Direct Familiarity Does Not Alter Mating Preference for Sisters in Male *Pelvicachromis taeniatus* (Cichlidae)

Timo Thünken, Theo C. M. Bakker, Sebastian A. Baldauf & Harald Kullmann

Institute for Evolutionary Biology and Ecology, University of Bonn, Bonn, Germany

**Introduction**

Close inbreeding has often been shown to be extremely harmful because of the fixation of deleterious recessive alleles in inbred offspring (Charlesworth & Charlesworth 1987). As a consequence, inbred offspring often show reduced fitness compared with outbred offspring (inbreeding depression, Charlesworth & Charlesworth 1987). The ability to identify relatives (kin recognition, Hepper 1991) offers individuals the opportunity to avoid them actively as mating partners (Pusey & Wolf 1996). In the animal kingdom, there are numerous examples that individuals avoid mating with close kin using kin recognition mechanisms (e.g. fishes: Frommen & Bakker 2006; Gerlach & Lysiak 2006; birds: Nakagawa & Waas 2004; humans: Wolf 2004; Lieberman et al. 2007). However, the specific mechanisms of kin recognition are still disputed and probably differ between and also within species, depending on social context (Tang-Martinez 2001; Mateo 2004; Frommen et al. 2007a). Kin recognition can base either on direct familiarity (Porter 1988), also mentioned as prior association (Mateo 2004), or on indirect familiarity (Porter 1988), which is caused by phenotype-matching (Holmes & Sherman 1982; Mateo 2004). In social species individuals who are familiar from birth are usually relatives (sibs or parents). Therefore, recognizing direct familiar individuals is usually a reliable kin recognition mechanism. In species, in which encounters with unfamiliar kin – for example with older or younger sibs of a different cohort – are possible, kin recognition mechanisms evolved which are not based on direct but on indirect familiarity (phenotype matching). During phenotype matching, phenotypic cues of an unknown individual are compared either with the own phenotype (self-reference) or with phenotypic cues of familiar individuals, e.g. of individuals the choosing individual grew up with, which are
normally relatives (Holmes & Sherman 1982; Mateo 2004; Hain & Neff 2006). This mechanism makes it possible to recognize even unfamiliar kin.

In a previous study, we have shown that females and males of the cichlid Pelvicachromis taeniatus discriminated unfamiliar kin from unfamiliar non-kin as mating partners (Thünken et al. 2007a). In that study, offspring had been separated 4–6 wk after hatching from the parents. The full-sib groups had been split into two groups of 20 individuals each and raised isolated from each other for 9–12 mo. It is very unlikely that the adult subjects are able to individually recognize a conspecific with whom they spent only a few weeks as larvae and juveniles in groups comprising from approx. 40 up to more than 100 individuals. Therefore, we concluded that the observed kin discrimination based rather on phenotype matching than on direct familiarity.

Against expectations, in those experiments both sexes revealed a preference for full-sibs over unrelated individuals as mating partners. However, we could not rule out the possibility that direct familiarity between the experimental fish would have led to inbreeding avoidance. In this case, the unfamiliar kin would not have been recognized as close kin in our experiment. Following the optimal outbreeding theory (Bateson 1978, 1982, 1983), the unfamiliar sibs could have appeared as optimal mates, because they were rather similar, but not identical to the individuals the test fish grew up with. This would then have unintentionally led to a preference for related mating partners in the design used.

In nature relatives probably stay together for a longer time period than the few weeks in our laboratory. Therefore, under natural conditions direct familiarity may play a major role in kin recognition of P. taeniatus. The relevance of direct familiarity on mate choice is supported in many species. For example, in females of the naked mole rat (Heterocephalus glaber) direct familiarity to males was more important for inbreeding avoidance than indirect familiarity (Clarke & Faulkes 1999). In guppies (Poecilia reticulata), novel females were more attractive to males than familiar ones (Kelley et al. 1999) and in humans the duration of sibling coresidence was positively correlated to the degree of individual’s disgust imagining sexual contact with the opposite sex sib (Lieberman et al. 2007).

The aim of this study was to investigate whether long-term direct familiarity causes inbreeding avoidance in male P. taeniatus or whether the previously observed inbreeding preferences are consistent. As in this species males provide a similar amount of parental investment as females, they are also expected to be very choosy (Kokko & Johnstone 2002). Therefore, we gave reproductively active males of P. taeniatus the choice between a sister they were associated with since hatching and an unfamiliar, unrelated female.

Materials and Methods

Experimental Animals

Pelvicachromis taeniatus is a small, stream-dwelling cichlid from Nigeria and Cameroon. Pairs are socially monogamous cave-breeders and show a conspicuous size and color dimorphism. The larger males develop a slightly yellow ventral colouration, whereas the female belly is brightly violet. Males occupy caves, while females compete among each other for access to males. During mutual mate choice, both sexes evaluate the potential partner. After spawning, the female cares for the eggs in the cave, while the male defends the territory against intruders. Free swimming offspring are guarded by both parents.

In winter 2003, wild-caught P. taeniatus of the Moliwe population (near Limbe, West Cameroon, 04°04N/09°16E) were bred under standardized laboratory conditions at the Institute for Evolutionary Biology and Ecology, Bonn. After 4 wk of parental care, offspring were removed from the parents and split into two mixed-sex, full-sib groups of 20 fish each. Holding tanks (60 × 40 × 30 cm) were separated by opaque plastic sheets to avoid visual contact of adjacent families. The water temperature was kept at 25 ± 1°C. Larvae were fed with living Artemia nauplii and adult fish with frozen Chironomus larvae and Artemia.

Male mate-choice experiments were conducted in winter 2005 with 20 different full-sib groups of 13 breeding pairs, thus of seven families both offspring groups were used. In the case of these seven families, individuals of two different families were presented as non-sisters, in order to ensure the independence of the statistical units. Test fish as well as stimulus fish were used in only one test. Mating preferences were estimated by measuring the time a reproductively active male spent with a familiar sister or an unfamiliar non-sister. This is a commonly used method to estimate mating preferences (Wagner 1998), which has been shown to reliably predict the mating decision in a mouthbrooding cichlid (Couldridge & Alexander 2001) and in the convict cichlid Archocentrus nigrofasciatus (Santangelo 2005) featuring a similar mating system as P. taeniatus.
In our previous experiments, male and female *P. tae- nius*, given the choice between two potential mating partners, spawned with the individual they spent more time with before (Thünken et al. 2007a, unpublished data).

The test aquarium (\(40 \times 45 \times 30\) cm) was divided into three sections. Two equal-sized female compartments (\(19 \times 25 \times 30\) cm) were arranged side-by-side at one end of the tank, and separated by opaque, grey plastic partitions to prevent female-female interactions. The male compartment (\(20 \times 40 \times 30\) cm) was equipped with a centrally placed breeding cave and separated from the female sections by a transparent, perforated plexiglass partition to allow olfactory and visual communication between the sexes. In front of each female, a choice zone (\(5 \times 19\) cm) was indicated by a black line on the bottom of the aquarium. The aquarium was filled with aged water up to 15 cm. To reduce disturbance, the walls of the test aquarium were lined with opaque, grey plastic plates and the whole set-up was covered by a black curtain. The tank was illuminated by a 37 W fluorescent lamp. During 1 h of acclimatization, an opaque, grey plastic partition visually separated the male from the females. The partition was then removed and the male’s behaviour was videotaped from above. Test males as well as stimulus females were reproductively active, brightly coloured and showed courtship behaviour. A naïve observer analysed the choice behaviour on the video-recordings. For 15 min, he measured the time the male spent in the choice zones in front of the females after the male had visited both choice zones.

To control for variation in attractiveness, stimulus females were visually matched for size and body colouration. After the tests, the body size of the experimental fish was measured. Male standard length ranged from 5.1 to 6.8 cm (\(n = 20\), on average \(\pm SD\) 5.7 \(\pm 0.43\) cm) and female standard length ranged from 3.8 to 4.8 cm (\(n = 20\), on average \(\pm SD\) 4.1 \(\pm 0.24\) cm). In order to control for potential differences in attractiveness among females invisible to the human observer, we used a paired experimental design. One test consisted of two trials with the same pair of stimulus females. Between the trials, only the male was exchanged, hence, the male of the first trial was a familiar brother of one of the two females and the male of the second trial was a familiar brother of the other female. In order to remove olfactory traces of the former male, the water of the male compartment was exchanged between the trials. During the water exchange the females remained in their compartments.

Before starting the experiment, the two test males and the two stimulus females were isolated in separate tanks (\(33 \times 19 \times 17\) cm; \(25^\circ\)C) for a period of 36 h. Isolation over this short period of time does not lead to the loss of individual familiarity (e.g. Grosenick et al. 2007). In order to acclimatize the males to the experimental conditions, the tanks of the males were equipped with a breeding cave each. All males occupied their cave.

In the statistical analysis, we averaged the relative association times the two males of one paired test trial spent with their respective sister to control for potential differences in attractiveness of the two stimulus females. We conducted 10 paired replicates. In the statistical test (paired t-test), we compared the averaged relative time which the males spent with the familiar sisters with the relative time which the males spent with the unfamiliar unrelated females.

Because data were normally distributed according to the Kolmogorov–Smirnov test with Lilliefors correction, we used parametric tests. All calculations were performed with the spss 12.0 statistical software package. p-values are two-tailed throughout.

**Results**

Males spent significantly more time with their familiar sisters than with their unfamiliar non-sisters (paired t-test, \(t_9 = 2.684, p = 0.025\); Fig. 1). This preference for familiar sisters did not significantly differ from that for unfamiliar sisters (on average \(\pm SD\), 64 \(\pm 16.5\)% vs. 61 \(\pm 17.8\)%; t-test, \(t_{16} = \cdots\)).

![Fig. 1: Mean proportion of time (±SD) males spent in the choice zones in front of unfamiliar non-sisters and familiar sisters. The relative times the two males of one paired test (n = 10) spent with the respective familiar sister and with the unfamiliar unrelated female were averaged.](image-url)
0.309, \( p = 0.76 \) reported in Thünken et al. (2007a). In order to test whether female size influenced male mate choice, we averaged the relative time the males spent in the choice zones over both trials. Males did not spend more time with the larger female, the heavier female, or the female in better body condition (paired t-tests, all \( p > 0.1 \)), suggesting that slight differences between the stimulus fishes are negligible.

**Discussion**

In many social species, familiar individuals are avoided as mating partners and hence the costs of inbreeding are prevented (Pusey & Wolf 1996). The aim of the present study was to test whether direct familiarity also leads to the avoidance of close kin as mating partners in the social cichlid *P. taeniatus*. The results clearly showed that reproductively active males did not avoid familiar sisters but even preferred them over unfamiliar non-sisters. As we used a paired experimental design and females were matched concerning common mate choice criteria, the results can be ascribed to the different degrees of relatedness. This result contradicts previous findings in many species, that familiar close kin are avoided over unfamiliar unrelated individuals as mates (fish: Frommen & Bakker 2006; Gerlach & Lysiak 2006; mammals: Bolhuis et al. 1988; Clarke & Faulkes 1999; Krackow & Matuschak 1991; birds: Bateson 1982; Burley et al. 1990).

In a non-sexual context, preferences for familiar kin are widely distributed in animals (e.g. fish: Ward & Hart 2003; Frommen & Bakker 2004), as grouping with familiar or related individuals has several advantages (Krause & Ruxton 2002). Normally, sexual maturity inverts this preference because of the potential costs of inbreeding (Frommen & Bakker 2006; Gerlach & Lysiak 2006). Fish used in our experiment were sexually mature, showed courtship colouration and courted. Thus, we conclude that male *P. taeniatus* actually prefer familiar sisters as mating partners. This result confirms the inbreeding preferences found in previous experiments with this species in which unfamiliar kin were preferred as mating partners (Thünken et al. 2007a).

Because in the previous experiments also unfamiliar sibs were discriminated, kin recognition is probably based on phenotype matching, although it is not clear whether this mechanism relies on self-reference. If phenotype matching relies on learned cues of previous association partners, i.e. shoal mates, this recognition mechanism is only reliable if these are relatives. Genetic data of the cichlid *Sarotherodon melanootheron* (Pouyaud et al. 1999) and the Eurasian perch *Perca fluviatilis* (Gerlach et al. 2001) actually suggest that shoals are often comprised of relatives. However, in case of brood adoption, as described in other cichlid species (Wisenden & Keenleyside 1992), or mixed paternity because of sneaking (Taborsky 1994), self-referent phenotype-matching would be more reliable (Hain & Neff 2006). It is not known whether brood adoption exists in *P. taeniatus*. The extraordinarily long sperm of *P. taeniatus* (Thünken et al. 2007b) could be interpreted as adaptation to sperm competition caused by sneaking. However, the small testes size (T. Thünken, T. C. Bakker & H. Kullmann; unpubl. data) suggests a monogamous mating system.

Experimental evidence for mating preferences for close kin is scarce. Incestuous mating preferences were reported in zebra finches *Taeniopygia guttata* (Slater & Clements 1981), although this study was criticized for methodological reasons (Burley et al. 1990). Recently, Schjørring & Jäger (2007) showed preferences for sibs in mate choice experiments with the cestode *Schistocephalus solidus*. Some genetic studies in birds also suggest mating preferences for kin. In the North American barn swallow *Hirundo rustica erythrogaster* extra-pair mates were more closely related than expected by chance (Kleven et al. 2005). In the great frigate bird *Fregata minor* breeding pairs showed a considerable degree of genetic similarity (Cohen & Dearborn 2004) and in the song sparrow *Melospiza melodia* relatively inbred birds paired with related individuals (Reid et al. 2006). In the latter case, however, it is possible that the observed inbreeding is not because of mating preference, but to a loss of the ability to recognize kin in inbred individuals, as it has been shown for example in sticklebacks (Frommen et al. 2007b).

Despite numerous arguments for inbreeding avoidance behaviour, theory suggests that excessive outbreeding can also be disadvantageous, mainly because of genetic costs, i.e. the disruption of beneficial gene-complexes or the loss of local adaptations (optimal outbreeding, Bateson 1983; Templeton 1986). Therefore, intermediately related individuals should be preferred (Bateson 1982). Fitness advantages for offspring of intermediate related parents have been shown in the bluegill sunfish *Lepomis macrochirus* (Neff 2004). Recent research on major histocompatibility complex (MHC)-dependent mate choice shows that at least sticklebacks (*Gasterosteus aculeatus*) do not always prefer dissimilar mates but rather a partner which ensures an optimal number of MHC alleles in the offspring (see Milinski 2006 for a...
review). In humans, some studies reveal preferences for MHC-similar individuals (Jacob et al. 2002; Roberts et al. 2005; but see Wedekind et al. 1995), but the interpretation of the adaptive value of these preferences remains ambiguous.

In addition to the potential genetic costs of outbreeding, kin selection theory also predicts advantages from mating with kin (Kokko & Ots 2006; Parker 2006). Furthermore, paternal care or group living might buffer inbreeding depression (Aviles & Bukowski 2006; but see Griffiths & Armstrong 2001). In P. taeniatus, full-sib pairs were more cooperative during parental care than unrelated pairs (T. Thünken et al. 2007a). Furthermore, inbred offspring of these pairs did not show any inbreeding depression.

The present study confirms kin discrimination based on phenotype matching and shows that familiarity does not affect the male preference for related females in P. taeniatus.

Acknowledgements

We thank J. Frommen, R. Modarressie, I. Rick, T. Schmoll and C. Seibt for discussion and two anonymous referees for useful comments on the manuscript. This study was funded by the Deutsche Forschungsgemeinschaft (DFG) (BA 2885/2-1).

Literature Cited


Kokko, H. & Johnstone, R. A. 2002: Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and
Santangelo, N. 2005: Courtship in the monogamous convict cichlid: what are individuals saying to rejected and selected mates? Anim. Behav. 69, 143—149.