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Parallel evolution, not always so parallel: comparison of small benthic charr, *Salvelinus alpinus*, from Grímsnes and Thingvallavatn, Iceland

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Synopsis

Iceland is unique in terms of geologically young freshwater systems and rapid adaptations of fresh water fishes to diverse habitats, e.g. lava with ground water flow. Iceland has six species of freshwater fishes, including Arctic charr, *Salvelinus alpinus*. Previous research has shown great diversity within this species. Four different morphs of Arctic charr are found in one lake, Thingvallavatn, including a small benthivorous charr. Similar populations of small benthic charr are known from several other Icelandic freshwater locations, including Nautavakir in Grímsnes. Our comparison of the small benthic charr morphs in Thingvallavatn and in Grímsnes showed that they are similar in morphology but distinguishable in several characteristics. Small benthic charr in Grímsnes and Thingvallavatn demonstrate similar adaptations and are an example of parallel evolution. However, subtle morphological differences between them indicate further specialized adaptations at each location.

Introduction

Parallel evolution where phenotypically similar morphs or species are found in similar ecological habitats is common (Schluter 2000) and has been observed in numerous freshwater fishes, including stickleback, *Gasterosteus* spp., charr, *Salvelinus* spp., whitefish, *Coregonus* spp. and sunfish, *Lepomis* spp. (e.g. Robinson & Wilson 1994, Smith & Skúlason 1995). The most common form of parallel evolution is the adaptation to benthic and pelagic habitats within lakes. A prime example is the stickleback in five small lakes in British Columbia, Canada that have diverged to the benthic and pelagic habitats to produce a species pair in each lake (McPhail 1994, Schluter 2000). Parallel evolution demonstrates the importance of natural selection in the evolution of resource polymorphism and sympatric speciation (Schluter 2000).

Iceland is an excellent location for studies of the importance of natural and parallel evolution in the origin of diversity. Iceland is geographically isolated, and the freshwater systems are young, formed after the most recent glaciation. This has resulted in only six freshwater fish species in Iceland (Jónsson & Noakes 2001). Because of the volcanic nature of Iceland, freshwater habitats are often diverse and complex. This has resulted in high intraspecific diversity among Icelandic freshwater fishes. This is clearly seen in the threespine stickleback, *Gasterosteus aculeatus*, where parallel evolution has been documented in relation to mud and lava habitats within lakes (Kristjánsson 2001, Kristjánsson et al. 2002a), and in the Arctic charr, *Salvelinus alpinus* (Skúlason et al. 1992, Snorrason & Skúlason 2004). Icelandic Arctic charr show very high diversity often with a parallel evolution of benthic and pelagic morphs (Snorrason & Skúlason 2004). The morphological diversity of these two species has evolved rapidly after the last glaciation (Kristjánsson et al 2002b, Snorrason & Skúlason 2004).

A striking feature of Icelandic freshwater habitats is the presence of lava which creates a complex three dimensional structure, offering shelter for small fishes and invertebrates. Relatively young lava rocks host a greater number and higher diversity of invertebrates than older, more eroded, rocks (Malmquist et al 1999). Another feature of the active volcanic zone in Iceland is that lakes and rivers are fed predominantly by groundwater flowing through the porous lava bedrock (Gardarsson 1979). Icelandic freshwater fishes show special adaptations to the lava habitat. Threespine stickleback in lava habitats differ from those in muddy habitats (Kristjánsson 2001, Kristjánsson et al 2002a). In habitats throughout Iceland with lava and groundwater small Arctic charr are commonly found with a distinct subterminal mouth and robust body (Skúlason et al. 1992, Sturlaugsson et al 1998, Snorrason & Skúlason 2004, Árni Einarsson, personal communication, Bjarni K. Kristjánsson, unpublished data). These charr seem to represent parallel evolution. They are similar in morphology and are not likely evolved from an immediate common ancestor as they occur within many different watersheds (Bjarni K. Kristjánsson, unpublished data), although that needs to be further studied. Two of the places where these charr are found are in Thingvallavatn and in Grímsnes, where a groundwater river flows into the glacial river Hvítá

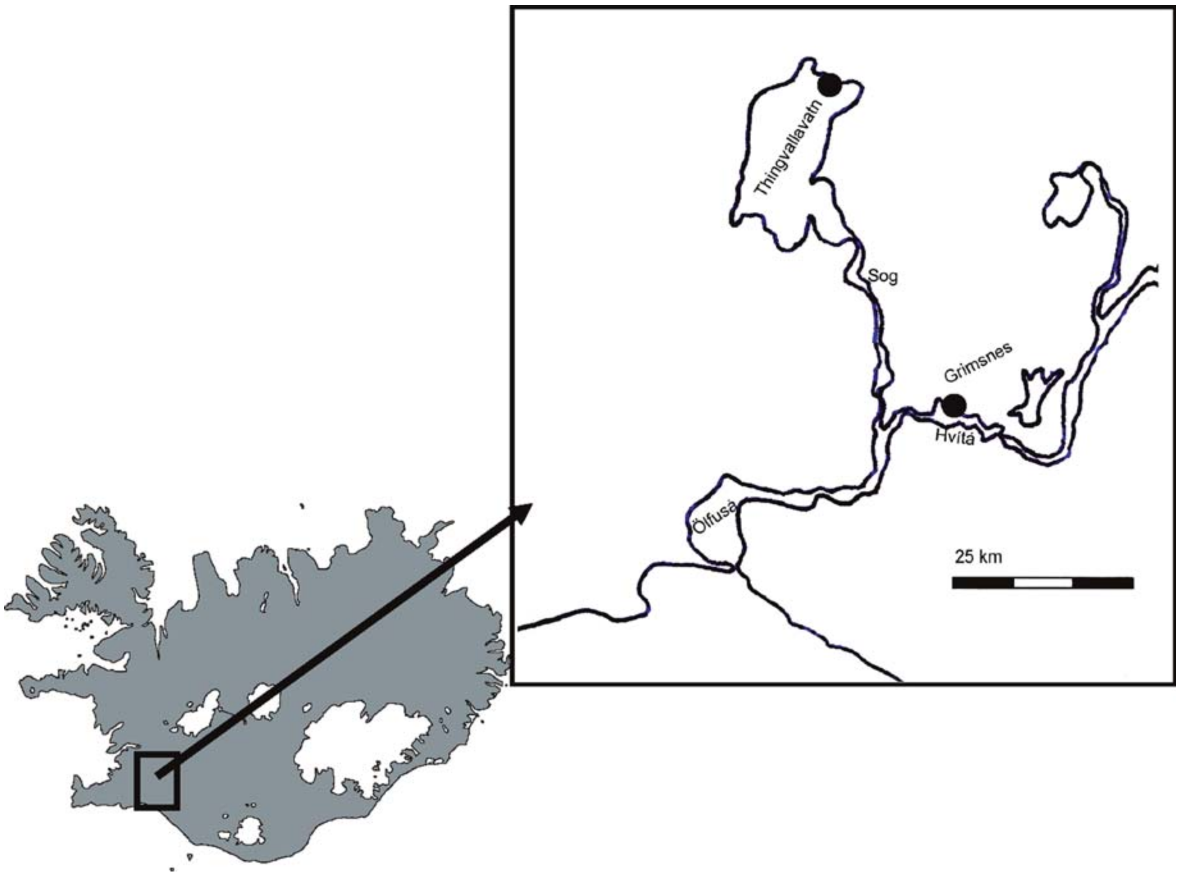

Figure 1. Location of the two sites in Iceland where small benthic arctic charr were sampled.

(Figure 1, Bjarni K. Kristjánsson, personal observation). The river Sog, which drains Thingvallavatn connects to river Hvitá about 2–3 km below the location in Grímsness. The two populations have, however, been isolated from each other for about 9600 years, when arctic charr in Thingvallavatn became isolated because of volcanic activities (Sæmundsson 1992). Although the small benthic charr from these two populations may represent a case of parallel evolution they are probably not identical, we therefore ask the question: can we detect morphological differences between these groups that may indicate special local adaptation to each habitat.

Material and methods

We caught 34 small benthic charr by electrofishing at Nautavakir in Grímsnes on 6 June 2002, and 30 charr the next day in Vatnsvik in Thingvallavatn (Figure 1). We anaesthetised the fish (CO_2 solution) and immediately froze them (−20°C). In the laboratory we thawed the fish and photographed the left side of each (Nikon CoolPix 990 digital camera). We put small plastic markers under the maxillary bone, the operculum and the pectoral fin to distinguish them from the body (Figure 2a, b). We digitized 22 landmarks on each digital image (tpsdig program[1]). Five of those landmarks were sliding landmarks (Figure 1c). We used relative warp analysis (tps-relw [2]) to analyze for differences in morphology, while controlling for geometric body size. The analysis scales the landmarks from each fish to a centroid size, position and rotation. An average shape of all the samples is then computed. The program then calculates partial warps which describe the amount of bending and stretching of a plane of scaled coordinates of all fish to fit them on the centroid size. The partial warps are then saved and used in further analysis. To visualize the observed differences we used tps-splin[3] to create thin plate spline images.

We dissected the fish to determine sex and maturity according to Dahl (1943). Each fish was scored from 1–5, where stage 1 is immature gonad development and stage 5 indicates fully matured gonads with possible signs of having spawned before. Age was determined from the otoliths. We read the otoliths under blue light in glycerol. We viewed the intact otoliths and counted the number of annuli (Jonsson 1976)

We compared morphology between fish from the two habitats by multivariate statistical analysis, discriminant analysis, using SPSS. We used an acceptance value of $p < 0.05$ in all comparisons.

Results

Charr from the two habitats look very similar (Figure 2a, b). However, when we compared their morphology in detail we found the fish differed significantly (DFA, Wilks-$\lambda = 0.07$, $F_{(39)} = 9.02$, $p < < 0.01$) and that the model correctly classified all the fish (Figure 3). When visualising this difference (Figure 4) the fish from the two locations differed most in the head region. The fish in Thingvallavatn had more sub-terminal mouths than the fish in Grímsnes.

Fewer of the fish from Grímsnes were mature than the fish from Thingvallavatn. In Grímsnes 17.4% of the males and 11.1% of the females were fully mature in comparison to 25.0% for males and 28.6% for females in Thingvallavatn. The fish from Thingvallavatn ranged in age from 1+ to 6+ years and in fork length from 61 to 121 mm, while the fish from Grímsnes ranged in age from 0+ to 4+ years and in fork length from 57 to 114 mm.

To test if the differences that we observed in morphology were related to age we regressed morphology on age using tpsRegr[4] (Rohlf 2004d). There was no significant relationship between morphology and age using a generalized Godall *F* test with 1000 random permutations.

[1] Rohlf 2004a – TpsDig program version 1.4 available free at http://life.bio.sunysb.edu/morph/

[2] Rohlf 2004b – TpsRelw program version 1.39 available free at http://life.bio.sunysb.edu/morph/

[3] Rohlf 2004c – TpsSplin program version 1.2 available free at http://life.bio.sunysb.edu/morph/

[4] Rohlf 2004d – TpsRegr program version 1.28 available free at http://life.bio.sunysb.edu/morph/

Discussion

Small benthic charr in Iceland may have evolved in a parallel way in lava and groundwater habitats. However, the small benthic charr from Thingvallavatn and Grímsnes are not identical and may be showing adaptations towards different local conditions in their respective habitats, although the observed differences may be caused by differences in founding populations or genetic drift. Although

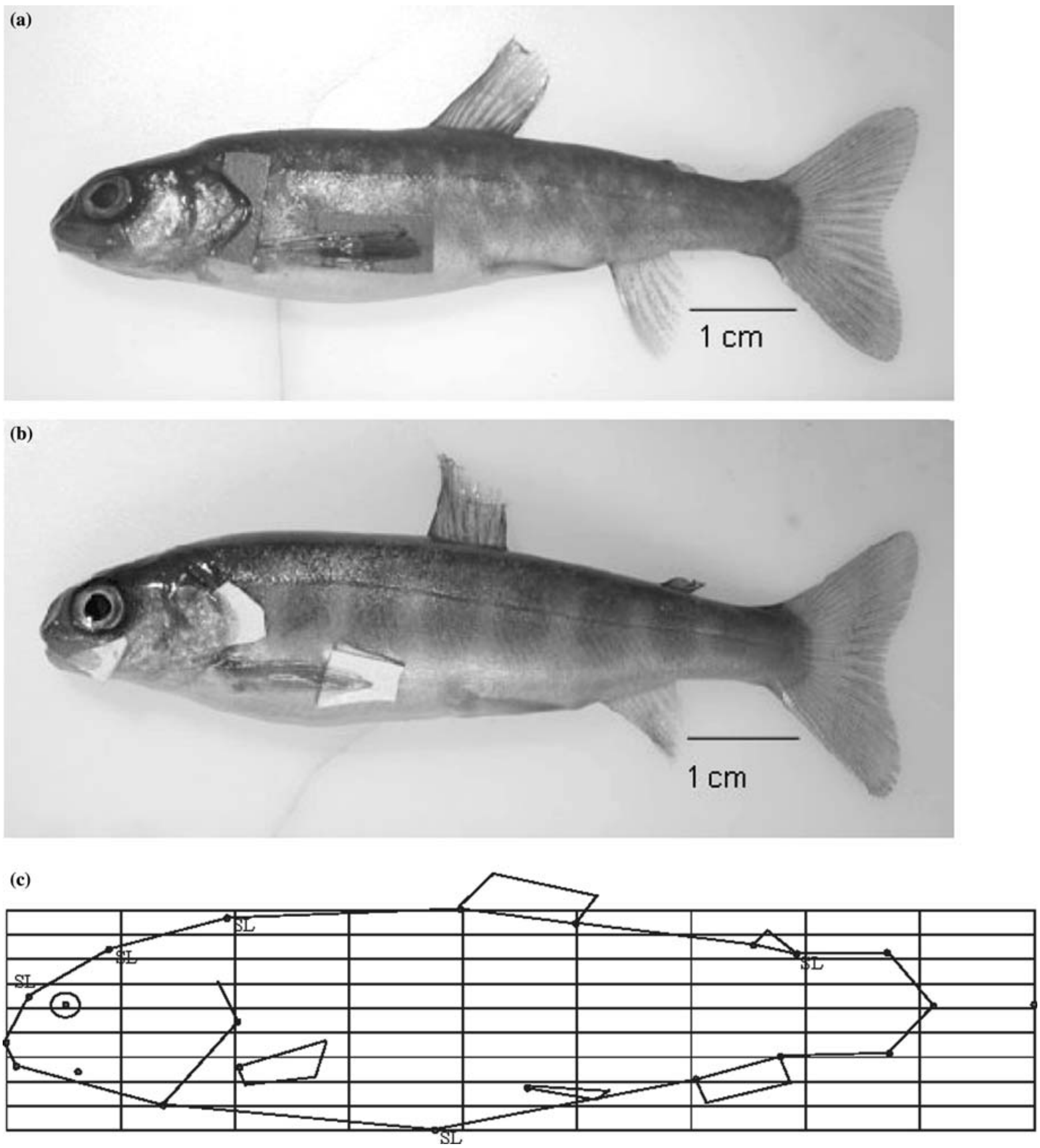

Figure 2. (a) A typical small, benthivorous charr from Thingvallavatn, southwest Iceland; (b) A typical small, benthivorous charr from Grímsnes, southwest Iceland.; (c) Landmarks used in this study are shown on the fish. Five sliding landmarks are specially marked.

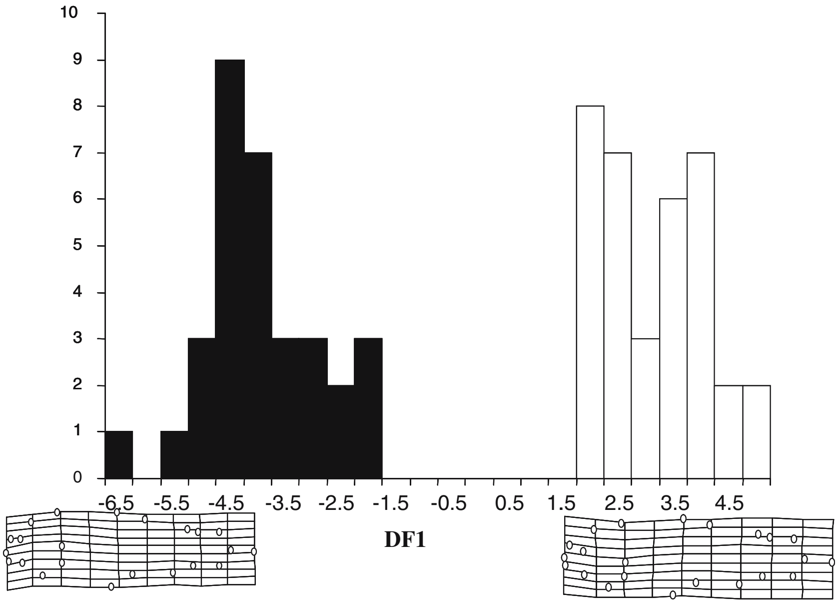

Figure 3. Distribution of scores from a discriminant analysis of two populations of small, benthivorous charr in Iceland, with deformation grids that show the shape of fish at the two extremes of the axis. Black bars represent fish from Vatnsvik in Thingvallavatn and white bars represent fish from Grímsnes.

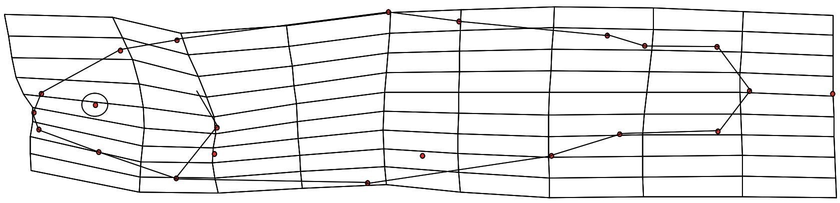

Figure 4. Deformation grids showing deformation from typical small benthic Arctic charr in Grímsnes to a typical small, benthic charr in Vatnsvik, Thingvallavatn. The figure is magnified two times to enhance the appearance of the differences.

similar in appearance, the charr in Grímsnes differ from the charr in Thingvallavatn in morphology, mainly in the structure of the head. The fish from Thingvallavatn have shorter jaws and more subterminal mouths. This could represent different feeding specializations of the two morphs, and needs to be studied further. The fish in Grímsnes were younger and less mature than the fish in Thingvallavatn but this does not explain the morphological differences detected.

Arctic charr with similar morphological features are found in other locations in Iceland, for example in Straumsvík, in southwest Iceland (Sturlaugsson et al. 1998), in lava caves close to Myvatn in northeast Iceland (Gardasson & Einarsson 1991, Skúlason et al. 1992, Ásgeirsdóttir 2002, Árni Einarsson, personal communication) and in at least five other places where there is a combination of lava and groundwater. These locations are both in the north and south of Iceland and in many different watersheds (Bjarni K. Kristjánsson, unpublished data). Often these populations have been isolated from each other for thousands of years (Sæmundsson 1992), as is the case for the two populations studied here. This indicates that habitat characteristics may represent an important force of natural selection acting on the evolution of these charr in a parallel way.

The small benthic fish from Thingvallavatn and Grímsnes are similar in morphology but differ in some key morphological features. This may suggests that special local habitat factors promote further local adaptations. Because of the numerous repeated occurrences of small benthic charr in Iceland there is an opportunity to test what factors these may be. Thus, detailed characteristics of habitats among locations where small benthic Arctic charr are found can be examined in relation to morphological variables of charr to test specific predictions as to the effects of particular environmental factors on their special local adaptations.

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