

# Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks

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Recently, models of sympatric speciation have suggested that assortative mating can develop between sympatric morphs due to divergence in an ecologically important character. For example, in sympatric pairs of threespine stickleback (*Gasterosteus aculeatus* L.) size-assortative mating seems to be instrumental in reproductive isolation. Here, we examine courtship behaviour and assortative mating of newly described sympatric stickleback morphs in Lake Thingvallavatn, Iceland. We find that the two morphs show strong positive assortative mating. However, the mechanism involved in mate choice does not seem to be as straightforward as in other similar systems of sympatric stickleback morphs and may involve variation in nest type.

**Keywords:** speciation; assortative mating; stickleback

## 1. INTRODUCTION

Reproductive isolation allows genetic differences between species to persist. In allopatric speciation, differences, which accumulate over time cause pre- or post-zygotic isolation that will prevent gene flow if the species come into secondary contact. For speciation to result from ecological selection, sexual and ecological traits involved must be connected, for example, the same character being involved in both ecological adaptation and mate choice, or an independent character can become recruited for the purpose of mate discrimination (Kondrashov & Kondrashov 1999). The simplest and probably the most convincing example of reproductive isolation being influenced by ecological factors is premating isolation caused by host or habitat shift. This is, for example, seen in some phytophagous insects that mate on their host plant (Feder 1998; Nosil *et al.* 2002). Size-assortative mating is another example of ecological divergence with the potential to directly influence sexual isolation. Size is an important ecological character in many species and differences in size are often observed between populations. Sympatric morphs of many fishes species differ in body size and size often influences premating isolation, e.g. in

sticklebacks (*Gasterosteus aculeatus* L.; Nagel & Schluter 1998; McKinnon *et al.* 2004), sockeye and kokanee salmon (*Oncorhynchus nerka*; Foote & Larkin 1988) and Arctic charr (*Salvelinus alpinus*; Sigurjónsdóttir & Gunnarsson 1989).

Size-assortative mating has been found to be important in at least two potential cases of sympatric stickleback, the Canadian limnetic–benthic stickleback pairs (Nagel & Schluter 1998) and in populations of anadromous and freshwater sticklebacks (Ishikawa & Mori 2000; McKinnon *et al.* 2004). Several other factors are also involved in stickleback mate choice, including variation in nest structure and nest building (Blouw & Hagen 1990), male nuptial colour (McKinnon 1995; Boughman 2001), small scale habitat choice (Vamosi & Schluter 1999) and direct selection on male preference by female cannibalistic behaviour (Albert & Schluter 2004).

Two stickleback morphs have recently been described in Lake Thingvallavatn, Iceland: a lava type, found in complex lava caves and crevasses in the north end of the lake and a nitella type, found in dense vegetation of the alga *Nitella opaca* at depths of 10–25 m. These morphs differ in several morphological characters, most importantly in antipredator morphology. The lava fishes have shorter spines, are deeper bodied and have larger heads and fins (Kristjánsson *et al.* 2002). More recently, these morphs have been found to be genetically distinct but with low genetic differences. They are likely to have diverged very recently, within the last 10 000 years (Ólafsdóttir 2004). Here, we examine the courtship behaviour of the Lake Thingvallavatn stickleback morphs. The morphs show strong positive assortative mating. The reduced likelihood of mating between morphs may depend on a more complicated mechanism than simple size-assortative mating, possibly including nest inspection.

## 2. MATERIAL AND METHODS

### (a) Sampling and maintenance

Experiments were conducted in 2001–2003. Fishes were collected using unbaited minnow traps or electrofishing and adults transferred to the aquarium. They quickly recovered and mortality did not occur. All were allowed to adapt to the laboratory for 18–24 h and fed dried tubifex worms.

### (b) Experimental procedure

A single male was transferred to one-half of a 0.25 m<sup>3</sup> aquarium separated with an opaque partition. In each compartment was a nesting disk (10 cm diameter) containing sand, mud and vegetation and a flowerpot that provided shelter. Males were presented with gravid females to stimulate nest building. Males were judged ready to mate either after they crept through their nests (Rowland 1994) or when they actively courted the females, leading and indicating a spawning site (lava males can successfully court with incomplete nests). Trials started when a gravid female was introduced, were videotaped and lasted 30 min. Afterwards fishes were anaesthetized and preserved in 5% formaldehyde. Experimental males were used 1–3 times (mean=1.4), females were used only once. Logistic regression was used to check for the effect of male trial number on the probability of spawning. This was done separately for each stickleback group. No significant effect was found. Moreover, tests for assortative mating were repeated using only the first trial of each male.

Several behaviours were recorded from videotapes. The behaviours used in this analysis are, for males: (i) zig-zag and (ii) bite and for females: (i) examine nest and (ii) spawn. Differences between groups were tested using two-way ANOVA on square root transformed counts. Nests were given a score from 0 to 4 depending on the amount of vegetation (1–2 points), the opening (1) and the presence of ornaments (1). A nest without vegetation or clear opening scored 0 and a fully structured nest 4. Nest sites were

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scored according to the position in the tank, 2 representing nests in the nesting disk or less than 10 cm from the flowerpot, 1 if 10–30 cm and 0 if more than 30 cm away and scores analysed with a Mann–Whitney test.

Fixed fishes were transferred to 70% ethanol and standard length, head length, body depth and ventral fin size measured then log transformed.

### (c) Assortative mating and mate choice

Logistic regression was used to test for significant departure from random mating between the groups, examining the effect of the interaction between male and female groups on mating probabilities. Sexual isolation is indicated by a significant interaction using a likelihood ratio test. Both spawning and nest inspection were used as a measure of mate choice. To test if the female morphs differed in their tendency to inspect nests of males of the opposite morphs, the effect of male morph on nest inspection was examined separately for each female morph. Logistic regression was also used to tests for potential female mate preferences for size, morphology (body depth, head size), courtship intensity (zig-zag  $\text{min}^{-1}$ ) and nests (nest type and site) separately for each female morph.

## 3. RESULTS

### (a) Courtship behaviour and nest building

Both male morphs showed typical threespine stickleback courtship behaviour. Mostly the males built typical nests using vegetation and sand (table 1). The most common nest score is three, with an occasional score of 4 when nest ornamentation was observed (three nitella males), 10 out of 21 lava males had a score of 0 or 1 corresponding to no or very little vegetation used in nests and these scores were never observed with nitella males. The lava and nitella males differed in mean nest structure score ( $U=182$ ;  $p<0.001$ ). Moreover, 86% of nitella males nested within 10 cm of the flowerpot provided for cover but only 65% of lava males. Nest site score differed significantly between these groups ( $U=382$ ;  $p=0.035$ ). In 2001 and the early season of 2002, lava males with unconventional nests were not used in experiments, therefore, their tendency for incomplete nest building might be underestimated with this data.

### (b) Assortative mating and mate choice

There was positive assortative mating between the lava and nitella morphs. Lava females spawned with nitella males in two out of 14 trials and lava males in 10 of 18 trials. Nitella females spawned with lava males in four out of 20 trials and nitella males in 18 of 25 trials (figure 1). The interaction between male and female morphs, therefore, had a significant effect on the probability of spawning ( $\chi^2_1 = 10.79$ ,  $p=0.013$ ; first male trial only,  $\chi^2_1 = 10.72$ ,  $p=0.013$ ). When using nest inspection as a mate choice indicator the model was not significant. However, when analysed separately the nitella females were less likely to inspect the nests of lava males ( $\chi^2_1 = 6.168$ ,  $p=0.013$ ).

We also tested for the influence of morphology (body length, head size and body depth), courtship intensity (zig-zag  $\text{min}^{-1}$ ) and aggressive behaviour (bites  $\text{min}^{-1}$ ) and nest type and site on the probability of spawning. Male behaviour did not affect the likelihood of mating. There were no significant effects of morphology on choice of the females, although there was a trend for females choosing larger males (figure 2). Nest position did not have a significant effect on the probability of spawning for the lava females but nitella females were more likely to mate

Table 1. Mean scores and variance of nest structure and nest site.

site	<i>n</i>	nest structure	nest site
lava	21	1.62 (1.08)	2.57 (0.50)
nitella	35	2.93 (0.27)	2.79 (0.49)

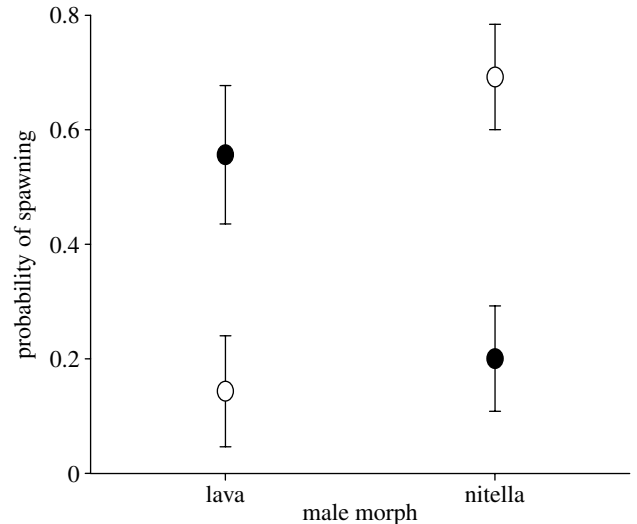


Figure 1. The probability of spawning and standard error for assortative mating trials of each morph pair. The closed circles represent lava females and open circles, nitella females.

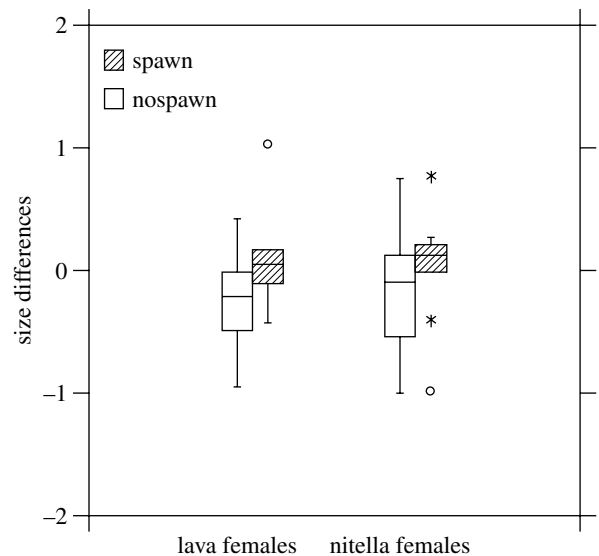


Figure 2. The effect of differences in body size of pairs on spawning probability. There is a trend for females mate with larger males but this was not significant.

with males that nested closer to cover ( $\chi^2_1 = 4.135$ ,  $p=0.042$ ). Results from all tests can be found in the electronic supplementary material.

## 4. DISCUSSION

There is positive assortative mating between the lava and nitella morphs. However, this is not simply size-assortative and more than one mate choice cue is implicated. In northern freshwater fishes ecological specialization often involves divergence in size with

large and small morphs coexisting (Snorrason & Skúlason 2004). Mate choice is often based on body size and assortative mating may evolve between fishes of different size groups (Foote & Larkin 1988; Sigurjónsdóttir & Gunnarsson 1989; McKinnon *et al.* 2004). The common observation of size-assortative mating in systems of sympatric morphs of freshwater fishes has important implications for models of speciation because it may indicate that divergence in nature is best described by single character models.

In Lake Thingvallavatn, there are slight differences in body size of the male morphs but the lava females are on average larger than females from the nitella habitat (Ólafsdóttir 2004). Size may be involved in mate discrimination but there is no size-assortative mating. Mate preferences and mate choice in the threespined sticklebacks are commonly based on multiple characters (Bakker & Rowland 1995). In the current study, nest partially explained the likelihood of spawning of nitella females and there may be a trend for females of both morphs choosing larger males (figure 2).

A notable difference between the males involved nest building and nest structure. The nests of Lake Thingvallavatn male sticklebacks are fairly typical of the species, but the unique habitat of the lava males seems to partly affect their nest building. Males from the lava morph are less likely to choose sites close to shelter and they invest less in nest structure than do the nitella males. The lava habitat offers abundant refuges from piscivorous fishes and does not seem to provide ideal conditions for nest building, with little available vegetation. The importance of nests in the evolution of these morphs is emphasized by the fact that lava females do not show significant initial preferences for male morph before inspecting their nest, rather their mate choice is made following nest inspection. However, the nitella females are less likely to inspect nests of lava males, therefore nitella females may be predisposed to use morphological or behavioural cues prior to nest inspection when evaluating potential mates.

To conclude, there is strong positive assortative mating between the Lake Thingvallavatn stickleback morphs. However, in contrast to other cases of ecological divergence of sticklebacks, the mechanism involved in this assortative mating does not seem to be a simple by-product of selection on body size. Popular models of sympatric ecological speciation imply that a neutral trait (e.g. colour) and an associated preference could become recruited into the ecological specialization (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). This model probably does not apply here either, as nest structure is probably an important component of the ecological specialization involved in adaptation to the lava habitat—this may therefore be a case where the specialization itself yields potentially important non-morphological cues to assortative mating.

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