

Active Inbreeding in a Cichlid Fish and Its Adaptive Significance

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Summary

Levels of inbreeding are highly variable in natural populations [1, 2]. Inbreeding can be due to random factors (like population size), limited dispersal, or active mate choice for relatives [3]. Because of inbreeding depression [4], mating with kin is often avoided [5], although sometimes intermediately related individuals are preferred (optimal outbreeding [6, 7]). However, theory predicts that the advantages of mating with close kin can override the effects of inbreeding depression [8–12], but in the animal kingdom, empirical evidence for this is scarce. Here we show that both sexes of *Pelvicachromis taeniatus*, an African cichlid with biparental brood care, prefer mating with unfamiliar close kin over nonkin, suggesting inclusive fitness advantages for inbreeding individuals. Biparental care requires synchronous behavior among parents. Since parental care is costly [13], there is a conflict between parents over care [12, 14], which can reduce offspring fitness [15]. Relatedness is expected to enhance cooperation among individuals [16]. The comparison of the parental behavior of in- and outbreeding pairs showed that related parents were more cooperative and invested more than unrelated parents. Since we found no evidence for inbreeding depression, our results suggest that in *P. taeniatus*, inbreeding is an advantageous strategy.

Results and Discussion

There are two, not necessarily mutually exclusive, theoretical approaches to predict the mating decision of an individual with respect to the relatedness of a potential partner. The first one considers the impact of parental genomic divergence, i.e., the genetic similarity of parents, on the fitness of offspring. Close inbreeding within species can result in inbreeding depression caused by an increased homozygosity of recessive, deleterious alleles and loss of heterosis [4]. However, extreme intra-specific outbreeding can also be disadvantageous (outbreeding depression) [17], because beneficial gene complexes or local genetic adaptations are disrupted [18]. There is numerous empirical support that animals avoid close kin as mating partner [5, 19, 20]. Optimal outbreeding theory [7] is supported by some experimental

behavioral studies showing mating preferences for intermediately related individuals [6] and by two genetic studies reporting stabilizing selection on genomic divergence in wild populations of animals [21] and plants [22]. Recent research on optimal diversity in individual major histocompatibility complex (MHC) of sticklebacks further confirms optimal outbreeding theory [23–25].

The second approach is based on Hamilton's inclusive fitness theory, stating that the reproductive success of relatives has to be considered when estimating individual fitness [16]. In this context, theoretical models stress the advantages of close inbreeding since the late 1970s [8–12]. Inbreeding tolerance depends on the one hand mainly on the strength of inbreeding depression and on the other hand on the mating system as well as on mate availability [11].

Here we investigate the mate-choice decision of female and male *Pelvicachromis taeniatus* with respect to relatedness. *P. taeniatus* is a small, socially monogamous cichlid with biparental brood-care that inhabits rivers and creeks in Cameroon and Nigeria. Males occupy caves, while females compete among each other for access to males. As in many cichlid species, brood care of both parents is necessary to efficiently protect the young against predators [26]. This requires highly synchronous behavior among parents. After spawning, the female cares for the eggs, while the male defends the territory against intruders. Free-swimming offspring are guarded by both parents. Our study fish originated from the Moliwe river in Cameroon, which is only a few kilometres long and totally isolated from other river systems.

In order to produce kin groups, wild-caught, randomly composed mating pairs were bred under standardized laboratory conditions. Offspring were separated from the parents 4 to 6 weeks after hatching. This duration of brood care complies with the observed natural conditions in cichlids [26]. Each kin group was split into two groups of 20 individuals and raised in olfactory and visual isolation from each other for 9–12 months until the start of the experiment. Since it is very unlikely that adult fish are able to individually recognize fish with whom they spent a few weeks as larvae or juveniles, unfamiliar kin were available. Mating preferences in reproductively active fish were estimated by measuring the time an individual spent with an unfamiliar full sib or an unfamiliar nonkin of the opposite sex in standard three-compartment aquaria [27]. We controlled for possible differences in attractiveness among stimulus fish by using a paired experimental design. One test consisted of two trials with the same pair of stimulus fish. Between the trials, only the test fish was changed. Hence, the test fish of the first trial was related to one of both stimulus fish, and the test fish of the second trial was related to the other stimulus fish. In the female- and male-choice experiments, fish of 14 and 8 families, respectively, were used. Each family was used in two tests but not in the same combination. Test fish, as well as stimulus fish,

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were used in only one test. Both sexes of *P. taeniatus* significantly preferred kin over nonkin (females: paired t test, $T_{13} = -2.822$, $p = 0.014$; males: Wilcoxon matched-pairs signed-ranks test, $n = 8$, $Z = -2.521$, $p = 0.012$; Figure 1). The mating decisions of females did not significantly differ from those of males (t test, $T_{20} = -0.575$, $p = 0.572$).

Our results indicate that adult *P. taeniatus* are able to discriminate unfamiliar kin from unfamiliar nonkin, suggesting phenotype matching [28–31] as recognition mechanism, and that they prefer to stay with kin. The time adult fish spent with the opposite sex generally correlates well with spawning probability [27, 32]. However, social preferences for kin in a nonsexual context are widespread in animals [33, 34]. To rule out this possibility, we conducted a further experiment in which fish were allowed to spawn. We simulated natural breeding conditions in which males with caves are probably limited: one male and two size-matched females, one of them an unfamiliar sister of the male, were placed in an aquarium (80 × 30 × 30 cm) equipped with a breeding cave and a hiding place for the rejected female. Females were individually distinguishable from each other by the number of spots on their dorsal and caudal fins. The experiment ended when one of the females spawned with the male. Until then, we noted daily with which female the male was associated. Males and females of 20 and 26 different families, respectively, were used. Again, all experimental fish were used only once. In 17 of the 23 experiments, sibs mated ($n = 23$, $\chi^2 = 5.261$, $p = 0.022$; Figure 2). On average, 93% of the male-female associations existed between the subsequent mating partners (binominal test, $n = 22$, $p < 0.001$). This indicates that the time an individual spends with the opposite sex is a reliable predictor of mating decision in *P. taeniatus*. Both sexes in the mating experiments thus actively favored to mate with close kin.

Fish experienced kin-only social groups in the laboratory. In future experiments, offspring will be reared in mixed kin/nonkin groups until sexual maturity in order to investigate the impact on early experience on mate choice and to test whether *P. taeniatus* use self-referential cues to recognize kin.

In the present experiments, the test fish were exposed to olfactory and visual cues of the stimulus fish, so we are not able to disentangle the relative significance of each factor in kin recognition. Previous research on cichlids highlights the importance of both visual and olfactory cues in species recognition [35–37]. Generally, in fish olfactory cues seem to play a fundamental role in kin recognition. MHC-related odors [38] may serve as a reliable recognition criterion [39] because MHC alleles are highly variable in cichlids [40] and relatives are likely to share alleles.

Assuming low inbreeding depression and limited mate availability, theory predicts benefits from mating with close kin even in monogamous species with biparental brood care [11, 41]. Previous work suggests a rather monogamous mating system in *P. taeniatus* (T.T., T.C.M.B., and H.K., unpublished data). Males with caves are probably limited and thus competition among females for mates is strong (H.K. and T.T., unpublished observation). Therefore, some females might lose their breeding opportunity. Since full sibs share on average 50% of their

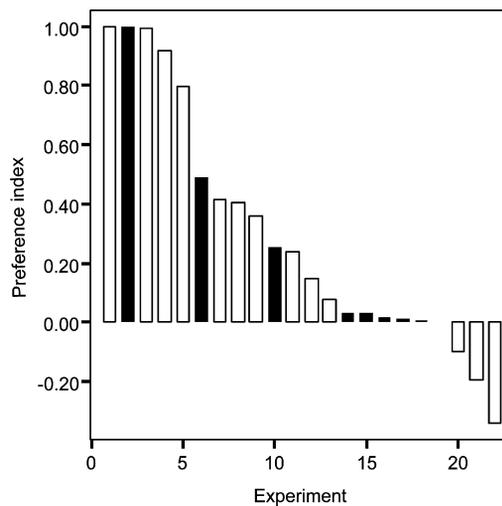


Figure 1. Preference Indices of the Male and Female Mate-Choice Experiments

The preference index was calculated by subtracting the relative time the test fish of the second trial spent with nonkin from the relative time the test fish of the first trial spent with the same stimulus fish (kin in this case). Values of the preference index can range from 1 (both stimulus fish spent all the time with their respective kin) to -1 (both stimulus fish totally avoided kin). Values of 0 indicate no preference. White bars represent the results of female ($n = 14$); black bars show male mate-choice experiments ($n = 8$). The experiments are ranked according to decreasing preference index.

genes, a male mating with his sister assures the transmission of his gene copies that are found in his sister to the next generation, enhancing his inclusive fitness.

Additionally, close inbreeding may be explained by relatives being better parents. Biparental care requires cooperative behavior between parents. Parental care is costly [13] and therefore, in species with biparental care, there is a conflict between parents over care [12, 14], which can reduce offspring fitness [15]. Relatedness is expected to facilitate the evolution of cooperation among individuals [16]. Thus, an individual has to trade

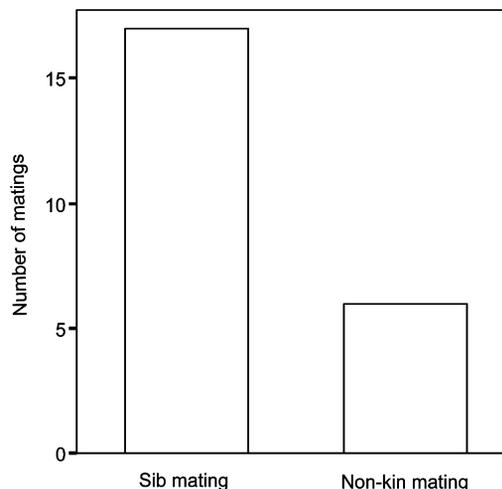


Figure 2. Spawning Experiment

Number of matings between sibs ($n = 17$) and nonsibs ($n = 6$) in spawning experiments with one male and two females, one related and the other unrelated to the male.

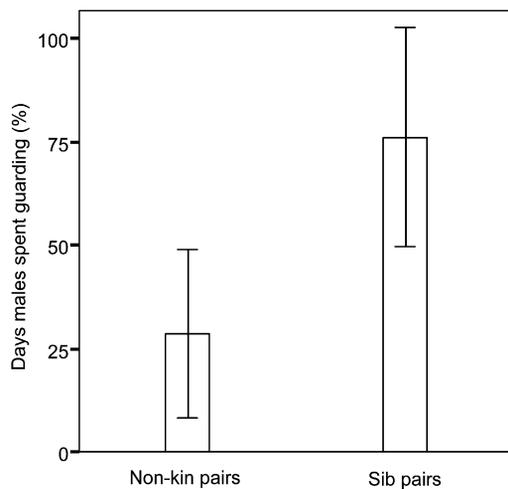


Figure 3. Cave Guarding of In- and Outbreeding Males
The average proportion of days (in percent \pm SD) in which males of in- ($n = 10$) and outbreeding ($n = 8$) pairs guarded the cave where the female cared for the eggs.

off the costs against the benefits of in- or outbreeding, i.e., the strength of inbreeding depression and the enhanced cooperation during biparental care.

To test the impact of parental relatedness on cooperation between parents and on offspring performance, we examined the parental behavior as well as the survival and growth rates of the offspring of 10 inbreeding and 8 outbreeding pairs. Inbreeding pairs consisted of unfamiliar full sibs, whereas outbreeding pairs consisted of unfamiliar nonkin. We found that males of inbreeding pairs spent significantly more time guarding the caves than males of outbreeding pairs (t test, $T_{16} = -4.161$, $p = 0.001$; Figure 3). When the fry swam free, inbreeding pairs spent significantly more time with the young than did outbreeding pairs (t test, $T_{15} = -2.631$, $p = 0.019$; Figure 4). This difference seemed to be due mainly to male guarding, which was significantly enhanced in inbreeding pairs ($78.19\% \pm 10.88\%$ in comparison to $61.79\% \pm 17.46\%$ in outbreeding pairs; t test, $T_{15} = 2.356$, $p = 0.032$). Females did not significantly differ concerning the time spent guarding the young (Mann-Whitney U test, $m = 8$, $n = 9$, $Z = -1.35$, $p = 0.200$). Further, when the young swam free, outbreeding males conducted twice as many attacks against their mates as did inbreeding males (Mann-Whitney U test, $m = 8$, $n = 9$, $Z = -1.98$, $p = 0.046$). No significant differences existed between in- and outbreeding females concerning attacks against their mates (Mann-Whitney U test, $m = 8$, $n = 9$, $Z = 0.000$, $p = 1.000$). Thus, inbreeding pairs were more cooperative and invested more into parental care than did outbreeding pairs. We did not find any evidence for strong inbreeding depression. Inbred and outbred offspring did not differ significantly concerning survival rate (median 61.76% [quartiles, 48.79 and 80.67] versus 43.75% [42.96 and 50.00]) and growth rate (Mann-Whitney U test, $m = 7$, $n = 7$, $Z = -0.958$, $p = 0.338$ and t test, $T_{12} = 1.040$, $p = 0.319$, respectively). However, in- and outbreeding may affect traits that were not measured in this study, e.g., fertility or fecundity of offspring. Whether the sound performance of inbred offspring is

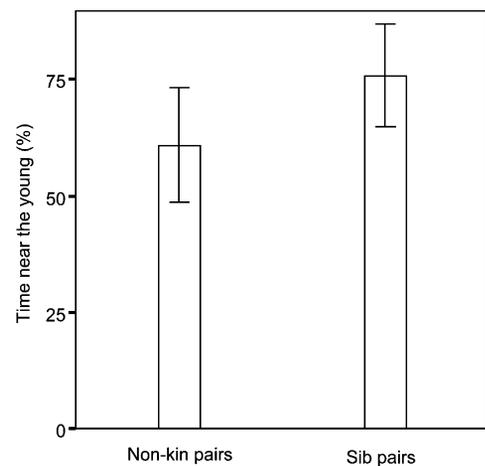


Figure 4. Young Guarding of In- and Outbreeding Parents
The average proportion of parent-offspring associations (in percent \pm SD) of related ($n = 9$) and unrelated ($n = 8$) parents.

due to a total lack of inbreeding depression or to a compensation of a low level of inbreeding depression by superior parental care of sib pairs will be the aim of future experiments. Because fish descended from a small population with probably regular inbreeding, it is possible that the genetic load is purged [42, 43].

Population-genetic studies suggest that in several African cichlid species, populations are often highly structured [44–47] and that within these subpopulations, mating between kin may occur [45]. A recent study in birds documents a considerable genetic similarity between mates [48]. Together with our presented results, this suggests that active inbreeding may thus be much more widespread than commonly assumed and has to be considered as an adaptive strategy.

Experimental Procedures

Experimental Animals

In 2001 and 2003, adult *P. taeniatus* were collected from Moliwe river near Limbe, Cameroon ($04^{\circ}04'N/09^{\circ}16'E$) and transferred to the laboratory in Germany. The fish used in the female and male mate-choice experiments were F1 offspring of the 2001 and 2003 parent generations, respectively. Spawning experiments were conducted with offspring of both parental generations. The fish of the parental care experiment descended from the same families as the fish in the male choice experiments. All test and stimulus fish were of reproductive age and showed courtship coloration as well as courtship behavior, i.e., quivering the curved body.

Mate-Choice Experiments

Female and male mate-choice experiments were conducted in dichotomous designed test tanks (see Supplemental Data available online) in spring 2003 and autumn 2005, respectively. During the experiments, olfactory and visual communication between females and males was possible. The behavior of the test fish was videotaped. The time individuals spent with sibs and nonsibs in the choice zones was measured by a naive observer. In order to compare the choice behavior of the test fish among trials, the time the test fish spent in each choice zone was calculated relative to the total time it spent in both choice zones.

Spawning Experiments

The experiments were conducted in the summers 2004 and 2005. Sisters did not differ significantly from nonsisters with respect to the number of spots in the fins, body length, body mass, and body

condition (Wilcoxon matched-pairs signed-ranks tests, all $n = 23$: $Z = -0.798$, $p = 0.42$; $Z = -0.175$, $p = 0.86$; $Z = -0.107$, $p = 0.92$ and $Z = -0.669$, $p = 0.50$, respectively). A male was defined as associated with a female when the distance between them was 10 cm or less. In 2004, experiments were stopped when no mating occurred after 3 months; in 2005, the duration was reduced to 2 months because in 2004 only one mating happened later than 2 months after starting the experiment.

Parental-Care Experiment

The parental-care experiment was part of a broader study concerning the reproductive behavior of *P. taeniatus*, which was conducted in spring 2006 (T.T., T.C.M.B., S.A.B., and H.K., unpublished data). 15 in- and 15 outbreeding pairs were randomly allocated to 30 aquaria (50 × 30 × 30 cm). 18 pairs spawned (10 inbreeding and 8 outbreeding), of which one pair (inbreeding) ate the wrigglers and 2 pairs cannibalized the free swimming offspring (1 inbreeding and 1 outbreeding pair). Inbreeding pairs did not significantly differ from outbreeding pairs concerning body length, mass, and condition as well as within-pair size differences (Mann-Whitney U test, all $m = 10$ and $n = 8$, all $p > 0.27$). After spawning, the cave was shortly removed and the number of eggs was counted. Two observers (S.A.B., T.T.), who were naive concerning the composition of the test pairs, recorded the behavior of each pair daily for 5 min over a period of 12 weeks. The observation order was random and determined daily. The following behavioral patterns were recorded in order to estimate intrapair cooperation and parental effort. During the egg-care period, it was recorded whether the male guarded the cave or not. We defined males as guarding when they stayed in front of the cave with erected fins. When the fry swam free, aggressive attacks among the mating partners were counted and the time each parent spent with the offspring was quantified. During the daily observations, we recorded in 30 s intervals which parent guarded the young. Behavior was scored as guarding when the distance of a parent to the young was one body length or less. 3 weeks after the young started to swim free, the parents were removed from the tank, and again 1 week later sib group size was standardized (30 ± 2 fish). Fry were fed standard with living *Artemia* larvae, and when they grew older with a mix of frozen *Artemia*, chironomid, and mosquito larvae. Sample sizes of offspring survival- and growth-rate analyses were reduced by 1, since for one pair, the number of eggs could not be assessed, and of another pair, only 22 young survived. Total body length of the offspring was measured 40 ± 1 and 80 ± 1 day after group reduction. Growth rate was calculated as absolute increase in body length between the two measurements in relation to the length at the first measurement. Because the growth rate was positively correlated with the former group size ($F_{1,13} = 6.884$, $p = 0.021$), comparisons between in- and outbred offspring were done with residual growth rates.

Statistical Analysis

All analyses were performed with SPSS 12.0 for Windows. Parametric statistics were applied only when data were normally distributed according to the Kolmogorov-Smirnov test with Lilliefors correction. Given p values are two-tailed throughout.

Supplemental Data

Supplemental Experimental Procedures can be found with this article online at <http://www.current-biology.com/cgi/content/full/17/3/225/DC1/>.

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