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Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.)

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ABSTRACT

In 1987, a fjord in the Snæfellsnes peninsula, north-west Iceland, was dammed and a freshwater lagoon formed. There is a large population of threespine stickleback in this lagoon. We compared morphological features of stickleback in the lagoon population to those of their marine ancestor, and morphological polymorphism within the lagoon in relation to mud and lava substrates. The freshwater stickleback have shorter spines and fewer armour plates than marine stickleback. There is also some morphological divergence between stickleback from the two substrates within the lagoon. Our results suggest that the threespine stickleback may adapt to a novel environment more rapidly than would be predicted from conventional models of biological differentiation.

Keywords: adaptation, evolution, Iceland, resource polymorphism, sexual dimorphism.

INTRODUCTION

Polymorphism in structures or behaviour for exploiting certain resources – resource polymorphism (Skúlason and Smith, 1995; Smith and Skúlason, 1996) – can give rise to discrete morphs or even new species (Robinson and Wilson, 1994; Skúlason and Smith, 1995). Thus, it offers unique opportunities to study the importance of ecological factors in evolution and speciation (Skúlason and Smith, 1995; Smith and Skúlason, 1996; Schluter, 1996, 1998a,b, 2000a; Robinson and Schluter, 2000). Resource polymorphism is common in northern freshwater fishes (e.g. Smith and Skúlason, 1996). These habitats are young (< 14,000 years old) and often still in the colonization phase (Skúlason *et al.*, 1999). They are usually depauperate, thus presenting invading species with a diversity of unexploited habitats and resources.

The threespine stickleback, *Gasterosteus aculeatus*, is a good candidate to study ecological factors that promote resource polymorphism and speciation. This species inhabits marine and freshwater habitats throughout its holarctic range (Wootton, 1984; Bell and Foster, 1994a) and marine threespine stickleback have repeatedly colonized freshwater habitats (Bell and Foster, 1994b). Freshwater stickleback are phenotypically variable, with

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apparently comparable adaptations in similar ecological surroundings (Bell and Foster, 1994a; McPhail, 1994). In many cases, morphs – or even new species – of stickleback can be found in sympatry or parapatry (Blouw and Hagen, 1990; Lavin and McPhail, 1993; McPhail, 1994; Ziuganov, 1995). Reproductive isolation has been found between sympatric forms in lakes (McPhail, 1994), between lake and river morphs (Lavin and McPhail, 1993) and between anadromous and freshwater stickleback (McPhail, 1994; Ziuganov, 1995).

The time required for marine stickleback to adapt to novel freshwater habitats is unclear. Genetic analysis based on rates of mutation (molecular clock) may indicate the time since two populations diverged (O'Reilly *et al.*, 1993; Orti *et al.*, 1994), but the precision (temporal resolution) of such estimates is on the order of hundreds of thousands of years. Habitat age can indicate the earliest date when stickleback were able to colonize the new environment (Bell and Foster, 1994b). For example, most freshwater areas now inhabited by stickleback were covered with ice until 15,000 to 10,000 years ago (Bell and Foster, 1994a; Skúlason *et al.*, 1999). In this short time, freshwater stickleback have evolved considerable phenotypic diversity (Bell and Foster, 1994a).

Some animals can diverge, and even evolve into new species, in a relatively short time (Rice and Hostert, 1993; Hendry *et al.*, 2000). In *Drosophila* species, reproductive isolation can evolve as a by-product of disruptive selection, even in sympatry (Rice and Salt, 1990; Rice and Hostert, 1993). Sockeye salmon (*Oncorhynchus nerka*) were introduced into Lake Washington, USA in 1937. Within 13 generations, two morphotypes in the lake spawn either in tributary rivers or along lake beaches (Hendry *et al.*, 2000). In cichlids (*Haplochromis* spp.), in the African Great Lakes, speciation can occur in than less 300 years (Owen *et al.*, 1990). Stickleback have been reported to evolve reproductive isolation as well as morphological changes from their marine ancestors in as few as eight generations (Ziuganov, 1995). A population of Norwegian stickleback isolated from their marine ancestors for about 40 years have a more compact body, with a reduction in lateral armour plates and a higher proportion of four spined individuals (Klepaker, 1993).

We compared a population of Icelandic stickleback recently isolated in a freshwater lagoon to its marine ancestor and evaluated the morphological diversity of stickleback within the lagoon, in relation to lava and mud substrates. We know that Icelandic freshwater stickleback often show distinct morphological adaptations to these habitat types (Kristjánsson, 2001; Kristjánsson *et al.*, 2002). This allowed us to examine morphological divergence over a very short period (about 12 generations).

MATERIALS AND METHODS

Hraunsfjördur, in the Snæfellsnes peninsula in north-west Iceland, is a narrow fjord about 4 km^2 (Fig. 1). About half way along the fjord, it is almost divided in two by a lava flow (5000–8000 years old) forming a narrow channel. The fjord has a salinity gradient as a result of fresh water flowing into the inland region. In 1987, the fjord was dammed for a salmon (*Salmo salar*) ranching operation that formed a freshwater lagoon (1.7 km^2), which is inaccessible to fish from the sea (J. Sturlaugsson, personal communication; Sturlaugsson, 1994). Some seawater enters the lagoon through the porous lava, which results in some brackish water in the lagoon close to the dam. Brown trout (*Salmo trutta*), rainbow trout (*Onchorynchus mykiss*) and Atlantic salmon are released into the lagoon for recreational fishing. Both trout species are likely predators of stickleback (Reimchen, 1994).

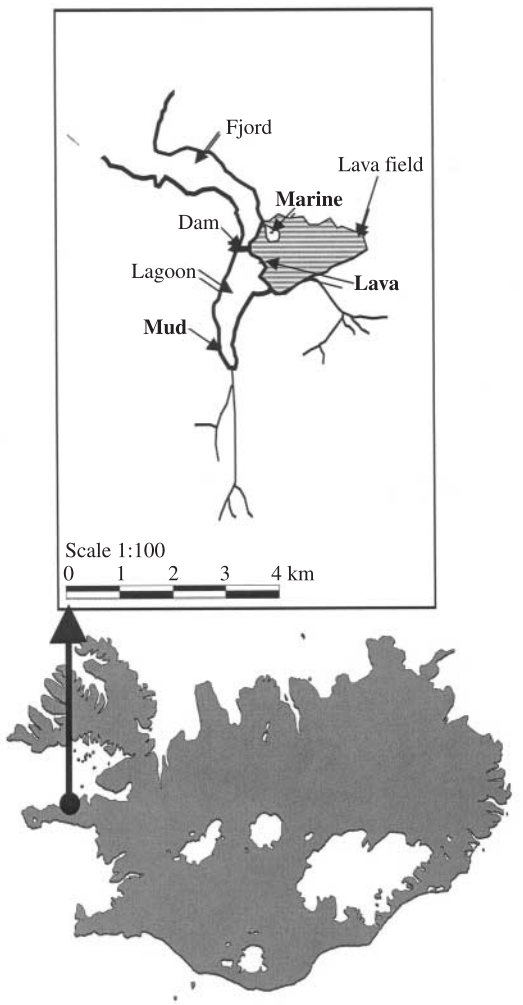

Fig. 1. The sampling location at Hraunsfjördur (64°55′N, 22°10′W), Iceland and habitats within the area.

The lagoon hosts a very dense population of stickleback (personal observation). A large population of marine stickleback spawns in marine pools in the lava in the fjord, as well as on vegetated mud substrate, before migrating back out to sea (personal observation; J. Sturlaugsson, personal communication).

We caught stickleback with minnow traps (Dynamic Aqua-Supply Ltd., mesh size 3.2 mm) on 9 July 1999 at two locations within the lagoon and one in the fjord (Fig. 1). The locations were:

1. A marine tide-pool, within the lava connected to the fjord by a narrow channel.
2. A lava substrate within the lagoon, about 150 m from the dam. The lava is a typical

ah-ah lava, very rough with holes and small crevices often creating small pools. Stickleback were caught at a depth of 1–2 m.

3. A mud substrate at the greatest distance from the sea in the lagoon. The bottom here consists of soft mud with some vegetation. Stickleback were caught at a depth of about 1–1.5 m.

We randomly selected 30 fish larger than 30 mm (Bell, 1981) from each sampling location. Each fish was photographed from the left and dorsal angles. The photographs were scanned into a computer and 11 morphological distances calculated from landmarks digitized on them (Fig. 2). Fork length was measured to the nearest 0.1 mm and the fish were dissected to determine sex and diet. The stomach was opened and all prey organisms identified to the lowest taxonomic level possible. The diet was grouped in seven categories: Mollusca, Ostracoda, Chironomidae, Pupae and flies, Copepoda, Cladocera and Other. The proportions of these seven groups were calculated within each stomach and compared using non-parametric tests.

The fish were stained with Alizarin Red in 1% potassium hydroxide (e.g. Bell, 1982), after which the following meristic characters were counted: gill rakers on the lower and upper branch of the first gill arch, armour plates (designated as regular or keeled; e.g. Wootton, 1984), dorsal spines and rays in all fins. The caudal fin was divided into upper and

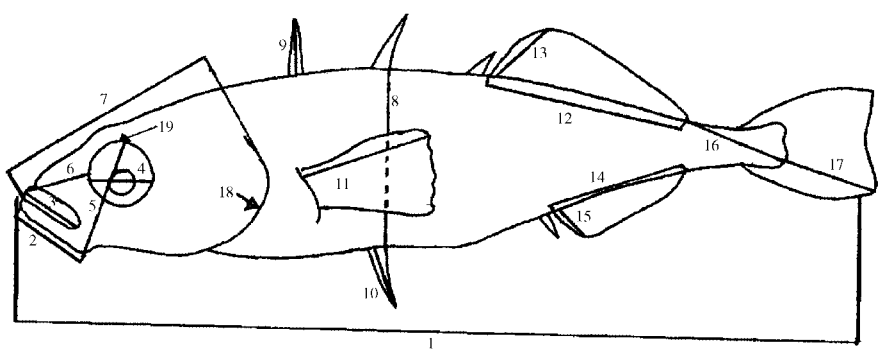

Fig. 2. Morphological characters measured from the left side of each fish. 1, fork length; 2, jaw length, the length of the lower jaw (P); 3, maxilla length, the length of the maxilla from the snout to the end of the maxillary bone (F); 4, eye diameter (P); 5, height to top of eye, the distance from the snout to the dorsal part of the eye (P); 6, snout length, the length from the snout to the anterior part of the eye (P); 7, head length, distance from the angle of the lower jaw to the end of the operculum (P); 8, body depth (P); 9, dorsal spine length (F); 10, ventral spine length (F); 11, pectoral fin length, the length of the longest ray in the pectoral fin (F); 12, dorsal fin length, the length of the dorsal fin base, measured from the origin of the first fin ray to the insertion of the last fin ray (P); 13, dorsal fin ray, length of first ray in the dorsal fin (F); 14, anal fin length, the length of the anal fin base measured from the origin of the first fin ray to the insertion of the last fin ray (P); 15, anal fin ray, the length of the first ray in the anal fin (F); 16, caudal peduncle length, the distance from the posterior end of the dorsal fin to the end of flesh in the caudal peduncle (P); 17, caudal fin length, the length of the longest ray in the caudal fin (F); 18, gill raker length, the length of the longest gill raker on the longer arm of the first gill arch (F); 19, distance between eyes, measured as seen from above (P). *Note*: (P) indicates that the measurements were from a photograph and (F) indicates that they were made directly on the fish.

lower halves and fin rays (principal and procurrant) were counted. We measured eight morphological characters directly from the fish using an ocular micrometer in a dissecting microscope (Fig. 2).

To standardize for the range of body sizes, each of the 19 morphological measurements was regressed against fork length and residual values calculated (Reist, 1985). This was done twice: (1) with all the fish combined for comparison of the marine and the freshwater fish; (2) with only the freshwater fish for comparison between the two habitats within the lagoon. Single factorial (analysis of variance) and multifactorial (discriminant function analysis) methods were used to compare morphology and meristic counts of the fish. Scores on the discriminant function axes (DF1 and DF2) were compared among groups using 2×2 analysis of variance (ANOVA) with *post-hoc* tests. Number of spines and number of pectoral fins rays were compared using the χ^2-test. In all cases where there were multiple comparisons, sequential Bonferroni (Rice, 1989) correction was used to minimize the possibility of type I errors.

To evaluate the rate of divergence, we calculated haldanes (Gingerich, 1993) according to the formula $h = (x_2/s_p) - (x_1/s_p)/g$, where x_2 and x_1 are mean trait values for each of the two populations (marine and freshwater), s_p is the pooled standard deviation and g is the number of generations.

RESULTS

The marine fish (51.1 ± 7.13 mm; mean $\pm$ *s*) were larger than freshwater fish (47.3 ± 6.53 mm; $F_{1,90} = 4272$, $P < 0.01$). The sex ratio of fish did not differ between these habitats. Sexual dimorphism was found in both habitats. Males had larger jaws (freshwater: $t_{58} = 5.3$, $P < 0.05$, mean difference = 0.5 mm; marine: $t_{27} = 5.1$, $P < 0.05$, mean difference = 0.7 mm), snouts (freshwater: $t_{58} = 5.3$, $P < 0.05$, mean difference = 0.4 mm; marine: $t_{27} = 4.4$, $P < 0.05$, mean difference = 0.5 mm) and maxilla (freshwater: $t_{58} = 4.3$, $P < 0.05$, mean difference = 0.4 mm; marine: $t_{27} = 3.9$, $P < 0.05$, mean difference = 0.6 mm) than females (Table 1). In the freshwater habitats, males also had larger heads ($t_{58} = 6.2$, $P < 0.05$, mean difference = 0.8 mm) and a greater distance to the top of their eyes ($t_{58} = 4.6$, $P < 0.05$, mean difference = 0.4 mm) than females. Fish in the marine environment had larger dorsal ($t_{87} = -3.6$, $P < 0.05$, mean difference = 0.3 mm) and ventral spines ($t_{87} = -3.8$, $P < 0.05$, mean difference = 0.6 mm) and longer anal fins ($t_{87} = -2.8$, $P < 0.05$, mean difference = 0.6 mm) than freshwater fish. The four groups (marine males, marine females, freshwater males and freshwater females) differed in their overall morphology (Wilk's $\lambda = 0.12$, $\chi^2_{54} = 160.2$, $P < 0.05$; Fig. 3). The DF1 explained about 61% of the variance and the model remained significant when that axis had been removed (Wilk's $\lambda = 0.39$, $\chi^2_{34} = 73.1$, $P < 0.05$). The model correctly classified 79% of the marine males, 87% of the marine females, 91% of the freshwater males and 74% of the freshwater females (Fig. 3). We examined whether the discriminant scores on DF1 and DF2 (which explained about 32% of the variance) differed among the groups. The scores on DF1 differed between habitats (ANOVA, $F_{1,85} = 46.1$, $P < 0.01$) and between the sexes (ANOVA, $F_{1,85} = 115.9$, $P < 0.01$) and the interaction of habitats and sex was also significant (ANOVA, $F_{1,85} = 7.5$, $P < 0.01$). *Post-hoc* tests with Bonferroni correction ($P < 0.05$) showed that, in both habitats, there was sexual dimorphism and both sexes differed between the habitats (Fig. 3). The scores on DF2 differed between habitats (ANOVA, $F_{1,85} = 67.6$, $P < 0.01$) and between the sexes (ANOVA, $F_{1,85} = 27.4$, $P < 0.01$), but the interaction of these two variables was not

Table 1. Eighteen morphological and eight meristic variables of threespine stickleback from a marine habitat and a freshwater lagoon in Hraunsfjördur, Iceland (mean ± *s*)

	Marine			Freshwater		
Trait[a]	Male	Female	Combined	Male	Female	Combined
Jaw length	0.41 ± 0.50	−0.34 ± 0.27	0.08 ± 0.55	0.32 ± 0.25	−0.19 ± 0.40	−0.01 ± 0.43
Anal fin length	0.83 ± 1.24	−0.04 ± 0.82	0.38 ± 1.12	0.08 ± 0.48	−0.32 ± 0.83	−0.18 ± 0.74
Dorsal fin length	−0.25 ± 1.31	0.08 ± 0.94	−0.08 ± 1.12	−0.12 ± 0.48	0.13 ± 0.70	0.04 ± 0.63
Snout length	0.40 ± 0.33	−0.13 ± 0.32	0.13 ± 0.42	0.18 ± 0.25	−0.19 ± 0.26	−0.06 ± 0.31
Eye width	0.06 ± 0.47	−0.25 ± 0.15	−0.10 ± 0.37	0.07 ± 0.23	0.03 ± 0.26	0.05 ± 0.25
Head length	0.46 ± 1.14	−0.39 ± 0.54	0.02 ± 0.97	0.52 ± 0.42	−0.29 ± 0.52	−0.01 ± 0.62
Top of the eye height	0.41 ± 0.84	−0.26 ± 0.37	0.06 ± 0.71	0.24 ± 0.33	−0.18 ± 0.34	−0.03 ± 0.39
Body depth	0.63 ± 1.73	0.10 ± 0.45	0.36 ± 1.25	−0.22 ± 0.70	−0.14 ± 0.96	−0.17 ± 0.87
Caudal peduncle	−0.08 ± 0.53	−0.13 ± 0.61	−0.10 ± 0.57	0.11 ± 0.70	0.02 ± 0.83	0.05 ± 0.78
Distance between eyes	−0.13 ± 0.38	−0.02 ± 0.20	−0.08 ± 0.30	0.10 ± 0.60	−0.05 ± 0.47	0.04 ± 0.51
Dorsal fin ray	0.29 ± 0.47	−0.19 ± 0.50	0.04 ± 0.53	0.20 ± 0.57	−0.14 ± 0.36	−0.02 ± 0.47
Anal fin ray	0.04 ± 0.47	−0.16 ± 0.64	−0.06 ± 0.56	0.00 ± 0.48	0.04 ± 0.44	0.02 ± 0.45
Caudal fin	−0.03 ± 0.73	−0.35 ± 0.97	−0.20 ± 0.86	−0.13 ± 0.51	0.23 ± 3.22	0.10 ± 2.61
Pectoral fin	0.40 ± 0.58	0.09 ± 0.99	0.24 ± 0.82	−0.15 ± 0.62	−0.10 ± 0.75	−0.12 ± 0.70
Dorsal spine	0.31 ± 0.27	0.11 ± 0.46	0.21 ± 0.38	−0.02 ± 0.46	−0.14 ± 0.33	−0.10 ± 0.38
Ventral spine	0.45 ± 0.96	0.35 ± 0.65	0.40 ± 0.80	−0.21 ± 0.65	−0.17 ± 0.61	−0.18 ± 0.62
Maxilla length	0.30 ± 0.44	−0.31 ± 0.40	−0.02 ± 0.52	0.28 ± 0.35	−0.13 ± 0.36	0.01 ± 0.41
Gill raker length	0.09 ± 0.16	0.08 ± 0.74	0.08 ± 0.53	0.04 ± 0.11	−0.09 ± 0.15	−0.04 ± 0.15
Lower gill rakers	13.4 ± 0.8	13.6 ± 1.1	13.5 ± 1.0	13.2 ± 0.9	13.1 ± 0.9	13.1 ± 0.9
Upper gill rakers	5.6 ± 0.6	5.9 ± 0.9	5.8 ± 0.8	5.8 ± 0.8	5.6 ± 0.7	5.6 ± 0.8
Regular armour plates	13.4 ± 7.6	15.1 ± 8.0	14.3 ± 7.7	8.5 ± 5.4	8.8 ± 5.5	8.7 ± 5.4
Keeled armour plates	4.9 ± 4.0	6.1 ± 3.0	5.6 ± 3.5	1.4 ± 2.2	2.1 ± 2.9	1.9 ± 2.7
Dorsal fin	12.1 ± 0.7	11.8 ± 0.7	12.0 ± 0.7	11.9 ± 0.6	12.0 ± 0.7	11.9 ± 0.7
Anal fin	8.8 ± 0.7	8.2 ± 1.1	8.5 ± 0.9	8.5 ± 0.7	8.3 ± 0.8	8.4 ± 0.8
Ventral caudal fin	11.9 ± 0.9	11.8 ± 0.7	11.8 ± 0.8	12.4 ± 0.8	12.4 ± 0.9	12.4 ± 0.9
Dorsal caudal fin	12.1 ± 1.1	12.0 ± 1.0	12.0 ± 1.0	12.6 ± 0.8	12.2 ± 0.8	12.3 ± 0.8

[a]All values in millimetres.

significant ($P > 0.05$) (Fig. 3). *Post-hoc* tests showed that, in the freshwater habitat, there was sexual dimorphism and both sexes differed between habitats.

Fish from the marine and freshwater habitats differed in their armour structure. Marine fish had more plates, both regular ($t_{88} = -4.0$, $P < 0.05$, mean difference = 5.6) and keeled ($t_{58} = -5.5$, $P < 0.05$, mean difference = 3.7), although the freshwater fish showed a wide range in plate number. Fish from the marine and freshwater habitats differed in their overall meristic counts (Wilk's $\lambda = 0.44$, $\chi^2_{24} = 50.2$, $P < 0.05$). The model correctly classified 29% of the marine males, 50% of the marine females, 57% of the freshwater males and 51% of the freshwater females (Fig. 3). The DF1 described about 73% of the variance and the model only remained significant when that axis was included. The discriminant scores on that axis were different between habitats (ANOVA, $F_{1,86} = 43.0$, $P < 0.01$) but not between the sexes and the interaction of these two variables was not significant (Fig. 3).

We calculated haldanes for those variables that were significant between fish from the marine and freshwater habitats. The largest haldanes observed were for the spines (dorsal spina −0.80, ventral spine −0.47). The other variables had lower values of haldanes (anal fin length −0.27, regular armour plate number 0.19, keeled armour plate number 0.16).

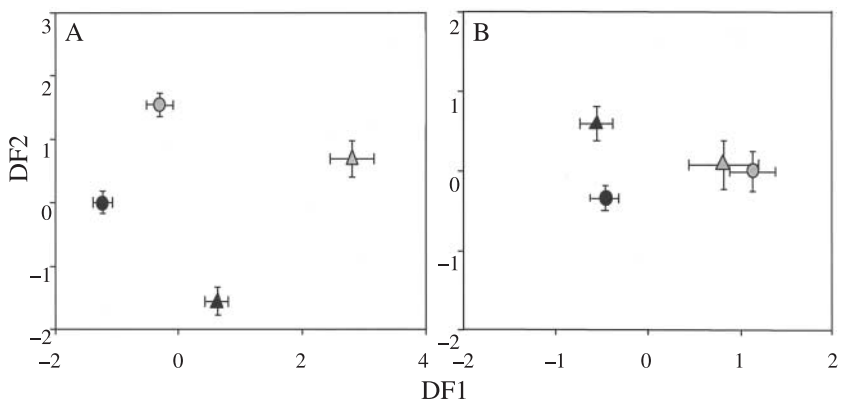

Fig. 3. Discriminant scores of (A) morphology and (B) meristic counts of stickleback from marine and freshwater habitats within Hraunsfjördur, north-west Iceland. Each graph shows the average of the four groups with one standard error. The groups are: △, marine males; ●, marine females; ▲, freshwater males; ●, freshwater females.

Similar proportions of stickleback from the two habitats had food in their stomachs (marine 70%, freshwater 80%). The marine fish (2.8 ± 1.48) had more species and prey groups in their stomachs than the freshwater stickleback (1.6 ± 1.19; $t_{67} = -3.3$, $P < 0.01$). All of the food groups except Mollusca differed between marine and freshwater stickleback. The marine fish had higher proportions of ostracods ($Z = -3.1$, $P < 0.05$), chironomids ($Z = -3.9$, $P < 0.05$), copepods ($Z = -4.1$, $P < 0.05$) and 'other' ($Z = -2.5$, $P < 0.05$), which were mainly fish eggs. The freshwater fish had higher proportions of pupae/flies ($Z = -2.5$, $P < 0.05$) and Cladocera ($Z = -2.5$, $P < 0.05$).

The fish in the freshwater lagoon did not differ in size between the two substrate types, but they differed in morphology. In the lava habitat, males had larger jaws ($t_{28} = 5.1$, $P < 0.05$, mean difference = 0.5 mm) and maxilla ($t_{28} = 3.6$, $P < 0.05$, mean difference = 0.3 mm), whereas in the mud habitat they had larger snouts ($t_{28} = -0.2$, $P < 0.05$, mean difference = 0.4 mm), than females (Table 2). The mud fish had longer caudal peduncles ($t_{58} = -3.4$, $P < 0.05$, mean difference = 0.6 mm) than the lava fish. The four groups differed in their overall morphology (Wilk's $\lambda = 0.12$, $\chi^2_{54} = 99.3$, $P < 0.05$). The model correctly classified 100% of the lava males, 85% of the lava females, 82% of the mud males and 74% of the mud females (Fig. 4). The DF1 explained about 63% of the variance and the model was only significant when that axis was included. The significant variables loaded highly on the DF1 axis, as well as head length, distance to the top of the eye and the length of fin rays in the dorsal fin, which are all sexually dimorphic. The scores on that axis differed between the sexes (ANOVA, $F_{1,56} = 121.3$, $P < 0.01$) but not between habitats and the interaction of habitat and sex was not significant.

No differences were seen in individual meristic counts between the groups. The groups did, however, differ in their overall meristic counts (Wilk's $\lambda = 0.49$, $\chi^2_{24} = 38.2$, $P < 0.05$). The model correctly classified 10% of the lava males, 75% of the lava females, 64% of the mud males and 63% of the mud females (Fig. 4). The DF1 explained about 60% of the variance and the model was only significant when that axis was included. The scores on that axis differed between fish from the two habitats (ANOVA, $F_{1,56} = 11.9$, $P < 0.01$) and

Table 2. Eighteen morphological and eight meristic variables of threespine stickleback from lava and mud habitats within a freshwater lagoon in Hraunsfjördur, Iceland (mean ± *s*)

	Lava			Mud		
Trait[a]	Male	Female	Combined	Male	Female	Combined
Jaw length	0.24 ± 0.19	−0.29 ± 0.30	−0.11 ± 0.37	0.42 ± 0.28	−0.07 ± 0.47	0.11 ± 0.47
Anal fin length	0.47 ± 0.52	0.16 ± 0.84	0.26 ± 0.75	−0.04 ± 0.52	−0.40 ± 0.59	−0.26 ± 0.58
Dorsal fin length	−0.04 ± 0.47	0.31 ± 0.69	0.19 ± 0.64	−0.29 ± 0.48	−0.13 ± 0.64	−0.19 ± 0.58
Snout length	0.19 ± 0.28	−0.12 ± 0.27	−0.02 ± 0.31	0.27 ± 0.25	−0.13 ± 0.24	0.02 ± 0.31
Eye width	0.02 ± 0.22	−0.06 ± 0.28	−0.04 ± 0.26	0.07 ± 0.24	0.02 ± 0.21	0.04 ± 0.22
Head length	0.37 ± 0.43	−0.30 ± 0.64	−0.08 ± 0.66	0.65 ± 0.41	−0.25 ± 0.37	0.08 ± 0.58
Top of the eye height	0.25 ± 0.28	−0.16 ± 0.38	−0.02 ± 0.40	0.30 ± 0.38	−0.13 ± 0.30	0.03 ± 0.39
Body depth	−0.06 ± 0.84	−0.02 ± 0.93	−0.04 ± 0.89	−0.07 ± 0.53	0.10 ± 1.02	0.04 ± 0.86
Caudal peduncle	−0.27 ± 0.55	−0.34 ± 0.90	−0.32 ± 0.79	0.37 ± 0.71	0.29 ± 0.61	0.32 ± 0.64
Distance between eyes	−0.06 ± 0.60	−0.15 ± 0.55	−0.12 ± 0.56	0.17 ± 0.60	0.09 ± 0.33	0.12 ± 0.44
Dorsal fin ray	0.14 ± 0.55	−0.13 ± 0.26	−0.04 ± 0.40	0.29 ± 0.61	−0.10 ± 0.45	0.04 ± 0.54
Anal fin ray	−0.15 ± 0.55	0.09 ± 0.50	0.35 ± 0.50	0.06 ± 0.38	−0.05 ± 0.39	−0.01 ± 0.38
Caudal fin	−0.34 ± 0.60	0.70 ± 4,44	0.17 ± 3.64	−0.18 ± 0.49	−0.45 ± 0.54	0.35 ± 0.53
Pectoral fin	0.12 ± 0.62	0.19 ± 0,81	0.03 ± 0.74	−0.23 ± 0.53	−0.13 ± 0.66	−0.17 ± 0.60
Dorsal spine	0.13 ± 0.51	−0.01 ± 0.35	0.03 ± 0.41	0.03 ± 0.44	−0.07 ± 0.30	−0.03 ± 0.35
Ventral spine	0.08 ± 0.76	0.16 ± 0.48	0.13 ± 0.57	−0.21 ± 0.57	−0.09 ± 0.63	−0.13 ± 0.60
Maxilla length	0.45 ± 0.31	0.00 ± 0.32	0.15 ± 0.37	0.11 ± 0.32	−0.30 ± 0.35	−0.15 ± 0.39
Gill raker length	0.12 ± 0.11	−0.05 ± 0.15	0.01 ± 0.16	0.02 ± 0.10	−0.02 ± 0.12	−0.01 ± 0.11
Lower gill rakers	13.3 ± 0.9	13.2 ± 0.9	13.2 ± 0.9	13.1 ± 0.9	12.9 ± 0.9	13.0 ± 0.9
Upper gill rakers	5.7 ± 0.8	5.6 ± 0.8	5.7 ± 0.8	5.8 ± 0.9	5.5 ± 0.7	5.6 ± 0.8
Regular armour plates	9.8 ± 6.4	9.8 ± 6.0	9.8 ± 6.0	7.3 ± 4.3	7.7 ± 4.8	7.6 ± 4.5
Keeled armour plates	2.2 ± 2.4	2.5 ± 3.0	2.4 ± 2.8	0.7 ± 1.8	1.8 ± 2.8	1.4 ± 2.5
Dorsal fin	12.1 ± 0.7	12.0 ± 0.9	12.0 ± 0.8	11.6 ± 0.5	11.9 ± 0.3	11.4 ± 0.4
Anal fin	8.8 ± 0.8	8.5 ± 0.9	8.6 ± 0.9	8.2 ± 0.6	8.1 ± 0.7	8.1 ± 0.6
Ventral caudal fin	12.1 ± 0.9	12.2 ± 0.9	12.2 ± 0.9	12.7 ± 0.6	12.7 ± 0.8	12.7 ± 0.7
Dorsal caudal fin	12.3 ± 0.9	12.2 ± 0.8	12.3 ± 0.8	12.9 ± 0.5	12.2 ± 0.8	12.4 ± 0.8

[a]All values in millimetres.

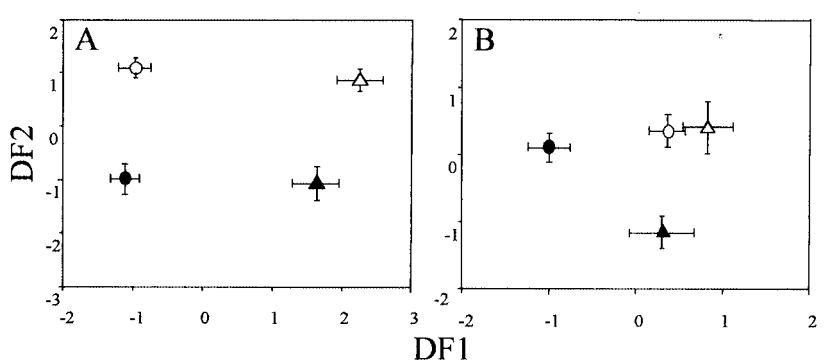

Fig. 4. Discriminant scores of (A) morphology and (B) meristic counts of stickleback from a freshwater lagoon in Hraunsfjördur, north-west Iceland. Each graph shows the average of the four groups with one standard error. The groups are: △, mud males; ○, mud females; ▲, lava males; ●, lava females.

between the sexes (ANOVA, $F_{1,56} = 10.6$, $P < 0.01$), but the interaction of habitat and sex was not significant (Fig. 4). *Post-hoc* tests showed sexual dimorphism in the mud area and the females differed between the habitats.

There was no difference in the proportion of fish that had food in their stomachs between the two habitats (lava 77%, mud 83%). The lava fish had more prey groups (2.2 ± 1.53) in their stomachs than the mud fish (1.2 ± 0.37; $t_{24} = 3.1$, $P < 0.01$). In the two habitats, the fish had similar diets.

DISCUSSION

The stickleback in the Hraunsfjördur lagoon have altered their morphology significantly since being isolated in 1987. The most obvious changes are in armour structure, with the freshwater fish having a reduced number of armour plates and shorter spines. The fish differ also in their overall morphology between habitats, differences that are consistent for both sexes. These differences are a case of rapid evolution, whereby traits show an evolution rate ranging from −0.80 haldanes for dorsal spines to 0.16 haldanes for keeled armour plates. This rate of evolution is higher than previously reported in stickleback (Klepaker, 1993; Hendry and Kinnison, 1999). In a Norwegian population isolated for 31 years, eye diameter (0.043 haldanes) and spine length (0.021 haldanes) were found to be different between the isolated population and their marine ancestors (Klepaker, 1993; Hendry and Kinnison, 1999). The rate of evolution in the present study is also among the highest rates reported (Hendry and Kinnison, 1999). Similar rates were calculated for Trinidadian guppies, *Poecilia reticulata* (coloration and decoration 0.27–0.74 haldanes; Endler, 1980; Hendry and Kinnison, 1999), and for various morphological characters of the finches of the Galapagos (−0.37 to 0.71; Grant and Grant, 1995; Hendry and Kinnison, 1999).

Threespine stickleback have become isolated in fresh water many times, and in all cases studied their morphology has diverged from that of their marine ancestors (Wottoon, 1984; Taylor and McPhail, 1986; Klepaker, 1993; Bell, 2001), usually after a long period of isolation (Bell and Foster, 1994a,b). As seen in Hraunsfjördur, the most common change in stickleback isolated in fresh water is a reduction in body armour. These differences could be due to some extent to phenotypic plasticity, but it is believed that differences in the number of lateral plates are based largely on genetic differences (Hagen and Gilbertson, 1972; Peichel *et al.*, 2001). Predation pressure is probably greater in the marine environment. Increased predation pressure selects for a higher number of lateral plates as well as larger spines (Reimchen, 1994). Another possibility is that the relatively low concentration of calcium in fresh water selects for fish with fewer plates (Klepaker, 1993; Bourgeois *et al.*, 1994) or even no pelvic girdle (a bony structure supporting the spines; Bell *et al.*, 1985, 1993; Bell, 1987; Bell and Ortí, 1994).

Sexual dimorphism is common in stickleback; not only do the sexes often differ in size, but also in the structures and coloration used in reproduction (Wootton, 1984; Bell and Foster, 1994b). In Hraunsfjördur, as well as in other Icelandic stickleback populations, sexual dimorphism in trophic morphology is common (Kristjánsson, 2001; Kristjánsson *et al.*, 2002). Males usually look more limnetic than females (Caldecutt and Adams, 1998; but see Bentzen and McPhail, 1984), but this is not reflected in the diet of males, which apparently take their food from the substrate. It is possible that the sexes are adapting to different microhabitats. The sexes show different behaviour during the breeding season (Wootton, 1984; Foster, 1994), which may require different feeding strategies.

The stickleback in the lagoon are morphologically more diverse than the marine population and show some morphological segregation between mud and lava habitats. This suggests discrete resource adaptations in the stickleback living in the different habitats. The differences are small and the only clear difference in body shape was that the mud fish had longer caudal peduncles than the lava fish. The fish also differed in their overall meristic counts and females were more variable than males. It is not easy to pinpoint which of the variables is the most important in separating mud and lava fish, as the DF1 axis is influenced by sexual dimorphism and no variable differed significantly among the groups. The mud fish tended to have more rays in their caudal fins, while the lava fish had more rays in their anal fins. Furthermore, the lava stickleback had more plates than the mud fish. It is possible that these differences in fin structure represent adaptive differences in locomotion in the microhabitats where these fish are living and that they may have different feeding and sheltering behaviour. These morphological differences are not clearly reflected in the diet of these fish. The only obvious difference was that the lava fish ate more Cladocera than the mud fish. Fish from both habitats were mainly feeding on pupae and flies. Pupae are usually very seasonal in availability. It is known that, in resource morphs of fish, dietary differences are often lost when seasonal food is available (Malmquist *et al.*, 1992; Snorrason *et al.*, 1994). This could be the case in the lagoon in Hraunsfjördur, where dietary differences may be more profound in the absence of pupae. This prediction has to be studied further by sampling at different times of the year.

Some might question if the freshwater population originated from the marine population. There are small rivers entering the lagoon (Fig. 1), where freshwater stickleback might have lived and then colonized the lagoon after the dam was formed. It is unlikely that the lagoon was colonized only by freshwater stickleback, since the lagoon population is a mixture of individuals with few and many lateral plates. There are also no records of stickleback in these tributary rivers and local people do not recall having seen them there (B. Jónsson, personal communication). Furthermore, the freshwater lake Seljavallavatn, which discharges into the fjord, does not have a stickleback population (personal observation). It is, therefore, probable that the lagoon population originated from a marine population.

Diverse resource adaptations are commonly found in northern freshwater fishes (Robinson and Wilson, 1994; Skúlason and Smith, 1995; Smith and Skúlason, 1996; Robinson and Schluter, 2000) and are common in threespine stickleback (McPhail, 1994). Such segregation to mud and lava habitats has been seen in some lakes in Iceland (Kristjánsson, 2001; Kristjánsson *et al.*, 2002). Resource polymorphism is promoted by many ecological factors (Skúlason and Smith, 1995; Smith and Skúlason, 1996; Schluter, 1996, 1998a,b, 2000a,b; Skúlason *et al.*, 1999; Robinson and Schluter, 2000; Kristjánsson *et al.*, 2002). Among other things, predation and intraspecific competition have been suggested to promote discrete resource adaptations in Icelandic threespine stickleback (Doucette, 2001; Kristjánsson, 2001; Kristjánsson *et al.*, 2002).

Sympatric or parapatric morphs are often genetically different and reproductively isolated (McPhail, 1994; Taylor and McPhail, 1999). In some cases, resource morphs will adopt different reproductive styles, either spawning at different localities within lakes or at different times of the year. This reduces interbreeding and may result in speciation (Skúlason and Smith, 1995; Smith and Skúlason, 1996). This appears to have occurred in several northern freshwater fish species, including arctic charr, *Salvelinus alpinus* (Skúlason *et al.*, 1999). This has been observed in stickleback (McPhail, 1994), where morphs or

species often spawn in different microhabitats (Jamieson *et al.*, 1992; McPhail, 1994). Stickleback in fresh water are often reproductively isolated from their marine ancestors.

Whether the segregation of the lagoon stickleback is based on genetic differences or phenotypic plasticity is not clear and requires further study. Stickleback are known to be phenotypically plastic, but plasticity varies among traits (Day *et al.*, 1994; Day and McPhail, 1996). For example, meristic characters such as gill raker number is not plastic, but morphometric characters such as head depth are (Day *et al.*, 1994). Plate number is highly heritable (Hagen, 1973; Peichel *et al.*, 2001) and is not likely to be plastic. The main differences observed between stickleback in the marine and freshwater habitats were in plate number and length of the spines. It is probable that differences in plate number are genetic, while those in spine length are more plastic. The stickleback within the lagoon differed in caudal peduncle length. We suggest that this difference is mainly due to a plastic response, but this remains to be tested.

We have shown that, after 12 years (at most 12 generations), stickleback in Hraunsfjördur freshwater lagoon show different morphotypes than their marine ancestors. As well as being different from the marine fish, the freshwater fish show habitat (mud *vs* lava) and morphological divergence within the lagoon. This divergence might have been promoted by high intraspecific competition. We are currently investigating the genetic basis for these morphological and behavioural differences as a part of the Icelandic Stickleback Project.

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