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The role of genetic relatedness among social mates in a cooperative breeder

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Breeding with relatives can have severe fitness consequences, so avoiding these costs is often evolutionarily favored. There are a number of mechanisms that reduce the likelihood of mating with relatives, including avoiding relatives as mates (through sex-biased dispersal and mate choice) and delayed sexual maturity in the presence of relatives. Here, we examine these mechanisms in *Neolamprologus pulcher*, a group-living cichlid fish that exhibits male-biased dispersal. Despite sex-biased dispersal in this species, mean relatedness between social mates was not different from that expected if pairs had formed randomly, suggesting individuals neither actively avoid nor prefer pairing with relatives. Furthermore, gonadal investment of subordinates living in social groups was not correlated with their relatedness to the opposite-sex dominant breeder in the group, suggesting that sexual maturation does not depend on the presence or absence of a relative. Highly related social pairs showed higher rates of within-pair aggression and lower rates of nonaggressive social affiliation than less-related social pairs. Breeder investment and indicators of female breeder and group quality were not correlated with relatedness values between social mates. However, scraping rates (a potential quality indicator) were lower in males paired with more closely related females. We consider whether the apparent lack of inbreeding avoidance reflects an evolutionary history of limited breeding opportunities in *N. pulcher* or a facultative strategy of more-fit individuals and discuss the behavioral results in light of the suggested nonassortative mating with regard to relatedness. *Key words:* cichlidae, inbreeding avoidance, Lake Tanganyika, mate choice, relatedness, reproductive suppression. *[Behav Ecol 19:816–823 (2008)]*

Breeding with relatives can lead to decreased heterozygosity, the exposure and expression of deleterious recessive phenotypes, and, in small populations, the potential fixation of harmful recessive alleles (Pusey and Wolf 1996; Ralls et al. 1998). Inbreeding avoidance is one factor that may drive the evolution of sex-biased dispersal; if one sex more commonly leaves the natal territory, the risk of inbreeding is decreased for both sexes (Greenwood 1980; Pusey 1987). In the absence of sex differences in the costs and benefits of philopatry, the sex for which inbreeding is most costly is expected to become the dispersing sex (Greenwood 1980). The aim of this study was to determine whether *Neolamprologus pulcher*, a cichlid fish endemic to Lake Tanganyika, avoids inbreeding. In *N. pulcher*, males disperse further and more often than females (Stiver et al. 2004, 2007). Furthermore, when individuals move to new social groups, they move away from both their same- and opposite-sex relatives (Stiver et al. 2007). This movement may reduce their risk of inbreeding or same-sex kin competition. However, not all individuals disperse, and the breeding vacancies (particularly female vacancies) may be filled through inheritance (Balshine-Earn et al. 1998; Dierkes et al. 2005; Stiver et al. 2006; Fitzpatrick et al. forthcoming). Hence, it is unclear whether the probability of pairings among related individuals is decreased by biased dispersal.

Sex-biased dispersal is only one mechanism that may reduce the cost of inbreeding and such a sex bias may or may not lead to complete avoidance of relatives as mates. Instead, individuals may choose to avoid related mates, basing mate choice decisions on cues of relatedness (e.g., spatial information, familiarity or recognition alleles, Blouin SF and Blouin M 1988; Pusey and Wolf 1996). In addition, individuals may delay sexual maturity in the presence of an opposite-sex parent or relative (Pusey and Wolf 1996) due to self-restraint (Hanby and Bygott 1987; Wolff 1992) or as a result of reproductive suppression by the opposite-sex relatives (Abbott 1993).

Relatedness to a mate can influence parental investment, as parents have been shown to modulate their investment on the basis of the investment of its partner or the quality of its partner/offspring (differential allocation hypothesis, Burley 1986, 1988; Sheldon 2000). An individual mated with a relative may increase care either to insure the survival of potentially less-fit young or in response to the increased inclusive fitness value of these offspring (Margulis 1997). Alternatively, as these offspring may be less fit, parental investment in them may be decreased (Margulis 1997).

However, inbreeding is not necessarily disadvantageous, and any associated costs are species/population specific. Some benefits of inbreeding include the maintenance of beneficial coadapted gene complexes (Bateson 1983) and purging of deleterious alleles (Waller 1993); accordingly, inbreeding tolerance has been found in conjunction with a lack of evidence for inbreeding depression (Keane et al. 1996; Koenig and Haydock 2004). Finally, inbreeding can increase an individual's inclusive fitness (Fisher 1941; Bengtsson 1978; Parker 1979; Lehmann and Perrin 2003), provided that alternative matings by both partners are not lost (Dawkins 1979) and that kin-selected benefits override potential inbreeding depression costs (Kokko and Ots 2006). Furthermore, as individuals

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vary genetically (and therefore in genetic load and potential inbreeding cost), they may also vary in their likelihood of inbreeding. Recently, Thünken et al. (2007) suggested that inbreeding is an advantageous strategy in the African cichlid *Pelviacachromis taeniatus*; more related pairs were more cooperative with one another, and such pairs not only increase their inclusive fitness but also gain a more caring partner (due to decreased conflict over care). Therefore, in certain situations, mating with related individuals may be tolerated, or even preferred (for recent empirical examples, see Cohen and Dearborn 2004; Kleven et al. 2005; Thünken et al. 2007), and failure to avoid inbreeding should not always be attributed to a lack of breeding opportunities or failure of kin recognition (Kokko and Ots 2006).

To address the role of relatedness in pair formation, we used genetic data from wild pairs of *N. pulcher*, a cooperatively breeding cichlid fish species from Lake Tanganyika in which nonbreeding subordinate group members help protect the offspring and the territory of a dominant breeding pair (Taborsky and Limberger 1981; Balshine-Earn et al. 1998). Based on 7 microsatellite loci, we compared the relatedness between established pairs of *N. pulcher* in our study population to relatedness between all potential pairs. We also investigated how gonadal investment (evidence of reproductive suppression) of subordinate helpers was associated with their relatedness to the opposite-sex breeder in their group (whether or not subordinates reproduce is a topic currently under investigation; Dierkes et al. 1999, 2005; Fitzpatrick et al. 2006). Inbreeding costs in this species are unknown, although jaw and mouth deformities are common among captive bred fish and may reflect a history of inbreeding. Evidence of inbreeding costs in other cichlid species is mixed; in convict cichlids (*Cichlasoma nigrfasciatum*), Piron (1978) found few skeletal deformities after 3 generations of inbreeding, whereas Winemiller and Taylor (1982) found significant deformities and decreased survival by the fourth and fifth generations. Inbreeding was documented in *P. taeniatus*, where it was suggested that potential inbreeding costs are balanced by the benefits of breeding with a relative (Thünken et al. 2007).

Reproductive ecology of *N. pulcher* suggests that individuals of this species should be capable of avoiding inbreeding. Limited dispersal between subpopulations (especially by large males) occurs, and dispersal within a subpopulation occurs widely and is apparently unrestricted by distance (as evidenced by a positive correlation between the relatedness between individuals from different groups and the distance between their groups; Stiver et al. 2007). *Neolamprologus pulcher* individuals respond differently and appropriately to familiar versus unfamiliar individuals (Hert 1985; Taborsky 1985; Balshine-Earn and Lotem 1998; Frostman and Sherman 2004) and adjust their cooperative effort in accordance with their relatedness to the dominant breeders in their social group (Stiver et al. 2005, 2006). These findings suggest that *N. pulcher* has the capacities to recognize relatives and avoid inbreeding by dispersal.

Pairing with a relative may be a "last chance" option of an individual that would otherwise be excluded from breeding or an avenue to increased inclusive fitness (potentially limited to high-quality individuals); hence, relatedness between a pair may be associated with traits that relate to individual or group quality (Kokko and Ots 2006). Furthermore, individuals in such pairings may show increased investment (Margulis 1997; Thünken et al. 2007). To assess these possibilities, we first examined whether individuals increase their parental effort as pair relatedness values between the pair increased (Margulis 1997; Kokko and Ots 2006; Thünken et al. 2007). Second, we examined how body size, parasite load (assessed by scraping behavior; Barber et al. 2000), group size, and average size of helpers in the group vary with pair relatedness values. These traits have previously been linked to individual and group quality (Taborsky and Limberger 1981; Barber et al. 2000; Balshine et al. 2001; Heg et al. 2004; Brouwer et al. 2005; Desjardins et al. forthcoming). Finally, to examine if there was a social response of an individual to their relatedness to their mate, we examined the association of pair relatedness to the social behavior between the breeding pair.

We predicted that if mechanisms of inbreeding avoidance are in operation, then 1) on average, individuals would be less related to their social mate than to another opposite-sex breeder who is not their social mate and 2) subordinates in the presence of a related opposite-sex breeder (potential mate) would invest less in gonadal tissues than subordinates with a less-related opposite-sex breeder. We also predicted that 3) more related social pairs would have higher parental investment (higher workloads, less feeding, and less time off territory) than less-related pairs and that 4) relatedness between pairs may be related to individual quality: negatively (if such pairings result from individuals who would otherwise not breed) or positively (if individuals breed with relatives only when their quality affords them the potential costs). Because inbreeding is likely to be more costly for females than for males (due to their higher gametic investment, increased parental effort, and fewer potential lifetime breeding partners relative to males; Bateman 1948; Taborsky and Limberger 1981; Balshine et al. 2001; Stiver et al. 2005, 2006; Heg et al. 2008; Desjardins et al. forthcoming; see Materials and Methods below), we expected that these patterns would be more evident among females than males and, whenever possible, examined behavioral and individual quality indicators in males and females separately.

MATERIALS AND METHODS

Study species

Neolamprologus pulcher, endemic to Lake Tanganyika, live in groups consisting of one dominant breeder of each sex and 1–20 subordinates (termed "helpers") of both sexes (Taborsky and Limberger 1981; Balshine et al. 2001; Heg et al. 2005). Each group inhabits a territory containing excavated rock cavities that serve both as shelters and as sites of fertilization (the group of rocks where eggs are spawned is termed the "brood chamber"). Territories are clustered into subpopulations containing 2 to 200+ territories (Fitzpatrick et al. 2006; Stiver et al. 2006). Whereas dominant female breeders and subordinate helpers reside in a single social group's territory, dominant male breeders may hold residence in multiple territories at once. Therefore, as males may be simultaneously dominant and breeding in multiple groups, they have a higher potential number of concurrent (and likely lifetime) breeding partners compared with breeding females, who can be dominant in only one group at a time (Limberger 1983; Desjardins et al. forthcoming).

Breeders and helpers all contribute to direct brood care, maintenance of the shelter system, and defense against predators, space competitors, and conspecifics (collectively termed work effort). Other behaviors commonly observed are aggressive and nonaggressive social interactions among group members and body scrapes. Scraping can remove ectoparasites (Barber et al. 2000), and scraping rates have been used as a proxy measure for parasite load (Desjardins et al. forthcoming). For further information on this species, see Taborsky and Limberger (1981) and Taborsky (1984); for a recent ethogram of the behaviors specifically examined here, see Buchner et al. (2004).

Whereas exact rates of dispersal between subpopulations are unknown, studies of recaptured individuals and pairwise relatedness between subpopulations suggest that movement between subpopulations is rare, restricted by distance, and largely constrained to large males (the maximum-recorded dispersal distance is 12 m; Stiver et al. 2004, 2007). Within a subpopulation, although many individuals do not disperse and are found in groups with their closest relatives, movement between groups is apparently unconstrained by distance (Stiver et al. 2007). Fish that do disperse to another group appear to maximize their distance from their closest relatives within that subpopulation (Stiver et al. 2007). However, male-biased dispersal does not necessarily imply that inbreeding is more costly for males than for females: sex-biased dispersal may be selected to enhance resources (e.g., territories or mates) or decrease kin competition (Greenwood 1980). Beyond differences in gametic investment, which increase the costs of inbreeding for females relative to males (Bateman 1948), other sex differences suggest that inbreeding is more costly for *N. pulcher* females. Females have higher workloads, invest more in young and in their defense, and have fewer potential lifetime partners than their male breeding partners (Taborsky and Limberger 1981; Limberger 1983; Balshine et al. 2001; Stiver et al. 2005; Heg et al. 2008; Desjardins et al. forthcoming).

Sample collection and behavioral observations

Fieldwork was conducted between January and April 2004 on the Zambian shores of Lake Tanganyika (Kasakalawe Bay, 8°46.87′S, 31°04.88′E). Field methods are described briefly here and further details of the study site, methods for individual/group capture, and collection of behavioral data may be found in Balshine-Earn et al. (1998); Balshine et al. (2001); Werner et al. (2003); Stiver et al. (2004, 2005, 2006, 2007); Fitzpatrick et al. (2006, forthcoming); Desjardins, Stiver, Fitzpatrick, and Balshine (2008); Desjardins, Stiver, Fitzpatrick, Milligan, et al. (2008); and Desjardins et al. (forthcoming).

Groups were located at 8.5–11.5 m depth using SCUBA, and all data were recorded on PVC plates. Groups used in this study were visited and observed on at least 2 occasions in the field season prior to conducting the behavioral focal watches, and dominance hierarchies (based on behavioral observations of social interactions) within each social group were known (for all group members). Individuals could be reliably identified on the basis of a combination of body size measures and unique natural artificial body markings. Artificial marks are created by injecting nontoxic acrylic paint into the scale pocket (such marks do not harm the fish; for further capture and marking details, see Balshine-Earn et al. 1998; Balshine et al. 2001; Stiver et al. 2006). In total, 238 fish from 59 groups were genotyped for this study (see Genetic methods), comprising 45 male breeders, 59 female breeders, 74 female helpers, and 60 male helpers. Fifty-nine pairs were examined in which each breeder could be compared with their mate (or mates, as was the case with 8 multiply mated males). Helper gonads were measured (to the nearest 0.001 g), and the gonadal investment of these helpers was compared with their relatedness to their opposite-sex breeder.

The behavior of 37 of the male breeders and 50 of the female breeders was observed for one to four 10-min focal observation periods (mean number of focal observations per individual was 2). During these focal observations, we recorded all territory defense, maintenance and brood chamber visits (jointly called work effort), and social behaviors (aggressive, submissive, and social interactions) performed by each individual, as well as their feeding and scraping rates and the total time they spent on the territory. When there were multiple observations per individual, a mean rate per 10 min was calculated for each behavior.

Two of the 59 pairs were temporarily captured on their territory. Their body length was measured, their sex determined by examination of the genital papilla (Balshine-Earn et al. 1998), and a small fin tissue sample cut from their dorsal fin before individuals were released back to their territory. The remaining 57 pairs were captured along with their entire group for a number of physiological studies (see Fitzpatrick et al. 2006; Stiver et al. 2006; Desjardins, Stiver, Fitzpatrick, and Balshine 2008; Desjardins, Stiver, Fitzpatrick, Milligan, et al. 2008; Desjardins et al. forthcoming). Body length (to the nearest 0.01 cm) and body mass (to the nearest 0.001 g) were recorded before sacrifice using an overdose of ethyl 4-aminobenzoate (Sigma–Aldrich, St. Louis, MO) and cervical severance. Sex was verified for these fish by examination of gonads, gonadal mass was measured (to the nearest 0.001 g), and a sample of muscle tissue was taken for genetic analyses. Tissue samples were preserved in 95% ethanol.

Genetic methods

Individuals were genotyped at the following 7 microsatellite loci: LOC101 (Brandtmann et al. 1999), ML007 (Kohler 1997), Pzeb1, Pzeb3 (Parker and Kornfield 1996), Ppun21 (Taylor et al. 2002), TmoM13 (Zardoya et al. 1996), and US783 (Schliewen et al. 2001). All individuals included in the data set were successfully genotyped at a minimum of 5 loci (the average number typed per individual was 6.73). Allele frequency estimates (calculated using CERVUS 2.0) were subpopulation specific and based on all individuals (breeders and nonbreeders) sampled within that subpopulation. Relatedness between 2 individuals was based on Queller's *r* value, estimated using KINSHIP 1.3.1 (Goodnight and Queller 1999; analyses using other relatedness estimators yielded similar results; for more details, see Stiver et al. 2007).

Queller's *r* is based on the likelihood of alleles being identical in kind rather than being identical by descent, and relatedness estimates reflect the genotypic similarity of microsatellite loci between a pair in comparison to the expected value between 2 individuals selected at random from the population. Negative values indicate that the relatedness between the pair was less than that expected between 2 random individuals (Queller and Goodnight 1989; Rousset 2002; for similar negative values using Queller's *r*, see also Kleven et al. 2005; Foerster et al. 2006). Hence, an inbred pairing may involve either individuals with shared ancestry or individuals that are more closely related than would be expected from random mating with regard to relatedness (Keller and Waller 2002); we were unable to differentiate between these 2 possibilities. For further details of genetic methods, see Stiver et al. (2007).

Statistical analyses

Relatedness values among social mates

Neolamprologus pulcher individuals regularly interact with individuals from other social groups in the same subpopulation while feeding in the water column above the territories (Taborsky and Limberger 1981) and while "visiting" other groups (Bergmüller et al. 2005; Heg et al. 2008). As a consequence of these regular social interactions and the fact that within subpopulation dispersal is unconstrained by the distance between groups (Stiver et al. 2007), we considered "potential" mates to be all opposite-sex breeders within a breeder's subpopulation (excluding their actual mates; for similar analyses and criterion, see Keller and Arcese 1998; Kleven et al. 2005). We tested whether or not individuals prefer or avoid pairing with their relatives by comparing the relatedness between pair partners (between "actual" mates) with the mean relatedness between all potential partners. If individuals avoid relatives as mates (negative assortment), their relatedness to their potential mates should be higher than that to their actual mate. If individuals prefer relatives as mates (positive assortment), their relatedness to their potential mates should be lower than that to their actual mate. We could calculate a mean relatedness to potential mates for females from 56 pairs and males from 57 pairs (males mated to multiple females were included more than once; please see Data analysis below for details of how we controlled for nonindependence). Additionally, as subordinates may vary in gonadal development based on their relatedness to the opposite-sex breeder of their group, we examined this potential inbreeding avoidance mechanism by comparing the relative gonad size (using gonadosomatic index [GSI] to control for body size) of 74 female and 60 male subordinates (gonad mass/body mass × 100) to the relatedness estimate of the subordinate to their opposite-sex breeder.

Pair relatedness and individual/group traits

The behavioral traits examined were work effort (including defense, brood chamber visits, and territory maintenance activities), time spent off territory, feeding rate, and scraping rate. Individuals that are feeding and or off the territory are not performing work, and these behaviors are negatively correlated with work effort. Also, individuals that are away from their territory have the opportunity to gain information about alternative pairings (by gaining information about breeding vacancies in other territories) and may engage in extrapair copulations. Finally, scraping, as a proxy measure for parasite load, is a measure of individual quality (Barber et al. 2000; Desjardins et al. forthcoming).

The physical traits examined were individual body length (standard length), body condition (mass/length), and gonadal investment (gonad mass/body mass × 100). These measures have also been previously used as indicators of individual quality (Balshine et al. 2001; Desjardins et al. forthcoming). Also, as ability to gain multiple mates may also indicate male quality, the relatedness of pairs involving multiply mated males was compared with the relatedness of pairs involving singly mated males. When male traits (e.g., standard body length) were the unit of analysis, the relatedness value used for multiply mated males was his mean relatedness to all of his social mates.

Groups with more helpers have larger territories, more shelters, and increased reproductive success; further, larger helpers are more effective at driving away predators (Balshine et al. 2001; Heg et al. 2004; Brouwer et al. 2005). Therefore, we examined how relatedness between a pair correlated with group size and the mean body length (standard length) and body condition (mass/length) of all helpers. We also examined aggressive (rams, bites, chases, puffed throats, and mouthfights) and nonaggressive (soft nudges, submissive displays/postures, parallel swimming, and following) behaviors performed by the male toward the female and by the female toward the male to determine whether social interactions between the breeders differed with regard to their relatedness to one another.

Data analysis

To control for correlation among the independent variables and to reduce the number of tests conducted, principle components analyses (PCAs, with varimax rotation) were preformed on all variables being compared with pair relatedness. Male and female *N. pulcher* differ in their behavior, mean size, and gonadal investment patterns (Taborsky and Limberger 1981; Balshine et al. 2001; Stiver et al. 2005; Desjardins et al. forthcoming); therefore, variables were included in 1 of the 3 PCAs in accordance with whether the variable was a female trait, a male trait, or a trait associated with both breeders (i.e., the social interaction of the pair with one another and traits of the group). The variables included in the PCAs were 1) female traits (work effort, time spent off territory, feeding rate, scraping rate, body length [standard length], body condition [mass/length], and gonadal investment [gonad mass/body mass × 100]), 2) male traits (see variable list for "female traits"), and 3) traits associated with both breeders (group size, the mean helper body length [standard length], mean helper body condition [mass/length], aggression of the male toward the female, aggression of the female toward the male, nonaggression of the male toward the female, and nonaggression of the female toward the male).

After determining how variables loaded onto the retained PCA factors, the correlation of pair relatedness to the variables in each component was examined using a forward stepwise regression (with pair relatedness as the dependent variable and all other variables as independents). This allowed us to examine the correlation of each variable with pair relatedness while removing the effect of correlations between independent variables. Partial correlations were reported for all stepwise regressions, as they provide information about the relationship between the dependent variable and each of the separate independent variables (sample sizes for the regressions are reported in Results).

As relatedness estimates are based on paired data, all individuals contribute to multiple data points in the comparison of the relatedness among actual mates to the relatedness between potential males. Similarly, males with multiple mates contribute to multiple estimates of relatedness between actual pairs. The resultant pseudoreplication is an issue when using traditional statistical tests, which use standard comparison distributions and partially base the calculation of P values on sample size. We controlled for this nonindependence of data by using randomization tests conducted with RUNDOM Projects 2.0 Lite (Jadwiszczack 2003; all tests used $N = 10\ 000$ randomizations). Randomization/permutation tests simulate a comparison frequency distribution based on the data being examined. This is done by drawing values from the entire population, randomly assigning them to one of the groups being examined (e.g., relatedness between actual mates and relatedness between potential mates), and calculating the mean difference/correlation between these simulated groups. The procedure is repeated N times to create a distribution of simulated means/correlation coefficients, which is then compared with the difference/correlation between the 2 real groups to generate a P value. All other tests were conducted using Statview 5.0. All P values reported are 2 tailed.

RESULTS

Relatedness values among social mates

Neolamprologus pulcher did not avoid relatives as social mates (see Figure 1 for a comparison of pairwise relatedness estimate distributions between actual and potential mates). Relatedness values between social pairs were not different from relatedness values calculated for all potential mates within a subpopulation (Fisher's paired comparison randomization test; females: $N = 56$, $P = 0.64$; males: $N = 57$, $P = 0.93$).

Subordinate helpers' investment in gonadal tissue (an indicator of reproductive ability) was uncorrelated with their relatedness to the opposite-sex breeder (randomized Pearson correlation, female subordinates: $r = 0.15$, $N = 74$, $P = 0.21$; male subordinates: $r = 0.13$, $N = 60$, $P = 0.33$). Similarly, there was no relationship between gonadal investment and

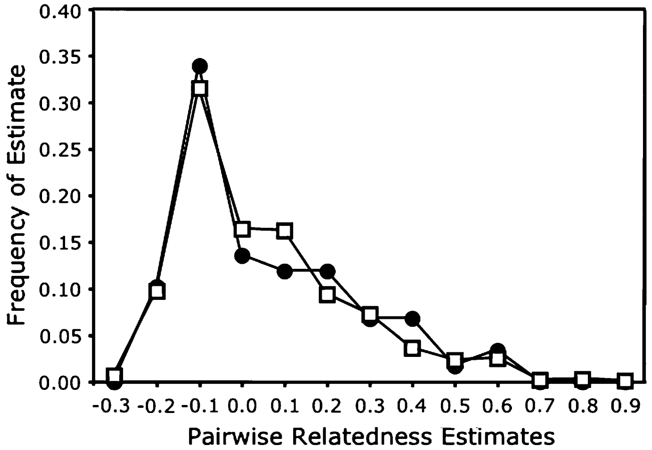

Figure 1
A comparison of the distribution of pairwise relatedness estimates between actual mates (filled black circles; mean r ± standard error = 0.011 ± 0.03; range = −0.27 to 0.55) and potential mates (open squares; 0.004 ± 0.006; range = −0.18 to 0.29).

relatedness to the opposite-sex breeder when we analyzed only the most dominant male and female helper in each group (female subordinates: $r = 0.13$, $N = 38$, $P = 0.42$; male subordinates: $r = 0.27$, $N = 33$, $P = 0.13$).

Pair relatedness and individual/group traits

The results of the PCAs and stepwise regressions are summarized in Table 1. There were 3 major components in PCA 1 ("female traits"; component 1 = female standard length and body condition, $N = 57$; component 2 = female GSI, time off territory, and work effort, $N = 50$; component 3 = female scraping and feeding rate, $N = 50$). None of these female trait variables correlated with relatedness between the pair.

There were 3 components in PCA 2 (examining "male traits"; component 1 = male standard length, body condition, and time off territory, $N = 34$; component 2 = male GSI and work effort, $N = 35$; component 3 = male scraping and feeding rate, $N = 37$). Whereas variables in the first 2 components did not correlate significantly with pair relatedness, there was a negative correlation between male scraping rate and pair relatedness (component 3; partial $r = -0.441$, $P = 0.006$; see Table 1). However, relatedness between pairs was not different between singly mated males ($N = 37$) and potentially higher quality multiply mated males ($N = 8$; 2-sample randomization test, $P = 0.49$).

The third PCA revealed 3 components among variables associated with both breeders (component 1 = mean helper standard length and mean helper body condition, $N = 56$; component 2 = nonaggressive behaviors of the male to the female and of the female to the male, $N = 50$; component 3 = group size and aggressive behaviors of the male toward the female and of the female toward the male, $N = 48$). Variables loading onto component 1 were not correlated with the relatedness between the breeding pair. However, females that were more related to their mate engaged in fewer mate-directed nonaggressive social behaviors than females that were less related to their mate (component 2; partial $r = -0.336$, $P = 0.02$; see Table 1). Finally, males who were more related to their mate were also more aggressive toward their mate (component 3; partial $r = 0.377$, $P = 0.008$; see Table 1).

Some researchers suggest that body condition is better assessed by the residuals of the regression of body mass on length and gonadal investment by the residuals of the regression of gonad mass on body mass (Jakob et al. 1996; Tomkins and Simmons 2002). Use of these alternative metrics did not change our findings (analyses are available from the authors on request).

DISCUSSION

Despite sex-biased dispersal in *N. pulcher* (Stiver et al. 2007), which is thought to decrease the likelihood of encounters between relatives, relatedness of breeding pairs did not differ from the level predicted if mating were random and high relatedness was observed between some breeding pairs. Gonadal investment by subordinate helpers was not correlated with their relatedness to the opposite-sex dominant, suggesting that subordinate maturity is not delayed as a mechanism of inbreeding avoidance. Recent work on gonadal investment patterns demonstrates that subordinate gonadal growth is suppressed in the presence of a same-sex dominant (Fitzpatrick et al. 2006, forthcoming). Therefore, it is possible that the influence of relatedness to the opposite-sex breeder could be obscured by the restricted range of gonadal size in subordinates.

Relatedness between breeding pairs was not associated with breeder work effort or to feeding and time off of territory. However, males displayed more aggression toward female partners they were more related to, and females performed less nonaggressive social behavior when more related to their social male partners. Related social mates may arise when both breeders inherit the breeding position in their natal territory or when both move to the same new group (possibly dispersing together; Heg et al. 2008) and eventually breed there (Balshine-Earn et al. 1998). On average, the likelihood of an individual large helper ascending in status to become a breeder through inheritance is 83% for females and 33% for males (Balshine and Buston forthcoming), suggesting that the likelihood of both individuals inheriting their breeding position is around 28% (Balshine-Earn et al. 1998; Stiver et al. 2006; Fitzpatrick et al. forthcoming). Related breeding pairs are likely to have been living together for longer, and from younger ages, than nonrelated breeders and thus have a longer history of social interactions. Long-term coresidency from a young age may act as a cue of relatedness (Blaustein 1983), and the fact that males were more aggressive to more related mates, while females showed less nonaggressive social behavior toward more related mates, suggests that these pairs responded to cues of relatedness. Other studies have similarly revealed altered behavioral responses to differences in relatedness (see Stiver et al. 2005, 2006), and taken together, these findings make it unlikely that failure of recognition is associated with the observation of related pairs.

If *N. pulcher* individuals can recognize relatives, why do they risk pairing with them? This nonavoidance of relatives as mates may reflect a lack of breeding opportunities (Kokko and Ots 2006) or could be because some individuals prefer to breed with relatives (as such pairings can increase inclusive fitness; Kokko and Ots 2006). In line with this latter suggestion, we found that more related pairs generally involved males who scraped less and presumably had fewer parasites. These males are potentially of higher genetic quality than other males, as parasite load has been associated with genetic quality (Hamilton and Zuk 1982). It may be that breeding with a relative is a facultative strategy, dependent on individual quality and potential inbreeding costs. Thus, the appearance of random mating with regard to relatedness may result from a difference in individual decisions: whereas some individuals avoid mating with relatives, others may tolerate or even prefer such pairings. Future work should further examine this possibility.

However, the lack of breeding opportunities should not be discounted. When breeding opportunities are rare, as in *N. pulcher*, individuals that risk breeding with relatives may

Table 1
Results of the PCAs and forward stepwise regressions

	PCA loading values			Partial *r* with pair relatedness		
	Component			Component		
PCA 1: female traits						
Variable	1	2	3	1	2	3
Standard length	*0.959*	0.023	0.118	−0.093	—	—
Body condition	*0.947*	0.053	0.001	−0.122	—	—
GSI	0.086	*0.417*	−0.00001	—	−0.113	—
Time off territory	0.016	*−0.714*	0.522	—	0.034	—
Work effort	−0.112	*0.819*	0.162	—	0.185	—
Scraping rate	0.320	−0.105	*0.520*	—	—	−0.081
Feeding rate	0.178	−0.133	*−0.856*	—	—	−0.238
PCA 2: male traits						
Variable	1	2	3	1	2	3
Standard length	*0.847*	0.266	−0.011	−0.225	—	—
Body condition	*0.852*	0.032	0.011	−0.047	—	—
Time off territory	*−0.663*	0.287	0.162	0.193	—	—
GSI	0.065	*0.807*	0.110	—	−0.098	—
Work effort	0.465	*−0.617*	0.347	—	0.119	—
Scraping rate	0.088	0.258	*0.662*	—	—	**−0.441** (0.006)
Feeding rate	0.348	0.209	*−0.843*	—	—	−0.173
PCA 3: traits associated with both breeders						
Variable	1	2	3	1	2	3
Mean helper standard length	*0.961*	0.010	0.011	0.043	—	—
Mean helper body condition	*0.950*	−0.051	0.018	0.071	—	—
Nonaggression (male to female)	−0.102	*0.850*	−0.090	—	−0.249	—
Nonaggression (female to male)	0.068	*0.824*	0.037	—	**−0.336** (0.02)	—
Group size	0.078	−0.208	*−0.655*	—	—	−0.205
Aggression (male to female)	0.243	−0.332	*0.523*	—	—	**0.377** (0.008)
Aggression (female to male)	−0.014	−0.096	*0.827*	—	—	0.001

See Materials and Methods for further details. Italics indicate factors loading together onto a particular component. Numbers in bold indicate a correlation that is significant at $P \leq 0.05$, and the associated P values for these correlations are reported in parentheses below the correlation coefficient.

have a selective advantage over those who do not (Smith 1979; Waser et al. 1986; Pillay 2002). Severe limitation in mating opportunities may select for relaxed mate choice. Thus, less choosy individuals may have increased reproductive success and avoidance of relatives as mates may not have been selected for, despite *N. pulcher*'s apparent ability to recognize relatives and respond to cues of relatedness (Stiver et al. 2005, 2006). In *N. pulcher,* mate choice is usually sequential rather than simultaneous, a situation which is predicted to lead to greater tolerance for inbreeding (Kokko and Ots 2006). However, even when presented with simultaneous mate choice opportunities between unfamiliar, unrelated and familiar, related fish in the laboratory, *N. pulcher* have failed to show preferences of any kind (there was no difference in the time that focal individuals spent with 2 potential mates; Hazelden 2004; Pacitto 2005; Sharland 2006). Finally, although inbreeding is likely to be more costly for females than males (as females have greater parental expenditure and fewer breeding opportunities), females are smaller than males and thus may have limited control over which male will eventually dominate and pair up with them. However, female *N. pulcher* paired with a relative could avoid inbreeding costs by spawning with unrelated neighboring males (Brooker et al. 1990; Mulder et al. 1994; Pusey and Wolf 1996; but see Kleven et al. 2005). In blue tits (*Parus caeruleus*), birds do not avoid socially pairing or copulating with relatives but extrapair paternity suggested that a postcopulatory mechanism that biases fertilization toward a nonrelated partner (Foerster et al. 2006). Broods of mixed paternity have been observed in wild-living *N. pulcher* groups (Dierkes et al. 2005), and, because helpers in the wild show diminished reproductive capability (Fitzpatrick et al. 2006), it is most likely that extrapair males in the wild are neighboring breeder males. Future work is planned to determine whether female *N. pulcher* reduce potential inbreeding costs by breeding with extrapair males more frequently when they are related to their social mate.

Studies examining other species have found mixed evidence for inbreeding avoidance, both with regard to social pair formation and actual mating behavior. Individuals are more likely to takeover a vacated breeding position when the social partner is a nonrelative in acorn woodpeckers, *Melanerpes formicivorus* (Koenig et al. 1998), and in Florida scrub jays, *Aphelocoma coerulescens* (Woolfenden and Fitzpatrick 1978, 1984). Female subordinates show low gonadal investment in the presence of related male breeders (acorn woodpeckers, Koenig et al. 1999; Damaraland mole-rats, *Coetomys damarensis,* Cooney and Bennett 2000; meerkats, *Suricata suricatta,* O'Riain et al. 2000). Furthermore, a number of studies support the idea that preferences of social and mating partners are based on cues that indicate that potential mates are nonrelatives (common voles, *Microtus arvalis,* Bolhuis et al. 1988; house mice, *Mus musculus,* Brown and Eklund 1994; humans, *Homo sapiens sapiens,* Wedekind et al. 1995). However, other studies have failed to find any evidence of inbreeding avoidance (dwarf mongooses, *Helogale parvula,* Keane et al. 1996; song sparrows, *Melospiza melodia,* Keller and Arcese 1998) or have revealed that relatives may be preferred as social or extrapair mates (Japanese quail, *Coturnix japonica,* Bateson 1982; great frigatebirds, *Fregata minor,* Cohen and Dearborn 2004; barn swallows, *Hirundo rustica,* Kleven et al. 2005).

In our study, we examined allelic similarity between pairs and thus could not determine if pairs with higher relatedness values were actually genetically similar by descent. In many species, familiarity and spatial location act as cues of relatedness, and individuals do not directly assess genetic similarity directly (Blouin SF and Blouin M 1988; Pusey and Wolf 1996). In such species, individuals can avoid "incest" by recognizing and avoiding close relatives, but they may still suffer "inbreeding" costs by mating with a genetically similar individual (e.g., great reed warblers, *Acrocephalus arundinaceus*, Bensch et al. 1994). Only full pedigree information on our study groups would allow us to examine whether social mates with a high estimated relatedness are, in fact, true relatives. Such a pedigree study, although certainly worthwhile, is currently logistically challenging, if not impossible. The development of technology allowing for long-term tracking of small tropical fish species like *N. pulcher* may allow future studies to specifically examine pair formation and risk of pedigree inbreeding and the costs of inbreeding. Such data would also allow us to address concerns about the use of microsatellite-based estimators in inbreeding studies (particularly when examining heterozygosity and inbreeding depression; Coltman and Slate 2003; Balloux et al. 2004).

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REFERENCES

Abbott DH. 1993. Social conflict and reproductive suppression in marmoset and tamarin monkeys. In: Mason WA, Mandoza SP, editors. Primate social conflict. Albany (NY): State University of New York Press. p. 331–371.

Balloux F, Amos W, Coulson T. 2004. Does heterozygosity estimate inbreeding in real populations? Mol Ecol. 13:3021–3031.

Balshine S, Buston PM. Forthcoming. Cooperation in fish. In: Magnhagen C, Braithwaite V, Forsgren E, Kapoor BG, editors. Fish behaviour ecology. Science Publisher.

Balshine S, Leach B, Neat F, Reid H, Taborsky M, Werner N. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). Behav Ecol Sociobiol. 50:134–140.

Balshine-Earn S, Lotem A. 1998. Individual recognition in a cooperatively breeding cichlid: evidence from video playback experiments. Behaviour. 135:369–386.

Balshine-Earn S, Neat FC, Reid H, Taborsky M. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behaviour in a cooperatively breeding fish. Behav Ecol. 9:432–438.

Barber I, Hoare D, Krause J. 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fish. 10:131–165.

Bateman AJ. 1948. Intra-sexual selection in *Drosophilia*. Heredity. 2:349–368.

Bateson P. 1983. Optimal outbreeding. In: Bateson P, editor. Mate choice. Cambridge: Cambridge University Press. p. 257–277.

Bateson PPG. 1982. Preferences for cousins in Japanese quail. Nature. 295:236–237.

Bengtsson BO. 1978. Avoiding inbreeding: at what cost? J Theor Biol. 73:439–444.

Bensch S, Hasselquist D, von Schantz T. 1994. Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler? Evolution. 48:317–326.

Bergmüller R, Heg D, Peer K, Taborsky M. 2005. Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. Behaviour. 142:1643–1667.

Blaustein AR. 1983. Kin recognition mechanisms: phenotypic matching or recognition alleles? Am Nat. 121:749–754.

Blouin SF, Blouin M. 1988. Inbreeding avoidance behaviors. Trends Ecol Evol. 3:230–233.

Bolhuis K, Strijkstra AM, Moor E, Lende K. 1988. Preferences for odours of conspecific non-siblings in the common vole, *Microtus arvalis*. Anim Behav. 36:1551–1553.

Brandtmann G, Scandura M, Trillmich F. 1999. Female-female conflict in the harem of a snail cichlid (*Lamprologus ocellatus*): behavioural interactions and fitness consequences. Behaviour. 136:1123–1144.

Brooker MG, Rowley I, Adams M, Baverstock PR. 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monofamous species? Behav Ecol Sociobiol. 26:191–199.

Brouwer L, Heg D, Taborsky M. 2005. Experimental evidence for helper effects in a cooperatively breeding cichlid. Behav Ecol. 16: 667–673.

Brown JL, Eklund A. 1994. Kin recognition and the major histocompatibility complex: an integrative review. Am Nat. 143:435–461.

Buchner AS, Sloman KA, Balshine S. 2004. The physiological effects of social status in the cooperatively breeding cichlid *Neolamprologus pulcher*. J Fish Biol. 65:1080–1095.

Burley N. 1986. Sexual selection for aesthetic traits in species with biparental care. Am Nat. 127:415–445.

Burley N. 1988. The differential allocation hypothesis: an experimental test. Am Nat. 132:611–628.

Cohen LB, Dearborn DC. 2004. Great frigatebirds, Fregata minor, choose mates that are genetically similar. Anim Behav. 68:1229–1236.

Coltman DW, Slate J. 2003. Microsatellite measures of inbreeding: a meta-analysis. Evolution. 57:971–983.

Cooney R, Bennett NC. 2000. Inbreeding avoidance and reproductive skew in a cooperative mammal. Proc R Soc Lond B Biol Sci. 267:801–806.

Dawkins R. 1979. Twelve misunderstandings of kin selection. Z Tierpsychol. 51:184–200.

Desjardins JK, Fitzpatrick JL, Stiver KA, Milligan N, Van Der Kraak GJ, Balshine S. Forthcoming. Costs and benefits of polygyny in the cichlid, *Neolamprologus pulcher*. Anim Behav.

Desjardins JK, Stiver KA, Fitzpatrick JL, Balshine S. 2008. Differential responses to territory intrusions in cooperatively breeding fish. Anim Behav. 75:595–604.

Desjardins JK, Stiver KA, Fitzpatrick JL, Milligan N, Van der Kraak GJ, Balshine S. 2008. Sex and status in a cooperative breeding fish: behavior and androgens. Behav Ecol Sociobiol. 2:785–794.

Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. Ecol Lett. 8:968–975.

Dierkes P, Taborsky M, Kohler U. 1999. Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. Behav Ecol. 10:510–515.

Fisher RA. 1941. Average excess and average effect of a gene substitution. Ann Eugen. 11:53–63.

Fitzpatrick JL, Desjardins JK, Milligan N, Stiver KA, Montgomerie R, Balshine S. 2008. Female-mediated causes and consequences of status change in a cooperatively breeding fish. Proc R Soc Lond B Biol Sci. 275:929–936.

Fitzpatrick JL, Desjardins JK, Stiver KA, Montgomerie R, Balshine S. 2006. Male reproductive suppression in the cooperatively breeding fish, *Neolamprologus pulcher*. Behav Ecol. 17:25–33.

Foerster K, Valcu M, Johnsen A, Kempenaers B. 2006. A spatial genetic structure and effects of relatedness on mate choice in a wild bird population. Mol Ecol. 15:4555–4567.

Frostman P, Sherman PT. 2004. Behavioral response to familiar and unfamiliar neighbors in a territorial cichlid, *Neolamprologus pulcher*. Ichthyol Res. 51:283–285.

Goodnight KF, Queller DC. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Mol Ecol. 8:1231–1234.

Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. Anim Behav. 26:645–652.

Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? Science. 218:384–387.

Hanby JP, Bygott JD. 1987. Emigration of subadult lions. Anim Behav. 35:161–169.

Hazelden M. 2004. Hormones, aggression, and kin recognition in a cooperatively breeding cichlid, *Neolamprologus pulcher* [BSc thesis]. Ontario (Canada): McMaster University.

Heg D, Bachar Z, Brouwer L, Taborsky M. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. Proc R Soc Lond B Biol Sci. 271:2367–2374.

Heg D, Brouwer L, Bachar Z, Taborsky M. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. Behaviour. 142:1615–1641.

Heg D, Heg-Bachar Z, Brouwer L, Taborsky M. 2008. Experimentally induced helper dispersal in colonially breeding cooperative cichlids. Environ Biol Fishes. doi: 10.1007/s10641-007-9317-3.

Hert E. 1985. Individual recognition of helpers by the breeders in the cichlid fish, *Lamprologus brichardi* (Poll 1974). Z Tierphychol. 68:313–325.

Jadwiszczack P. 2003. Rundom Projects 2.0 LITE. [Internet]. [cited 2006 May]. Available from: http://pjadw.tripod.com.

Jakob EM, Marshall SD, Uetz GW. 1996. Estimating fitness: a comparison of body condition indices. Oikos. 77:61–67.

Keane B, Creel SR, Waser PM. 1996. No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. Behav Ecol. 7:480–489.

Keller LF, Arcese P. 1998. No evidence for inbreeding avoidance in a natural population of song sparrows (*Melospiza melodia*). Am Nat. 152:380–392.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. Trends Ecol Evol. 17:230–241.

Kleven O, Jacobsen F, Roberston RJ, Lifjeld JT. 2005. Extrapair mating between relatives in the barn swallow: a role for kin selection? Biol Lett. 1:389–392.

Koenig WD, Haydock J. 2004. Incest and incest avoidance. In: Koenig W, Dickinson J, editors. Ecology and evolution of cooperative breeding in birds. Cambridge: Cambridge University Press. p. 142–156.

Koenig WD, Haydock J, Stanback MT. 1998. Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition. Am Nat. 151:243–255.

Koenig WD, Stanback MT, Haydock J. 1999. Demographic consequences of incest avoidance in the cooperatively breeding acorn woodpecker. Anim Behav. 57:1287–1293.

Kohler U. 1997. Zur Struktur und Evolution des Sozialsystems von Neolamprologus multifasciatus (Cichlidae, Pisces), des kleinsten Schneckenbuntbarsches des Tanganjika-Sees [PhD thesis]. München (Germany): Ludwig-Maximilians-Universität.

Kokko H, Ots I. 2006. When not to avoid inbreeding. Evolution. 60:467–475.

Lehmann L, Perrin N. 2003. Inbreeding avoidance through kin recognition: choosy females boost male dispersal. Am Nat. 162:638–652.

Limberger D. 1983. Pairs and harems in a cichlid fish, *Lamprologus brichardi*. Z Tierpsychol. 62:115–144.

Margulis S. 1997. Inbreeding-based bias in parental responsiveness to litters of oldfield mice. Behav Ecol Sociobiol. 41:177–184.

Mulder RA, Dunn PO, Cockburn A, Lazenby-Cohen KA, Howell MJ. 1994. Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. Proc R Soc Lond B Biol Sci. 255:223–229.

O'Riain MJ, Bennett NC, Brotherton PNM, McIlrath G, Clutton-Brock TH. 2000. Reproductive suppression and inbreeding avoidance in wild populations of co-operatively breeding meerkats (*Suricata suricatta*). Behav Ecol Sociobiol. 48:471–477.

Pacitto M. 2005. Kin preferences and body colouration in relation to dominance status in a co-operatively breeding cichlid fish [BSc thesis]. Ontario (Canada): McMaster University.

Parker A, Kornfield I. 1996. Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. Environ Biol Fishes. 47:345–352.

Parker GA. 1979. Sexual selection and sexual conflict. In: Blum MS, Blum NA, editors. Sexual selection and reproductive competition in insects. New York: Academic Press. p. 123–166.

Pillay N. 2002. Inbreeding in Littledale's whistling rat *Parotomys littledalei*. J Exp Zool. 293:171–178.

Piron RD. 1978. Breeding the convict cichlid (*Cichlasoma nigrofasciatum*) for use in laboratory fish toxicity tests. J Fish Biol. 13:119–122.

Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. Trends Ecol Evol. 11:201–206.

Pusey AE. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends Ecol Evol. 2:295–299.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. Evolution. 43:258–275.

Ralls K, Ballou JD, Templeton A. 1998. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conserv Biol. 2:185–193.

Rousset F. 2002. Inbreeding and relatedness coefficients: what do they measure? Heredity. 88:371–380.

Schliewen UK, Rassmann K, Markmann M, Markert J, Kocher TD, Tautz D. 2001. Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. Mol Ecol. 10:1471–1488.

Sharland K. 2006. To choose or not to choose? Mate selection in *Neolamprologus pulcher* [BSc thesis]. Ontario (Canada): McMaster University.

Sheldon BC. 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol Evol. 15:397–402.

Smith RH. 1979. On selection for inbreeding in polygynous animals. Heredity. 43:205–211.

Stiver KA, Desjardins JK, Fitzpatrick JL, Neff B, Quinn JS, Balshine S. 2007. Evidence for age and sex specific dispersal in a cooperatively breeding fish. Mol Ecol. 16:2974–2984.

Stiver KA, Dierkes P, Taborsky M, Balshine S. 2004. Dispersal patterns and status change in a cooperatively breeding fish; evidence from microsatellite analyses and behavioral observations. J Fish Biol. 65:91–105.

Stiver KA, Dierkes P, Taborsky M, Gibbs HL, Balshine S. 2005. Relatedness and helping in fish: examining the theoretical predictions. Proc R Soc Lond B Biol Sci. 272:1593–1599.

Stiver KA, Fitzpatrick J, Desjardins JK, Balshine S. 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. Anim Behav. 71:449–456.

Taborsky M. 1984. Brood care helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. Anim Behav. 32:1236–1252.

Taborsky M. 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. Behaviour. 95:45–75.

Taborsky M, Limberger D. 1981. Helpers in fish. Behav Ecol Sociobiol. 8:143–145.

Taylor MI, Meardon F, Turner G, Seehausen O, Mrosso HDJ, Rico C. 2002. Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a *Pundamilia pundamilia* × *Pendamilia nyererei* hybrid. Mol Ecol Notes. 2:443–445.

Thünken T, Bakker TCM, Baldauf SA, Kullmann H. 2007. Active inbreeding in a cichlid fish and its adaptive significance. Curr Biol. 17:225–229.

Tomkins JL, Simmons LW. 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. Anim Behav. 63:1009–1016.

Waller DM. 1993. The statistics and dynamics of mating system evolution. In: Thornhill NW, editor. The natural history of inbreeding and outbreeding. Chicago: University of Chicago Press. p. 97–117.

Waser PM, Austad SN, Keane B. 1986. When should animals tolerate inbreeding? Am Nat. 128:529–537.

Wedekind C, Seebeck T, Bettens F, Paepke AJ. 1995. MHC-dependent mate preferences in humans. Proc Biol Sci. 260:245–249.

Werner NY, Balshine S, Leach B, Lotem A. 2003. Helping opportunities and space segregation among helpers in cooperatively breeding cichlids. Behav Ecol. 14:749–756.

Winemiller KO, Taylor DH. 1982. Inbreeding depression in the convict cichlid, *Cichlasoma nigrofasciatum* (Baird and Girard). J Fish Biol. 21:399–402.

Wolff JO. 1992. Parents suppress reproduction and stimulate dispersal in opposite-sex juvenile white-footed mice. Nature. 359:409–410.

Woolfenden GE, Fitzpatrick JW. 1978. The inheritance of territory in group-breeding birds. Bioscience. 28:104–108.

Woolfenden GE, Fitzpatrick JW. 1984. The Florida scrub-jay: demography of a cooperative-breeding bird. Princeton (NJ): Princeton University Press.

Zardoya R, Vollmer D, Craddock C, Streelman J, Karl S, Meyer A. 1996. Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (*Pisces: Perciformes*). Proc R Soc Lond B Biol Sci. 263:1589–1598.