Lava, habitat stability and instability: specific factors in the evolution of Arctic charr and three-spine stickleback in Iceland.

Resource polymorphism is common in animals and is believed to be an important step in sympatric speciation (Skúlason & Smith 1995). This polymorphism is found in morphology, ecology and behaviour of animals, and is related to the use of specific resources. For example, it has been suggested that the diversity of cichlid species in the great African rift lakes is promoted by resource polymorphism. The best-studied cases of resource polymorphism can, however, been found in freshwater lakes in the northern hemisphere (Skúlason & Smith 1995, Robinson & Schluter 1999, Schluter 2000, Snorrason & Skúlason 2002), particularly in Thingvallavatn, Iceland (Wilson 1992).

Northern freshwater systems are young, have only been colonized after the last glaciation some 10 000 years ago, and are considered to be still in the colonization phase (Skúlason et al 1999). These habitats are species-poor and the colonizating species are presented with a diversity of unexploided habitats and resources. As a result the combined low interspecific competition and high intraspecific competition have promoted resource polymorphism within the colonizing species (Robinson & Wilson 1994, Skúlason & Smith 1995, Smith & Skúlason 1996). It is believed that this has taken place repeatedly and independently in many habitats and in several fish species such as whitefish (*Prospium*, *Corogonus*, spp), sunfish (*Lepomis* spp.), arctic charr (*Salvelinus* *alpinus*) and stickleback (*Gasterosteus aculeatus*) (Robinson & Wilson 1994, McPhail 1994, Skúlason & Smith 1995, Smith & Skúlason 1996).

Phenotypically similar morphs or species of fishes are often found in similar ecological habitats in different lakes. They have originated through parallel evolution caused by common selection pressure in different lakes (Schluter & Nagel 1995, Robinson & Schluter 2000, Rundle et al. 2000). Parallel evolution is common in northern freshwater fishes (Robinson & Schluter 2000). One of the best examples of phenotypic polymorphism and parallel evolution in northern fishes is the three-spine stickleback (McPhail 1994, Bell & Foster 1994, Kristjánsson et al 2002a,b), but similar patterns can be seen in other fishes such as the arctic charr (Skúlason et al 1999, Snorrason & Skúlason 2003).

Iceland is clearly one of the best places to study ecological factors that may promote diversification and resource polymorphism in freshwater fishes (Wilson 1992). As a mid-oceanic island, the native fauna of Iceland is species-poor. On the other hand, Icelandic freshwater systems are numerous and diverse (Gardarsson 1979, Skúlason et al. 1999) and are often shaped by the volcanic nature of the island. These volcanic lava habitats often have specific fauna not found elsewhere (Kristjánsson & Svavarsson 2003, Malmquist et al 1999). In older parts of the country, the bedrock is hard, resulting in little groundwater but high surface water, much influenced by seasons and weather. In younger parts of the country the neo-volcanic lava in lakes provides numerous microhabitats for fishes. The lava substrate has not yet eroded and so it hosts more benthic invertebrates which may serve as food for fish than the highly eroded stones of geologically older areas of the country (Malmquist et al. 1999). The lava is often highly complex, with numerous holes and fissures, as well as subterranean caves. This provides numerous sheltering spaces for small fishes. Subterranean inflow through porous rocks in the catchment area promotes stability in water level, temperature and chemistry. This may result in special local adaptations of populations enabling them to better use these microhabitats.

Arctic charr in Iceland is a very heterogeneous species, and numerous obvious resource morphs are found that often show parallel patterns in adaptations towards bottom and pelagic habitats of the lakes (Skúlason et al 1993, 1999, Jónsson & Skúlason 2000). This is well documented in Thingvallavatn in southwest Iceland, where four sympatric charr morphs are found: two benthic and two pelagic (Skúlason et al 1992). One of the benthic morphs in Thingvallavatn is specially adapted to the lava substrate that is common in the lake (Snorrason et al 1994). This is called the dwarf morph and it has likely evolved through paedomorphosis because it has retained the juvenile morphology. Dwarf morphs of arctic charr can be found in association with lava in a number of other areas in Iceland. Their morphology has seldom been studied, but these dwarf phenotypes appear to represent a clear case of parallel evolution (Sigursteinsdóttir & Kristjánsson in prep). In fact, the association between arctic charr and lava has not been studied in any detail. It is important for our understanding of diversification and speciation of animals to examine how ecological variables may promote this process.

Studies on the diversification and evolution of sticklebacks in Iceland are relatively recent (Snorrason et al 2002). The morphology and behaviour of sticklebacks have been studied in relation to distinct benthic habitats (Doucette 2001, Kristjánsson 2001). The morphology of these sticklebacks shows some parallel patterns in relation to mud and lava habitats in different lakes, although the level of morphological divergence is very different among the lakes (Kristjánsson et al 2002a). It has also been shown that the sticklebacks in Iceland evolve rapidly after being isolated in freshwater. In as short time as 13 generations divergence can occur in relation to mud and lava habitats (Kristjánsson et al. 2002b).

My studies on the three-spine sticklebacks in Iceland show that lava habitats in lakes are important in the evolution of the species. This habitat may be considered to be rather specific for Iceland in comparison to other freshwater habitats in the northern hemisphere. This habitat offers small fish species such as sticklebacks a unique habitat. The numerous holes, crevices and fissures offer good shelter. The relatively young lava rock offers higher densities of invertebrates than the eroded rocks common in other habitats (Malmquist et al. 1999). My findings indicated that this habitat promotes the evolution of sticklebacks (Kristjánsson 2001, Kristjánsson et al. 2002a). This is seen by the parallel patterns in the morphology of sticklebacks in lava habitats. Although parallels were observed in morphology, the fish in the lava habitats were not identical. In a study on two dwarfish arctic charr morph that looked very similar and represented a clear case of parallel evolution the fish were not identical but differed in important morphological features related to foraging (Sigursteinsdóttir & Kristjánsson in preparation) . It is likely that specific ecological factors are affecting the evolution of these fish resulting in small differences in morphology. It is, therefore, important to examine how ecological factors in the lava habitat promote the divergence of fish.

My Ph.D. thesis will test the hypothesis that lava habitat is critical to the evolution of freshwater fishes in Iceland. Lava is a unique benthic habitat in freshwater. It is a highly structural complex, with numerous small holes and crevices that can offer shelter for small fishes. The rough lava rocks allow for a higher density of invertebrate prey organisms (Malmquist 1999), which provide food for small fishes. Although the structure of lava is similar among and within lakes it is not identical, and can differ quite a lot depending on its composition and age. The lava habitats differs in age, and degree of erosion, they differ in the amount of groundwater that flows through them, as well as the type and roughness of lava. I will assess these different habitat types of lava and the degree to which they promote different evolution of fish through three interrelated approaches.

1 – Description of the habitat.

A – Ecology of lava in freshwater.

To test my hypothesis, I have outlined my thesis as a series of questions. Is the lava habitat in lakes (and rivers) uniform in its composition of fish and invertebrate animals? Are there differences in species composition in lava habitats that can be related to differences in the structure of the lava and to other physical characteristics of the lava habitat?

To examine these questions, I will do detailed studies on lava habitats in two lakes; Hredavatn in Borgarfjördur and Thingvallavatn. These two lakes are easily accessible, they both have extensive lava fields, but differ in the type of lava. First I will map the lava areas along the shorelines. This mapping will include such key features as areas of groundwater inflow, as well as detailed descriptions of the physical characteristics of the lava itself. Secondly I will select replicate areas in each lake, with and without groundwater. Then I will divide each area into grids. I will quantitatively sample each grid by electro fishing to determine the abundance and spatial distribution of fishes within each area. I will sample selected grids for substrate and determine the physical characteristics of the particles (ranging from solid lava, through lava stones to fine mud). I will preserve all biological samples (formalin), sort all organisms from each sample and then count and identify all organisms. This will provide quantitative data on species composition and density within lava areas, and will also give me information on a range of physical scales that is needed to determine the importance of the lava habitat.

B - Stability, measurement of stability in different lava habitats.

It is commonly assumed that areas of lakes with groundwater flow are more stable than areas where there is no groundwater flow. I will test this assumption, and determine if it is possible to measure stability in these habitats.

To examine this I will select replicate areas within Hredavatn and Thingvallavatn, with and without groundwater flow. I will collect substrate samples at regular intervals during the year at each area and will carry out the same estimates of species abundance and distribution as described above. I will also record selected physical characteristics (depth, temperature, total dissolved solids).

2 – Relationships of habitat structure and fish morphology

Variation in morphology of fishes in relation to habitat structure.

Research has shown that the habitat is key to the evolution of resource polymorphism and sympatric speciation. Numerous studies have shown specially adapted forms in the benthic and the limnetic habitats in lakes (e.g. Skúlason & Smith 1995, Schluter 2000). Researchers have also shown that these forms often have adapted in a parallel fashion (e.g. Schluter 2000). The parallel way the fishes adapt to the environment is, however, not an endpoint and different populations can be found at different level of specialization (Kristjánsson et al 2002a). These differences may be related to differences in the structure of the habitat the fish inhabit or time since the habitat became available to the fish. Adaptations to benthic or limnetic habitats are commonly described in freshwater fishes. They are, however, not the only ones that occur. Adaptations towards distinct bottom habitats can also be commonly seen. The cichlids in the great African rift lakes are a good example of this. In the northern hemisphere this is probably best seen where fishes have adapted to soft and hard substrates in lakes. One of the hard substrates fishes have adapted to is lava, which is common in lakes in Iceland.

Research has previously shown that lava is a unique habitat in lakes, promoting special evolution of fishes (Kristjánsson et al 2002a, Sturlaugsson et al 1998). These habitats promote parallel evolution of a special lava form of the three-spine stickleback, although the sticklebacks in lava habitats across lakes show different levels of specialization (Kristjánsson et al. 2002a). In Arctic charr lava habitat seems to promote the evolution of special dwarf morphs (Snorrason & Skúlason 2002, Sturlaugsson et al. 1998). There are indications that these forms also show different levels of specialization (Sigursteinsdóttir and Kristjánsson in preparation).

My hypothesis is that the different level of specialization seen among fishes in lava habitats is related to different habitat structure. I will test this hypothesis by examining the habitat in more detail and relating the structure of the habitat to variations among morphs within and between lakes.

I will test my hypothesis this in two ways:

a) I will collect “dwarf” arctic charr at four locations within Thingvallavatn. Two of these locations are within the lava area in the north of the lake, one where there is groundwater and one where there is no groundwater. Two other locations are where there is older, more eroded substrate material. At each location I will collect samples of lava particles and mud samples from the substrate. I will also measure the various environmental factors described above. I will analyze the samples as described above. I will collect charr and sticklebacks by electrofishing. I will freeze the fish in the field for later examination in the laboratory. In the laboratory I will thaw the fish and photographed them with a digital camera. I will transfer these digital photographs to a computer where I will define and analyze a series of morphological measurements. I will dissect the fish, remove stomach contents and identify those for all prey. I will remove otoliths and prepare them for age determination. I will determine the sex and sexual maturity of each fish, and estimate fecundity for females.

b) I will collect “dwarf” arctic charr in as many locations as possible in addition to Thingvallavatn. I already know of “dwarfs” in five other locations, but I will search for them in other locations. I will collect charr and comparable habitat measurements from a series of lakes where there are no dwarf morphs. I will collect environmental variables, lava and mud samples, and will process them as above from all lakes where I sample charr. I will collect charr and sticklebacks by electrofishing and will process the fish as described previously.

I will use these data to examine intralacustrine variation of “dwarfs” in Thingvallavatn, and within each other lake. I will also compare these data between all the lakes in my study. I will assess patterns of correlated morphological variation of dwarfs with habitat structure. My predictions are that the dwarf morphs will be more likely to occur in habitats with greater complexity and stability, both within and between lakes.

3 – Through functional measurements of fish biology and development.

The relation of habitat structure and stability to morphological and behavioural plasticity.

Ecological and evolutionary theory predicts that increased specialization is attained at the cost of losses in phenotypic plasticity (e.g. Skúlason et al 1999). Increased stability of the environment is believed to be directly related to the evolution of specialization. Unstable environments maintain the need for phenotypic plasticity and thus prevent the population from becoming specialized. The dwarf arctic charr are considered to be a specialized morph, but there are indications that not all dwarf populations show the same adaptations and are not likely to be at the same level of specialization (Sturlaugsdóttir and Kristjánsson in prep). The results of the morphological and diet measurements in my project will test this prediction.

I will then proceed to test the two competing hypotheses to account for the phenotypic plasticity of dwarf charr morphs. One hypothesis is that the level of plasticity in dwarf arctic charr is related to how specialized they are. The other hypothesis is that their plasticity is a function of the stability of the environment where they live.

To test these hypotheses I will select 4 – 5 populations of dwarf arctic charr that live in areas that differ the most in habitat structure, and show the most and the least variation in morphology. I will capture adults of these populations during the breading season. I will fertilize the eggs from each female with sperm from one male, thus creating a series of 5 - 10 full family groups from each population. I will rear the families in the Hólar University College aquaculture facility. At the onset of first feeding I will divide each group in two halves. I will feed half of the offspring floating food while I will feed the other half food of the same composition that is formulated to sink quickly to the bottom. I will sample fish from each group 3 and 6 months after the onset of feeding (20 per population). I will preserve all individual fish and examine their morphology in detail as for those sampled directly from the lakes. The results of this experiment will show whether the level of morphological plasticity differs among the different population, families or food treatments. These results will allow me to decide which of my two hypotheses I can accept, or reject, as the explanation for the evolution of dwarf charr morphs.