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# BRIEF COMMUNICATIONS

## Mixed parentage in *Neolamprologus pulcher* groups

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Genetic data collected on co-operatively breeding *Neolamprologus pulcher* groups from Lake Tanganyika revealed mixed parentage in 80% of the groups examined. A case (1/11) of shared maternity was detected where a subordinate female bred alongside the dominant female in a social group. Extra-pair paternity was assigned to other dominant males who held their own social groups, but subordinate males were not found to father young in any group (0/9).

Key words: Cichlidae; exclusion; maternity; paternity; reproductive skew.

In the past, dominant individuals in a social group were often assumed to be the exclusive parents of young in that group. However, easy access to genetic parentage data has challenged this view, and both subordinate group members and individuals from other social groups have been identified as the true parents of offspring in many social groups (Richardson *et al.*, 2001). Mixed parentage within social groups can occur as a result of (1) reproductive concessions by dominant individuals (Johnstone, 2000), (2) tug-of-war over reproduction within the group (Johnstone, 2000) or (3) parasitic spawning by other sexually mature individuals (Taborsky, 1998). This study used microsatellite markers to examine mixed parentage in *Neolamprologus pulcher* (Trewavas & Poll), a co-operatively breeding cichlid fish endemic to Lake Tanganyika. This species lives in mixed-sex groups comprising a dominant pair and one to 20 subordinate individuals; all individuals contribute to care of young

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(Taborsky & Limberger, 1981; Balshine *et al.*, 2001; Desjardins *et al.*, 2008). Past work has suggested that a conflict over reproduction exists between dominants and subordinates (Skubic *et al.*, 2004; Heg *et al.*, 2006), and laboratory studies have shown mixed parentage among group members (Dierkes *et al.*, 1999; Heg & Hamilton, 2008). However, in these laboratory studies, dominant individuals were unable to enter other groups and were therefore unable to attempt to gain parentage outside their group.

Unlike the laboratory, assessing parentage in the wild fish presents a logistical challenge as *N. pulcher* spawns underneath complex rock formations, 3–45 m underwater (Taborsky & Limberger, 1981; Balshine *et al.*, 2001). Young cannot be reliably collected until they are free swimming. Groups are closely clustered into subpopulations, and although group members aggressively defend their territory from non-group members, individuals have been observed to 'visit' other groups (Bergmüller *et al.*, 2005; Heg *et al.*, 2008). Therefore, it is possible that in the wild, dominant individuals may lose parentage not only to subordinate group members as previously argued (Dierkes *et al.*, 1999, 2008; Bender *et al.*, 2008; Heg & Hamilton, 2008) but also to visiting large dominant individuals from other groups (Taborsky, 1998; Fitzpatrick *et al.*, 2006). Additionally, mixed parentage within a group could result from a recent breeder turnover, a fairly common event in this species (Balshine *et al.*, 2001; Stiver *et al.*, 2004; Dierkes *et al.*, 2005). Dierkes *et al.* (2008) found mixed parentage in wild sampled broods; however, the true parents of these extra-pair young could not be identified.

In this study, wild fish were genetically sampled between February and April 2005 on the Zambian shores of Lake Tanganyika (Kasakalawe Bay 8°46·87′ S; 31°04·88′ E). The parentage of all fish under 10 mm in size was assessed in five groups [see Dierkes *et al.* (2008) for similar size classification]. Groups with young were identified as soon as young emerged from the brood chamber and monitored up to 1 week prior to collection (Desjardins *et al.*, 2008). In addition to genotyping all group members in these five groups, 97 dominant individuals from the same subpopulation were sampled (see Table I for details of all sampled individuals). Collection and genetic techniques are described in Stiver *et al.* (2007). Briefly, individuals were genotyped at three to 12 microsatellite loci developed for other cichlid species and optimized for use in *N. pulcher* (LOC101, ML007, NP773, Ppun21, Pzeb1, Pzeb3, Pzeb4, TmoM13, TmoM11, UME003, UNH154, US783; Heg *et al.*, 2006; Stiver *et al.*, 2007).

Parentage assignment was primarily based on exclusion, with allowance for a single tandem repeat-unit mismatch to account for errors arising from variation in DNA sequence, quality of DNA and scoring error (Pompanon *et al.*, 2005). A manual comparison of individual genotypes was employed and LOD scores were calculated in CERVUS 3.0 (Marshall *et al.*, 1998). (LOD scores are the natural log of the overall likelihood ratio of parentage for a candidate parent). Individuals considered as potential parents of young were the dominant and subordinate group members as well as sexual mature individuals from the same subpopulation as the group being examined. In cases where only one allele was recovered at a locus (Pompanon *et al.*, 2005), that locus was not considered in the comparison unless there was an allelic match. If both the group dominant and another sampled dominant individual could be

TABLE I. Demographic information on the sampled groups of *Neolamprologus pulcher* and number of dominant males and females sampled from the subpopulation each group was located in. The parentheses in the 'Number of subordinates' column indicate the sex of the subordinates, ordered from largest to smallest. F, female; M, male and U, sex unknown. Subordinate sex is in bold when that subordinate was 'breeding' or 'dominant' sized [falling within the 95% confidence interval of size for dominants of their sex; see Stiver *et al.* (2007) for a similar cut-off]. While sample sizes precluded examination of how individual size related to parentage, the sizes of dominant males and females and size range of subordinates are reported to allow for reader comparison (all sizes in centimetre)

| Group | Number of dominants sampled in the subpopulation (females, males) | Number of young sampled | Number (and sex) of subordinates in each sampled group | Size of dominants (females, males) | Subordinate size range |
|---|---|---|---|---|---|
| A | 26, 27 | 1 | 6 (**MF**FUUU) | 5·33, 6·16 | 2·01–5·50 |
| B | 26, 27 | 14 | 4 (**F**MFM) | 5·08, 5·61 | 3·54–4·61 |
| C | 26, 27 | 12 | 6 (MFFFFU) | 5·24, 5·49 | 2·63–4·65 |
| D | 9, 8 | 6 | 6 (**F**MMFFU) | 5·34, 6·10 | 2·45–4·73 |
| E | 14, 13 | 10 | 4 (**M**MMU) | 5·21, 6·00 | 2·54–5·59 |

assigned as a parent, parentage was assigned to the dominant group member except when the LOD score to the other potential parent was significant and exceeded that to the dominant group member. This approach reduces the likelihood of falsely rejecting the null hypothesis that the dominant individuals are the parents of the young (Taborsky & Limberger, 1981; Dierkes *et al.*, 2008). Occasionally, both the dominant male and female were potential parents of the young but could not both be parents (as they each shared the same allele with the offspring). In those cases, parentage was assigned to that individual who had the higher probability of parentage based on LOD scores. These analyses also allowed for examination of genetic similarity between other group members (dominants and subordinates).

To control for pseudoreplication arising from multiple young being compared with the same dominant individuals, statistical tests were conducted RUNDOM 2.01 LITE using 10 000 randomizations (Jadwiszczack, 2003; Stiver *et al.*, 2007). All tests were two-tailed.

Parentage results are summarized in Table II (Fig. 1). Young had a higher mean LOD score to their assigned father (mean LOD score ± S.E. = 1·18 ± 0·23; $n = 36$) than to the excluded dominant male, supporting the exclusion assignment (−3·38 ± 0·72; $n = 17$; two-sample randomization–permutation test, $P < 0·0001$). Similarly, the mean LOD score to their assigned mother (1·02 ± 0·60; $n = 35$) was greater than that to the excluded dominant female (−3·01 ± 0·83; $n = 11$; $P < 0·01$).

In four of five groups, multiple full sibling sets could be identified. Multiple maternity was found in two of five groups and multiple paternity in three of five groups. Dominant females were mothers of the majority (74·4%) of young in their groups and 83·3% of the sibling sets (broods). Dominant males sired 70·7% of the young (70·0% of the sibling sets or broods). There was no evidence that male subordinates gained paternity, but there was confirmation of female

TABLE II. Parentage summary, with per cent young assigned to the dominant female and dominant male of each sampled group. Details of the parentage for each set of siblings within each group are also given. Data in bold represent groups where the dominant female or the dominant male were not the sole mother or father of all young

| Group | % Young of dominant female | % Young of dominant male | Sibling set | Young in set | Mother of set | Father of set |
|---|---|---|---|---|---|---|
| A | 100 | 100 | A-1 | 1 | Dominant female | Dominant male |
| B | 100 | **36** | B-1 | 6 | Dominant female | Dominant male in another group* |
| | | | B-2 | 4 | Dominant female | Dominant male |
| | | | B-3 | 4 | Dominant female | Dominant male in another group* |
| C | 100 | **58** | C-1 | 7 | Dominant female | Dominant male |
| | | | C-2 | 5 | Dominant female | Unidentified |
| D | **50** | **67** | D-1 | 3 | Subordinate female | Dominant male |
| | | | D-2 | 2 | Dominant female | Unidentified |
| | | | D-3 | 1 | Dominant female | Dominant male |
| E | **20** | 100 | E-1 | 8 | Unidentified | Dominant male |
| | | | E-2 | 2 | Dominant female | Dominant male |

*The sizes of these dominant males (standard length) were 5·72 and 5·46 cm.

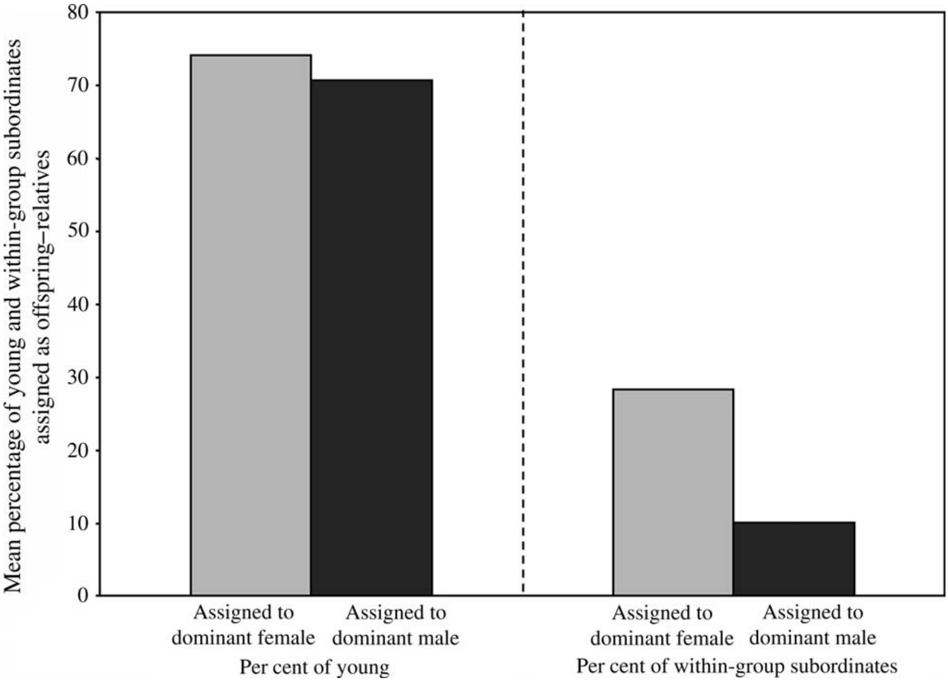

FIG. 1. Mean per cent of young and subordinate group members that were assigned as offspring or relatives of the dominant female and male of their group.

subordinates sharing reproduction with dominant females. In group A, the dominant male and female were the parents of the sole young collected. The dominant female in group B reproduced with three different males: the dominant male as well as two different dominant males from other groups in the same subpopulation. In Group C, the dominant female had young fathered by both the dominant male and the another unidentified male. In Group D, reproduction was shared between the dominant female and the largest subordinate female. In this group, the dominant male fathered the young of the subordinate female but was excluded as the father of two of the three young belonging to the dominant female. Finally, in Group E, the dominant male fathered young with both the dominant female and an unidentified female.

While there was reproductive sharing between a dominant and subordinate female, it was unclear whether similar reproductive sharing occurs in the wild between male group members. Male subordinates in the laboratory have fathered young in the group, and mixed paternity in the field has been suggested to result from subordinate males stealing fertilizations from the dominant male breeder, before being expelled from the group for this transgression (Dierkes *et al.*, 1999, 2008; Bender *et al.*, 2008). However, examination of a large sample of wild-caught group members suggests that male subordinates have decreased reproductive capabilities relative to the dominant individuals (Fitzpatrick *et al.*, 2006, 2008). Coupled with the identification of two extra-pair fathers who were dominant males from different groups in the subpopulation, this suggests that other dominant males in the population represent a greater threat to male paternity in the wild than subordinate male group members do. Reproductive competition from other dominant individuals has been documented in several species (Taborsky, 1998), and one striking example can be seen in *Telmatochromis vittatus* Boulenger, where the largest and most dominant males in the area exclude territorial males during spawning (Ota & Kohda, 2006).

All young in a social group were treated as a single cohort, as they were followed from emergence to collection and appeared to be of a uniform size. However, their relative age was not known with certainty and it cannot be determined absolutely whether or not these young represent a single, or multiple, reproductive events. As shared maternity most often results from broods laid on different days (Heg & Hamilton, 2008), and young of two distinct size classes have been observed in wild groups of *N. pulcher* (pers. obs.), the two sibling groups in groups D and E are most likely from two distinct broods. In D, the subordinate and dominant females each produced a brood (although two of the three surviving young of the dominant female were not fathered by the dominant male). In E, it is possible that one sibling set was produced either by an unidentified dominant female who was subsequently replaced by the dominant female sampled (Balshine *et al.*, 2001; Stiver *et al.*, 2004; Dierkes *et al.*, 2005) or by a female subordinate who dispersed or died prior to observations and sampling (Dierkes *et al.*, 2008).

While mixed paternity may also result from male breeder turnover, at least two of the sibling groups from group B may result from mixed paternity within a single brood laid by the female. The fathers of the sibling sets in this group could all be identified as holding dominant positions in the subpopulation at the time of fry emergence and sampling. Female *N. pulcher* may continue laying eggs over the period of several hours (unpubl. obs.), allowing the opportunity for other males to perform extra-pair fertilizations if the dominant male is not effectively guarding the brood chamber (Taborsky, 1998).

Comparison of the genetic profiles revealed kin relationships between nine subordinates and their associated dominants. As expected from previous work (Dierkes *et al.*, 2005; Stiver *et al.*, 2005), the number related to their dominant female ($n = 7$) exceeded the number related to their dominant male ($n = 2$; Fig. 1). Subordinates in Group B (one female and two males), Group C (one male, one female and one individual of unknown sex) and Group D (one male) were relatives of the dominant female. Only in Group E were subordinate relatives of the dominant male detected (two males). As breeder turnover can be rapid in this species (Balshine *et al.*, 2001; Stiver *et al.*, 2004; Dierkes *et al.*, 2005), the size of these subordinates relative to their dominant relatives suggests that the relationship is more likely one of half sibling or full sibling rather than a parent–offspring relationship. The presence of sibling sets among the larger group members can be explained by either philopatry or group dispersal, both of which have been documented in this species (Dierkes *et al.*, 2005; Heg *et al.*, 2008). Which of these processes led to the observed sibling groupings cannot be concluded from this study, although evidence of sex differences in philopatry and dispersal (Dierkes *et al.*, 2005; Stiver *et al.*, 2004, 2007) suggests that the three dominant females living with subordinate siblings may have inherited the breeding position within their natal group, while the male dominant living with two male subordinate siblings may represent a group dispersal event. The findings from this study reveal new details of the natural history, parentage and mating patterns of a wild population of *N. pulcher*, highlighting the sex differences in reproduction and competition.

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