MOLECULAR ECOLOGY

Molecular Ecology (2012) doi: 10.1111/j.1365-294X.2012.05573.x

Brood mixing and reduced polyandry in a maternally mouthbrooding cichlid with elevated among-breeder relatedness

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Abstract

Uniparental maternal brood care often coincides with multiple paternity and single maternity of broods, possibly reflecting benefits of polyandry and costs of uniparental care. Genetic data from the maternally mouthbrooding cichlid fish *Simochromis pleurospilus* revealed the opposite pattern – low polyandry and allomaternal care. More than 70% of the investigated females had mated with a single male, and 14% of the females had unrelated fry in their broods. Broods with foreign fry were in the late stage of brood care, in which females guard free-swimming fry and recall the broods into their mouths for protection. With one exception, fostering females were related to their adopted fry at the level of first cousins ($R_{QG} > 0.12$), but relatedness between fosters and adopted fry was not significantly higher than between fosters and fry tended by other females. Relatedness among breeders extended to the level of first-order relatives. Mean relatedness among contemporaneously breeding dams ($R_{QG} = 0.08$) was significantly higher than among dams breeding in different seasons ($R_{QG} = -0.04$), which suggests a temporal or spatial concentration of mouthbrooding relatives. Indeed, females sometimes brood in small groups. This behaviour may reduce brood predation but will increase the risk of brood mixing, which is possibly mitigated by low costs of brood care and indirect benefits accrued by relatedness among the breeders in the group. Remarkably, the apparent inbreeding potential did not give rise to bet-hedging polyandry or active avoidance of relatives, as half of the mated individuals were related at $R_{QG} > 0.13$ and polyandry did not coincide with high within-pair relatedness.

Keywords: brood care, conspecific brood parasitism, inclusive fitness, mating system, parentage, relatedness

Received 4 November 2011; revision received 13 February 2012; accepted 20 February 2012

Introduction

Multiple maternity and multiple paternity can co-occur in species with brood care. In species with biparental and maternal care, rates of multiple maternity are typically lower than rates of multiple paternity, while this pattern is reversed in species with paternal care (Avise *et al.* 2002; Bennett & Owens 2002; Liebgold *et al.* 2006; Kupfer *et al.* 2008; Coleman & Jones 2011). Several different mechanisms underlie mixed parentage, including multiple mating and brood parasitism. Multiple mating may be elicited, for example, to avoid inbreeding costs (Tregenza & Wedell 2002) or may be a consequence of alternative reproductive behaviours. For example, alternative male mating tactics such as sneaking are frequently employed in species with external fertilization (Oliveira *et al.* 2008) and may not only secure reproductive success for the parasitic male, but also provide genetic benefits to females (Reichard *et al.* 2007). Brood

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parasitism is presumably most successful and consequently adaptive for the parasite when brood care costs are low (Sorenson 1992), and hosts will fail to evolve defences if they are too costly (Krüger 2011) or if there are fitness advantages to adoption (Eadie *et al.* 1988; Wisenden 1999; Tallamy 2005). The presence of conspecific foster young is often detrimental to the reproductive success of alloparental birds (Lyon & Eadie 2008), and in fish, it can even elicit cannibalism of the entire brood by the caring parent (Neff 2003; Rios-Cardenas & Webster 2005). Particularly in fish, however, alloparenting can also increase the reproductive success of fostering parents through predation dilution and increased attractiveness at low extra costs (McKaye & McKaye 1977; Taborsky 1994; Smith & Wootton 1995; Wisenden 1999; Stiver & Alonzo 2011).

Parental care in oviparous fishes typically includes guarding and fanning of eggs and fry, while provisioning of young is rare (Smith & Wootton 1995), and slight increases in brood sizes will have mild if any effects on the costs of brood care (Wisenden 1999). Alloparental care becomes more costly for fish parents for instance when brooding space is limited (Stiver & Alonzo 2011), which should lead to defences against foreign fry. In uniparental species, a high investment in brood care is also expected to prompt a careful choice of mating partners and has an effect on which and how many individuals of the noncaring sex will reproduce. For example, the risk of fitness losses due to inbreeding and variation in mate quality could be countered by the selection of a single high-quality partner (Lebigre *et al.* 2007) or by polygamous mating as a bet-hedging strategy (Tregenza & Wedell 2002).

Mouthbrooding is an energetically expensive form of brood care in which the fish parents incubate eggs and fry in their buccal cavity for varying lengths of time until the young can either be released and guarded for some additional time or have reached complete independence (Sefc 2011). Guarded fry are recalled into the parents' mouths when threatened by predation. Mouthbrooders in the freshwater fish family Cichlidae sometimes guard con- and heterospecific foreign fry and retrieve them into their mouths along with their own young (e.g. Ribbink 1977; Lewis 1980; Ribbink *et al.* 1980; Kuwamura 1988; Kellogg *et al.* 1998). While heterospecifics stand out among guarded fry by their distinct coloration and body shape (Ribbink 1977), the detection of conspecific foreign fry is less straightforward. It requires direct observations of a mixing event (Yanagisawa 1986; Ochi *et al.* 1995), indirect evidence such as pronounced size differences between fry of the same brood (Yanagisawa 1986; Kuwamura 1988; Ochi *et al.* 1995) or the increase in fry numbers with time (Yanagisawa 1986), or genetic parentage data (Kellogg *et al.* 1998). An increased brood size can increase the competition among fry for sheltering space and retard fry retrieval without offering any conceivable benefits to the mouthbrooder. Consequently, adoption has been considered maladaptive in mouthbrooders, and in the rare occasions that have been reported, it has been assumed to be either elicited by parasitic parents (Ribbink 1977; Yanagisawa 1986; Ochi & Yanagisawa 2005) or caused by an accidental mixture of fry (Lewis 1980). Theoretical models suggest that under certain conditions inclusive fitness benefits may be gained from sheltering the offspring of relatives, however (Zink 2000; López-Sepulchre & Kokko 2002; Tallamy 2005), and molecular techniques have provided evidence for kin adoption in several bird species (e.g. McRae & Burke 1996; Andersson & Ahlund 2000; Andersson & Waldeck 2007; Anderholm *et al.* 2009; Tiedemann *et al.* 2011). Compared to the widespread occurrence of brood care in animals, the taxonomic coverage of studies addressing intraspecific brood parasitism in relation to kinship is narrow. It has been mostly confined to waterfowl and thus it is not yet possible to assess whether relatedness and inclusive fitness are of general importance for adoption behaviour. With their enormous diversity of brood care patterns and the pervasiveness of alloparental care (Wisenden 1999), fishes are promising model organisms to study the relationship between kinship and adoption in more breadth. Genetic analyses of maternal and biparental mouthbrooding cichlid species in the field (Kellogg *et al.* 1995, 1998; Parker & Kornfield 1996; Taylor *et al.* 2003; Egger *et al.* 2006; Sefc *et al.* 2009; Haesler *et al.* 2011) have so far revealed alloparental mouthbrooding of conspecific fry in only one species, *Protomelas spilopterus* from Lake Malawi, but relatedness among the involved individuals was not examined (Kellogg *et al.* 1998). In agreement with the expected pattern for species with maternal care, the previous genetic studies also showed that single maternity was typically associated with high rates of multiple paternity of the maternally mouthbred broods (Kellogg *et al.* 1995; Parker & Kornfield 1996; Sefc *et al.* 2009; Haesler *et al.* 2011; but see Kellogg *et al.* 1998; Egger *et al.* 2006). Contrasting with this pattern, our present study on the maternal mouthbrooder *Simochromis pleurospilus* (Tropheini) from Lake Tanganyika provides genetic evidence for conspecific brood mixing and a low frequency of multiple paternity. We use microsatellite data to test whether fostering mouthbrooders are related to their adopted fry and whether this level of relatedness suggests kin-biased adoption or random brood mixing. Prompted by the occurrence of several closely related breeders in the population, we also investigate whether the risk of inbreeding is diminished by mate choice of unrelated individuals or by polyandrous mating. Together, these tests add to our understanding of the role of relatedness in mating and brood care decisions.

Material and methods

Sampling and DNA extraction

Simochromis pleurospilus is an algae-feeding inhabitant of the shallow rocky littoral in southern Lake Tanganyika, where territorial adult males and nonterritorial females and juveniles establish genetically structured populations (Kotrschal & Taborsky 2010; Kotrschal *et al.* 2012). Fourteen mouthbrooding females with a total of 283 offspring were collected in October 2004 (broods A and B; Tonga Village, 8°43.83′S, 31°8.4′E), October 2005 (broods C-F), March 2006 (broods G-L) and October 2006 (broods M and N; C-N at Kalambo Lodge, 08°36.51′S, 31°11.65′E). The sampling site at Kalambo Lodge comprises approximately 400 m^2, and all of the mouthbrooding females spotted within this area at each season were captured.

The broods were taken from the females' mouths and sacrificed by an overdose of clove oil. Their developmental stages ranged from egg to fully developed (Table 1). In addition, a population sample ($n = 16$ in Oct. 2005, $n = 15$ in March 2006 and $n = 4$ in Oct. 2006; total $n = 35$) was collected at Kalambo Lodge to estimate microsatellite marker polymorphism and allele frequencies in the Kalambo population. Mothers and fish caught for the population sample were released after fin clipping. The population sample contained numerous subadult individuals and noninvasive sex identification was not possible. Eggs, embryos, fry as well as fin clips of the mothers and the population sample were preserved in 99% ethanol. After measuring the total length of the fry, DNA was extracted with a standard Chelex protocol (Walsh *et al.* 1991).

PCR amplification and fragment analysis

Eight microsatellite markers were genotyped: UNH1009, UNH908 (both Carleton *et al.* 2002), UNH130 (Lee & Kocher 1996), UNH2016 (Albertson *et al.* 2003), Hchi6, Hchi36 (both Maeda *et al.* 2008), Ppun9 (Taylor *et al.* 2002) and Pmv3 (Crispo *et al.* 2007). The UNH markers were amplified as in Egger *et al.* (2006) and Sefc *et al.* (2009). Amplification of the remaining four loci employed a locus-specific reverse primer, a locus-specific forward primer with a 5′ CAG-tail (5′-CAG-TCGGGCGTCATCA-3′) and a fluorescent-labelled CAG oligonucleotide, which binds to the fragment created by the reverse primer and produces a labelled PCR product (Schuelke 2000; Mullen *et al.* 2006). The PCR parameters were as follows: 94 °C for 5 min; followed by 35 cycles of 94, 57 and 72 °C, each temperature for 30 s; followed by eight cycles with a touchdown reduction in annealing temperature of 0.5 °C per cycle; 7 min final extension at 72 °C. PCRs contained 0.4 μM of the reverse primer, 0.1 μM of the CAG-tagged forward primer, 0.4 μM of the labelled CAG oligonucleotide, as well as 0.5 U DNA

Table 1 Brood and fry sizes, collection sites and dates, level of polyandry and dam–sire relatedness coefficients (R_{QG})

Brood	Number of fry in brood	Fry size (TL)	Collection site, date	Sire number*	Number of offspring by primary sire (according to GERUD)	Dam–primary sire relatedness (R_{QG})
A	13	Egg	Tonga, Oct. 2004	1	13	n.a.
B	17	Egg	Tonga, Oct. 2004	1	17	n.a.
C	19	3 mm	Kalambo, Oct. 22, 2005	1	19	0.429
D	22	8 mm	Kalambo, Oct. 22, 2005	1	21	−0.435
E	7	5 mm	Kalambo, Oct. 22, 2005	2–3	6	n.a.
F	29	8 mm	Kalambo, Oct. 23, 2005	1	29	0.184
G	25	15 mm	Kalambo, March 25, 2006	1	17	0.094
H	19	15 mm	Kalambo, March 25, 2006	1–2	19	−0.214
I	13	10 mm	Kalambo, March 28, 2006	2	9	−0.083
J	13	15 mm	Kalambo, March 28, 2006	2	11	0.131
K	32	12 mm	Kalambo, March 29, 2006	1	32	0.409
L	27	2 mm	Kalambo, March 29, 2006	1	27	0.374
M	20	14 mm	Kalambo, Oct. 21, 2006	1	20	−0.042
N	27	4 mm	Kalambo, Oct. 23, 2006	1	27	0.306

*Sire numbers were estimated only from the mouthbrooder's own offspring, excluding adopted fry, to reflect the number of mates per female breeder.

polymerase (Biotherm), 1 × *Taq*-Puffer (Biotherm) with 1.5 mM $MgCl_2$ and 0.5 mM of each dNTP. PCR fragments were sized against an internal size standard (GeneScan-500 ROX; Applied Biosystems) using an ABI 3130xl automatic sequencer (Applied Biosystems) and GENEMAPPER 3.7 software (Applied Biosystems).

Parentage analysis

Marker polymorphism and Hardy–Weinberg equilibrium (Table 2) were analysed in GERUD 2.0 (Jones 2005) and Arlequin (Excoffier *et al.* 2005). Parentage analyses employed the program GERUD 2.0, which determines the minimum number of sires explaining the genotypes of offspring in a brood, and COLONY 2.0 (Jones & Wang 2010), which includes population allele frequencies and Mendelian segregation probabilities in a maximum-likelihood model for sibship reconstruction. COLONY analyses were first run with a genotyping error rate of 10% to identify potential errors. Electropherograms with putative erroneous alleles were double-checked, and analyses were rerun with the error rate set to zero. Replicate PCRs confirmed the incompatible genotypes of fry and mouthbrooders in mixed broods. Paternity reconstruction in GERUD requires that all offspring share the same mother; accordingly, each brood was analysed separately, and adopted fry (identified by alleles not present in the mouthbrooding female, from which they were collected) were removed from the data. In contrast, COLONY simultaneously infers full-sibs, maternal and paternal half-sibs from all female and offspring genotypes and hence reveals multiple paternity and multiple maternity of broods and the sharing of sires and dams across broods. In COLONY, the two broods from Tonga Village (A and B) were analysed separately from the broods sampled at Kalambo Lodge, without the inclusion of population allele frequencies as these were not available for this population.

Table 2 Marker polymorphism and exclusion probabilities. Mothers collected at Kalambo Lodge were added to the population sample for this analysis (total $n = 47$)

Locus	No. of alleles	Gene diversity	H_o	E_1	E_2
UNH2016	5	0.50	0.45	0.273	0.131
UNH1009	10	0.81	0.89	0.612	0.436
UNH130	11	0.73	0.73	0.527	0.343
UNH908	3	0.64	0.76	0.343	0.197
Hchi6	6	0.74	0.72	0.512	0.336
Hchi36	5	0.65	0.68	0.404	0.238
Ppun9	13	0.73	0.74	0.533	0.344
Pmv3	8	0.75	0.79	0.520	0.342
All Loci	mean 7.6	mean 0.69	mean 0.72	0.9943	0.9435

H_o, observed heterozygosity; E_1, exclusion probability when one parent is known; and E_2 when neither parent is known.

Analysis of pairwise relatedness and kinship

Pairwise relatedness coefficients (R_{QG} values) in the sample from Kalambo Lodge were calculated according to Queller & Goodnight (1989) in RELATEDNESS 5.0.8 (Goodnight Software, Houston; available at http://gsoftnet.us/GSoft.html). Using the allele frequency estimates from the population sample plus the females collected at Kalambo Lodge (Table 2), close relatedness among several breeding females and individuals of the population sample was detected. Following the recommendation to exclude potential relatives from the calculation of allele frequencies (RELATEDNESS manual), 11 individuals were removed from the data to eliminate 21 pairs with $R_{QG} > 0.4$, and allele frequencies calculated from this reduced sample ($n = 36$) were then used for final calculations of pairwise relatedness among all individuals. While this procedure avoids the underestimation of the R_{QG} values between related pairs, the conclusions from statistical tests using R_{QG} values obtained by this procedure did not differ from those with R_{QG} values based on allele frequencies from the full sample. Because adult breeders and subadults from three different seasons were pooled for the estimation of allele frequencies, the origin of these groups from the same gene pool was tested. There was no differentiation between individuals from October 2005 to March 2006 (FST = −0.0099, $P = 0.91$) or between breeders (i.e. breeding females and reconstructed sire genotypes) and the population sample (FST = 0.0006, $P = 0.36$), and each of these groups was in Hardy–Weinberg equilibrium at each locus. Moreover, one individual of the population sample in 2005 was recaptured as mouthbrooding female in 2006.

The relatedness of fostering dams to their adopted fry was compared to the relatedness between dams and the broods bred by other females to test for preferential adoption of relatives. Our broods consisted of one to three groups of full-sibs each. As all fry within a full-sib group share the exact same degree of relatedness to a given female, the variance of pairwise R_{QG} values between a female and the fry within a full-sib group is due to stochasticity. To reduce stochasticity and arrive at a more accurate estimate of the relatedness between a female and a full-sib group of fry, we calculated the mean of the pairwise R_{QG} values between the female and each of the full-sibs. For example, two relatedness estimates were calculated for female D and the brood of female I, which had mated with two males (Fig. 1), by taking the means of the pairwise R_{QG} values between

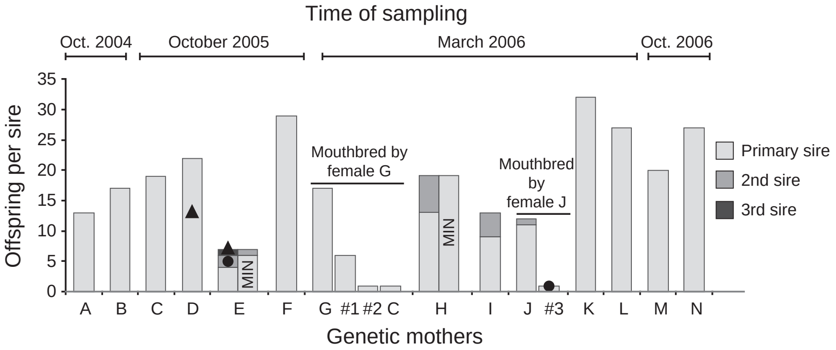

Fig. 1 Sibship analysis of broods collected from mouthbrooding females A–N. COLONY reconstructed 23 full-sib groups originating from a total of 17 different mothers (on the *x*-axis) and 21 different sires. Deviating paternity reconstructions by GERUD in broods E and H are labelled 'MIN'. Bar height and vertical divisions correspond to the number of offspring per sire–dam combination. Paternal half-sib groups spread across two different broods are marked by triangles (broods D and E) and circles (broods E and J). #1, #2 and #3 are reconstructed maternal genotypes not matching any of the sampled mouthbrooders.

(i) female F and the 'I' fry sired by the first male; and (ii) between female F and the 'I' fry sired by the second male. This approach also removes the problem of dependence between members of a full-sib group.

In addition to the estimation of relatedness coefficients between pairs and groups of individuals, we also tested for the presence of close relatives using the programs KINSHIP version 1.3.1. (Goodnight & Queller 1999) and ML-Relate (Kalinowski *et al.* 2006). For each pair of individuals, KINSHIP calculates the likelihood ratios for two hypotheses of kinship, in our analyses a null hypothesis of 'unrelated' versus primary hypotheses of 'half-sib', 'full-sib' and 'parent–offspring', and uses simulations to determine the thresholds for the rejection of the null hypothesis. ML-Relate compares the likelihoods of being unrelated, half-sibs, full-sibs and parent–offspring and returns the most likely relationship and its likelihood relative to the likelihoods of the other possible kinship types. Parent–offspring relationships were counted only when at least one allele per locus was shared between individuals.

Statistical analyses of relatedness coefficients

Differences in relatedness between different groups of individuals, for example among dams versus among sires, were tested by two-tailed Mann–Whitney *U*-tests. A resampling method was used to test whether the mean relatedness within a particular group of individuals, for example adopted fry or mated individuals, was higher (or smaller) than expected in a random sample taken from a larger group of individuals, for example all available fry or all possible sire–dam dyads. The proportion of the resampled data sets with means equal to or larger (smaller) than the observed one was considered to represent the probability of obtaining the observed difference by chance.

The distributions of relatedness coefficients were illustrated by boxplots showing medians, quartiles, as well as means and pairwise R_{QG} values. The R PACKAGE 2.12.2 (R Development Core Team, 2011) was used for statistical tests and graphs.

Results

Parentage reconstruction

Single paternity and maternity prevailed among the fourteen analysed broods (Fig. 1). Minimum (GERUD) and maximum-likelihood (COLONY) sire number estimates were largely congruent and demonstrated monogamous spawning by 10 or 11 females and multiple mating by three or four females, each with two or three different males (Fig. 1). Congruence between the two methods for sire number estimation provides good support for the accuracy of the result (Sefc *et al.* 2008; Sefc & Koblmüller 2009). Broods G and J contained eight and one offspring, respectively, whose alleles were not present in the mouthbrooding female. Because the mouthbrooders and/or the fry were heterozygous at most mismatching loci, these incompatibilities cannot be attributed to null alleles (Table S1, Supporting information). Both of the mixed broods consisted of well-developed fry (15 mm TL; fully developed fins), and there was no size difference between own and foreign fry. Maximum-likelihood sibship reconstruction by COLONY assigned the eight foreign young of brood G into three different full-sib groups with six, one (S8) and one (S22) individuals per group, respectively (Fig. 1). While shared maternity between S8 and S22 cannot be excluded, neither of them could have the same mother as the six other foreign fry. Therefore, at least two additional mothers contributed offspring to this brood.

There is no compelling support for shared paternity or maternity across broods. COLONY suggested three half-sibships across broods, that is, shared paternity between broods D and E and between E and J, and shared maternity between brood C and one of the adopted fry in brood G, which was collected 5 months later (Fig. 1). In each case, however, the assignment of half-sibs is based on a single individual in one of the broods, which naturally provides insufficient information for accurate sibship and parental genotype reconstruction. It is noteworthy that the broods, from which the adopted fry originated, could not be identified. Although mouthbrooding females were found to be stationary (B. Taborsky, personal communication), it is possible that the donor females were not present in our study site at the time of sampling. Alternatively, given that the mixed broods were very close to becoming independent and the adopted fry originated from broods of the same developmental stage, the missing mothers may already have released their broods and were therefore not comprised in the sample of mouthbrooding females.

For analyses of relatedness among breeding individuals, genotypes of 11 sires, which had sired at least nine offspring (to achieve a high probability of detecting both alleles of a heterozygous male; Sefc *et al.* 2009), were reconstructed from the brood genotypes (Table 1). Sire genotype reconstructions were congruent between COLONY and GERUD.

Relatedness among breeders and broods

Fostering mothers were related to three of the four adopted full-sib groups with $R_{QG} > 0.1$ (Fig. 2, Table S2, Supporting information), and relatedness between foster female G and the six fry of the adopted full-sib group #1 (Fig. 1) was significantly higher than zero (mean $R_{QG} = 0.13$; one sample $t = 2.409$, d.f. = 5, one-tailed $P = 0.03$). Rather than indicating selective adoption of related broods, the relatedness between fosters and adopted fry was not significantly higher than the relatedness between fosters and other contemporaneous broods (Fig. 2, Table S3, Supporting information). However, fosters were somewhat more closely related to contemporaneous foreign broods than were nonfostering females (Fig. 2, Table S3, Supporting information). Overall, the relatedness between female breeders and offspring of other contemporaneously

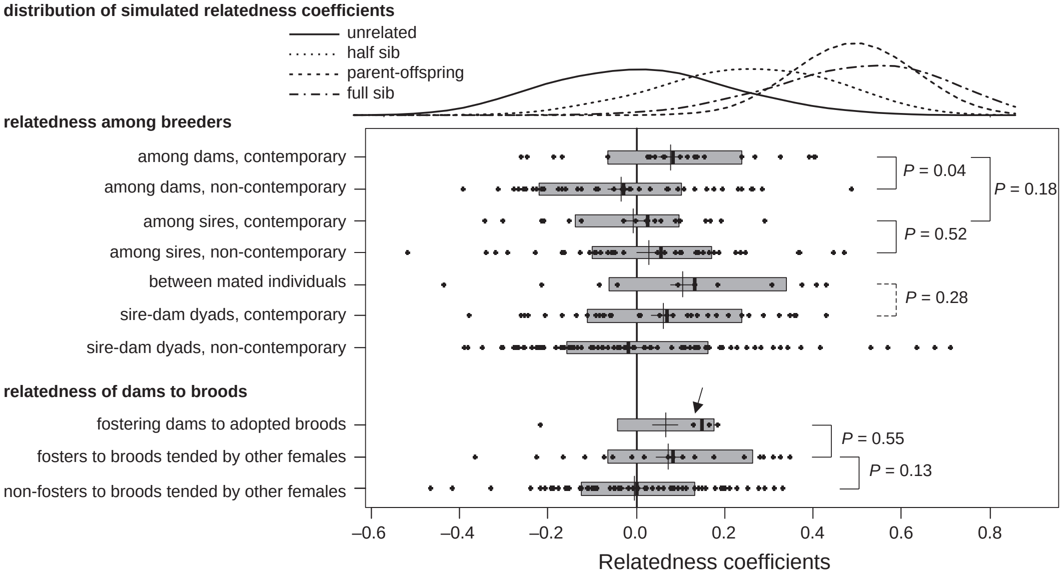

Fig. 2 Pairwise relatedness among dams, sires, and between dams and fry in comparison with simulated distributions of R_{QG} values for different degrees of kinship. Relatedness between breeders is shown separately for individuals collected at the same time (contemporary breeders) and individuals breeding at different sampling periods (compare Table 1). Relatedness between dams and broods was calculated only with contemporaneously collected samples, as noncontemporaneous fry are no candidates for adoption. Boxes, vertical lines and crossed lines indicate quartiles, medians and means, respectively; dots represent the pairwise R_{QG} values among breeders, whereas relatedness to broods is given as the mean R_{QG} values between dams and all fry pertaining to the same full-sib group. In the category 'fostering dams to adopted broods', the arrow marks the value, which was calculated as mean of the R_{QG} values between dam G and the six adopted full-sibs, whereas the other dots represent pairwise R_{QG} values between foster females and single adopted fry (see Table S2, Supporting information). Differences between sets of data were tested by Mann–Whitney *U*-tests (solid brackets) and by resampling (hatched bracket). The *P* value obtained by resampling corresponds to the proportion of random samples taken from all sire–dam dyads having larger mean R_{QG} than the mated individuals.

breeding females was significantly higher than zero in 23 of 60 breeder–brood comparisons after Benjamini–Hochberg correction for multiple testing (one sample *t*-tests, Table S4, Supporting information). This implies an elevated level of relatedness among some of the contemporaneously breeding individuals, and indeed, the distributions of pairwise relatedness coefficients among dams and of dam–sire dyads were shifted towards positive values and extended into the range of first- and second-order relatives (Fig. 2). Kinship analyses by the program KINSHIP identified more close relatives among contemporaneously breeding individuals, among all breeding individuals and among the full sample (breeders plus population sample) than expected as false positives at a 0.05 significance level (Table S5, Supporting information). Some of these relationships received strong statistical support in KINSHIP, and all but one of the related pairs detected by KINSHIP were also found to be related by ML-Relate (Table S5, Supporting information).

There was no difference in mean relatedness among contemporaneously breeding sires and mean relatedness among contemporaneously breeding dams, or among all dams and all sires (Fig. 2, Table S3, Supporting information). However, mean relatedness was significantly higher among contemporaneously breeding dams than between dams breeding at different times, whereas no such difference was detected for sires (Fig. 2, Table S3, Supporting information).

Despite the elevated level of relatedness between dams and sires, relatedness between mates did not differ from overall dam to sire relatedness (Fig. 2, Table S3, Supporting information), and potential close kin was also detected among the mated pairs (Table S5, Supporting information). High pair relatedness did not coincide with polyandry (Table 1).

Discussion

Foreign fry in two broods and a low rate of polyandry contrast with the maternity and paternity patterns observed in other polygamous maternal mouthbrooders. In *S. pleurospilus*, continuous mouthbrooding is followed by a period of guarding, during which the fry are recalled in response to threat stimuli (Taborsky 2006a,b). The fry in our mixed broods were in this second phase of brood care and approached independence. Foreign young have been detected among the guarded fry of several maternal mouthbrooders (Ribbink 1977; Lewis 1980; Ribbink *et al.* 1980), and occasional mix-ups of late-stage fry during the guarding phase may be accidental, for example when adjacently guarding breeders recall fry for protection. Only five of the captured broods contained fry large enough to have reached the guarding phase (fry sizes >10 mm; Table 1), such that the detection of two mixed broods – one of them with fry adopted from at least two other dams – suggests that mixing among late-stage broods is not infrequent. In several cichlid species, the uptake of foreign fry was not prevented by discrimination of the recalling female or the responding fry against unfamiliar signals (Baerends & Baerends-Van Roon 1950; Mrowka 1986), although increased brood sizes might hamper rather than promote predation avoidance in maternal mouthbrooders (McKaye & McKaye 1977; Ribbink *et al.* 1980). Foreign fry could therefore obtain buccal shelter because *S. pleurospilus* females are incapable of kin recognition (which is unlikely in a cichlid, see McKaye & McKaye 1977), or because the net balance of costs and benefits favours adoption over rejection. With one exception, fosters in our study were related to their adopted fry at or above the level of first cousins and their alloparental care may hence increase their inclusive fitness. Adoption of relatives has so far mainly been observed in birds. In waterfowl species with strong natal philopatry, the spatial pattern of relatedness is sometimes sufficient to explain the relatedness between hosts and parasites without requiring a specific preference for related hosts (McRae & Burke 1996; Anderholm *et al.* 2009; reviewed by Hatchwell 2010). In other cases, however, the bias towards related hosts is stronger than predicted by the spatial relatedness structure alone and therefore other mechanisms, such as kin recognition and breeding synchronization of relatives, must be involved (Andersson & Ahlund 2000; Andersson & Waldeck 2007; Waldeck *et al.* 2008; Jaatinen *et al.* 2009). In *S. pleurospilus*, fry adoption appeared to be independent of relatedness, and the elevated relatedness between fosters and adopted fry apparently follows from the relatedness among some of the contemporaneous breeders and between some of the breeding females and foreign broods. The detection of male and female kin in the population (Table S5, Supporting information) indicates natal philopatry of both sexes, which is supported by distinct genetic population structure across short geographic distances (Kotrschal *et al.* 2012).

Beyond the effect of philopatry on the relatedness structure in the population, higher relatedness among contemporaneously breeding females than among females breeding at different seasons suggests an additional temporal and/or spatial concentration of mouthbrooding relatives. In the field, it has been repeatedly observed that several mouthbrooding females (up to eight individuals) form shoals, which remain stationary near the lake bottom (A. Kotrschal, personal communication; B. Taborsky, personal communication). Nonbrooding females were never part of shoals. Possibly, these females profit from joint predator defence and/or predation dilution when guarding their offspring (Kruuk 1964; Keenleyside 1972; Westneat 1992). At the same time, this behaviour increases the risk of picking up a neighbour's fry, especially when a quick retrieval of the brood is vital. Kin-synchronized breeding or kin-based breeder aggregation may relax the pressure to avoid brood mixing and may allow *S. pleurospilus* females to brood and guard their offspring in close vicinity. *S. pleurospilus* breed throughout the year, and kin synchronization could be facilitated by social stimulation (Keenleyside 1972; Liley *et al.* 1986; Ochi 1986; Evans *et al.* 2009), in this case via interaction between related females. Alternatively, even if the timing of breeding was random, relatives breeding at the same time by coincidence might join in breeding aggregations to the exclusion of nonkin, and be over-represented in our data if not all contemporaneous groups were sampled. Both kin synchronization and kin aggregation would require some mechanism of kin recognition. Our data provide no evidence for strict kin-synchronized breeding, because the relatedness between dams is not correlated with the size differences of their fry (not shown). The spatial distribution of breeders was not recorded. As a third possibility, the elevated relatedness among contemporaneously breeding females, although statistically significant, may be purely coincidental. In this case, the timing of breeding and the aggregation of mouthbrooders would be independent of relatedness, but owing to the naturally occurring proportion of relatives in the population and the low costs of late-stage brood care, mouthbrooders could afford the risk of occasional brood mixing in exchange for benefits accrued by breeding close together.

The mating system of *S. pleurospilus* combines characteristics of an exploded lek with those of a resource defence system (Kotrschal & Taborsky 2010). In compliance with a lek system, male territories serve a courtship function and do not concentrate critical resources, but by their territorial behaviour, males provide a high-quality feeding patch and therefore nutritional benefits for the females (Kotrschal & Taborsky 2010). The low rate of polyandry detected in the present study is more similar to the exclusive pair-spawning of mouthbrooding species, in which males and females use a common territory permanently (Taylor *et al.* 2003) or temporarily (Egger *et al.* 2006), than to the often high levels of multiple paternity in broods of promiscuous mouthbrooders (Kellogg *et al.* 1995; Parker & Kornfield 1996; Sefc *et al.* 2009; Haesler *et al.* 2011). Moreover, unlike expected in a lek, our data provide no evidence that reproductive success was monopolized by a small number of males, as none of the reconstructed sires had offspring in more than one brood.

While the occurrence of relatives among the breeders in the population can mitigate the impact of brood mixing, it could have a negative effect on reproductive success if inbreeding was associated with fitness losses. A number of behavioural strategies have evolved to avert inbreeding, including sex-biased dispersal, active avoidance of kin and polygamy as a bet-hedging strategy (e.g. Pusey & Wolf 1996; Tregenza & Wedell 2002; Archie *et al.* 2007; Randall *et al.* 2007; Costello *et al.* 2008; Lebigre *et al.* 2010), but none of these appear to be employed by the studied population. Both sexes appear to be philopatric, as relatives were found within and between both sexes and mean relatedness did not differ between males and females. There is no evidence for the avoidance of kin as mates, as relatedness estimates were high between some of the mated individuals and mean relatedness between mated individuals did not differ from that in randomly drawn sire–dam dyads. Finally, bet-hedging polyandry is unlikely, because multiple mating was infrequent and close relatedness was indicated between some individuals, which had spawned exclusively with each other.

In conclusion, while the ecological and behavioural components of the mating system of *S. pleurospilus* blend attributes of exploded leks with characteristics of resource defence systems, the distribution of genetic parentage is more compatible with resource-based reproduction than with lek polygamy. Our data furthermore suggest that the inbreeding potential caused by natal philopatry of both sexes did not give rise to bet-hedging polyandry or the active avoidance of relatives. Finally, we propose the following explanation for the occurrence of mixed broods. By guarding near to each other, mouthbrooding females create a situation that is susceptible to brood mixing but perhaps profitable in terms of predation avoidance. Brood mixing most likely occurs by accident during the guarding of late-stage fry, and rejection has not evolved because brood care costs at this advanced stage of care are low and at least occasionally balanced by inclusive fitness gains when relatives are involved. Our study suggests a novel pathway by which brood predation can influence the occurrence of brood adoption (in addition to predation dilution). Furthermore, our study raises questions about the mechanisms behind the aggregations of related breeders, in particular whether related females aggregate preferentially or may even strategically synchronize their breeding activities.

Acknowledgements

We thank Alexander Kotrschal for sharing his field data on aggregations of mouthbrooding females with us. The study was supported by the Austrian Science Fund (FWF; grant

P20883-B16 to KMS and P18647-B16 to BT) and the Swiss National Science Foundation (SNF; 31003A-133066 to BT).

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Data accessibility

Microsatellite data: DRYAD entry doi:10.5061/dryad.9f392s7r.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite genotypes of mouthbrooding females J and G and adopted fry (indexed 'S'). Mismatching fry genotypes are shown in bold print. The full sib group identifiers correspond to Fig. 1.

Table S2 Relatedness coefficients R_{QG} between fostering females G and J and their adopted fry (indexed 'S'). The full sib group identifiers correspond to Fig. 1.

Table S3 Relatedness coefficients for different groups of individuals (number of pairwise comparisons, means ± SD and medians of pair-wise relatedness coefficients) and tests for differences between sets of data.

Table S4 One-tailed one-sample *t*-tests comparing the relatedness coefficients between dams and the offspring of other females to a null hypothesis of $R_{QG} = 0$.

Table S5 Half sib, full sib and parent-offspring pairs detected by KINSHIP and ML-Relate analyses.