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Inbreeding avoidance through non-random mating in sticklebacks

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Negative effects of inbreeding are well documented in a wide range of animal taxa. Hatching success and survival of inbred offspring is reduced in many species and inbred progeny are often less attractive to potential mates. Thus, individuals should avoid mating with close kin. However, experimental evidence for inbreeding avoidance through non-random mating in vertebrates is scarce. Here, we show that gravid female three-spined sticklebacks (*Gasterosteus aculeatus*) when given the choice between a courting familiar brother and a courting unfamiliar non-sib prefer to mate with the non-sib and thus avoid the disadvantages of incest. We controlled for differences in males' body size and red intensity of nuptial coloration. Thus, females adjust their courting behaviour to the risk of inbreeding.

Keywords: stickleback; mate choice; inbreeding depression; *Gasterosteus aculeatus*

1. INTRODUCTION

Inbreeding depression is a well-known phenomenon even discussed by Darwin (1868). It has been demonstrated in several groups of animals and plants (Crnokrak & Roff 1999; Armbruster & Reed 2005) and is a problem receiving growing attention in conservation biology and animal breeding (Kristensen & Sørensen 2005). The most plausible mechanism of inbreeding depression is that through an increased level of homozygosity deleterious recessive alleles are unmasked (Charlesworth & Charlesworth 1987). This leads to a wide range of disadvantages for the inbred progeny and thus to a reduced fitness of parents (Crnokrak & Roff 1999; Armbruster & Reed 2005). In fishes for example, inbred salmonids showed a higher rate of body deformations, a reduced fry survivorship and a reduced growth rate while inbred guppies (*Poecilia reticulata*) displayed an altered reproductive behaviour (Waldman & McKinnon 1993 and citations therein).

Inbreeding depression should thus facilitate the evolution of inbreeding avoidance mechanisms. Dispersal of individuals from their natal group (Pusey 1987) or avoidance of grouping with kin (Arnold 2000) is one well-documented way to avoid inbreeding. Others are extra-pair (Foerster *et al.* 2003) or extra-group copulations as well as delayed maturation or reproductive suppression (Pusey & Wolf 1996). Kin recognition and subsequent rejection of kin as

mate would be another mechanism to avoid inbreeding. Such mechanism has been studied in a number of vertebrate taxa (Pusey & Wolf 1996), mostly in mouse species and a few bird species. Data are lacking for ectothermic vertebrates.

Three-spined sticklebacks are a popular vertebrate model system in ecology and evolution and their mating system is well studied (Wootton 1976). Females prefer to mate with larger males (Kraak *et al.* 1999) as well as with redder ones (Bakker & Milinski 1993), and they adjust their choice in relationship to the male's MHC alleles (Reusch *et al.* 2001). Furthermore, adult sticklebacks are able to recognize familiar kin (Frommen & Bakker 2004). The risk of inbreeding in sticklebacks is high in newly established and small populations (Heckel *et al.* 2002; Aeschlimann *et al.* 2003). Inbred sticklebacks have greater body asymmetries (Mazzi *et al.* 2002) making them less attractive as mating partners (Mazzi *et al.* 2003; but see Morris *et al.* 2005). Inbred eggs have a lower fertilization and hatching rate compared to outbred fishes, and fewer survive to reproductive age (J. G. Frommen 2005, unpublished data). Thus, in sticklebacks mating with kin results in severe loss of fitness. This raises the question of whether female sticklebacks are able to circumvent the disadvantages of inbreeding by avoiding mating with close kin. In choice experiments, gravid females were given the opportunity to court with either a familiar brother or an unfamiliar non-sib while controlling for differences in body size and red coloration between males. Inbreeding may also affect female mating preferences (Mazzi *et al.* 2004). Because about half of the females tested had been inbred for one generation, we could study whether this also applied to the avoidance of mating with close kin.

2. MATERIAL AND METHODS

Trials were conducted between December 2004 and January 2005. Sticklebacks used were laboratory-bred outbred offspring of anadromous fishes caught during their spring migration in April 2003 on the island of Texel, The Netherlands, and offspring of fishes caught at the same location in April 2002, which had been inbred during one generation using brother–sister matings. Eggs were taken out of the nests after fertilization. At the age of two months, group sizes were reduced to 15 full-sibs. We tested fishes from 16 different full-sib groups, seven of them had been inbred, nine outbred. Only one female from each group was used while some groups provided the brother in one test and the non-brother in another. However, all individuals were used only once.

Males that showed breeding coloration were isolated into 10 l glass tanks equipped with a Petri dish filled with sand and 2 g of java moss *Vesicularia dubyana* for nest building. Males were stimulated daily by presenting a gravid female. Males that failed to build a nest within one week were discarded.

The test tank was divided into two compartments measuring 25 × 40 × 25 cm and 20 × 40 × 25 cm, respectively, using perforated clear Plexiglass. The larger part was again divided by grey PVC partitions into two compartments each measuring 19 × 20 × 25 cm. The larger and the smaller compartments were additionally separated by a grey PVC partition which could be lifted from the outside. A black curtain was tightened around the test tank so that no light from outside could shine into the set-up. Fifty centimetres above the tank we placed a fluorescent tube wrapped with a red filter (Rosco, Supergel 73), which filtered out all red light between 550 and 750 nm and a webcam (Creative, model CT6840) connected to a laptop computer.

A gravid female was placed in the larger part while a female brother and an unrelated individual were transferred together with their nests to the two smaller compartments. After a familiarization time of 1 hour, the PVC partition was lifted. Now the female had

visual and olfactory contact with both males. After the female had entered each choice zone (measuring 7×19 cm in front of the males' compartments), the time she spent in front of each male's compartment was measured for 30 min. If at least one of the three fishes did not start courting within 10 min the trial was discarded. This was to ensure that females chose sides based on mate choice rather than a mere shoaling preference. In order to ensure that gravid females were used, only individuals that spawned with a third male within 24 h after the test were considered for statistical analysis (Mazzi *et al.* 2003). Therefore, five females were excluded. Because the time a female stickleback spends near a courting male or his odour is positively correlated with the probability of spawning with that male (McLennan & McPhail 1990; Milinski *et al.* 2005), this standard set-up (Wagner 1998) provides a good measure of a female's mating preference.

All data were normally distributed according to the Kolmogorov–Smirnov test with Lilliefors-correction, and thus parametric statistics was applied. Given p -values are two-tailed throughout.

3. RESULTS

The time spent near non-kin did not differ significantly between inbred (mean time \pm s.d., $1031.9 \text{ s} \pm 457.0$) and outbred (mean time \pm s.d., $997.1 \text{ s} \pm 410.1$) females (figure 1; two sample t -test, $N_{\text{inbred}} = 7$, $N_{\text{outbred}} = 9$, $t = -0.16$, $p = 0.875$). The data were therefore pooled. Females spent significantly more time courting in front of the unrelated male than in front of her brother (figure 1; table 1). Twelve out of 16 females spent more time in front of unrelated fishes (figure 2; Chi-square test, $\chi_1^2 = 4.0$, $p = 0.046$).

Differences in male body mass and nuptial coloration were excluded as potential factors influencing female choice by size-matching the males in a pair to the nearest 2 mm, and performing the tests under green light (Milinski & Bakker 1990), respectively. Thus, the males in a pair did not significantly differ in body size, body mass or condition factor (Bolger & Connolly 1989; paired t -test, $N = 16$, all t between -1.58 and -0.56 , all $p > 0.135$). A comparison between the time spent near the larger or smaller, heavier or lighter and better or worse conditioned male did not reveal any significant results (table 1).

4. DISCUSSION

Our results show that female sticklebacks are able to adjust their mate choice to the danger of inbreeding. An unsolved issue is whether females' mating decisions are based on 'true' kin recognition through recognition alleles or on familiarity effects like phenotype matching (Blaustein 1983; Grafen 1990). However, it is extremely difficult to rule out familiarity effects (Mateo 2004). Fishes reared in separated kin groups are still familiar with the smell of a fraction of their siblings. Even if they are reared totally isolated they are able to get familiar with their own cues ('self-referent phenotype matching'; Mateo & Johnston 2000). Sticklebacks live at least during the early larval phase close to their kin (Wootton 1976). In this time, they have got the opportunity to get familiar with their siblings' phenotypes. Reaching adulthood they can use this template to avoid mating with individuals they shared a nest with and thus avoid inbreeding. However, functionally it does not matter whether kin is recognized by recognition alleles or phenotype matching as long as individuals are treated according to coefficients of relationship (Mateo 2004).

Table 1. Mean time females spent courting in front of different males. (Females preferred non-kin over brothers as a mate while differences in body mass, body size and condition did not have a significant influence. Because body size was matched to the nearest 2 mm there were only 11 pairs of males that differed in size.)

criteria of choice	mean time in seconds (s.d.)	n	t	p
kin/non-kin	503.0 (213.6) 1012.3 (416.6)	16	-3.44	0.004
larger/smaller	898.1 (476.9) 598.2 (414.2)	11	1.20	0.256
heavier/lighter	649.6 (367.6) 865.7 (444.4)	16	-1.14	0.273
better/worse condition	968.7 (417.7) 816.6 (419.1)	16	-0.60	0.556

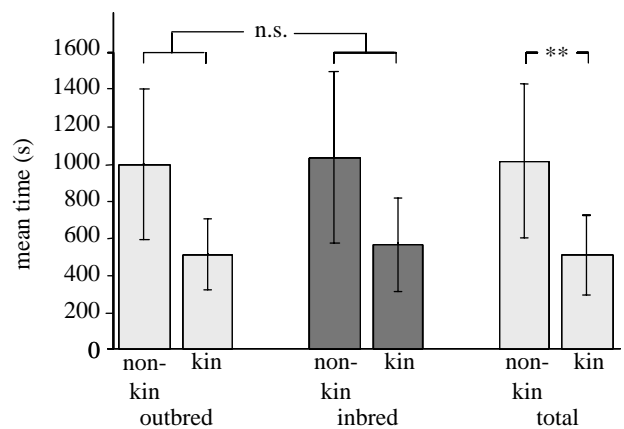


Figure 1. Mean time (\pm s.d.) females spent courting in front of the non-kin (left bars) and brothers (right bars). Given is the time females spent in the choice zone measuring 7×19 cm in front of the males' compartments for outbred ($n = 9$) and inbred fish ($n = 7$) as well as for all females ($n = 16$). Each test lasted 1800 s. n.s., non-significant, $**p < 0.01$.

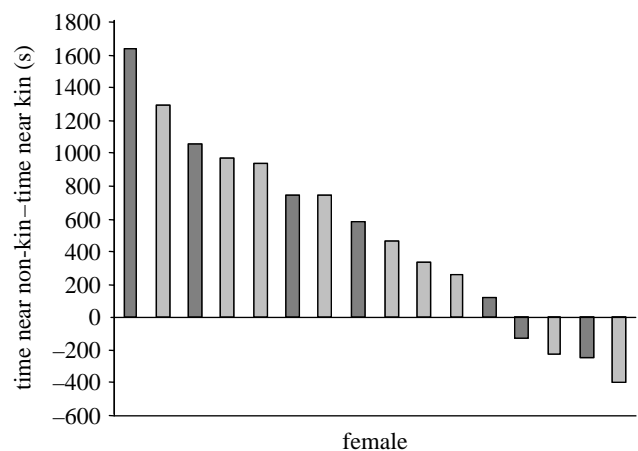


Figure 2. Relative time single females spent in front of the different males. Positive values indicate that the female spent more time in front of the unrelated fish, negative values that it spent more time near her brother. The 16 females are arranged in order of decreasing preference for the unrelated male. Dark bars indicate inbred fish.

In sticklebacks olfactory cues play a role in shoaling decisions (Ward *et al.* 2004, 2005). Whether sticklebacks use olfactory or/and visual cues to discriminate between kin and non-kin in mate choice is unknown. Sticklebacks are able to 'count' and compare MHC alleles of potential mates and they prefer to spawn with individuals with many but not too many different MHC alleles (Reusch *et al.* 2001). Siblings share part of their MHC alleles, so a possible way to avoid inbreeding is to spawn with individuals with dissimilar MHC alleles (Penn & Potts 1999; but see Reusch *et al.* 2001). Thus, kin recognition might rely first of all on olfactory cues (but see Steck *et al.* 1999). If this is true, one might expect that inbred females would have a more pronounced preference for non-kin because inbreeding will reduce individual MHC-heterozygosity. We did not find, however, a significant change in female mating preference with inbreeding.

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