

# Intraspecific brood mixing and reduced polyandry in a maternal mouth-brooding cichlid

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Microsatellite loci were used to evaluate the level of polyandry and intraspecific brood mixing in *Protomelas c.f. spilopterus*, a paedophagous, maternal mouth-brooding cichlid from Lake Malawi, Africa. We found that broods were fertilized by one to three males, which was a reduced level of multiple paternity compared to other mouth-brooding cichlids. Low density of breeding males and the risk of intraspecific predation are likely explanations for reduced polyandry. Intraspecific brood-mixing was found in four out of the six broods examined, with the proportions of foreign fry ranging from 6% to 65%. The potential origins of brood mixing are discussed, although no firm conclusions can be drawn given the limited behavioral observations for this species. **Key words:** cichlids, foreign fry, intraspecific brood mixing, Lake Malawi, microsatellites, mouth brooding, multiple paternity, *Protomelas*. [*Behav Ecol* 9:309–312 (1998)]

The life history of species in the family Cichlidae is marked by a long period of parental care. In the more than 1000 species currently recognized, progeny are protected by one or both parents until they are free swimming (approximately 3–4 weeks), and many species continue to provide parental care beyond this period. Cichlid fishes are primarily either substrate brooders or mouth brooders. Substrate brooders maintain their broods on or close to the substrate, whereas mouth brooders carry offspring in their mouths until the fry are free swimming. In some mouth-brooding species, parents continue to guard fry after they are initially released by taking them back into the mouth. In both substrate- and mouth-brooding species, examples of male, female, and biparental care can be found (see Keenleyside, 1991, for a review).

The prolonged period of parental care in cichlids increases the opportunity for brood mixing between conspecifics as well as heterospecifics (see Keenleyside, 1991; Taborsky, 1994, for reviews). The pattern of parental care will obviously influence the odds of brood mixing. For example, intra- and interspecifically mixed broods have been well documented for numerous species of substrate brooders (e.g., Fraser, 1996; Fraser and Keenleyside, 1995; McKaye, 1977; McKaye and McKaye, 1977; Ochi and Yanagisawa, 1996; Ribbink et al., 1981; Ward and Wyman, 1977; Wisenden and Keenleyside, 1992;). The upper limit of the number of fry that can be incorporated into a school of mixed parentage might be expected to be flexible considering that it is only limited by the defense capabilities of the parents and in some cases by the availability of shelter for the young (Keenleyside, 1991). In contrast, maximum brood size in mouth-brooding species is more rigid considering the limiting factor is the size of the buccal cavity (mouth) of the guarding parent (Keenleyside, 1991). Despite this restriction, there have been several documented cases of mouth brooding intra- and interspecifically mixed broods, although many of these examples involved species with reduced

mouth-brooding periods (Lewis, 1980; Ochi and Yanagisawa, 1996; Ochi et al., 1995; Ribbink, 1977; Ribbink et al., 1980, 1981; Sato, 1986; Mrowka, 1987; Yanagisawa, 1985; 1986).

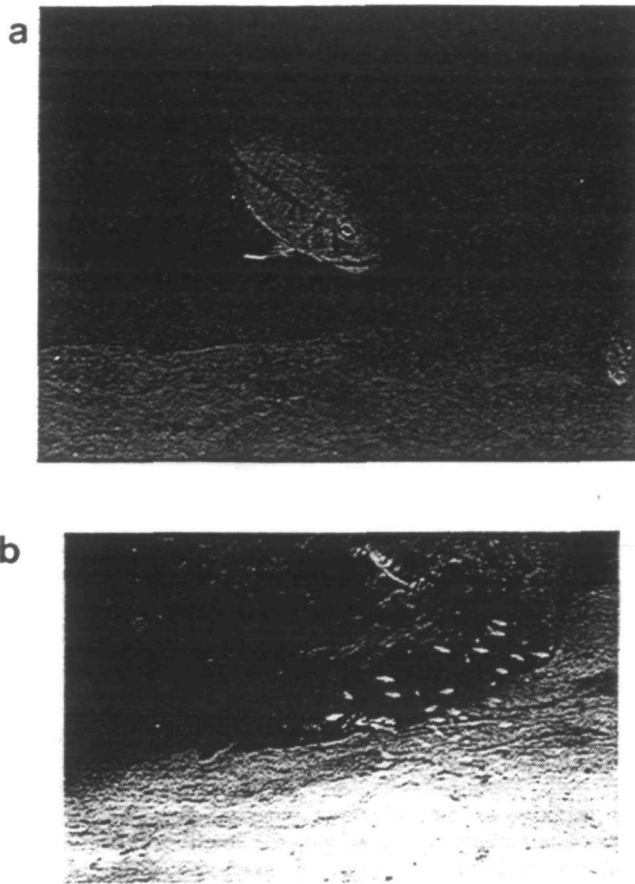
In addition to the pattern of parental care, the diet of the host species might also influence the likelihood of foreign fry adoption (Ribbink et al., 1980). Conceivably, different trophic types (i.e., piscivores) might be more prone to consuming foreign fry, and hence, females and/or fry that avoid such trophic types might be selected for. To date, however, mixed brooding has been demonstrated for piscivores (Lewis, 1980; Mrowka, 1987; Ochi and Yanagisawa, 1996; Ribbink et al., 1980; Ward and Wyman, 1977), insectivores (Mrowka, 1987; Ochi and Yanagisawa, 1996; Ribbink et al., 1980; Ward and Wyman, 1977; Yanagisawa 1985, 1986), herbivores (Fraser, 1996; Fraser and Keenleyside, 1995; McKaye, 1977; McKaye and McKaye, 1977; Ochi and Yanagisawa, 1996; Ribbink, 1977; Ribbink et al., 1980; Ward and Wyman, 1977; Wisenden and Keenleyside, 1992), and scale eaters (Ochi and Yanagisawa, 1996).

*Protomelas c.f. spilopterus* (Trewavas), a female mouth-brooder, is widely distributed throughout Lake Malawi in intermediate habitat (at the rock and sand interface) at average depths of 10 m (Konings, 1990). The structural resemblance of its mouth and pharyngeal dentition to known paedophages (feeding specialists that either ram brooding females to dislodge their eggs/fry for consumption or dart between breeding pairs to consume eggs) in lakes Malawi and Victoria strongly suggest that it is also a paedophage (Eccles and Trewavas, 1989). In addition, we have observed *P. c.f. spilopterus* exhibiting paedophagous feeding behaviors. Territorial males in breeding coloration have been seen in June (Konings, 1990) and March (Stauffer JR Jr., personal observation) and are often encountered singly (Konings, 1990). One of us (J.R.S.), however, has observed four to five territorial males in a loose cluster spanning an area of approximately 25 m<sup>2</sup>. Males typically construct semicircular bowers (sand structures used strictly for courtship and spawning; McKaye et al., 1990) against or underneath rocks (Konings, 1990). Females orally brood eggs for an unknown period of time (Figure 1a) and continue to guard the fry by taking them back into the mouth after the initial release (Figure 1b; Konings, 1990).

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**Figure 1**  
 (a) A brooding female *Protomelas c.f. spilopterus*. Note the distended gular (chin) region which is characteristic of a brooding individual.  
 (b) A female cichlid [species *Tyrannochromis macrostoma* (Regan)] gathering fry back up into her mouth.

The original objective of this study was to evaluate the level of polyandry in *P. c.f. spilopterus* using hypervariable microsatellite sequences (DNA fingerprinting). In an earlier study, we found this species to be a rare exception to multiple paternity in Lake Malaŵi cichlids (Kellogg et al., 1995; see also Parker and Kornfield, 1996), and we wanted to further explore this phenomenon. On examination of several additional broods, however, we discovered that intraspecific mixing of broods was quite common. Considering that intraspecifically mixed-broods had been infrequently demonstrated in maternal mouth-brooders, and furthermore never demonstrated for a paedophage, the focus of the research was expanded to evaluate mixed brooding.

## METHODS

We captured six families of *P. c.f. spilopterus* using monofilament gill nets while scuba diving on Mazinzi Reef in Lake Malaŵi (see Stauffer et al., 1997, for a map of the location). A family consisted of a brooding female and all the fry that she was holding. We witnessed no loose fry during the capture of each female. All fry and tissue samples (muscle from above the lateral line) of the female were stored in liquid nitrogen for transport to the laboratory. The remaining portion of each adult specimen was fixed in 10% formalin and preserved in 70% ethanol for long-term storage of the vouchers in The Pennsylvania State University Fish Museum (catalog numbers PSU3163, PSU3164, and PSU3165).

Microsatellite loci were isolated using the methods of Puli-do and Druyk (1994), which are described in detail by Lee and Kocher (1996). We used loci UNH001 in combination with either UNH002 or UNH050 (see GenBank accession numbers U17044, U17045, and AF036714 for sequences; see also Kellogg et al., 1995; Lee and Kocher, 1996). Before DNA extraction, all fry were inspected to identify species and assess developmental stage using the scheme of Anken et al. (1993) for approximation. DNA digestion, extraction, amplification, and analysis methods followed those in Kellogg et al. (1995). Within each family, fry were first evaluated for maternal alleles that matched those of the female carrying them. Those individuals that did not possess matching maternal alleles were assumed to be offspring from a foreign brood. The subset of fry within each family that displayed matching maternal alleles was included in the analysis of multiple paternity. To estimate paternity levels within a brood, we assumed that all paternal individuals were heterozygous (based on the observed heterozygosities of 0.75 or greater; see below); the number of paternal individuals per brood was inferred to be the smallest whole number greater than or equal to one-half the number of paternal dilocus genotypes in that brood (Kellogg et al., 1995; Parker and Kornfield, 1996). Alleles were assigned to paternal genotypes to minimize deviations from expected patterns of Mendelian inheritance (see Parker and Kornfield, 1996, for illustration).

## RESULTS

In 6 females and their 200 offspring, we observed 13 alleles at locus UNH001 ( $n = 183$ ), 7 at locus UNH002 ( $n = 103$ ), and 12 at locus UNH050 ( $n = 86$ ). Observed heterozygosities were 87.43% (UNH001), 74.76% (UNH002), and 97.67% (UNH050). The number of paternal alleles for the six broods that were examined ranged from two to five indicating that one to three males per female contributed fertilizations (Table 1).

Four of the six broods that we examined were composed of host as well as foreign fry (all visually identified as *P. c.f. spilopterus*), and the proportion of foreign fry ranged from 6% to 65% (Table 1). The development stage of mixed broods ranged from 14 (categorized by pectoral fin buds and lower jaw movement, approximately 5.5 days postfertilization) to 20 (categorized by the visibility of the pelvic fin anlage, approximately 16.5 days postfertilization) (Anken et al., 1993). There were no noticeable differences in the length or developmental stage of the foreign versus host fry. A general trend of increased proportions of foreign fry with advanced developmental stages was evident; however there were exceptions (i.e., brood 4 in Table 1).

## DISCUSSION

High levels of multiple paternity have been demonstrated for numerous cichlid species (Kellogg et al., 1995; Parker and Kornfield, 1996). Many cichlids of Lake Malaŵi, in particular the species that inhabit the sand, are truly lekking (Höglund and Alatalo, 1995). Large numbers of males (in some cases up to 50,000) aggregate and defend territories for mating (McKaye, 1991). Among the sand dwellers, females mate with two to six different males, which is not surprising given that there are many potential mates clustered at high densities (Kellogg et al., 1995). The cichlids that inhabit rocky areas of Lake Malaŵi also show multiple paternity, with two to six males contributing to broods (Kellogg et al., 1995; Parker and Kornfield, 1996). Male rock-dwelling cichlids aggregate in a leklike nature, and hence, also occur in relatively high densities.

Table 1

Maternal and inferred paternal genotypes at microsatellite loci UNH001 and UNH002 or UNH050 for six broods of *Protomelas c.f. spilopterus*

Brood	Maternal genotype	Paternal genotypes			No. of fry examined	Proportion of foreign fry (%)	Developmental stage
		1	2	3			
1 UNH001	207/213	194/206	183/187	191/?	32	6	14
UNH002, UNH050	333/333	323/331	351/359				
2 UNH001	167/183	177/201			35	0	15
UNH002, UNH050	188/198	193/197					
3 UNH001	167/167	183/191	194/?		31	65	19
UNH002, UNH050	198/200	197/199	204/?				
4 UNH001	189/221	193/207			36	0	19–20
UNH002, UNH050	337/353	321/327					
5 UNH001	176/184	188/190	183/217		32	56	19–20
UNH002, UNH050	198/198	199/215	197/?				
6 UNH001	191/193	183/193			34	53	19–20
UNH002, UNH050	321/321	300/350					

Question marks indicate that only one paternal allele was observed in the juvenile genotype, hence the other is unknown or possibly a homozygote.

*Protomelas c.f. spilopterus* displayed reduced levels of multiple paternity with typically one or two males contributing to broods and a single family showing contributions from three males. This species inhabits intermediate habitats at the rock/sand interface. Males are often encountered singly (Konings, 1990) or in a loose aggregation of four to five males (Stauffer JR Jr., personal observation). Therefore, the reduced polyandry that has been identified is perhaps a function of low male densities.

An alternative explanation centers on the specialized feeding mode of this species. Again, this species is a paedophage, feeding heavily on the eggs and fry of brooding females. It could be that females mate with only one male because searching and interacting with other males might result in egg predation on the eggs already being carried (Kellogg et al., 1995). A better understanding of male spatial densities as well as field observations of mating behaviors would help shed light on the causes of reduced polyandry in this species.

In addition to reduced multiple paternity, intraspecific brood-mixing appeared to be quite common (four out of the six broods examined), even in early fry development. As mentioned previously, both intra- and interspecific brood-mixing have been demonstrated for several cichlid species (see Keenleyside, 1991; Taborsky, 1994, for reviews). The origin of brood mixing has traditionally been explained in several ways (Ochi et al., 1995): (1) parents carry the offspring in their mouths and put some under the care of foster parents (termed "farming-out"; McKaye et al., 1992; Yanagisawa, 1985, 1986); (2) foster parents kidnap the young (McKaye, 1985; McKaye and McKaye, 1977); (3) aggressive behavior between parents of two adjacent broods results in the winner's acquisition of the loser's brood (Lewis, 1980); (4) young of guarded broods close to each other simply mix (McKaye, 1977); and (5) deserted or stray young intrude into unrelated broods (Ribbink, 1977; Ribbink et al., 1980). In addition, kinship relationships of brooding females might influence the likelihood of mixed brooding, as suggested by kin selection theory (Maynard Smith, 1964). Each of the above explanations carry implications regarding whether or not there are potential benefits to the parties involved. For example, farming-out might benefit parents that are relocating their young. With reduced parental effort, they are ensuring the survivorship of at least some of their offspring (McKaye et al., 1992;

Yanagisawa, 1985, 1986). Both kidnapping and aggressive behavior have been shown to benefit the kidnapping parents due to reduced predation on their offspring in mixed broods (Lewis, 1980; McKaye, 1985; McKaye and McKaye, 1977). Accepting closely related offspring into a brood could result in gene proliferation. Finally, the benefits, if any, of accidental mixing (explanations 4 and 5 above) are unclear.

Unfortunately, kinship relationships could not be established with the current molecular data. In addition, because there are so few field observations for *P. c.f. spilopterus*, we cannot comment extensively on the origin of brood mixing in this species. For several sand-dwelling species we have located dense clusters of brooding females in what are termed "nursery areas." *Protomelas c.f. spilopterus* has not been observed in such dense clusters (Konings, 1990), although a group of four to six brooding females has been observed at densities of approximately 1 female per m<sup>2</sup> (Stauffer JR Jr., personal observation). Aggressive behavior and accidental mixing seem somewhat unlikely given the low densities of *P. c.f. spilopterus* females. In addition, two broods in late developmental stages [one brood in developmental stage 19–20 and another brood from a previous study in developmental stage 25 (categorized by complete embryonic development, approximately 24 days postfertilization; Kellogg et al., 1995)] did not contain foreign fry, indicating that brood mixing is not a universal phenomenon in this species. If brood mixing were the result of aggressive behavior and accidental mixing, which is in turn influenced by high densities of guarding females, one might expect mixed broods to be ubiquitous. Finally, one brood that was early in development (developmental stage 14; see Table 1) included foreign fry. If brood mixing was indeed accidental, one would expect foreign fry to be in later developmental stages, presumably closer to the free-swimming stage (approximately developmental stage 23; about 22 days postfertilization; Anken et al., 1993). Again, more field data are needed before any conclusions can be drawn.

In general, the true origin of brood mixing remains unclear because of the difficulty of observing the process of mixing and in particular of detecting intraspecific mixing. For studies of intraspecific mixing, investigators have relied on length differences to delineate among cohorts (Ochi et al., 1995). Length differences, however, may not be a reliable indicator

of brood mixing considering that we observed no noticeable differences in the broods examined for this study. Microsatellites are an ideal tool for exploring the prevalence, conditions, and origins of brood mixing.

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