Kin discrimination in sticklebacks is mediated by social learning rather than innate recognition

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Introduction

Kin recognition has been a topic of behavioural studies since over 40 yr (Holmes 2004). The ability to discriminate kin from non-kin is of advantage in several situations during an individual’s lifecycle (see Waldman 1988 for a review). During social interactions altruistic behaviour towards kin may increase an individual’s indirect fitness (Hamilton 1964). During mate choice individuals that recognize kin are able to avoid the negative effects of inbreeding (Charlesworth & Charlesworth 1987; Waldman & McKinnon 1993). Furthermore, in species with paternal care and frequent occurrence of cuckoldry males which are able to assess the degree of relatedness with their brood may adjust the parental effort accordingly (Neff 2003; Frommen et al. in press).

The ability to discriminate kin from non-kin has been developed in a broad array of taxa (Waldman 1987; Ward & Hart 2003). In fish several studies show the impact of kin recognition on shoaling decisions, mate choice and parental care (reviewed in Ward & Hart 2003). Female sticklebacks for example avoid to mate with familiar brothers (Frommen & Bakker 2006) while brood-caring males recognize unrelated eggs in their nests and accordingly adjust their caring behaviour (Frommen et al. in press).

While the existence of kin recognition is well demonstrated, its mechanism remains poorly understood. Following Holmes & Sherman (1982) and Waldman (1987) kin recognition based on spatial cues is called indirect recognition. Here individuals adjust their behaviour towards others according to their physical location. For example, in species in which the dispersal of offspring is limited, individuals are surrounded by relatives. Therefore, social interactions towards neighbours are reliably interactions towards kin. Our experimental animals, sticklebacks, form large shoals during their non-reproductive period, which often migrate to the ocean during autumn (Wootton 1984). Thus, spatial cues would be an unreliable indicator of kinship. Therefore, other explanations...
are needed to explain sticklebacks’ ability to recognize kin.

Cases in which kin recognition is mediated by learned cues (Tang-Martinez 2001) or by recognition alleles are called direct recognition (Holmes & Sherman 1982; Waldman 1987). Recognition of related familiar or unfamiliar individuals by learned cues (‘phenotype matching’) requires at least a short phase of familiarization. The weakness of this mechanism is that in species with close contact between kin and non-kin during growing up, individuals may learn the cues of relatives as well as non-relatives and treat all individuals bearing these cues equally. In shoaling species this scenario is plausible because males often collect clutches of several females (Goldschmidt & Bakker 1990) and foreign males often steal fertilizations by sneaking (Largiadèr et al. 2001). Thus, the degree of relatedness among fry in one nest may vary between totally unrelated and full sibs. Here, learning cues of all fry in the nest lead to a scenario where completely unrelated individuals are treated as relatives (Hain & Neff 2006). Therefore, an alternative mechanism would be more reliable. Innate mechanisms allow an individual to recognize kin without any prior experiences with relatives. Self-referent phenotype matching is such an innate mechanism. Here, an individual learns its own cues and uses them to build up a recognition template (Mateo & Johnston 2000). Later in life, conspecifics that match this template are treated as kin. Alternatively, an individual may bear recognition alleles, described as ‘green-beard alleles’ by Dawkins (1976). These recognition alleles cause the expression of a special phenotypic cue and the ability to recognize others bearing this cue (Holmes & Sherman 1982; Waldman 1987). However, this mechanism, often called ‘true’ kin recognition (Grafen 1990), will lead to cooperation with non-kin bearing the same alleles by chance. It is therefore expected that recognition alleles are unlikely to spread (Dawkins 1976; Blaustein 1983; but see Jansen & van Baalen 2006).

In shoaling species, kin recognition may lead to an increased amount of altruism among shoal members. Additionally, shoaling is of advantage for an individual in multiple ways. Members of a shoal benefit from enhanced anti-predator protection (Magurran 1990) and improved foraging efficiency (Pitcher et al. 1982). On the other hand, individuals joining a shoal experience a higher degree of competition (Krause 1994) and face an increased amount of aggressive interactions (Krause & Ruxton 2002). Shoaling with familiar fish has been shown to result in more stable dominance hierarchies and thus to a reduction of aggressive behaviour between the members of a shoal (Gómez-Laplaza 2005). In addition, shoaling with familiar individuals facilitates the evolution of altruistic behaviour (Utne-Palm & Hart 2000). For shoals composed of related individuals there exist similar benefits. For example, in different salmonid species the level of aggression was lower in kin groups than in non-kin groups (Brown & Brown 1993). In addition to direct benefits, individuals that join kin may increase their inclusive fitness (Hamilton 1964) by shoaling with kin. If a shoaling fish benefits from an increased number of group members, then an individual gains additional indirect benefits of shoaling when choosing a shoal composed of relatives (Ward & Hart 2003).

The influence of kinship on shoaling decisions is well documented (see Ward & Hart 2003 for a review). For example, perch larvae form kin-groups in the wild (Behrmann-Godel et al. 2006). Female rainbowfish prefer to shoal with sisters while they avoid their brothers (Arnold 2000). The latter is interpreted as a form of inbreeding avoidance. Similar effects are shown in zebrafish, where juveniles prefer to shoal with unfamiliar kin, while adult females avoid to group with unfamiliar brothers (Gerlach & Lysiak 2006). Recent works in sticklebacks have shown that adult, non-reproductive individuals prefer to shoal with familiar (Frommen & Bakker 2004) as well as unfamiliar siblings (Frommen et al. 2006). However, the mechanism of kin recognition in sticklebacks remains unknown. The aim of this study was to determine whether the ability to recognize kin is based on social learning or on innate mechanisms like self-referent phenotype matching or recognition alleles. We therefore reared sticklebacks in groups where they were allowed to get familiar either only with kin (expt 1) or with kin and non-kin (expt 2). If kin is recognized by social learning, then fish reared with kin and non-kin are expected to be unable to differentiate between these two groups. In contrast, an innate mechanism would allow sticklebacks to distinguish between familiar kin and non-kin even if they were reared together (Hain & Neff 2006).

Materials and Methods

Experimental Subjects

Sticklebacks used in the experiments were laboratory-bred offspring of anadromous fish that had been caught during their spring migration in April 2004.
from a large, genetically heterogeneous (Heckel et al. 2002) population on the island of Texel, the Netherlands (Kemper 1995). Eggs were spawned in May 2004. Six additional clutches used in expt 1 were spawned in Dec. 2004. Clutches were taken out of the nests immediately after fertilization. For expt 1, clutches were split into two sub-groups. Both sub-groups were placed in a 1 l container that was divided into two equal-sized compartments using a black mesh with a mesh size of about 1 mm. Thus, visual and olfactory contact between sub-groups was possible. One sub-group was placed in each compartment. In this way we gained familiar full-sib groups (see Fig. 1). For expt 2, a kin group was placed in one compartment of a container similar to expt 1, while another group of kin unrelated to the former one was placed in the second compartment (see Fig. 1). By doing this we gained two different kin groups that were familiar to each other. Fry hatched at an age of 9 ± 1 d. After 29d group sizes were reduced to 20 full sibs in each compartment. At an age of 36 ± 1d groups were transferred to larger aquaria measuring 30 × 20 × 20 cm. Here, the tank was divided into two compartments by using perforated (hole diameter 1.7 mm) clear Plexiglas. Fish were kept in an air-conditioned room under standardized summer light regime (day length 16L:8D, temperature 15 ± 1°C). Water in the small aquaria was changed daily, in the large aquaria weekly. Each compartment was aerated by an airstone. The aquaria were separated by opaque grey partitions in order to prevent interactions between fish of neighbouring aquaria. Fry were fed daily ad libitum with living Artemia nauplia. In expts 1 and 2 we tested fish from 35 and 22 full-sib groups, respectively. All fish were used only once, however, some groups provided the kin group in one test and the non-kin group in another. After the experiments fish were kept in the lab for further studies.

**Experimental Design**

Fish in expt 1 were tested at an age of 43 ± 2 d; fish in expt 2 were 14d older. Shoaling preferences were tested in a glass aquarium measuring 30 × 20 × 20 cm. It was divided into two stimulus sections measuring 4.5 cm on each side and a test section measuring 21 cm in the middle using perforated clear Plexiglas. This enabled the testfish to have visual and olfactory contact to each of the two stimulus groups. The aquarium was filled with 1d-old tap water and lit by a 36 Watt fluorescent tube placed 10cm above the aquarium. The water temperature at the time of testing was 15 ± 1°C. Interactions of the fish with the environment of the aquarium were prevented by making the side and back walls of the aquarium opaque using grey plastic plates. Additionally, a black curtain was tightened around the test aquarium.

In the two stimulus sections we placed seven fish, each a random sample of two different full-sib groups. In expt 1, testfish were taken out of one compartment of a rearing aquarium while one of the stimulus shoals consisted of familiar full-sibs taken out of the second compartment. We used familiar full-sibs reared without physical contact to the testfish in order to test whether a possible influence of physical contact had to be considered in
expt 2. Unfamiliar non-sibs were taken from a second aquarium (Fig. 1a). In each trial of expt 2 all fish were taken out of the same aquarium: the testfish and one stimulus shoal consisting of familiar kin out of one compartment and the second consisting of familiar non-kin out of the other (Fig. 1b). In both experiments the fish in the shoals were size-matched by visual judgement. Fish in expt 1 were small and fragile. We therefore did not measure their standard body mass and standard length after the experiments as we did for fish in expt 2. Shoals in expt 2 did not differ significantly in mean body mass, standard body length and condition factor (Bolger & Connolly 1989) (Wilcoxon matched-pairs signed-ranks test, n = 22, all z between −0.357 and −1.156, all p > 0.17). The position of the kin groups alternated between tests to circumvent side effects. The testfish was transferred to a perforated, transparent plastic enclosure (10 × 7 cm, height 16.5 cm) that was placed in the middle of the test compartment. After 2 min the enclosure was lifted by a string from behind the curtain. After the testfish crossed a line drawn on the front and back wall dividing the test compartment into two halves, movements were recorded for 30 min. Recording was performed using a webcam (made by Creative, model CT6840, Creative Labs, Dublin, Ireland) that was placed in front of the aquarium and connected to a laptop computer behind the curtain.

The digital film recordings were analysed afterwards. The time testfish spent in each half of the test compartment was quantified. The half in which the testfish spent more than 50 percent of the test time was defined as preferred. The time spent on each side has been shown to be a good indicator of shoal choice in sticklebacks (Frommen & Bakker 2004) and was highly correlated with the time spending directly in front of each shoal in former experiments (J. G. Frommen unpubl. data) we did not use choice zones in front of each shoal to measure shoaling preferences. The observer was naïve with respect to the side of the related fish.

Statistical Analysis

Time variables were normally distributed according to Kolmogorov–Smirnov tests with Lilliefors-correction. Thus, parametric statistics were used. Some body characteristics did not show a normal distribution. In these cases we used non-parametric statistics. Given test probabilities are two-tailed throughout. Analyses were performed using spss 11.0.1 statistical package (SPSS Inc., Chicago, Illinois, USA).

Results

Experiment 1

Significantly more testfish preferred the side of their familiar siblings (chi-square test, n_sibling = 24, n_non-sibling = 11, $\chi^2_1 = 4.829, p = 0.028$, Fig. 2). However, because three testfish chose the non-sib side for nearly the whole 30 min, testfish did not spend significantly more time near familiar kin (mean time in seconds ± SD: 956.3 ± 380.6) than near unfamiliar non-kin (843.7 ± 380.6) (paired t-test, n = 35, t = 0.875, p = 0.388).

Experiment 2

The number of testfish that preferred the side containing familiar kin (n = 9) did not differ significantly from the number that preferred the side containing familiar non-kin (n = 13) (chi-square test, $\chi^2_1 = 0.727$, p = 0.394, Fig. 2). Furthermore, the time testfish spent near kin (837.1 ± 342.5) and non-kin (962.9 ± 342.5) did not differ significantly (paired t-test, n = 22, t = −0.862, p = 0.399).

Significantly more testfish chose the kin group when fish in the non-kin group were unfamiliar (expt 2) than when the non-kin group consisted of familiar fish (expt 1) (chi-square test, $\chi^2_1 = 4.241, p = 0.039$, Fig. 2).

Body mass, size and body condition of the testfish or its relatives in the group did not significantly correlate with the time that the testfish spent near its kin. This was true when they were expressed as group mean or difference between the testfish and group mean (Pearson’s correlation, n = 22, all r between −0.347 and 0.754, all p > 0.265). Group variability in size or condition did not correlate with time spent near kin ($r_p = −0.321$ and −0.123, $p = 0.145$ and 0.585, respectively) but there was a weak negative correlation between group variability in body mass and time spent near kin ($r_p = −0.447, p = 0.037$). However, this correlation does not remain significant after Bonferroni correction.

Discussion

The results of expt 1 show that juvenile sticklebacks recognize familiar kin and prefer to shoal with their siblings. These results are in concordance with the findings of FitzGerald & Morrissette (1992) and Frommen & Bakker (2004); but see Steck et al. (1999). Additionally, several studies have shown that sticklebacks of different age are also able to
recognize unfamiliar kin (FitzGerald & Morrissette 1992; Frommen et al. 2006). However, the mechanism of kin recognition remained unknown. If sticklebacks recognize relatives by learned cues, one would not expect any preference when kin and non-kin were reared in close contact, while the use of innate mechanisms would lead to a preference for familiar kin over familiar non-kin. Because the results of expt 2 show that the ability to recognize kin is lost when kin and non-kin were reared together, learned cues seem to be a more plausible mechanism than recognition alleles.

Sticklebacks spend the first days of their life in the nest in close contact to each other. Here, they have ample opportunities to get familiar with siblings' cues. However, fry in one nest often differ in the level of relatedness because males collect clutches of different females (Goldschmidt & Bakker 1990) and sneaking occurs frequently (Largiadèr et al. 2001). Thus, learned cues will lead to a mistreatment of familiar non-kin as kin, which may result in a loss of fitness. For example, the reproductive success of an individual may strongly be reduced if it refuses to mate with familiar non-kin. Furthermore, an individual may express altruistic behaviour like food-sharing (Utne-Palm & Hart 2000) towards an unrelated familiar. However, this might be valuable in the context of reciprocal altruism (Trivers 1971).

Furthermore, the advantages of group-living might outweigh the costs of sharing food with non-kin. Additionally, in cases where full sibs carry different recognition cues, learning might be the more reliable kin-recognition mechanism (Penn & Potts 1999).

As a solution to this problem Waldman (1987) suggested that an individual might use different mechanism in different social contexts. For example, Belding’s ground squirrels use different kinds of recognition mechanisms during their life cycle (Mateo 2004). Young recognize kin using direct familiarity as early as 15 d of age while they are not able to recognize unfamiliar kin via phenotype matching until 30 d. Honeybees may use shared genetically or environmentally determined cues when defending the nest, but a self-template when discriminating between full- and half-siblings during brood care or mate choice (Waldman 1987 and citations therein). In bluegill sunfish, a species characterized by a complex mating system including dominant and satellite males, a recent study has shown that descendants of satellite males are able to recognize unfamiliar kin by self-referent phenotype matching. Descendents of dominate males in contrast do not express such an ability (Hain & Neff 2006). Sticklebacks may also use different recognition mechanisms in different contexts. In shoaling decision, where the costs of a mistreatment of
non-kin may be low, they possibly use the quite uncertain social learning, while they may use innate mechanisms or even a combination of both in mate choice (Frommen & Bakker 2006).

An alternative explanation of the results of exp2 2 would be that fish were able to recognize kin but did not adjust their behaviour accordingly (Waldman 1987). However, results of exp1 as well as several other studies using sticklebacks of different ages like fry (FitzGerald & Morrissette 1992), sub-adults (Frommen et al. 2006), adults (Frommen & Bakker 2004) have shown that sticklebacks adjust their shoal choice according to familiarity and kinship. Thus, the sticklebacks in exp2 should behave similarly.

The causal mechanism of kin recognition in sticklebacks is unknown, but a major role of olfactory cues seems plausible (Ward et al. 2004, but see Steck et al. 1999). Ward et al. (2005) showed that sticklebacks recognize individuals reared in the same habitat as well as those fed on the same food on olfactory cues only. Furthermore, sticklebacks are able to distinguish between the smell of different Major Histocompatibility Complex (MHC) alleles and use this information in mate choice (Milinski et al. 2005). Thus, it is conceivable that sticklebacks learn the smell of their nestmates during the first days of their life and use this template during the rest of their life. Additionally, recent studies have shown that female sticklebacks were able to ‘count’ MHC alleles of foreign individuals and compare them with their own MHC diversity (Reusch et al. 2001; Aeschlimann et al. 2003). Thus, at least during mate choice sticklebacks have the ability to perform some kind of self-reference. The question whether this self-reference is also used in kin recognition during sticklebacks’ mate choice (Frommen & Bakker 2006) should be addressed in future studies.

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