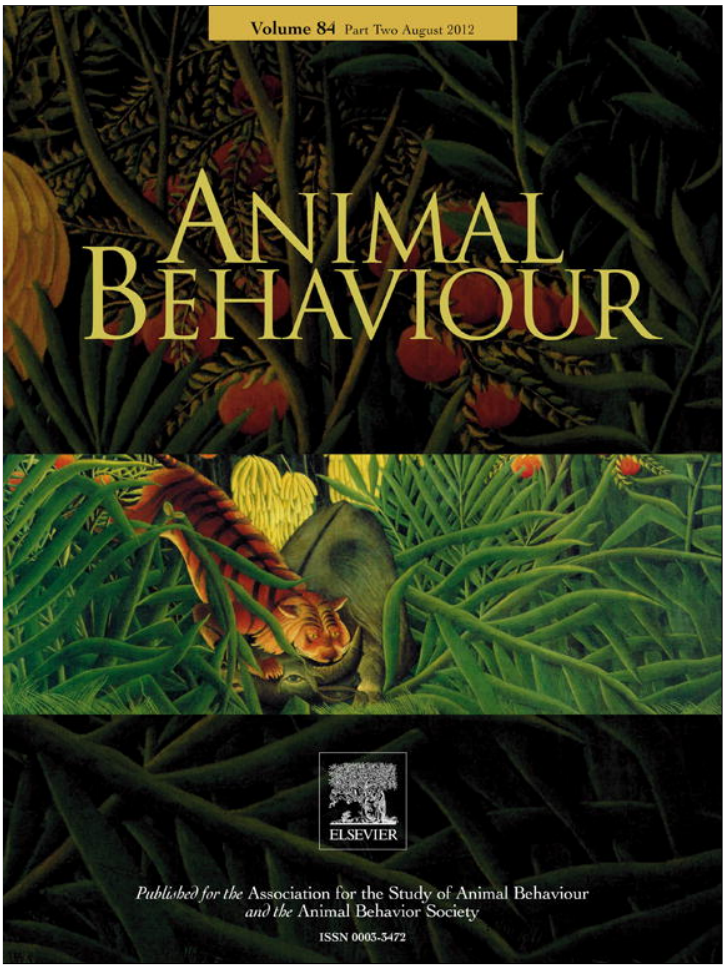

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Kin recognition by phenotype matching is family- rather than self-referential in juvenile cichlid fish

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The ability to differentiate between kin and nonkin is of importance in nepotistic as well as in mate choice contexts. Phenotype matching is a significant kin recognition mechanism, which is widespread in animals. However, the underlying proximate mechanisms are still poorly understood. Phenotype matching can be based on either self-reference or familial imprinting. We investigated phenotype matching in juvenile *Pelvicachromis taeniatus* based on chemical cues. *Pelvicachromis taeniatus* is a socially monogamous cichlid fish with biparental brood care. Previous studies indicate that the adults use phenotype matching to recognize kin. Juvenile fish were reared under three different conditions to manipulate recognition templates: (1) reared with kin, (2) reared in isolation or (3) reared with foster siblings. *Pelvicachromis pulcher* families served as foster families. In the experiments, test fish had to choose between olfactory cues obtained from two stimulus shoals differing in relatedness to the test fish. Test fish reared with kin discriminated unfamiliar kin from unfamiliar nonkin indicating that juvenile *P. taeniatus* also use phenotype matching to recognize kin. Focal fish reared in isolation or with foster siblings did not significantly discriminate unfamiliar kin from unfamiliar nonkin suggesting that juveniles did not imprint on their own phenotypic traits. However, individuals reared with foster siblings preferred unfamiliar olfactory stimuli of the foster species over those of their own indicating they used rearing partners as reference. Thus, phenotype matching is probably based on familial imprinting rather than self-reference in juvenile *P. taeniatus*.

Kin recognition, that is, the cognitive ability to distinguish between kin and nonkin, as well as kin discrimination, that is, the differential treatment of kin and nonkin, have been studied in various taxa (mammals: Mateo 2003; birds: McGregor 1989; reviewed in Nakagawa & Waas 2004; anuran amphibians: Blaustein & Waldman 1992). Kin discrimination is essential for kin selection theory (Hamilton 1964), which predicts indirect fitness benefits for helping or cooperating with close kin. Phenotype matching is an important kin recognition mechanism (Holmes & Sherman 1982; Waldman 1987; Mateo 2004; also referred to as 'indirect familiarity', Porter 1988) because it enables an individual to recognize unfamiliar kin. In phenotype matching an individual learns phenotypic cues from itself and/or relatives it was reared with and forms a kin template. Later on, phenotypic cues of conspecifics are compared with this kin template and classified either as kin or nonkin. If an individual includes only cues from itself in that recognition template, the mechanism is referred to as self-referent phenotype matching (Holmes & Sherman 1982).

A kin template may include visual, olfactory and acoustical learned traits of relatives or the individual itself (Brown & Brown 1996; Nakagawa & Waas 2004; Sharp et al. 2005; Mehlis et al. 2008; Kaminski et al. 2009). Self-reference is advantageous in species in which multiple mating occurs and siblings differ in relatedness in one litter/clutch. Imprinting on nestmates, for example, would result in integrating phenotypic traits of half-siblings into kin recognition templates and lead to an inaccurate template. Kin recognition by phenotype matching has been demonstrated in various taxa, for example mammals (Holmes 1986a, b; Sun & Müller-Schwarze 1997; Wahaj et al. 2004), amphibians (Blaustein & Waldman 1992) and fishes (Gerlach & Lysiak 2006). However, the reference on which the kin template is based often remains unclear. Self-referent kin recognition has been suggested for instance in rodents (Mateo & Johnston 2000), birds (Schielzeth et al. 2008) and fishes (Hain & Neff 2006) but definitive evidence is still scare because any contact with kin has to be excluded during development (Hauber & Sherman 2001; Hare et al. 2003). Demonstration of self-reference is suggested if an individual reared in isolation or cross-fostered is able to discriminate unfamiliar kin (Mateo 2004, 2010; Mateo & Holmes 2004). In contrast to self-reference, imprinting on nestmates, also called

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familial imprinting, is indicated when unfamiliar foster siblings are recognized (mammals: Mateo 2003; birds: Nakagawa & Waas 2004; fishes: Olsen & Winberg 1996).

In this study, we aimed to identify the mechanism underlying kin recognition by phenotype matching in juveniles of the cichlid fish *Pelvicachromis taeniatus*, which is a small, socially monogamous cave breeder from West Africa. After hatching, the fry are herded by both parents for several weeks (Thünken et al. 2010). Juvenile *P. taeniatus* live in shoals after their parents have left them, which is common in juvenile cichlids (Lamboj 2006). In previous experiments, adult *P. taeniatus* discriminated unfamiliar kin from unfamiliar nonkin in a mate choice context when both visual and chemical cues were available, suggesting phenotype matching as the kin recognition mechanism (Thünken et al. 2007b). The results of subsequent experiments suggest that, as in other animal species, olfactory cues play an important role in this process (Thünken et al. 2009, 2011). To investigate how the kin template for phenotype matching is formed in juvenile *P. taeniatus*, that is, whether it relies on self-reference or whether the juveniles imprint on their rearing partner, we raised juveniles with kin, in isolation and with foster siblings. In a series of experiments juvenile *P. taeniatus* were then given the choice between differently scented water in a fluviarium (Y-maze).

METHODS

Experimental Fish

The experimental fish were bred between February and April 2010 in the laboratory at the Institute for Evolutionary Biology and Ecology in Bonn, Germany under standardized conditions. To create different families, each of 16 size-assorted breeding pairs of *P. taeniatus* was introduced into a breeding tank (45 × 40 cm and 30 cm high), which was equipped with a standard breeding cave, an aquarium heater and a filter (model: Hobby gully filter). The bottom was covered with 500 ml of autoclaved sand and java moss, *Taxiphyllum barbieri* (2.5 g) to provide shelter. The water temperature was kept at 25 ± 1 °C and the experimental subjects were held under a light:dark regime of 12:12 h. They were fed daily with a mixture of defrosted *Chironomus* larvae and *Artemia*. Until the breeding pairs spawned, approximately 30% of the tank water was changed weekly to enhance the spawning probability of fish; after they had spawned the same amount of water was changed once a month. Additionally, breeding pairs of *Pelvicachromis pulcher* were established under the same breeding conditions for the cross-fostering experimental design (see below). Breeding caves were checked for eggs daily. The eggs were then transferred to the different rearing conditions. The day the eggs hatched and the first day of free swimming was noted for every family. Free-swimming fry were fed once a day with living *Artemia* nauplii in the morning hours before the experiments started. Experimental fish were not tested before their 14th day of free swimming.

Rearing Conditions

(1) Reared with kin. We placed 10–20 eggs of one family together in a plastic tank (16 × 9 cm and 10 cm) filled with 850 ml tap water and equipped with an airstone for oxygen supply. Tanks were surrounded by grey plastic sheets on each side to prevent visual contact with neighbouring individuals. Approximately two-thirds of the water in each tank was changed daily and refilled with aged tap water. Water temperature was 22 ± 1 °C. The tanks were checked daily for unfertilized eggs and dead individuals, which were removed. Two sibling groups of each family were established. If a female's clutch size was sufficiently large (at least 60 eggs), a third sibling group was left with their parents. Since sibling groups were reared separately from each other, this design allowed us to test kin recognition independent from direct familiarity (prior association).

(2) Reared in isolation. One egg was placed alone in a plastic tank (16 × 9 cm and 10 cm high) and raised under the same conditions as described above. No visual or olfactory contact with any other individual except itself was possible. Since only the individual's own cues were available for imprinting, this experimental design allowed to test whether kin recognition is self-referent in this species.

(3) Reared with foster siblings. Since interspecific as well as intraspecific brood adoption is common in cichlids (e.g. Greenberg 1963; Wisenden & Keenleyside 1994; Fraser 1996; Ochi & Yanagisawa 2005), cross-fostering provides an elegant opportunity to determine on which reference phenotype matching is based. On the one hand, it excludes or minimizes experience with kin but maintains a normal social environment for the growing individual. On the other hand, it allows an individual to imprint on nonkin. We used *P. pulcher* as the foster species because they show a similar shoaling behaviour but have a slightly different body coloration and morphology compared to juvenile *P. taeniatus* enabling us to identify *P. taeniatus* in a *P. pulcher* group. Cross-fostering was conducted by either rearing one *P. taeniatus* from the egg stage in a group of 10 *P. pulcher* (age difference ± 2 days) kept in plastic tanks or by introducing wrigglers (larval state after hatching and before free swimming) of *P. taeniatus* into the brood of a *P. pulcher* breeding pair. To set the wrigglers directly into the breeding cave of the foster family, the breeding pair was carefully netted and kept in one corner of the tank. The *P. taeniatus* wrigglers were then sucked individually into a plastic tube (diameter = 4.5 mm) and subsequently released into the breeding cave. Breeding pairs were set free after all foreign fry were transferred. Cross-fostering was chosen to demonstrate self-reference alongside rearing fish in isolation to control for possible social deficits. Furthermore, this design allowed us to test which references were used to set up kin recognition templates in this species since familial imprinting can be demonstrated by species preferences of cross-fostered individuals.

Four different experiments were conducted. Fish reared with kin were given the choice between the odour from unfamiliar kin and unfamiliar nonkin to test whether they use phenotype matching to discriminate kin (experiment 1). Fish reared in isolation or with foster siblings, respectively, were also given the choice between unfamiliar kin and unfamiliar nonkin to determine whether phenotype matching is self-referent (experiments 2 and 3). In a fourth experiment fish reared with foster siblings were given the choice between odour of unfamiliar heterospecifics (i.e. the foster species *P. pulcher*) and unfamiliar unrelated conspecifics to examine whether the focal individual imprinted on the odour of the foster species. The sides on which the odour was introduced were switched during each experiment. Therefore, each experiment consisted of two trials (1 and 2). Kin recognition experiments 1, 2 and 3 were of a paired design to control for potential differences in general attractiveness in stimulus odours. One paired experiment consisted of two single experiments with the same pair of stimulus shoals but a different test fish between successive experiments. Hence, the focal fish in the first experiment and the focal fish in the second experiment were related to different stimulus shoals. The experiment to determine species preferences in cross-fostered fish had an unpaired design. All experiments were conducted between March and May 2010.

Experimental Set-up

Experiments were conducted in a dichotomous choice Y-maze (Fig. 1). The Y-maze was made of white PVC tubes with an internal

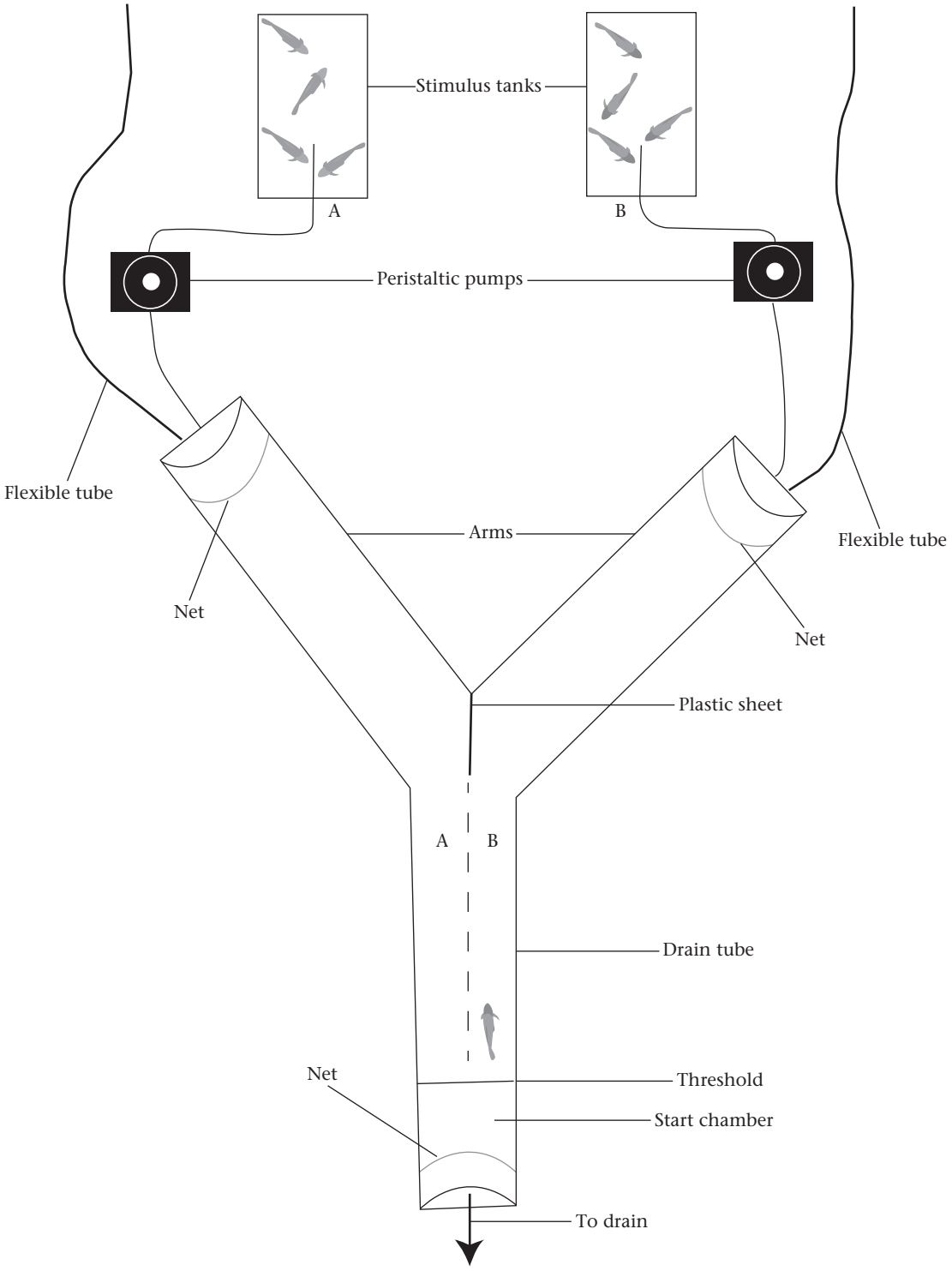

Figure 1. The Y-maze viewed from above. In the middle, a plastic sheet was installed to ensure laminar flow in the drain tube. The open ends of the tubes were sealed with grey plastic sheets. The plastic sheet attached to the drain tube had a cutting (0.5×2 cm) to drain the water. Nets were fixed 3.5 cm before each end of the Y-maze to prevent the experimental subjects from being flushed out at the draining end or from reaching the direct source of the olfactory cues, the tubes of the peristaltic pumps. The start chamber was defined as a neutral zone. The drain tube was divided into the choice zones A and B since water flow was laminar. Arms connected to the stimulus tanks A or B, respectively, were also declared as choice zones. Therefore, the choice area A included the arm connected to stimulus A and section A in the drain tube; choice area B included the arm connected to stimulus B and section B in the drain tube.

diameter of 9.5 cm and a total length of 72 cm. The tubes were cut in half to create a trough. The drain tube contained a start chamber (11×9.5 cm), in which the test fish could habituate. A perforated gate could be removed using a pulley. This start chamber was declared a neutral zone. Water level in the Y-maze was 3 cm and the total water volume was approximately 3.2 litres. The whole maze was surrounded by white Styrofoam to minimize disturbance and secure isolation of test fish from any outer stimuli. Two tanks containing the stimulus fish were located outside the Styrofoam surroundings. Each was connected to a peristaltic pump (Ismatec,

tube diameter 2.54 mm), which added water scented by a stimulus shoal at a flow rate of 45 ml/min to one arm of the maze. Flow rate was checked regularly and adjusted if necessary. Additionally, two separate flexible tubes (diameter 4.5 mm) siphoned water at a rate of 70 ± 2.83 ml/min from a reservoir tank (60×45 cm and 29.5 cm high) to either arm of the maze to ensure two parallel-flowing water masses (A and B, see Fig. 1). Laminar flow was checked with dye. The reservoir tank was equipped with an aquarium heater and filtered with charcoal. Temperature was 25 ± 1 °C. The reservoir tank provided tempered and filtered water that was used to fill the Y-maze before the experiments. It was refilled with tap water after each experiment. A video camera (CCD Ever Focus, model EQ150 Video Camera, 1/3′ BW High Resolution Camera with Ever Focus CCTV Lenses) was attached to a wooden frame 90 cm above the Y-maze to record fish behaviour. The Y-maze was evenly illuminated by a fluorescent tube (Osram lumilux L 58 W) installed approximately 2 m above the experimental tank.

To create the odour used in the experiments, we placed stimulus fish in a tank (24.5×15 cm and 15 cm high) equipped with an airstone for oxygen supply. Pretests showed that a concentration of one fish per litre of water is sufficient to elicit a response: juveniles significantly preferred water enriched with odour of familiar kin over blank water (S. Hesse, T. C. M. Bakker, S. A. Baldauf & T. Thünken, unpublished data). In the kin recognition experiments (experiments 1–3) stimulus water had to suffice for two experiments (owing to the paired design). Therefore, a minimum of 4 litres of stimulus water was produced. In the familial imprinting experiment (experiment 4) we produced only 3 litres of stimulus water since this experiment was unpaired. Stimulus tanks were surrounded by white Styrofoam to avoid visual contact between the stimulus fish groups and were placed in front of the peristaltic pump before the experiment started. During the experiments, stimulus fish remained in the tanks in a net breeder placed in the centre of the tank ensuring that no stimulus fish was sucked into the tubing of the peristaltic pump.

Experimental Procedure

After the Y-maze was filled with tap water from the reservoir tank, the peristaltic pumps and the additional water flow from the reservoir tank were started. After a continuous flow had been established, the experimental fish was placed in the start chamber using a flexible tube into which it was carefully sucked. As the test fish were very small (total length < 2 cm), this was the gentlest way to handle them. After 1 min, the tubing of the peristaltic pumps was transferred to the stimulus tanks, one pump supplying one arm of the maze with stimulus water. After 5 min the video recording was started and the gate was lifted using a pulley. The experiment started as soon as the test fish left the start chamber. It was assumed to have left the start chamber if more than half of its body was over the threshold. If a test fish did not leave the start chamber within 30 min, the experiment was stopped and the fish was tested at the earliest 2 days later. After 10 min the recording was stopped and the water supply of the peristaltic pumps was changed to blank water again. This trial is referred to as trial 1. The test fish remained in the Y-maze. After another 5 min the supply was changed to stimulus water again but the sides were switched, with the stimulus that was on the left-hand side in trial 1 now on the right-hand side and vice versa. The video recording was started and the experiment started 3 min later when the test fish was outside the start chamber. If it was within the start chamber, experiments started when the test fish left the start chamber. Pretests with dye showed that 3 min were sufficient for the odour to reach the drain at the drain tube. After 10 min the experiment was stopped. This trial is referred to as trial 2. The experimental fish was removed from the Y-maze using a flexible tube. Its total length was measured using a digital calliper. Total lengths of the stimulus fish were also measured. Since the kin discrimination experiments followed a paired design, we used the same stimulus fish in both experiments. If the test fish in the second experiment refused to run the maze, the stimulus fish were kept apart from their tank mates until the next experiment. Between two experiments the Y-maze was washed with ethanol (70%) to eliminate all odours from the previous trial. It was then rinsed with clear water and refilled with tap water from the reservoir tank. Hydrogen peroxide (4%) was run through the tubing of the peristaltic pump for 2 min followed by clear water for 4 min to eliminate all remaining odours and wash out the chemical. Hydrogen peroxide as well as ethanol is commonly used for this purpose (e.g. Olsen & Winberg 1996; Mehlis et al. 2008).

Data Analysis

Choice behaviour of the experimental fish was recorded using Windows media encoder 9 series. The videos were encoded to ensure that the films could be analysed blindly with respect to the identity of the test fish. The time the test fish spent in the two association zones (Fig. 1) was measured over a period of 4 min (according to Wisenden & Dye 2009). The test fish entered one association zone if more than half of its body was in it. If the experimental fish swam directly on the line between the association zones it was recorded as making no choice. Test fish that spent the whole time in one association zone only were excluded from the analysis since we could not be sure that the test fish had really experienced both stimuli.

Statistical calculations were performed with the R. 2.9.1 statistical software-package (R, The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). Parametric tests were used because data were normally distributed. *P* values are two tailed throughout. For all four experiments, the two trials were analysed separately. Because kin recognition experiments 1–3 were of a paired design (see above) a kin preference index was calculated in the following way: the relative time the focal fish in the first experiment spent with one of the two stimuli minus the relative time the focal fish in the second experiment spent with the same stimulus. A linear model (LM) was fitted to analyse kin preferences in experiments 1 and 2. The kin preference index was the dependent variable. Some families were used multiple times but family combinations in paired experiments were unique. We considered a trial as valid only if the test fish had visited both association zones to make sure that both stimuli were experienced. Experiments in which the test fish stayed in only one preference zone were excluded from analysis and when the design was paired both experiments were invalid. Owing to this constraint, in trial 2 of experiment 1 the sample size ($N = 3$) was too small for statistical analysis. In the third experiment preferences for kin were analysed using a linear mixed-effect model (LME). The kin preference index was the dependent variable. Family combination was entered as a random factor. Tests in the species recognition experiment were unpaired and analysed using a linear mixed-effect model (LME). The relative time the focal fish spent with the stimulus odour was the dependent variable and stimulus (conspecific/heterospecific odour) the explanatory variable. Family and individual were entered as random factors.

Ethical Note

The experiments comply with the current laws of Germany. No animal was harmed or died during the experiments. Experimental fish were bred between February and April in the laboratory at the

Institute of Evolutionary Biology and Ecology in Bonn. The parental fish were either F1 or F2 offspring from wild-caught *P. taeniatus* from the Moliwe River, Cameroon. No licences were required for the collection or import of fish or for the study. Juveniles of *P. pulcher* were also bred in the laboratory. The Institute of Evolutionary Biology and Ecology maintained some wild-caught *P. pulcher* from a population in Nigeria, which were obtained from a fish importer (Mimbon, Cologne). Other parental fish were obtained from various fish stores in Germany in 2010. After the experiments, the fish of both species were kept in the laboratory and took part in several other behavioural experiments.

RESULTS

Experiment 1: Focal Fish Reared with Kin

Experimental subjects discriminated significantly between unfamiliar kin and unfamiliar nonkin. They spent significantly more time on the side on which odour from unfamiliar nonkin was added than on the side on which odour from unfamiliar kin was added in trial 1 (LM: $t_5 = -3.899$, $P = 0.011$; Fig. 2).

Experiment 2: Focal Fish Reared in Isolation

Experimental subjects did not discriminate significantly between unfamiliar kin and unfamiliar nonkin. They spent a mean ± SD 47.5 ± 22.9% of time with odour from unfamiliar kin and 52.5 ± 22.9% with odour from unfamiliar nonkin in trial 1 (LM: $t_7 = -0.311$, $P = 0.765$) and 50.0 ± 13.1% of the time with odour from unfamiliar kin and 50.0 ± 13.1% with odour from unfamiliar nonkin in trial 2 (LM: $t_5 = 0.435$, $P = 0.682$).

Experiment 3: Cross-fostered Focal Fish (Kin Recognition)

As in experiment 2, focal fish did not discriminate significantly between unfamiliar kin and unfamiliar nonkin. Experimental fish did not spend more time on the side on which odour from unfamiliar kin was added (trial 1: 47.8 ± 9.3%; trial 2: 49.2 ± 20.2%) than on the side on which odour from unfamiliar nonkin was added (trial 1: 52.2 ± 9.3%; trial 2: 50.8 ± 20.2%) in both trials (trial 1: LME: $t_4 = 0.003$, $P = 0.998$; trial 2: LME: $t_4 = -0.094$, $P = 0.93$).

Experiment 4: Cross-fostered Focal Fish (Species Recognition)

Experimental subjects discriminated significantly between the cross-foster species and conspecifics. Although they showed no significant discrimination in trial 1 (conspecifics: 54.9 ± 22.3%; heterospecifics: 45.1 ± 22.3%; LME: $\chi^2_1 = 1.696$, $P = 0.193$), in trial 2 test fish had a strong preference for the odour of the cross-fostered species (LME: $\chi^2_1 = 7.737$, $P = 0.005$; Fig. 3).

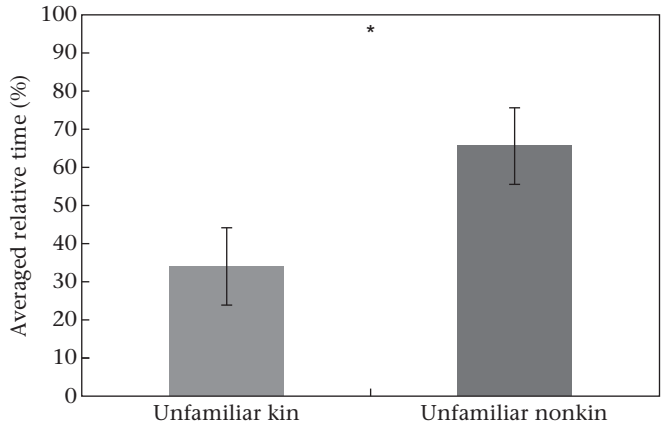

Figure 2. Mean relative time ± SD that test fish ($N = 12$) reared with kin spent with odour from unfamiliar kin and unfamiliar nonkin in trial 1. *$P < 0.025$.

DISCUSSION

Our experiments show that juvenile *P. taeniatus* reared with kin discriminated between unfamiliar kin and unfamiliar nonkin. Discrimination of unfamiliar kin suggests phenotype matching as the kin recognition mechanism. Phenotype matching has been shown in different fish taxa, for example in zebrafish, *Danio rerio* (Gerlach & Lysiak 2006), guppies, *Poecilia reticulata* (Hain & Neff 2007), three-spined sticklebacks, *Gasterosteus aculeatus* (Frommen et al. 2007a) and cichlids (Le Vin et al. 2010). It was also indicated in *P. taeniatus* since both males and females prefer familiar and unfamiliar kin as mating partners (Thünken et al. 2007a, b). In this species inbreeding seems to be adaptive as breeding pairs consisting of siblings show increased investment in offspring and less aggressive interactions compared to nonkin breeding pairs (Thünken et al. 2007b; see also Langen et al. 2011). Furthermore, male *P. taeniatus* discriminated between odour of an unfamiliar unrelated male, odour of a familiar brother and odour from itself indicating that some sort of self-recognition must exist in this species (Thünken et al. 2009).

Juvenile fish in this study reared in isolation or with foster siblings did not discriminate significantly between unfamiliar kin and unfamiliar nonkin. However, juvenile *P. taeniatus* reared with foster siblings discriminated conspecifics from heterospecifics and showed a preference for the latter. This preference for the cross-foster species in connection with the lack of discrimination between unfamiliar kin and unfamiliar nonkin suggests that juveniles imprint on their nestmates rather than on traits from themselves. Still, as the sample size in the experiments was relatively low, we cannot completely rule out that juveniles might additionally use self-reference. In the animal kingdom in general, definitive evidence for self-referent kin recognition is still scarce. Self-referent phenotype matching has been demonstrated in the bluegill sunfish, *Lepomis macrochirus*, a species in which two mating strategies for males exist (Hain & Neff 2006). Only offspring from 'cuckolder males', which sneak fertilizations, but not offspring from 'parental males', which build a nest and care for the fry, used self-reference. Since most bluegill sunfish fry (approximately 80% according to Hain & Neff 2006) were sired by the parental male,

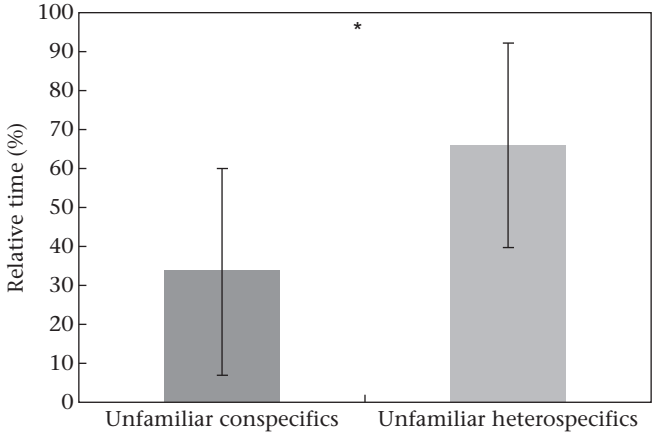

Figure 3. Mean relative time ± SD that the test fish ($N = 11$) reared with the foster species spent with odour obtained from unfamiliar conspecifics and unfamiliar heterospecifics in trial 2. *$P < 0.025$.

imprinting on nestmates would result in an inadequate kin template for 'cuckolder' fry. Thus, learning one's own phenotypic traits is the only reliable possibility to obtain a correct kin template for offspring of 'cuckolder' males.

Pelvicachromis taeniatus is likely to be genetically monogamous during a single breeding event (K. Langen, T. Thünken & T. C. M. Bakker, unpublished data). Thus imprinting on nestmates and/or parents provides a reliable kin recognition template. Furthermore, a kin recognition template based on cues obtained from closely related individuals (full-siblings) provides a broad framework of phenotypic cues whereas imprinting on one's own cues results in a very strict and restricted kin template. A very strict kin recognition template may not allow for recognition of distantly related kin, such as cousins or half-siblings (Penn & Potts 1998).

In our study, recognition templates were manipulated in a way that is not possible under natural conditions. Eggs and fry are guarded by the female until they are free swimming while the male defends the territory against intruders (Thünken et al. 2007b, 2010). Therefore, imprinting on nestmates may provide a correct kin recognition template in this species. Gerlach et al. (2008) showed that there is a temporal window for imprinting in zebrafish larvae, which coincides with when larvae disperse from their spawning ground and therefore an increased possibility of meeting nonkin. Fry of *P. taeniatus* stay in the breeding cave for about 7 days until they are free swimming (Thünken et al. 2007b). Brain structures associated with olfaction develop early in cichlids (Villani 1999). Under natural conditions intermingling with nonkin or heterospecifics is unlikely to occur before the fish are free swimming and therefore imprinting on nestmates before the stage of free swimming provides an adequate kin recognition template in juvenile *P. taeniatus*.

Unexpectedly, kin discrimination of juveniles resulted in avoidance rather than preference for kin as in adults. Why did juveniles avoid instead of prefer their unfamiliar kin when kin selection theory predicts several advantages from associating with kin? Since studies concerned with kin avoidance in fishes are scarce (apart from inbreeding avoidance, but see Frommen et al. 2007b) possible answers to this question are somewhat hypothetical. Despite the advantages assumed by kin selection theory, field studies do not often find evidence for kin aggregations (Brodeur et al. 2008), which may indicate that there are some circumstances influencing benefits and costs of kin discrimination that must also be considered. Competition between relatives, for example, can negate the advantages of kin selection (West et al. 2002). Griffiths et al. (2003) demonstrated avoidance of kin because of competition in a field study on juvenile coho salmon, *Salmo salar*. Crevices used for shelter were a scare resource and individuals competed among each other for access. If such a shelter was occupied by more than one individual, the risk of being discovered by predators increased as juveniles relied on crypsis to reduce predation risk. Therefore, young juvenile salmon avoided sharing shelters with kin to avoid inflicting these costs on relatives. Thus, juvenile *P. taeniatus* may also avoid unfamiliar kin to reduce the cost of competition. In this case, competition for food rather than shelter may be the reason for avoidance of unfamiliar relatives. Usually, cichlids do not provide food for their fry. Therefore, juveniles search for food on their own and competition among shoal members may be high. Another reason for kin avoidance may be heterogeneous advantage, a theory that opposes kin selection theory (Griffiths & Armstrong 2001). Heterogeneous advantage theory predicts that competition among individuals is high when genetic diversity is low. For example, different genotypes may exploit a homogeneous resource in different ways and therefore use resources more effectively. Griffiths & Armstrong (2001) showed that the advantages of genetic diversity outweigh the benefits of kin association in juvenile salmon. In this study, kin and nonkin groups of juveniles were introduced into a river and their increase in body mass and condition index was measured. Both were significantly higher in nonkin groups. Therefore, this study was the first to provide evidence for heterogeneous advantage in fish. Juvenile *P. taeniatus* are not territorial as young salmon are but live in shoals. Here, different genotypes may exploit the food resources provided by the environment in different ways and maximize their direct fitness benefit. Possible causes for the avoidance of unfamiliar kin are therefore avoiding competition with relatives and advantages provided by genetic diversity. These causes are not mutually exclusive but might act in concert. However, further experiments are required to elucidate the adaptive advantages of juvenile kin avoidance.

In summary, we could show in our study that juvenile *P. taeniatus* use phenotype matching based on olfactory cues to discriminate kin from nonkin. Furthermore, our results suggest that kin recognition templates are formed by familial imprinting rather than self-reference in juveniles of this species.

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References

Blaustein, A. R. & Waldman, B. 1992. Kin recognition in anuran amphibians. *Animal Behaviour*, **44**, 207–221.

Brodeur, N. N., Noel, M. V., Venter, O., Bernatchez, L., Dayanandan, S. & Grant, J. W. A. 2008. No evidence of kin bias in dispersion of young-of-the-year Atlantic salmon *Salmo salar* L. in a natural stream. *Journal of Fish Biology*, **73**, 2361–2370.

Brown, G. E. & Brown, J. A. 1996. Kin discrimination in salmonids. *Reviews in Fish Biology and Fisheries*, **6**, 201–219.

Fraser, S. A. 1996. The influence of predators on adoption behaviour in adult convict cichlids (*Cichlasoma nigrofasciatum*). *Canadian Journal of Zoology*, **74**, 1165–1173.

Frommen, J. G., Mehlis, M., Brendler, C. & Bakker, T. C. M. 2007a. Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*): familiarity, kinship and inbreeding. *Behavioral Ecology and Sociobiology*, **61**, 533–539.

Frommen, J. G., Luz, C. & Bakker, T. C. M. 2007b. Nutritional state influences shoaling preference for familiars. *Zoology*, **110**, 369–376.

Gerlach, G. & Lysiak, N. 2006. Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour*, **71**, 1371–1377.

Gerlach, G., Hodgins-Davis, A., Avolio, C. & Schunter, C. 2008. Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. *Proceedings of the Royal Society B*, **275**, 2165–2170.

Greenberg, B. 1963. Parental behaviour and recognition of young in *Cichlasoma biocellatum*. *Animal Behaviour*, **11**, 578–582.

Griffiths, S. W. & Armstrong, J. D. 2001. The benefits of genetic diversity outweigh those of kin association in a territorial animal. *Proceedings of the Royal Society B*, **268**, 1293–1296.

Griffiths, S. W., Armstrong, J. D. & Metcalfe, N. B. 2003. The cost of aggregation: juvenile salmon avoid sharing winter refuges with siblings. *Behavioral Ecology*, **14**, 602–606.

Hain, T. J. A. & Neff, B. D. 2006. Promiscuity drives self-referent kin recognition. *Current Biology*, **16**, 1807–1811.

Hain, T. J. A. & Neff, B. D. 2007. Multiple paternity and kin recognition mechanisms in a guppy population. *Molecular Ecology*, **16**, 3938–3946.

Hamilton, W. D. 1964. Genetical evolution of social I & II. *Journal of Theoretical Biology*, **7**, 1–52.

Hare, J. F., Sealy, S. G., Underwood, T. J. J., Ellison, K. S. & Stewart, R. L. M. 2003. Evidence of self-referent phenotype matching revisited: airing out the armpit effect. *Animal Cognition*, **6**, 65–68.

Hauber, M. E. & Sherman, P. W. 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends in Neurosciences*, **24**, 609–616.

Holmes, W. G. 1986a. Kin recognition by phenotype matching in female Belding ground-squirrels. *Animal Behaviour*, **34**, 38–47.

Holmes, W. G. 1986b. Identification of paternal half-siblings by captive Belding ground-squirrels. *Animal Behaviour*, **34**, 321–327.

Holmes, W. G. & Sherman, P. W. 1982. The ontogeny of kin recognition in 2 species of ground-squirrels. *American Zoologist*, **22**, 491–517.

Kaminski, G., Dridi, S., Graff, C. & Gentaz, E. 2009. Human ability to detect kinship in strangers' faces: effects of the degree of relatedness. *Proceedings of the Royal Society B*, **276**, 3193–3200.

Lamboj, A. 2006. *Die Cichliden des westlichen Afrikas*. Bornheim: Schmettkamp.

Langen, K., Schwarzer, J., Kullmann, H., Bakker, T. C. M. & Thünken, T. 2011. Microsatellite support for active inbreeding in a cichlid fish. *PLoS One*, **6**, e24689.

Le Vin, A. L., Mable, B. K. & Arnold, K. E. 2010. Kin recognition via phenotype matching in a cooperatively breeding cichlid, *Neolamprologus pulcher*. *Animal Behaviour*, **79**, 1109–1114.

McGregor, P. K. 1989. Bird song and kin recognition: potential, constraints and evidence. *Ethology Ecology & Evolution*, **1**, 123–127.

Mateo, J. M. 2003. Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy*, **84**, 1163–1181.

Mateo, J. M. 2004. Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici*, **41**, 729–745.

Mateo, J. M. 2010. Self-referent phenotype matching and long-term maintenance of kin recognition. *Animal Behaviour*, **80**, 929–935.

Mateo, J. M. & Holmes, W. G. 2004. Cross-fostering as a means to study kin recognition. *Animal Behaviour*, **68**, 1451–1459.

Mateo, J. M. & Johnston, R. E. 2000. Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society B*, **267**, 695–700.

Mehlis, M., Bakker, T. C. M. & Frommen, J. G. 2008. Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. *Animal Cognition*, **11**, 643–650.

Nakagawa, S. & Waas, J. R. 2004. 'O sibling, where art thou?' A review of avian sibling recognition with respect to the mammalian literature. *Biological Reviews*, **79**, 101–119.

Ochi, H. & Yanagisawa, Y. 2005. Farming-out of offspring is a predominantly male tactics in a biparental mouthbrooding cichlid *Perissodus microlepis*. *Environmental Biology of Fishes*, **73**, 335–340.

Olsen, K. H. & Winberg, S. 1996. Learning and sibling odor preference in juvenile arctic char, *Salvelinus alpinus* (L.). *Journal of Chemical Ecology*, **22**, 773–786.

Penn, D. & Potts, W. 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society B*, **265**, 1299–1306.

Porter, R. H. 1988. The ontogeny of sibling recognition in rodents: superfamily Muroidea. *Behaviour Genetics*, **18**, 483–494.

Schielzeth, H., Burger, C., Bolund, E. & Forstmeier, W. 2008. Assortative versus disassortative mating preferences of female zebra finches based on self-referent phenotype matching. *Animal Behaviour*, **76**, 1927–1934.

Sharp, S. P., McGowan, A., Wood, M. J. & Hatchwell, B. J. 2005. Learned kin recognition cues in a social bird. *Nature*, **434**, 1127–1130.

Sun, L. X. & Müller-Schwarze, D. 1997. Sibling recognition in the beaver: a field test for phenotype matching. *Animal Behaviour*, **54**, 493–502.

Thünken, T., Bakker, T. C. M., Baldauf, S. A. & Kullmann, H. 2007a. Direct familiarity does not alter mating preference for sisters in male *Pelvicachromis taeniatus* (Cichlidae). *Ethology*, **113**, 1107–1112.

Thünken, T., Bakker, T. C. M., Baldauf, S. A. & Kullmann, H. 2007b. Active inbreeding in a cichlid fish and its adaptive significance. *Current Biology*, **17**, 225–229.

Thünken, T., Waltschyk, N., Bakker, T. C. M. & Kullmann, H. 2009. Olfactory self-recognition in a cichlid fish. *Animal Cognition*, **12**, 717–724.

Thünken, T., Meuthen, D., Bakker, T. C. M. & Kullmann, H. 2010. Parental investment in relation to offspring quality in the biparental cichlid fish, *Pelvicachromis taeniatus*. *Animal Behaviour*, **80**, 69–74.

Thünken, T., Baldauf, S. A., Kullmann, H., Schuld, J., Hesse, S. & Bakker, T. C. M. 2011. Size-related inbreeding preference and competitiveness in male *Pelvicachromis taeniatus* (Cichlidae). *Behavioral Ecology*, **22**, 358–362.

Villani, L. 1999. Development of NADPH-diaphorase activity in the central nervous system of the cichlid fish, *Tilapia mariae*. *Brain Behavior and Evolution*, **54**, 147–158.

Wahaj, S. A., Van Horn, R. C., Van Horn, T. L., Dreyer, R., Hilgris, R., Schwarz, J. & Holekamp, K. E. 2004. Kin discrimination in the spotted hyena (*Crocuta crocuta*): nepotism among siblings. *Behavioral Ecology and Sociobiology*, **56**, 237–247.

Waldman, B. 1987. Mechanisms of kin recognition. *Journal of Theoretical Biology*, **128**, 159–185.

West, S. A., Pen, I. & Griffin, A. S. 2002. Conflict and cooperation–cooperation and competition between relatives. *Science*, **296**, 72–75.

Wisenden, B. D. & Dye, T. P. 2009. Young convict cichlids use visual information to update olfactory homing cues. *Behavioral Ecology and Sociobiology*, **63**, 443–449.

Wisenden, B. D. & Keenleyside, M. H. A. 1994. The dilution effect and differential predation following brood adoption in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Ethology*, **96**, 203–212.