introduced in a population, either by mutation or gene flow, three evolutionary scenarios are likely. First, and most common, the new trait is selectively inferior to the previously adapted trait, and will not proliferate and subsequently will disappear due to selection and genetic drift. Second, if the novel trait is selectively favourable it can replace the original trait. Then gene flow will lead to a subsequent spread to other populations. This brings about evolutionary change, but not necessarily increased variability if one trait replaces another (e.g. Hendry and Taylor, 2004; Garant *et al.*, 2007). The third scenario considers selection that is not always unidirectional; selection can favour a novel trait under certain conditions, while the original trait has the advantage in others. Such adaptive differentiation is most often observed when gene flow between populations is restricted and different populations adapt to their local environment. Organisms that are geographically constrained by barriers to dispersal, such as on remote islands or in freshwater lakes, are likely to evolve differentiation at the population level. Divergent natural selection can, over time, enhance phenotypic and ecological differentiation, ultimately leading to speciation (Schluter, 2000). Divergent selection is also possible within a population: if novel gene-variants (structural or regulatory) are introduced by gene flow or mutation, divergent selection can result in population sub-structuring into divergent niches where each form has a selective advantage. To develop into a stable polymorphic population, some form of (frequency-dependent) divergent selection and probably also assortative mating is necessary. This paper originates from the discovery of a polymorphic freshwater population of threespine stickleback (*Gasterosteus aculeatus* L.) in which a significant proportion of the fish have an unusual morphological trait (Klepaker and Østbye, 2008). Our aim is to determine the evolutionary implications of the presence of a novel character: how did it enter the population, what can explain its proliferation within the population, and what maintains the sympatric presence of the two forms?

The threespine stickleback has a Holarctic distribution with marine, brackish water, and freshwater populations (Wootton, 1984; Bell and Foster, 1994). Freshwater populations have originated from marine stickleback on numerous occasions, representing cases of convergent evolution (Bell and Foster, 1994; Jones *et al.*, 2012). After freshwater colonization, a general reduction in body armour usually occurs (Giles, 1983; Klepaker, 1993; Bell *et al.*, 2004). Marine stickleback have a complete row of lateral plates from the head to the caudal fin, providing protection against predators (Reimchen, 2000). The anterior plates are large and strong and provide the dorsal and pelvic spines with structural support. The three dorsal and two pelvic spines can be locked in a raised position, and help protect against gape-limited predators (Hoogland *et al.*, 1957). A strong pelvis supports the pelvic spines and protects the vital internal organs. For most freshwater populations, the reduction in body armour has resulted in stickleback with shorter and weaker dorsal and pelvic spines, as well as a smaller pelvis and 3–6 remaining anterior lateral plates. This suggests that the morphological predator defence is reduced in strength. In some populations, reduction has gone much further, with the loss of all lateral plates reducing the structural support of the spines (Reimchen, 1983).

Pelvic reduction in the threespine stickleback has been reported from the Pacific coast of North America (Bell, 1974, 1987; Moodie and Reimchen, 1976; McPhail, 1992; Bell and Ortí, 1994; Cresco *et al.*, 2004), Scotland (Giles, 1983; Campbell, 1984; Coyle *et al.*, 2007), Iceland (Shapiro *et al.*, 2004), and Norway (Klepaker and Østbye, 2008). Lake-specific independent mutations in the regulatory region of the *Pitx1* gene appear to be responsible for the reduction or total loss of the pelvic structure (Shapiro *et al.*, 2004, 2006; Coyle *et al.*, 2007; Chan *et al.*, 2010). This regressive evolution appears to be derived independently in different populations (genetically studied in at least 10 lakes) (Chan *et al.*, 2010). Due to the likely dominant action of the *Pitx2* gene in fish with the *Pitx1* regulatory mutation, a fish will be asymmetric; usually the left spine is developed and the right spine is lost first (Campione *et al.*, 1999; Shapiro *et al.*, 2004; Bell *et al.*, 2007). In some areas, especially in Alaska, the occurrence of pelvic reduction is relatively common (Bell and Ortí, 1994), although it is a rare event overall in the distribution range of the species (Münzing, 1963; Bell, 1974; Wootton, 1976, 1984; Mori, 1987; Klepaker and Østbye, 2008).

In Norway, pelvic reduction has to date only been reported from four populations, and at low frequency in three of them (Klepaker and Østbye, 2008). These are distant populations; the shortest distance between two populations is 530 km. Only Lake Storvatnet, on Langøya Island, Northern Norway (Fig. 1), holds a population with a significant proportion of individuals with pelvic reduction. Here, 60% of the population lack one or both pelvic spines and 25% have a reduced pelvic girdle. We investigate this system to address central questions related to pelvic reduction. First, on the Pacific coast of North America, pelvic-reduced populations are frequently clustered (Bell, 1974; Moodie and Reimchen, 1976; Bell and Ortí, 1994), as also seen in Scotland (Giles, 1983; Campbell, 1984). To test the hypothesis that pelvic-reduced populations are geographically clustered, we screened a total of 36 populations on Langøya Island for pelvic reduction. Second, we wished to determine whether factors special to this lake and this population explain why pelvic reduction has occurred there. Previous studies have focused on the absence of fish predation and predation from invertebrates (see Reimchen, 1980; Vamosi, 2002; Marchinko, 2009), or limited essential minerals, as causes of a reduction of the pelvis and other parts of the armour defence (Giles, 1983; Bell *et al.*, 1993). Here, we contrasted water chemistry and fish species in lakes with and without pelvic reduction to test this hypothesis. Third, large year-to-year variation in pelvic spine numbers has been reported from Western Canada (Reimchen and Nosil, 2002). Thus, we tested if the distribution of the different pelvic morphs within the Lake Storvatnet population was stable over years. Fourth, we tested if different pelvic morphs occupied different lake habitats and at different times of the day as an indication of preference for divergent niches. Previous research has reported different habitat use and diets during ontogeny in symmetric and asymmetric pelvic morphs of stickleback (Reimchen and Nosil, 2001). Furthermore, we used a set

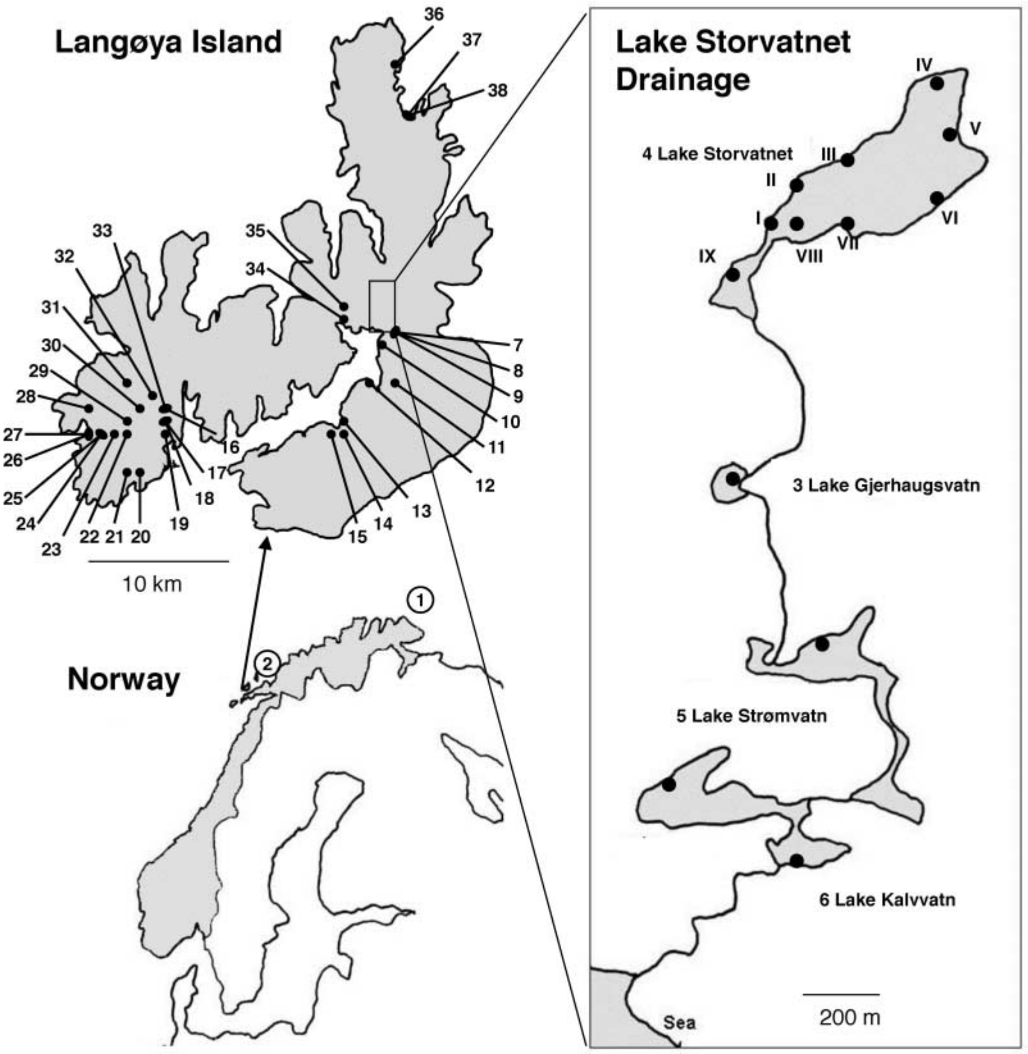

Fig. 1. Sampling locations at Langøya Island where numbers refer to Supplementary Table 1 (see Appendix at www.evolutionary-ecology.com/data/2719appendix.pdf). The two marine reference locations are marked with circles. The position of Lake Storvatnet drainage with the four lakes is shown, where dots indicate sampling locations. Nine locations (numbered I–IX) were sampled in Lake Storvatnet.

of microsatellites to compare pelvic morphs within Lake Storvatnet, and to assess genetic relatedness with the lakes downstream and two representative ancestral marine populations. In these four populations, detailed pelvic measurements were used to contrast patterns of genetic structure and phenotypic variation, and to estimate gene flow. The benthic/limnetic species pair in Lake Paxton (Bell, 1974; Reimchen, 1980) displays variation in pelvic structures that are maintained by the reproductive isolation. Similarly, if two different pelvic morphs in Lake Storvatnet experience differential fitness by adapting to different habitats and niches, their hybrids could suffer from reduced fitness due to ecological selection, and it is likely that some kind of assortative mating mechanism yielding reproductive isolation could develop.

METHODS AND MATERIALS

Study area and collection of sample

In an earlier study (Klepaker and Østbye, 2008), we found that four of 200 distantly located lakes on the Norwegian coast harboured stickleback with pelvic reduction. In one of these lakes, Lake Storvatnet, located on Langøya Island (850 km^2) in Vesterålen, Northern Norway, pelvic reduction was especially frequent. This lake was subjected to a more detailed study during 2007–2009. In the lake we sampled stickleback from nine locations (Fig. 1). Lake Storvatnet has a surface area of 0.3 km^2 and is relatively shallow with depths not exceeding 10 m in most parts of the lake. The lake is 80 m above sea level, which means at this latitude the lake experiences a long period of ice cover (late October to mid-June). There are three lakes downstream from Lake Storvatnet (Fig. 1). Before sampling in Lake Storvatnet, we also sampled stickleback from these three lakes as well as from 32 other lakes on Langøya Island in 2006. All sampling was done in August and September. Two marine populations were considered representative for the marine ancestoral state with regard to population genetics and pelvic development – one pelagic population from the Barents Sea and one from a coastal habitat in the harbour of Andøya [Fig. 1; see Supplementary Table 1 (for all supplementary material, see the Appendix at www.evolutionary-ecology.com/data/2719appendix.pdf)]. The purpose of this sampling design was to screen for the possible spread of pelvic reduction by gene flow within the Lake Storvatnet drainage, to search for local clusters of pelvic-reduced populations, and to compare the marine pelvic ancestor with derived freshwater ones.

All freshwater samples were collected using un-baited Plexiglass traps (Breder, 1960), while the marine samples were collected with hand-held dip-nets, as well as small mesh-sized gill-nets. Sampling was performed during 2006 to 2009, with one additional sample from Lake Storvatnet collected in 1989. After capture, all stickleback were stored on 70% EtOH prior to morphological and genetic analysis. Measurements of conductivity were obtained in the four lakes in the Lake Storvatnet drainage. The conductivity measurements were: Lake Storvatnet 43 $\mu S \cdot cm^{-1}$, Lake Gjerhaugsvatn 51 $\mu S \cdot cm^{-1}$, Lake Strømvatn 63 $\mu S \cdot cm^{-1}$, and Lake Kalvvatn 91 $\mu S \cdot cm^{-1}$.

In the threespine stickleback, four lateral plate morphs can be recognized (Hagen and Gilbertson, 1972): (1) a completely plated morph with a full cover of lateral plates along the flank of the body; (2) a partially plated morph with reduced lateral plate cover along the flank of the body, but with a fully or partly developed keel on the tail; (3) a low plated morph with only a few anterior lateral plates along the flank of the body and lacking a keel; and (4) a no-plated morph lacking all the lateral plates along the flank of the body. All of the fish were categorized based on these definitions (see Supplementary Table 1).

To record putative mortality factors for stickleback within Lake Storvatnet, we sampled macro-invertebrates and predatory fish. The search for invertebrates was performed in 2007, using a manual dip-net at the nine sampling sites in the lake (Fig. 1). Each site was searched twice. Nine multi-mesh gill-nets [Nordic type (Jensen and Hesthagen, 1996)] were used to catch potential predatory fish during one night in August 2007. We dissected a total of 86 brown trout (*Salmo trutta*) to determine the food preferences of that species.

Occurrence of pelvic reduction on Langøya Island

To get an overview of the occurrence of pelvic reduction on Langøya Island, we sampled stickleback from 36 lakes, including Lake Storvatnet and the three lakes downstream of it. Samples ($N = 3643$) were collected in 2006–2009 (Fig. 1; see Supplementary Table 1). The pelvic apparatus was classified using the nominal five-grade scale of Bell (1987; for details, see Klepaker and Østbye, 2008) (Fig. 2a). Each side of the fish was given a pelvic score (PS) between 0 (defined as a total loss of the pelvic apparatus) and 4 (defined as normal development of the pelvic apparatus), with a score of 1–3 representing intermediate pelvic reduction. The combined pelvic score (CPS), arrived at by summing the two sides of the fish, can range between 0 and 8. Thus, a CPS category of 8 represents a normally developed pelvic apparatus, whereas CPS categories 7 to 0 represent gradually reducing development of the pelvic apparatus with $\text{CPS} = 0$ representing complete loss. When scoring pelvic development, only adult fish (exceeding 30 mm in standard length) were used. However, when we investigated the ontogeny of pelvic development within Lake Storvatnet, the minimum standard length was 15 mm (Bell and Harris, 1985).

Spatio-temporal CPS patterns in Lake Storvatnet

Detailed sampling in Lake Storvatnet was performed to assess annual temporal stability, spatial patterns, and diurnal activity patterns of CPS categories. The annual temporal stability of CPS categories was studied using all the material from the nine sampling localities in 1989 and 2007–2009 ($N = 2325$) (Table 1). The spatial distribution of CPS categories was studied by comparing samples from the nine localities in 2007 ($N = 897$) (Fig. 1; see Supplementary Table 2). To study the vertical distribution of CPS categories, sampling was performed at four different depths near locality 2 in 2008 at depths of 0.5, 1.5, 3.0, and 8.0 m. The bottom vegetation was classified as dense, scattered or no vegetation ($N = 1041$) (see Supplementary Table 3). Moreover, any diurnal activity pattern of CPS categories was analysed by comparing day and night catches for the years 2007–2009 ($N = 2325$). Day samples were collected between 10.00 and 22.00 h and night samples between 22.00 and 10.00 h (see Supplementary Table 4). To test for possible selective

Table 1. The mean pelvic score (left and right PS and combined PS) distribution between years in the threespine stickleback in Lake Storvatnet (based on fish with total length >30 mm)

		Left pelvic score (PS)					Right pelvic score (PS)					Combined pelvic score (CPS)						
Year	*N*	0	1	2	3	4	0	1	2	3	4	2	3	4	5	6	7	8
1989	44	0.00	0.34	0.02	0.21	0.43	0.00	0.32	0.02	0.34	0.32	0.30	0.05	0.02	0.00	0.18	0.16	0.30
2007	897	0.00	0.14	0.08	0.19	0.59	0.00	0.19	0.09	0.24	0.48	0.13	0.02	0.07	0.05	0.14	0.14	0.45
2008	1041	0.00	0.13	0.05	0.25	0.56	0.00	0.20	0.06	0.28	0.47	0.12	0.02	0.07	0.04	0.17	0.14	0.43
2009	360	0.01	0.16	0.06	0.19	0.58	0.01	0.17	0.08	0.22	0.52	0.14	0.02	0.07	0.04	0.13	0.13	0.47
Total	2325	0.00	0.15	0.07	0.22	0.57	0.00	0.19	0.07	0.26	0.48	0.13	0.02	0.07	0.04	0.15	0.14	0.44

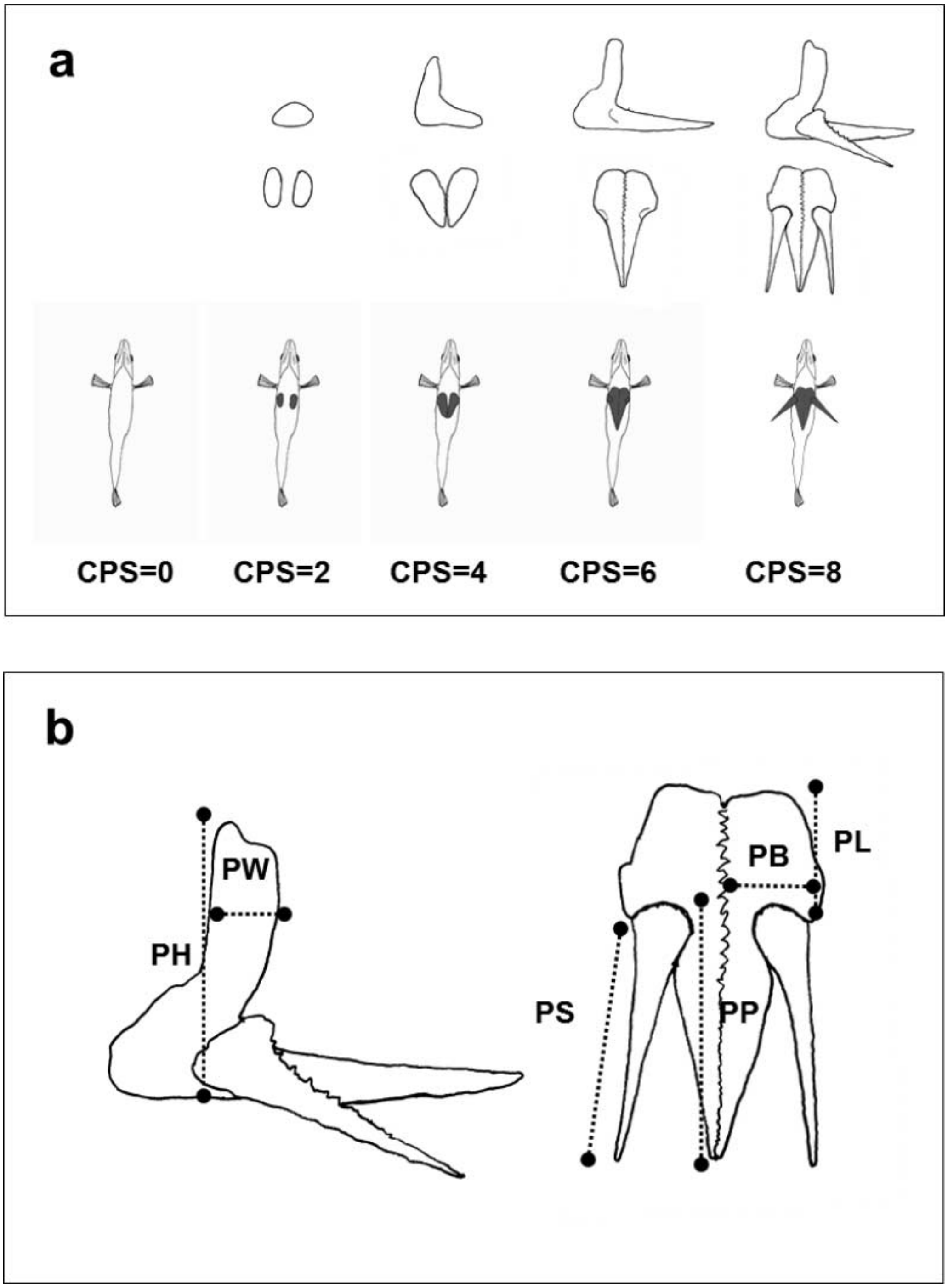

Fig. 2. (a) Definition (dorsal and lateral view) of combined pelvic scores (CPS) modified after Bell (1987) and (b) the six pelvic traits measured: the length of the basal plate in the pelvis (PL), the half breadth of the pelvis (PB), the length of the post-basal plate (PP), the height of the ascending branch (PH), the width of the ascending branch (PW), and the length of the pelvic spine (PS) on the left side if no reduction (or alternatively the right side).

mortality among CPS categories, we used the frequency of CPS categories compared between juveniles and adults sampled in both 2007 and 2009.

Pelvic traits from ancestors to derived freshwater populations

To test for putative phenotypic changes in the pelvic apparatus during the colonization of Lake Storvatnet from the marine ancestor, we compared stickleback from four locations: one off-the-coast pelagic marine sample (pelagic trawl catch in the Barents Sea; $N = 20$), one coastal marine sample (Andøy Harbour; $N = 37$), Lake Storvatnet ($N = 149$ drawn from the sample in 2007), and Lake Gjerhaugsvatn ($N = 22$) located downstream from Lake Storvatnet (see Fig. 1 and Supplementary Table 1).

In addition to classifying the fish into CPS categories, we measured a set of six pelvic traits (Fig. 2b): the length of the basal plate of the pelvis (PL), the half breadth of the pelvis (PB), the length of the post-basal plate (PP), the height of the ascending branch (PH), the width of the ascending branch (PW), and the length of the pelvic spine on the left side (PS). These traits were selected to maximize successful measurements on most CPS-category individuals. A fish with a CPS category of 7 has lost one of its pelvic spines, either the right or the left spine. Due to the action of the *Pitx2* gene in fish with *Pitx1* regulatory mutation, resulting in asymmetric individuals, usually the left spine is developed and the right spine is lost first (Campione *et al.*, 1999; Shapiro *et al.*, 2004; Bell *et al.*, 2007). Thus, in most samples, the length of the pelvic spine was measured on the left side ($N = 19$, CPS = 7; the sample size of fish having the left spine intact when asymmetric), but in the few cases when the left side was missing we had to measure the right side ($N = 2$, CPS = 7; the sample size of fish having the right spine intact when asymmetric). When a structure was missing completely, the length was set to 0. All the measurements were made using a stereomicroscope with 2–10× magnification.

The pelvic traits were regressed against body size (all regressions, $P < 0.05$) and the residuals from these regressions were used as size-corrected traits in a principal component analysis (PCA) [an independent size metric, size correction approach, following the notation of Berner (2011)]. Then, the first and second principal components (PC1 and PC2) were used as response variables in analyses of variance (ANOVAs) with population as the predictor variable. Second, on a subset of the data, we performed the same analyses using the first and second principal components (PC1 and PC2), but now only using measurements for fish in CPS category 8. This was done to determine whether size with a fully developed pelvic structure differed among these four populations. Finally, within the Lake Storvatnet data subset we applied the same set of analyses using the first and second principal components (PC1 and PC2), but now using all five observed CPS categories: 4 + 4, 4 + 3, 3 + 3, 2 + 2, 1 + 1 (regardless of sampling sites; here assuming a panmictic population structure). This latter analysis links the nominal CPS categories with the detailed metrics of the trait. As such, separate principal components were extracted from the different subsets in the three analyses performed.

DNA isolation, PCRs, and basic population genetics

DNA was extracted from pectoral fins using the proteinase K phenol chloroform protocol (Sambrook *et al.*, 1989). Here, we used 12 *a priori* neutral microsatellites and 11 microsatellites *suggested to be* quantitative trait loci (QTL) (Shapiro *et al.*, 2004, 2006) (see Supplementary Table

5). The locus *Stn336* has previously been shown to be a QTL associated with the *Pitx1* gene, a gene involved with pelvic reduction in threespine stickleback in laboratory crosses (Coyle *et al.*, 2007). We used *Stn336* as a putative QTL for pelvic reduction. The microsatellites were amplified using fluorescently labelled primers (*FAM*, *HEX* or *TET*), in ten PCR set-ups of simplex and multiplex reactions (labelled a–j) (for more specifications on PCR run conditions, see the Appendix at www.evolutionary-ecology.com/data/2719appendix.pdf).

Electrophoresis was conducted on an ABI 3100 automated sequencer using the size standard Genescan-500 LIZ (Applied Biosystems, Foster City, CA). PCR products were diluted for electrophoresis in groups of loci based on their molecular mass and attached fluorescent label. We added 1 μL of diluted PCR products to 10 μL of de-ionized formamide and 0.15 μL of the size standard Genescan-500 LIZ (Applied Biosystems). Finally, fluorescent DNA fragments were analysed using GENESCAN and GENOTYPER 3.7 (Applied Biosystems).

The microsatellites were binned prior to further population genetic analysis using TANDEM (http://evolution.unibas.ch/salzburger/software/tandem.htm) (Matschiner and Salzburger, 2009). Microsatellites were then analysed in MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.*, 2004) on an *a priori* populations × locus basis (4 populations × 23 microsatellites) for signs of stutter and null alleles. Observed heterozygosity (H_o), expected heterozygosity (H_E), allele counts, *F*-statistics, and genotypic linkage disequilibrium for the four locations (two marine and two freshwater) were estimated in GENEPOP 4.0.10 (Raymond and Rousset, 1995). Deviations from Hardy-Weinberg equilibrium were tested by the exact (probability) test for each locus and group (Guo and Thompson, 1992), using sequential Bonferroni adjustments (new $a = 0.05/23 = 0.0022$). Rarefaction of alleles was performed in HP-RARE (Kalinowski, 2005). To test for loci potentially under either directional or balancing selection, microsatellites were run in LOSITAN (Beaumont and Nichols, 1996; Antao *et al.*, 2008) under the stepwise mutation and the infinite allele models.

The results from the initial analyses of the 23 microsatellites showed that deviations from Hardy-Weinberg equilibrium were present in six population/locus combinations (out of a total of 92 tests), showing signs of heterozygote deficiency (see Supplementary Table 6): Andøy Harbour (*Stn61*, *Stn180*, *Stn263*, *Stn271*), Barents Sea (*Stn9*), and Gjerhaugsvatn (*Stn9*). The coastal Andøy Harbour population had 4 of 23 loci showing heterozygote deficiency.

In MICRO-CHECKER, 8 population/locus combinations (out of 92 tests) showed evidence of null alleles: Andøy Harbour (*Stn61*, *Stn180*, *Stn211*, *Stn265*, *Stn271*), Storvatnet (*Stn186*), Barents Sea (*Stn336*), and Gjerhaugsvatn (*Stn9*). One locus, *Stn186*, had significant stutter and was removed from the analysis. In no case were the null alleles in the same locus across populations. Using similar arguments as Østbye *et al.* (2006), all loci with significant null alleles were evaluated further. Here, the difference between the observed allele frequencies and the adjusted allele frequencies (from MICRO-CHECKER), following estimation of null alleles, was minor in most cases and exceeded one standard deviation of the allele frequency estimate in only 5 of the 98 adjustments of allele frequencies. Thus, null alleles were unlikely to alter our main conclusions.

Using LOSITAN, a total of three loci (*Stn52*, *Stn175*, *Stn271*) were removed from further analyses as candidates for directional selection under either the stepwise or the infinite allele model. Since the putative pelvic apparatus QTL *Stn336* was not under directional selection under the infinite allele and stepwise mutation models, it was considered neutral. Apparently, this locus is not linked to pelvic development in our study population, although it has been reported to be so elsewhere (e.g. Coyle *et al.*, 2007). Thus, the 19 microsatellite loci used in the genetic analysis were interpreted as neutral.

Genetic structure from the ocean to freshwater

Genetic differentiation was estimated between the four populations (two marine and two freshwater; grouping all pelvic morphs in Lake Storvatnet together) using log-likelihood-based exact tests on alleles, across groups, and combined across loci using Fisher's combined probabilities in GENEPOP 4.0.10 (Raymond and Rousset, 1995). Genetic relationships were also analysed using STRUCTURE 2.2.3 (Pritchard *et al.*, 2000), where the most likely numbers of genetic clusters were analysed using an admixture model with 2.5 million burn-in steps and 7.5 million MCMC replicates and 10 replicates for each of the runs for $K = 1–10$, based on methods in Evano *et al.* (2005) and the online program STRUCTURE-HARVESTER, version 0.6.1 [http://taylor0.biology.ucla.edu/struct_harvest/ (Earl and vonHoldt, 2012)].

To estimate historical gene flow (M: the migration rate m per generation divided by the mutation rate μ per generation) among sites, we used MIGRATE 3.0.3 (Beerli and Felsenstein, 1999, 2001) on microsatellite repeats, a Brownian microsatellite mutation model, default and ML-strategy where mutation rates were treated as constant for all loci. We also estimated contemporary gene flow (m: immigration rate per generation) among sites with BAYESASS 1.3 (Wilson and Rannala, 2003), which calculates migration rates over the past two generations based on the proportion of immigrants. Three independent runs with 6×10^7 MCMC steps, where the first 10^6 steps were burn-in, were considered sufficient to obtain convergence.

Genetic structure within Lake Storvatnet

Genetic differentiation and population structure were investigated in Lake Storvatnet using similar methods as above using GENEPOP 4.0.10 and STRUCTURE 2.2.3. Here, we set $K = 1–10$ (with 10 replicates) for resolving putative population structure between the five CPS categories (CPS 4 + 4, 4 + 3, 3 + 3, 2 + 2, 1 + 1). In addition, we tested if three geographical sampling localities were differentiated and if they showed genetic structure when comparing localities I, II, and VIII (Fig. 1). Using sampling locality II only, we also tested for genetic differentiation between CPS group 4 + 4 (i.e. no regulatory mutation) and the other four CPS groups combined (i.e. those that have a regulatory mutation: 4 + 3, 3 + 3, 2 + 2, and 1 + 1).

Relatedness was tested among the CPS categories [when applying the estimator of Queller and Goodnight (1989)] using the bootstrap procedure in COANCESTRY (Wang, 2011). Here, assuming some sort of assortative mating, and thus a build-up of reproductive isolation, we expected relatedness to decrease between CPS categories with increased CPS divergence. We also applied a factorial correspondence analysis (FCA) in GENETIX 4.05 (Belkhir *et al.*, 1996–2004) to determine similarity of allelic composition among the five CPS categories. Here, GENETIX performs an FCA where composite axes are generated maximizing differences among individuals of different CPS categories based on allele frequencies, and plots individuals in three dimensions according to genotypes. We tested if FCA-factors were correlated with morphology, using the extracted principal components of the six pelvic trait metric measurements.

RESULTS

Occurrence of pelvic reduction on Langøya Island

We surveyed 36 lacustrine populations for the occurrence of pelvic reduction on Langøya Island. Pelvic reduction was only found in Lake Storvatnet (see Fig. 1 and Supplementary Table 1). Thus, no fish with a reduced pelvis was found in any of the three lakes downstream from Lake Storvatnet or any other lake on the island.

Distribution of CPS and pelvic spine asymmetry in Lake Storvatnet

During 2007–2009, more than 50% of the stickleback had a reduced pelvic structure with at least one pelvic spine missing (Table 1). About 40% lacked both pelvic spines, and more than 25% showed a further reduction in the remaining pelvic structure. Loss of the whole pelvic structure was very rare and was found in only one of 2325 adult fish. The CPS distribution was apparently bimodal around $PS = 1$ and $PS = 4$ for both the left and right side (Table 1). Lateral asymmetry was common; ~25% of fish analysed had an asymmetric pelvic structure. Pelvic spine asymmetry was directional (paired sample t-test: $t_{2324} = 13.81$, $P < 0.0001$), in that it was five times more likely to observe a larger reduction on the right than the left side.

Annual, diurnal, and spatial stability of CPS in Lake Storvatnet

The CPS distribution was very stable among years (2007–2009) (ANOVA: $F_{2,2278} = 0.15$, $P = 0.86$) (Table 1). The 1989 sample differed slightly by having a higher frequency of CPS 2 and a stronger bimodal distribution around CPS 2 and CPS 8. However, the 1989 sample was limited in size. The day and night sampling showed no difference in the diurnal activity of fish with different CPS (ANOVA: $F_{1,2275} = 0.15$, $P = 0.67$) (see Supplementary Table 4). The distribution of CPS did not differ among the nine sites in Lake Storvatnet in 2007 (ANOVA: $F_{8,888} = 1.07$, $P = 0.38$) (see Fig. 1 and Supplementary Table 2). Furthermore, the CPS distribution did not differ among depths (0.5 m, 1.5 m, 3.0 m, 8.0 m; ANOVA: $F_{3,1037} = 0.97$, $P = 0.40$; see Supplementary Table 3) or among types of vegetation cover (nominal categories of dense, scattered, and no vegetation; ANOVA: $F_{2,1038} = 0.33$, $P = 0.72$).

Age structure and pelvic reduction in Lake Storvatnet

Based on the observed length distribution in the samples from 2007 and 2009 (in 2008 only adult fish were sampled), the Lake Storvatnet population appeared to consist of three age classes (some older individuals might also occur), with the two youngest age classes most abundant in the autumn samples (Fig. 3). Juvenile (0+) individuals did not overlap in standard body length with the adult specimens. When comparing pelvic-score categories, we found that the frequency of low pelvic scores was higher in juveniles than adults (Fig. 4). This was due to a higher frequency of $PS = 1$ juveniles. The pelvic score distribution differed significantly between juveniles and adults both in 2007 and 2009 (chi-square tests, 2007: left: $\chi^2_4 = 19.36$, $P = 0.001$; right: $\chi^2_4 = 30.91$, $P < 0.0001$; 2009: left: $\chi^2_4 = 9.92$, $P = 0.042$; right: $\chi^2_4 = 25.79$, $P < 0.0001$).

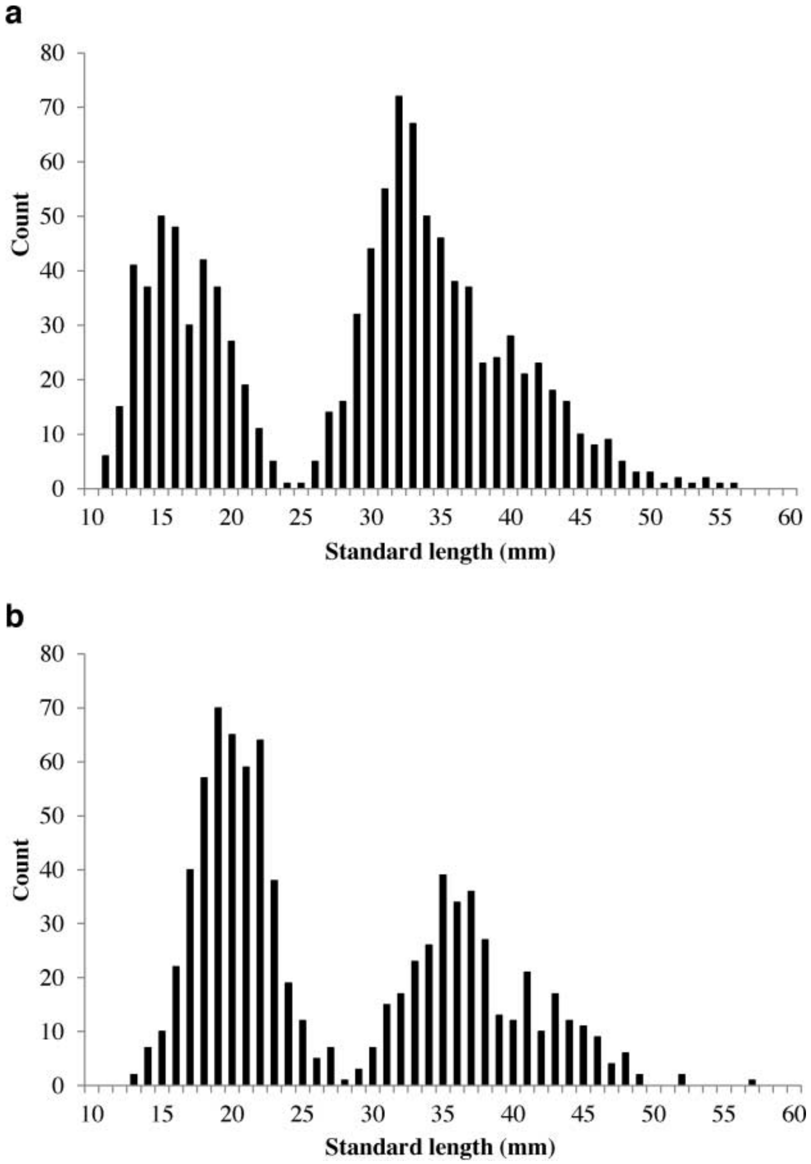

Fig. 3. Standard body length distribution (counts) showing a bimodal distribution between juvenile and adult stickleback in Lake Storvatnet. Juveniles and adults were sampled on two occasions: (a) 4–9 August 2007 and (b) 9–12 September 2009. The distinction between juveniles and adults is set to 25 mm standard length in 2007 and 28 mm standard length in 2009.

Predation and CPS in Lake Storvatnet

The nine different sampling sites in the lake (Fig. 1) were searched twice for potential insect predators by sweeping a hand-held dip-net. No such insects were caught, indicating that the abundance of large insect predators is low. Gillnet fishing revealed that Lake Storvatnet has

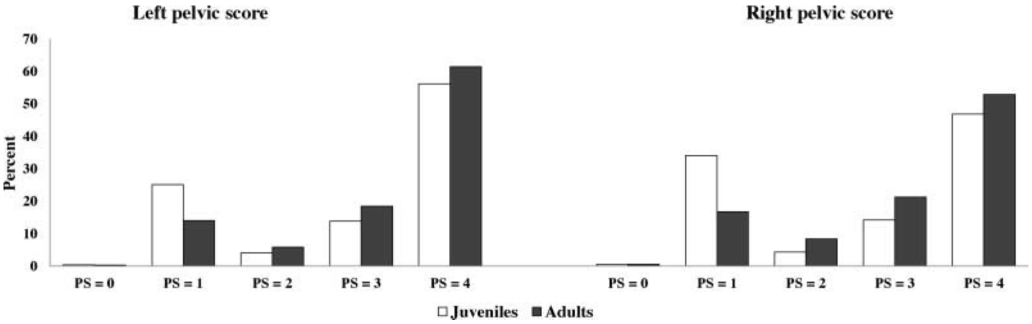

Fig. 4. The frequency distribution of pelvic scores (left and right) of juvenile (open bars) and adult fish (solid bars) for 2007 and 2009 combined. The distributions are based on 848 juveniles and 1022 adult fish.

an abundant population of brown trout; 86 trout were caught (mean ± S.D.: total length 25.3 ± 5.8 cm, weight 187 ± 136 g). Sixteen trout had eaten stickleback, with the smallest predatory trout being 24 cm long. Both juvenile and adult stickleback were found in the trouts' stomachs, but only the adult stickleback could be used for analysing pelvic development. In total, 28 individual adult stickleback were identified. However, the digestion made exact CPS difficult to score, but we were able to discriminate between normal versus reduced pelvis in 26 of 28 stickleback eaten. The proportion of stickleback eaten with a normal pelvis was 0.35, close to that observed in the population as a whole (0.45) (chi-square test: $\chi^2_1 = 0.03$, $P = 0.92$).

Pelvic trait reduction from the ocean to freshwater

When comparing the four localities, PC1 and PC2 explained 72.4% and 15.2% of the variation in pelvic size measurements (with eigenvalues of 4.35 and 0.91), respectively. All six pelvic traits loaded positively with PC1 [the length of the basal plate of the pelvis (PL) least so] and weakly and negatively with PC2 except PL, which loaded strongly and positively with PC2. The PC1 score differed among populations, with the Lake Storvatnet population having smaller PC1 than the other populations (ANOVA: $F_{3,227} = 74.28$, $P < 0.0001$; Tukey Kramer *post hoc* test, $P < 0.05$). PC2 also differed among populations, but here the Lake Storvatnet population differed only from the Barents Sea population (ANOVA: $F_{3,227} = 3.49$, $P < 0.0001$; Tukey Kramer *post hoc* test, $P < 0.05$). When using only CPS category 4 + 4, the same pattern of PC-loadings was seen as in the previous analyses for the four locations. Here, PC1 and PC2 explained 66.9% and 15.3% of the variation (with eigenvalues of 4.02 and 0.92) respectively. PC1 (ANOVA: $F_{3,118} = 88.15$, $P < 0.0001$) was larger in the marine populations (which did not differ from each other) than in the freshwater populations (which differed significantly) (Tukey Kramer *post hoc* test, $P < 0.05$). For PC2 (ANOVA: $F_{3,118} = 2.96$, $P = 0.035$), only the Andøy Harbour population was larger than that of the Barents Sea (Tukey Kramer *post hoc* test, $P < 0.05$). Finally, within Lake Storvatnet, PC1 and PC2 explained 64.1% and 15.9% of the variation (with eigenvalues of 3.84 and 0.96) respectively. Here, all six traits loaded positively with PC1, least so for the length of the basal plate of the pelvis (PL), which loaded strongly and negatively with PC2. The remaining traits loaded weakly to moderately strongly (pelvic spine length) and negatively with PC2. PC1 was in very close agreement with the subjective CPS groups (ANOVA: $F_{4,147} = 148.49$, $P < 0.0001$), where a *post hoc* test (Tukey Kramer, $P < 0.05$) separated CPS groups 1 + 1, 2 + 2, and 4 + 4 from each other. However, CPS group 4 + 4 was not different from CPS group 3 + 4. Neither was CPS group 3 + 3 different from 3 + 4. For PC2 (ANOVA: $F_{4,147} = 15.25$, $P < 0.0001$), the pattern was less clear, as the CPS groups overlapped to a larger extent; however, CPS groups 4 + 4, 3 + 3, and 2 + 2 were clearly differentiated from each other. Pelvic bone investment clearly decreases from marine environments (and Lake Gjerhaugsvatn, which did not differ from the marine populations) into Lake Storvatnet, which has the lowest investment. When comparing only fully developed pelvic individuals (CPS 4 + 4), marine populations show a larger investment than Lake Gjerhaugsvatn, which again has a larger investment than Lake Storvatnet.

Genetic structure from the ocean to freshwater

The four *a priori* populations (two marine and two freshwater) were highly significantly genetically differentiated in all tests, with F_{st} values between 0.023 and 0.216 (see Supplementary Table 7). However, the STRUCTURE analysis suggested only three main population clusters ($K = 3$), with the two marine populations (Barents Sea and Andøy Harbour) belonging to the same cluster and Lake Gjerhaugsvatn and Lake Storvatnet forming two different genetic entities (Fig. 5) (see also Supplementary Figure 1).

MIGRATE analyses revealed that estimates of historic gene flow (M) ranged between 3.21 (Barents Sea to Andøy Harbour) and 0.39 (Lake Gjerhaugsvatn to Barents Sea) when comparing the four localities. The long-term gene flow estimate was approximately twice as high from Lake Gjerhaugsvatn into Lake Storvatnet ($M = 1.32$; 5–95 percentiles = 1.21–1.44) as in the opposite direction ($M = 0.60$; 0.55–0.66). In the analysis of contemporary gene flow (m) in BAYESASS (see Supplementary Table 7), the confidence intervals overlapped with 0, so the null hypothesis of no contemporary gene flow between locations was not rejected.

Genetic structure within Lake Storvatnet

No overall significant genetic differentiation was found among the five CPS categories in Lake Storvatnet using GENEPOP (all $P > 0.05$; F_{st} values ranged from 0.0009 to 0.0057). When comparing sampling localities I, II, and VIII, sampling localities I and II differed significantly ($F_{st} = 0.004$), while I vs. VIII ($F_{st} = -0.0009$) and II vs. VIII ($F_{st} = 0.0003$) were not significantly different. Furthermore, there was no genetic differentiation between CPS 8 and the other CPS-categories pooled (tested at sampling locality II) ($P > 0.05$, $F_{st} = 0.0034$). Based on the analysis in STRUCTURE, there was only one panmictic population within Lake Storvatnet (results not presented).

On the other hand, the factorial correspondence analysis (FCA) in GENETIX demonstrated that there was some genetic structure among the Lake Storvatnet pelvic morphs. The four factors resolved between 28.8% and 19.2% of the variation (FCA1 = 28.75%, FCA2 = 27.85%, FCA3 = 24.25%, FCA4 = 19.15%; see Fig. 6a for the most divergent pattern between CPS categories). Furthermore, there were apparent associations between genetic FCA scores and pelvic apparatus PCA scores. PCA1 was positively and significantly correlated with FCA1 ($r = 0.37$, $P < 0.0001$, $N = 148$), FCA2 ($r = 0.22$, $P = 0.0075$, $N = 148$), FCA4 ($r = 0.41$, $P < 0.0001$, $N = 149$), and negatively correlated with FCA3 ($r = 0.32$,

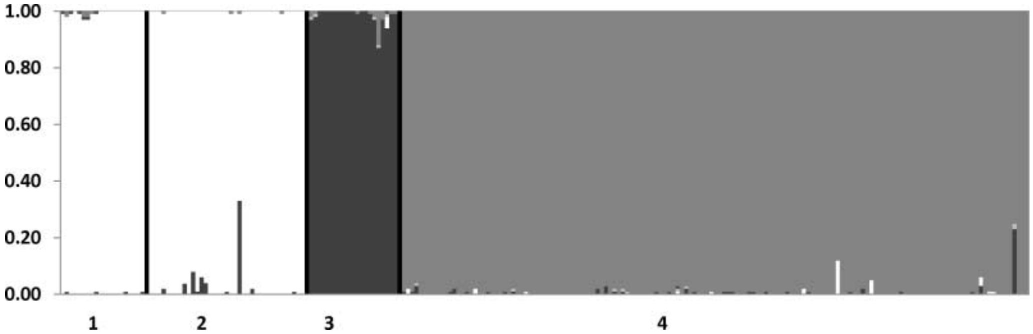

Fig. 5. The STRUCTURE analysis of the four populations reveals that three genetic clusters are evident ($K = 3$). 1 = Barents Sea (marine), 2 = Andøy harbour (marine), 3 = Lake Gjerhaugsvatn, 4 = Lake Storvatnet.

$P < 0.0001$, $N = 148$). For PCA2, a positive and significant correlation was observed with FCA1 ($r = 0.26$, $P = 0.0016$, $N = 148$), and a negative association with FCA4 ($r = 0.29$, $P = 0.0003$, $N = 148$). See Fig. 6b for an example of a pattern in these five CPS categories (PCA1 vs. FCA3). In the COANCESTRY analyses, the five pelvic morphs did not differ in relatedness values, nor was there any reduced relatedness associated with an increased degree of pelvic reduction as seen in these five pelvic morphs (all the confidence intervals overlapped).

DISCUSSION

The results of this study show that the pelvic reduction in threespine stickleback in Lake Storvatnet on Langøya Island cannot be explained by zoogeographical or climatic factors, as a reduction was not observed in other populations on the island. Even in the nearby lakes downstream of Lake Storvatnet, no pelvic reduction was observed. In Lake Storvatnet, the divergent pelvic morphs did not have different habitat preferences, as they were caught in equal frequencies and at the same times. Also, the frequency of these pelvic morphs was temporally stable over years. Moreover, there was no apparent genetic structuring in Lake Storvatnet. However, we did find some possible indications of early steps towards reproductive isolation, based on the observed associations between the allelic composition and patterns of pelvic spine variation. There was also some indication of selective mortality of pelvic morphs, whereby a higher frequency of pelvic-reduced morphs was found in the youngest age class compared with the older year classes. The size and development of the pelvic apparatus of the threespine stickleback is reduced when moving from the marine environment (off-the-coast marine sample and the coastal population) into freshwater (Lake Gjerhaugsvatn and Lake Storvatnet). The genetic analysis suggested three clusters, with the two marine populations grouped together. Below, we discuss the relevance of our results in relation to the occurrence of pelvic reduction.

Is pelvic reduction a result of experienced predation regimes?

The spine defence structure protects the threespine stickleback against gape-limited predators (Hoogland *et al.*, 1957), primarily piscivorous fish and birds (Reimchen, 1994). Furthermore,

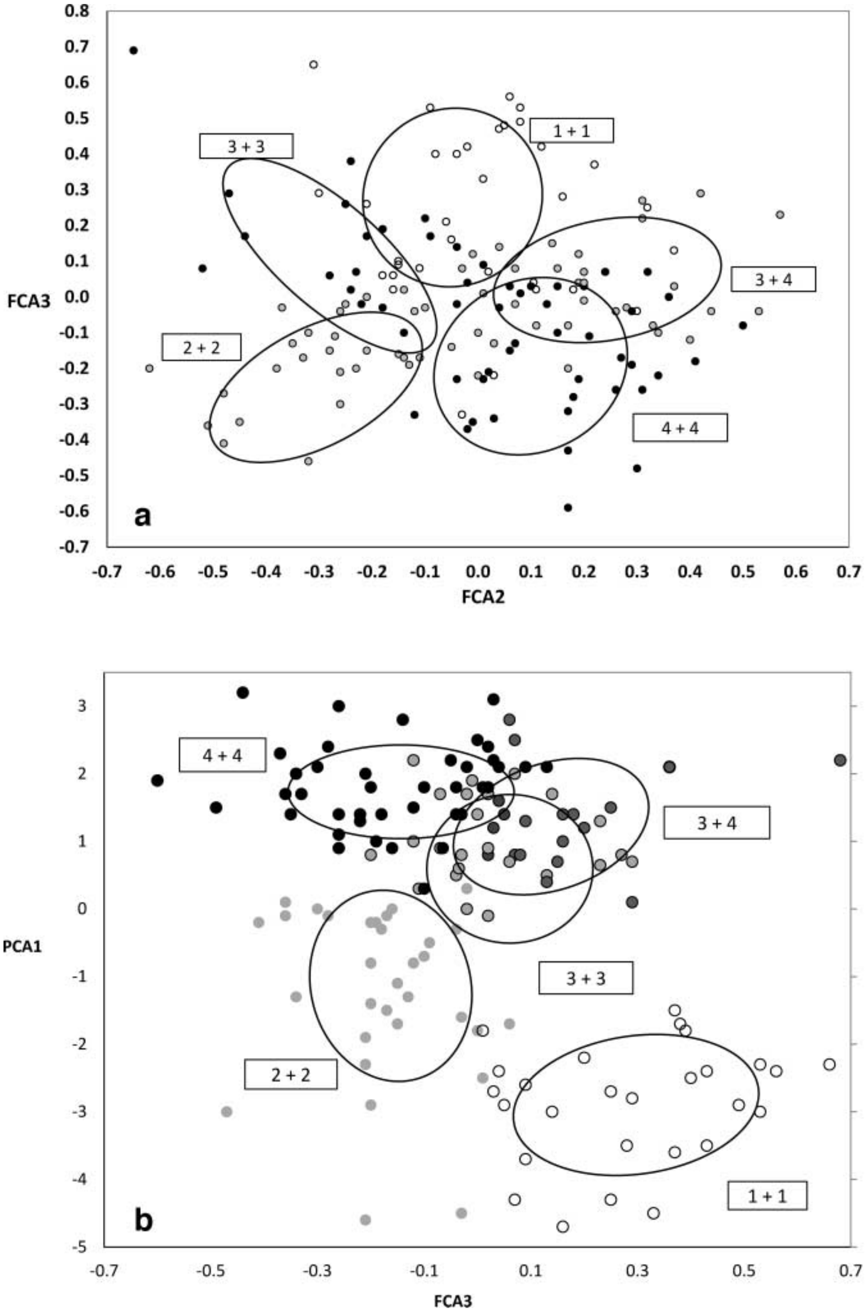

Fig. 6. (a) The factorial correspondence analysis (FCA) on alleles between the five pelvic morphs within Lake Storvatnet. Plot of FCA3 on the y-axis (explaining 24.3% of variation) versus FCA2 on the x-axis (explaining 27.9% of variation). (b) Association between PCA1 of the six metric pelvic traits (y-axis, explaining 80% of five subjective CPS groups) versus FCA3 (explaining 24.3% of variation). In (a) and (b), 50% density ellipses are plotted around each of the five pelvic morphs.

juvenile stickleback are also vulnerable to predation from large invertebrates such as *Notonecta* sp., *Aeshna* spp., and *Dytiscus* spp. These predators do not swallow their prey whole, and the spines do not offer any special protection. In contrast, the spines can increase predation risk by providing the invertebrate predator with structures to grab and hold onto the stickleback (Reimchen, 1980). Pelvic-reduced populations in North America often inhabit lakes and ponds where piscivorous fish are rare or absent and invertebrate predators common (Bell *et al.*, 1993; Reimchen, 1994). Thus, a high density of invertebrate predators might select against a well-developed pelvic structure. But repeated searches in all parts of the lake did not produce any possible invertebrate predators, showing that large invertebrates are rare in the lake, and unlikely to be a significant explanatory factor for pelvic reduction. The only candidates for piscivorous freshwater predators in the area are brown trout and Arctic charr (*Salvelinius alpinus*). Indeed, there is an abundant population of brown trout in Lake Storvatnet, many of which feed on both juvenile and adult stickleback. Thus, fish predation must be considered a significant factor. The stickleback found in trout stomachs were both with and without a normal pelvis and no selectivity for either morph was evident. Therefore, pelvic reduction in Lake Storvatnet is probably not due to lack of fish predation or a high abundance of invertebrate predators.

Is the lake calcium concentration a driver for pelvic reduction?

Calcium limitation as a selection agent leading to reduction of the pelvis and other parts of the armour defence was proposed by Giles (1983), who studied populations with highly reduced armour in Scotland. In Alaska, Bell *et al.* (1993) found that pelvic reduction was associated with low calcium concentrations (below 12 $\mathrm{mg \cdot L^{-1}}$), but only when predatory fish were absent. This contrasted with the observations in Scotland where a low calcium concentration alone was able to explain armour reduction (Giles, 1983). We have no data on specific calcium content, but we recorded the conductivity of the water in all the lakes in the Lake Storvatnet drainage. Conductivity is a good predictor of calcium in Norwegian lakes, especially at low concentrations of calcium [$r_{1327} = 0.688$, $P < 0.0001$; based on a large number of samples from Norwegian lakes (ln-ln scale) (D.O. Hessen, unpublished results)]. Lake Storvatnet has the lowest conductivity in the drainage, and the conductivity increases downstream due to marine sediments and the surrounding agricultural land. The conductivity differences are in accordance with Lake Storvatnet holding the only pelvis-reduced population in the drainage. Furthermore, the pelvic trait reduction in Lake Storvatnet versus Lake Gjerhaugsvatn and the two marine samples is also in accordance with developmental stress due to lack of calcium. However, the difference in conductivity between the two freshwater lakes is small, and the conductivity in Lake Storvatnet is not likely to be different from Norwegian freshwater in general. Norwegian lakes and ponds are in general low in calcium, and the majority of lakes are well below the threshold of 12 $\mathrm{mg \cdot L^{-1}}$ (Skjelkvåle *et al.*, 1996). So, based on the low calcium hypothesis, we should expect pelvic reduction to be a common phenomenon throughout Norway, which is clearly not the case. Thus, it is unlikely that low calcium concentration is the only explanation for pelvic reduction in Lake Storvatnet.

Stochasticity and dynamics of regulatory pelvic mutations

An alternative explanation, related to the low calcium hypothesis, is that regulatory mutations or combinations of a set of mutations necessary for pelvic reduction are extremely rare. Thus, the reason for the general absence of pelvic-reduced populations in low calcium lakes would not necessarily be that selection driven by low calcium would not favour it, but rather the absence of necessary genetic variants. As such, the frequency of pelvic reduction in freshwater populations could reflect the likelihood of necessary mutations occurring and spreading in the population. If genetic variants for pelvic reduction enter a population, due to mutation or gene flow, they might be selected for, and pelvic-reduced fish would proliferate in the population. However, no pelvic reduction was seen in Lake Gjerhaugsvatn downstream of Lake Storvatnet or in the other lakes further down in the drainage. The historical gene flow estimate shows a higher and asymmetric gene flow from Lake Gjerhaugsvatn into Lake Storvatnet, and thus suggests that most of the variation in Lake Storvatnet is derived from Lake Gjerhaugsvatn, or alternatively a common ancestral gene pool. The contemporary gene flow estimates showed that none of the four localities studied exchange effective migrants. As no apparent effective contemporary migration was detected between Lake Storvatnet and Lake Gjerhaugsvatn, it suggests that no fish migrate, that all pelvic-reduced migrants from Lake Storvatnet are selected against (ecologically misfit) in Lake Gjerhaugsvatn, and/or that such migrants will fail to reproduce (mate choice barriers) or survive (more liable to predation than the original population with normally developed pelvic apparatus) in the latter lake. Finally, the mutation responsible for the pelvic reduction in Lake Storvatnet may be so recent that it has not yet had the chance to invade the downstream lakes (invasion dynamics is a function of time and selection pressure).

Temporal stability of pelvic polymorphism

The observed proportion of the pelvic morphs was stable over time. Of course, the time span of our data is short when measured on an evolutionary time scale, as shown by Hunt *et al.* (2008). A slow directional selection process towards any of the extreme forms of the trait cannot be excluded. Yet, the observed stability suggests some factor maintaining the variation. Except for heterozygote advantage, stable polymorphism within a population may result from frequency-dependent selection (Hedrick, 2006). Adaptation to different habitats is common where frequency-dependent selection is observed, and natural for us to examine. In Paxton Lake and Little Quarry Lake in Canada, stickleback with pelvic reduction are found together with stickleback with a normal pelvis (McPhail, 1992). However, stickleback with a normal pelvis are more confined to open water (limnetic lifestyle) than the pelvic-reduced stickleback, which have a benthic/littoral lifestyle. However, these two lakes are not directly comparable with Lake Storvatnet, as the two sympatric morphs (or species-pairs) in Canada probably originated from double invasions from marine ancestors. Still, it would be a plausible explanation for the co-existence of stickleback with normal and reduced pelvis; the pelvic-reduced fish are distributed in parts of the lake with a lot of cover, where the risk of predation is lower. The benefits of reduced investment in morphological predator defence are balanced with an increased risk of being preyed upon when in open water, and of staying in potentially suboptimal habitats where the predation risk is lower, spending more time in or near cover, and less time searching for food. However, we were not able to detect any differences in the distribution of pelvic morphs between the different habitat types surveyed in the lake.

Signs of selection targeting pelvic morphs

The spine defence is a highly integrated system, with dorsal and pelvic spines being supported structurally by the pelvic girdle with its bilateral ascending branches and overlapping lateral plates. The loss of any part of this structure is likely to result in severe weakening or breakdown of the morphological anti-predator defence. As such, pelvic-reduced stickleback have lost a functional trait of great selective importance.

The late summer and autumn populations in Lake Storvatnet mainly consist of two year-classes, with a small number of older individuals. That the frequency of pelvic morphs changed during ontogeny suggests that the advantage of the pelvic apparatus is related to fish age and size. Pelvic-reduced juveniles may have a high survival the first summer, which is reduced during the winter and the following summer. An explanation for the high success of low-CPS juveniles is a higher growth rate, since they can allocate essential minerals to other body parts during growth. This is comparable to what has been suggested by Marchinko and Schluter (2007) and Barrett *et al.* (2008) regarding the reduction in the number of lateral plates in freshwater. This would be especially relevant in lakes where essential minerals are scarce. In addition to slower growth, individuals developing a complete pelvis may exhibit poorer swimming abilities, increased drag, and lower manoeuvrability (Andraso, 1997; Bergstrøm, 2002; Myhre and Klepaker, 2009). The benefit of juveniles investing in a normal pelvis may be less because their small size makes them vulnerable to most predators regardless of the defence given by the armour. However, at some stage in the population ontogeny trajectory, the advantage of predator protection balances the costs of producing and carrying armour. In Lake Storvatnet, this shift seems to happen during the first winter or following summer, when stickleback reach adult size.

In a polymorphic population where selective forces act divergently during ontogeny, we would expect either the population to be driven towards fixation of either extreme or that a stable polymorphism would develop. Looking at the Lake Storvatnet population in the three years 2007–2009, where the most extensive sampling was done, the distribution is remarkably stable from year to year. This holds both for the ratio of normal to reduced pelvis and for the different CPS groups within the pelvic-reduced stickleback. Also, the degree of left–right asymmetry was stable over the three years. This is different from what Reimchen and Nosil (2002) found in Lake Boulton, where mean pelvic spine number varied considerably between successive generations. These authors related this variation to differences in predation pressure. However, the sample we obtained in 1989 indicates that pelvic morph distribution can vary in Lake Storvatnet too. The distribution observed in 1989 was clearly more dominated by pelvic reduction than the three samples obtained two decades later. While a small sample size makes it less conclusive, the pelvic reduction observed in 1989 was larger than any sub-sample in 2007–2009. This may indicate a sort of stability in balancing selective forces over generations, although some fluctuations over a longer time span also occur.

Reproductive isolation of pelvic morphs driven by assortative mating?

The Lake Storvatnet stickleback population has an apparent bimodal distribution with regard to pelvic development. The largest part of the population has a normal or slightly reduced (one spine missing) pelvis, while the second largest part has only rudimentary remains of the pelvis. One hypothetical explanation for the bimodal nature of the population trait distribution could be the occurrence of opposing ontogenetic selection pressures that produce two adaptive peaks within the population. This could promote the build-up and development of reproductive isolation by some sort of assortative mating between morphs. This was suggested by the results of the factorial correspondence analyses, which indicated some small genetic differences among some of the CPS categories (see Supplementary Figure 2), suggesting weak reproductive isolation. Moreover, there were some correlations between genetic factorial correspondence axes and pelvic apparatus principal component axes. This implies that phenotypically similar individuals are parts of similar genetic clusters compared with more dissimilar individuals. Thus, it is theoretically plausible that the pattern we see in the Lake Storvatnet pelvic morphs could be attributable to two opposing ontogenetic selection pressures where fitness reduction is largest in the intermediate pelvic-developed individuals. This would be further enhanced if there was additional selection on life-history variables whereby the two strategies are being favoured; either mature at a small size and with a low pelvic investment, or mature at larger size, but with more investment in pelvic armour.

CONCLUDING REMARKS

The study of pelvic reduction in Lake Storvatnet showed that pelvic reduction has most likely originated in the lake, since no other populations with that trait are found in the area. The proliferation of pelvic-reduced morphs in Lake Storvatnet must be caused by some factors or events specific to the lake, but it is difficult to fit with the hypotheses of predation and calcium concentration that have been inferred previously. Also, the stability of pelvic morph distribution among years suggests some kind of stabilizing mechanism could be at play in this lake. This could hypothetically involve a balance between antagonistic selective forces on pelvic development during ontogeny rather than differences in habitat use. Indeed, as the proportion of pelvic morph categories differs during ontogeny, we suggest that selection operates in different directions during ontogeny. Here, protection offered by spines is not achieved until stickleback reach a critical body size when gape-limited predators experience problems handling stickleback with erected spines. Before this size is reached, individuals not investing in a complete pelvic structure may benefit from allocation of resources to other more vital parts of the body than the pelvis. Thus, the observed pattern may result from a complex interplay between dynamics of mutation invasion, fluctuating selective forces, and opposing fitness gains during ontogeny.

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