

Original Article

Size-related inbreeding preference and competitiveness in male *Pelvicachromis taeniatus* (Cichlidae)

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Sexual selection is an important force in the evolution of body size. Both intersexual selection, that is, preference for large individuals, and intrasexual selection, that is, increased competitiveness of large individuals, are involved in this process. Furthermore, preferences based on body size of the choosing individual might also influence body size evolution. Here, we investigated male mate choice and competitiveness in relation to male body size in *Pelvicachromis taeniatus*, a size-dimorphic cichlid. In previous experiments, both sexes showed mating preferences for larger and genetically related individuals. First, we examined male inbreeding preferences based on olfactory cues. Males that highly varied in body size were given the choice between the odor of a familiar sister and the odor of an unfamiliar unrelated female that were presented in combination with a computer-animated image of a female *P. taeniatus* as a visual stimulus. Male preference for the odor of their sisters was correlated with male body size. Only larger males were choosy concerning related odors and preferred their sisters, whereas smaller males were unselective. Second, we showed that large males outcompete smaller males in contest over a breeding site. The extent of aggression was negatively correlated with the size difference between the 2 males. Variation in male choice may reflect an adaptive strategy: small, less competitive and less attractive males might avoid the risk of failing to mate at all by reducing choosiness. Consequently, only large competitive males should obtain the benefits of choice, which may further contribute to the selective advantages of large body size. **Key words:** computer animation, inbreeding, kin recognition, mate choice, olfaction, sexual selection. [*Behav Ecol* 22:358–362 (2011)]

Sexual selection plays an important role in the evolution of male body size in many species (reviewed in Andersson 1994). The advantages of large body size result on the one hand from larger males being usually more successful in competition for females and on the other hand from being preferred by females as mating partners. In species with male mate choice, size-related male mating preferences might also contribute to the evolution of male body size. Size-related female choice has been reported, for example, in stalk-eyed flies (Hingle et al. 2001; Cotton, Rogers, et al. 2006). However, variation in male mate choice in general has received, relative to female choice, less attention (Jennions and Petrie 1997; Cotton, Rogers, et al. 2006; Cotton, Small, et al. 2006).

In this paper, we focus on size-related male mate choice and male–male competition in the fish *Pelvicachromis taeniatus*. It is a socially monogamous cichlid with biparental brood care from Western Africa, which features a conspicuous size dimorphism with males being larger than females. Furthermore, both sexes are brightly colored (Baldauf et al. 2010). During the breeding season, males occupy suitable breeding sites, usually caves, and defend them against rivals, whereas females

compete among each other for access to males with good breeding opportunities. Broods are intensively cared for by both parents (Thünken et al. 2010). As expected, because of considerable biparental investment, this species shows mutual mate choice (Thünken et al. 2007a; Baldauf, Kullmann, Schroth, et al. 2009). As in other species, larger males have been shown to be preferred by females over smaller males as mating partners (Baldauf, Kullmann, Schroth, et al. 2009), suggesting that female mate choice contributes to the evolution of male body size in *P. taeniatus*. However, in contrast to other species, both sexes of *P. taeniatus* showed mating preference for kin (Thünken et al. 2007a, 2007b). Breeding pairs consisting of related individuals were more cooperative during brood care than pairs consisting of unrelated individuals (Thünken et al. 2007a), which might be a decisive advantage of inbreeding in a species with biparental brood care requiring highly synchronous behavior between parents. As the costs of inbreeding seem to be insignificant (Thünken et al. 2007a, 2009), the benefits of inbreeding may exceed its costs.

In our first experiment, we investigated male inbreeding preferences in relation to male body size. Although olfactory cues generally play an important role in kin recognition in many species (Ward and Hart 2003; Mateo 2009), only a few studies examined olfactory kin discrimination in the context of mate choice in fishes (Gerlach and Lysiak 2006; Mehliis et al. 2008). Therefore, in the experiments, males of *P. taeniatus* varying greatly in body size were given the choice between the odor of a familiar sister and the odor of an unfamiliar unrelated female. The female odors were presented in

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combination with 2D computer animations of a female *P. taeniatus* as a visual stimulus. The use of computer animations, providing the opportunity to present highly standardized stimuli (see Baldauf et al. 2008), is well established in our model species (Baldauf, Kullmann, Schroth, et al. 2009; Baldauf, Kullmann, Winter, et al. 2009; Baldauf et al. 2010). Previous studies found a positive correlation between body size and preference in 2-spotted gobies and stalk-eyed flies (Amundsen and Forsgren 2003; Cotton, Rogers, et al. 2006). Thus, one might predict particularly pronounced mating preference in large males of *P. taeniatus*.

The aim of our second experiment was to examine the role of body size in male–male competition in *P. taeniatus*. In cichlids, evidence for the impact of intrasexual selection on the evolution of male body size is ambiguous. Some studies found significant correlations between male body size and competitiveness (e.g., Maan et al. 2001; Maan and Taborsky 2008), whereas others did not (Neat et al. 1998; Schütz and Taborsky 2005). We performed staged contests in which 2 reproductively active males differing in body size could compete over a breeding cave. The sequential assessment game theory (Enquist et al. 1990) predicts that fights should be more severe when asymmetry between opponents increases.

MATERIALS AND METHODS

Experimental fish

The experiments were conducted in summer 2008 with reproductively active *P. taeniatus*, that is, individuals that were brightly colored and showed courtship behavior. The fish used were outbred F2 offspring of a parental generation, which was collected from the Moliwe River, Cameroon, in the year 2003.

Male mate choice experiment

Before the choice experiments, the male test fish were isolated in tanks (30.5 × 20 × 10 cm, water level 10 cm). These isolation tanks were equipped with sand, an air stone for air supply, and a flowerpot, which was fixed at the bottom. The flowerpot should serve as hiding place and potential breeding cave. It was occupied by all males. Visual isolation was ensured by opaque Styrofoam sheets between the isolation tanks. To habituate the test fish to the background of the computer animation used in the following trials (see below), the broad sides of the isolation tanks showed a picture of a water plant that was used as background for the animation. The trials started after at least 3 days of habituation. The day before

the trial, approximately 50% of the water in the tank was changed. The stimulus females were isolated in tanks (25 × 15.5 × 15.5 cm, water level 10 cm), which were equipped with a hiding place and an air stone for air supply. Water from these tanks served as odor stimuli. Opaque plastic sheets prevented interaction of the stimulus fish. The water temperature was kept 25 ± 1 °C, and the day/night period was 12L/12D. Test fish as well as stimulus fish were fed once a day with frozen *Chironomus* larvae.

The experiments were conducted in the males' isolation tanks, thus reducing stress by leaving the fish in their familiar habitat. The tank containing the test fish was placed between 2 cathode ray tube monitors of the same model (EIZO Flex Scan F520, 85 Hz, connected to a Matrox G550 PCIe graphic board; Figure 1). The setup was illuminated by a fluorescent tube (37 W) installed 1 m above the middle of the tank. Additionally, white Styrofoam surrounded the setup. In the experiment, the test males were given the choice between 2 identical computer-animated females, which were presented in combination with different odors. On one side, water conditioned by a familiar sister was released into the tank by a peristaltic pump (flow rate: 16 ml/min). On the other side, water conditioned by an unfamiliar unrelated female was added in the same way. Pretests had revealed that animated females presented in combination with a female odor were more attractive to males than animations presented without odor stimulus, that is, tap water (Thünken T, Baldauf SA, Hesse S, Kullmann H, and Bakker TCM, unpublished data). The computer animation showed a brightly colored female that moved a horizontal pathway from one side of the monitor to the other for a period of 15 s, including a 2-s stop in the middle. After that, it recurred horizontally and moved back in the same time frame. For details and preparation of the computer animation, see Baldauf, Kullmann, Schroth, et al. (2009); Baldauf, Kullmann, Winter, et al. (2009).

After the test tank was placed between the monitors, the male was given 25 min to habituate to the experimental conditions. Afterward, the conditioned waters were introduced. Two hundred milliliters of conditioned water was taken from the isolation tank of the respective females 5 min before. The side on which the related odors were presented was determined randomly. Two minutes before the computer animations of the females were simultaneously started on both broad sides of the isolation tank, the olfactory stimulus was added. That allowed the male to perceive the 2 odors before the start of the experiment. After the experiment, the tubes of the peristaltic pumps and the tanks containing the stimulus water were cleaned using a 3% solution of hydrogen peroxide and afterward rinsed with distilled water. After the experiment,

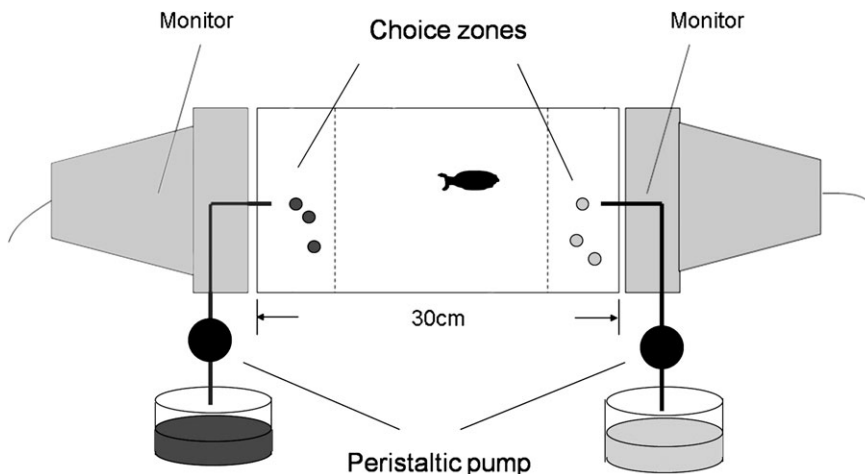


Figure 1

The experimental setup. Individual male *Pelvicachromis taeniatus* was placed between 2 display units in their habitat aquarium. Both displays showed the same computer animation of a female, whereas peristaltic pumps introduced the odor of a sister and that of an unrelated female. An association zone of 5 cm was marked in front of the monitor to measure male preferences for the olfactory cues.

the standard length of the test fish was measured. Fish behavior was recorded using a webcam. A naive observer analyzed the video recordings. Mating preferences were measured as association time near a stimulus of the opposite sex, which reliably predicts mating decisions in *P. taeniatus* (Thünken et al. 2007a). The time spent in each choice zone (see Figure 1) was calculated over a period of 2 min only after the fish had first visited an association zone. A time frame of 2 min is a standard duration in experiments using computer animations in fishes (see Baldauf, Kullmann, Schroth, et al. 2009; Baldauf, Kullmann, Winter, et al. 2009; and references therein).

Male–male competition experiment

Before the experiment, male test fish were individually isolated in small tanks ($19 \times 34 \times 19$ cm), which were equipped with a standard breeding cave Java moss, and an air stone. The water temperature was kept at approximately 23 °C. Opaque plastic sheets prevented visual contact between the males. The light/dark period was 12 h, and the test fish were fed with frozen *Chironomus* larvae. Once a day, a nuptially colored female was presented as a stimulus to each male for 5 min in a net breeder ($16.5 \times 12 \times 13$ cm) that allowed visual and olfactory contact.

The contest tank ($30 \times 50 \times 30$ cm) was equipped with a single standard breeding cave, a thin layer of sand as substrate, and 2 tufts of Java moss as a hiding place for the males. The temperature was kept at 23 ± 1 °C. Three sides of the tank were covered with opaque plastic sheets; to observe the contest behavior of the 2 males, the front glass pane was uncovered. After every trial, the Java moss, the breeding cave, and the contest tank were cleaned and the tank refilled with tap water. We composed male pairs of different size differences. In order to minimize the disturbance of males, size differences between males were estimated before the experiment and measured afterward. All contest pairs were of a similar age. To acclimatize to the new environment, each of the 2 test males was put in a net breeder ($16.5 \times 12 \times 13$ cm), and they were introduced simultaneously in the contest tank for 1 h. After 50 min of acclimation, a nuptially colored female was presented in a small tank ($15 \times 8 \times 9.5$ cm) in front of the contest tank in order to increase males' motivation. After acclimation, the males were carefully set free. From this moment on, male aggressive behavior, that is, attacks against the rival was recorded for 1 h. Natural variation of spots in the caudal fin of each male allowed the observers to discriminate between the 2 fighting males. After the observation time, we removed the tank with the stimulus female. The contest pair remained in the tank until the next morning when it was recorded, which male had successfully occupied the breeding cave. Afterward, the standard length of both test males was measured.

Statistical analysis

Parametric tests were only used when data were normally distributed according to the Kolmogorov–Smirnov test with Lilliefors correction; otherwise, nonparametric tests were applied. All calculations were performed with the R 2.9.1 statistical software-package. *P* values are two tailed throughout. To analyze mating preferences, we conducted a linear mixed effect model (“lme”). As response variable, we used the kin preference index. The index was calculated by subtracting the time the test male spent at the side with the unrelated odor from the time spent at the side with the related odor. To investigate the relationship between male's body size and kin preference, we entered male standard length as covariate into the model. For an additional analysis, we subdivided the test males into 2

size classes, that is, large ($N = 15$) and small males ($N = 15$), and entered size class as factor into the model. All test males were only tested once. However, to control for multiple testing of families, we entered family origin of the males as random factor into the model (males originated from 16 different families). Likelihood ratio tests (“LRT”) with maximum likelihood assessed whether the removal of a variable caused a significant decrease in the model fit. Reported *P* values of models refer to the increase in deviance when the respective variable was removed. Hence, degrees of freedom differ by 1. In the case of intercept models, the degrees of freedom result from the number of families involved.

RESULTS

Male mate choice experiment

Overall, males did not significantly discriminate between the odor of a familiar sister and that of an unfamiliar unrelated female (intercept, $t_{16} = 0.240$, $P = 0.813$; Figure 2). However, preference of males for familiar sisters was positively correlated with male body size (“LRT”, $N = 30$, $\chi^2 = 4.404$, $P = 0.036$; Figure 2). When subdividing the test males into 2 size classes (small males: $N = 15$, mean standard length 4.55 ± 0.15 standard deviation [SD] and large males: $N = 15$, mean standard length 5.55 ± 0.55 SD), on average large males significantly preferred the odor of their sisters (intercept, $t_{12} = 2.274$, $P = 0.042$; Figure 2), whereas small males were unselective (intercept, $t_{10} = -1.536$, $P = 0.156$; Figure 2). The choice of large males significantly differed from that of small males (“LRT”, $\chi^2 = 4.408$, $P = 0.036$).

Male–male competition experiment

Standard length of the test males ($N = 48$) was on average 5.2 ± 0.76 cm SD. Size differences within contest pairs ($N = 24$) were on average 0.7 cm, ranging from 0 to 1.7 cm. The total number of attacks was significantly negatively correlated with the size difference between the 2 males (Spearman rank correlation: $N = 24$, $r = -0.508$, $P = 0.011$). In 19 of 24 trials, the breeding cave was occupied by 1 of the 2 rivals the next day. In 17 cases, the breeding cave was occupied by the larger of the 2 males

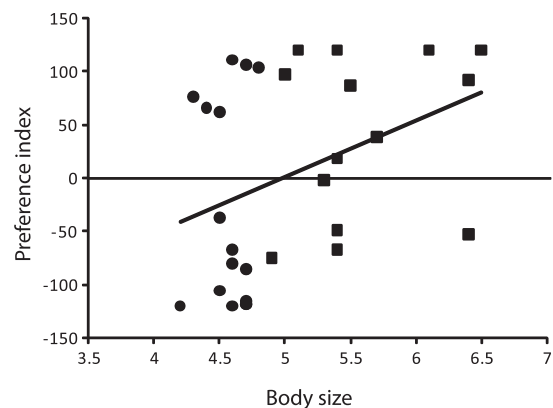


Figure 2 Relationship between male body size, that is, standard length (centimeter) and preference for the odor of females differing in relatedness to the male, expressed as preference index (time spent near the odor of a sister minus time spent near the odor of a nonsister). Positive values reflect male preference for a related female, whereas negative values show preference for unrelated stimuli. The preferences of large males ($N = 15$) are indicated by squares and those of the small males ($N = 15$) by circles.

(binominal test: $17/2$, $P < 0.001$). In 12 trials, aggressive interactions between males occurred during the observation period; the larger male conducted on average more attacks (median 8.5 attacks, inter quartile range, 3–15) than the smaller male (median 1.5 attacks, inter quartile range, 1.5–4.25, paired Wilcoxon signed-rank test, $N = 12$, $V = 64$, $P = 0.051$).

DISCUSSION

In this paper, we provide empirical evidence for size-related male mating preference and male competitiveness in the cichlid *P. taeniatus*. Only large males showed a significant preference for the odor of a sister. Status-dependent mate choice has been shown in several species (e.g., Bakker et al. 1999; Cornwallis and Birkhead 2006; Engqvist 2009). For example, in painted reed frogs, *Hyperolius marmoratus*, Jennions et al. (1995) found that female sensitivity to distinguish between different male advertisement calls was size dependent. They suggested that lack of choosiness in small individuals could reflect a lesser ability to perceive the differences between potential mating partners. In *P. taeniatus*, size-related differences in perceptual abilities concerning olfactory-based kin recognition are unlikely because *P. taeniatus* of different developmental stages are capable of olfactory kin recognition (Thünken et al. 2009; Hesse S, Baldauf SA, Bakker TCM, and Thünken T, unpublished data). It is more likely that differences between males reflect adaptive variation in preferences. The results of the male–male competition experiment indicate that small males are inferior in intrasexual competition. Furthermore, small males are less attractive for females (Baldauf, Kullmann, Schroth, et al. 2009). Therefore, it could be adaptive for small males to court females irrespective of their quality and to use any mating opportunity instead of remaining unmated. As a consequence, the greater choosiness of large males might further reinforce the selective advantages of large males. In mutual mate choice systems, it can be expected that this trade-off may also apply to females, thus potentially leading to some kind of assortment concerning quality traits (Baldauf, Kullmann, Schroth, et al. 2009).

The mating preferences of the large males confirm the inbreeding preferences reported in previous experiments (Thünken et al. 2007a, 2007b). Large males in the present study had a similar size as the test males from the studies of Thünken et al. (2007a, 2007b), in which size of the males ranged from 5.1 to 7 cm, on average 5.7 cm. At a proximate level, the results further suggest that olfactory cues play an important role in kin recognition and thus support a previous experiment in which males were able to distinguish between the odor of a familiar brother and that of an unfamiliar unrelated male in a competitive situation (Thünken et al. 2009).

Larger males outcompeted smaller ones in contests over a limited resource, in this case, a breeding cave supporting the findings in other cichlids that body size is associated with dominance (e.g., Maan and Taborsky 2008; Stiver et al. 2009). Furthermore, they conducted more attacks than smaller males against the contestant, suggesting that size is correlated with aggressiveness or with the extent to inhibit aggression in the opponent. Furthermore, we found a negative correlation between the total number of attacks and size difference within a contest pair. This result is in accordance with the sequential assessment game theory predicting that the more similar opponents are the more difficult is the assessment of differences in fighting ability resulting in extended fights (Enquist et al. 1990). Similar results were found, for example, in the cichlid *Nannacara anomala* (Enquist et al. 1990).

In conclusion, the results of the present study suggest that beside female mate choice (Baldauf, Kullmann, Schroth, et al. 2009) and intrasexual competition, size-related preferences might play a role in the evolution of male body size in

P. taeniatus. Only large males were selective which might reinforce the selective advantages of large males. Status-dependent preferences might also explain how genetic variation is maintained in a species with inbreeding preferences.

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