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Reproductive parasitism of broodcare helpers in a cooperatively breeding fish

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Large male helpers in the cooperatively breeding cichlid *Neolamprologus pulcher* gain reproductive success by parasitizing the reproductive effort of male territory owners. Under controlled, experimental conditions we examined the genetic relatedness between the members of brood pairs ($n = 14$), their male helpers ($n = 8$), and offspring ($n = 292$) in seven families. We used multilocus DNA fingerprinting to check for potential reproductive parasitism by male helpers and to assess their fertilization success. Of offspring produced in these families, 10.3% were sired by helpers. In parasitized broods, helper fertilization success varied between 12.5% and 35.8%. Male helpers parasitized parental reproduction when their body size exceeded 4.5 cm standard length (SL), even though sexual maturity may be reached much earlier (3.5 cm SL). Two of three parasitic helpers were punished by severe aggressive attacks when parasitizing the reproduction of breeders, which led to their expulsion from the territory. This study demonstrates a potential fitness benefit to broodcare helpers that is often neglected. It also points to the delicate balance that may exist between cooperative and competitive behavior in cooperatively breeding species. *Key words:* Lake Tanganyika, cichlids, cooperative breeding, DNA fingerprinting, *Neolamprologus pulcher, N. brichardi,* reproductive competition. *[Behav Ecol 10:510–515 (1999)]*

Cooperative breeding is well represented in birds (an estimated 220 species; Brown, 1987) and mammals (an estimated 120 species; Riedman, 1982), but apparently rare in fish (8 species; Taborsky, 1994). Numerous studies have aimed to explain why reproductively mature individuals would refrain from reproduction and instead help conspecifics to raise their offspring (see Emlen, 1991; Koenig et al., 1992; Komdeur, 1992). Helpers frequently gain indirect fitness benefits by rearing kin (Hamilton, 1964; Koenig et al., 1992; Reyer, 1984; Taborsky, 1984). However, helpers also gain direct fitness benefits, for example, by obtaining protection and access to food in a territory (Gaston, 1978; Taborsky, 1984), gaining parental experience (Brown, 1987; Komdeur, 1996), or inheriting the territory or a mate (Balshine-Earn et al., 1998; Reyer, 1980; Wolfenden and Fitzpatrick, 1984). Helpers may also benefit by siring offspring within the group they are staying (Brooker et al., 1990). Direct reproduction of helpers has been difficult to detect by behavioral observation alone, but DNA profiling (Jeffreys et al., 1985) has made detection possible. In cooperatively breeding tropical song birds, male helpers may sire some proportion of the offspring produced in a group (Haydock et al., 1996; Mulder et al., 1994; Rabenold et al., 1990).

In many fish species, males often take the opportunity to parasitize the investment of territory owners by simultaneous parasitic spawning (SPS; Taborsky, 1994, 1998). However, except in salmon, little is known about the relative reproductive success of males that participate in SPS. In Atlantic salmon (*Salmo salar*) the proportion of eggs fertilized by mature parr varied between 5 and 40% dependent on the number of parr present at each spawning (Hutchings and Myers, 1988; Jordan and Youngson, 1992; Thomaz et al., 1997). In the Pacific salmon *Oncorhynchus keta,* Schroder (1981) found that 25% of the eggs were fertilized by parasitic males. To our knowledge, it is as yet unknown whether broodcare helpers successfully parasitize the reproductive effort of breeders in fish.

The endemic Lake Tanganyika cichlid *Neolamprologus pulcher* (we regard *N. pulcher* and *N. brichardi* as synonymous; see below) is a highly social fish species. Usually, a pair of breeders and offspring of up to five different size classes resulting from previous broods share the duties of broodcare, territory defense, and maintenance. Broodcare helpers of both sexes clean and fan eggs and larvae, clean the substrate, perform digging, and take part in territory defense (Taborsky and Limberger, 1981).

As observed in a northern population of *N. pulcher* in the field (near Magara, Burundi), large helpers may leave their natal territory and join a nearby aggregation of conspecifics. The probability of joining an aggregation increases with a helper's size. Laboratory experiments revealed that helpers do not leave the territory on their own accord, but are usually expelled by the breeding pair, especially when there is no need for help (Taborsky, 1985). The permanent aggregations joined by helpers consist of non-reproducing, sexually mature fish. Between the helper stage and the acquisition of their own, reproductive territory, most or all fish live for some period of time within an aggregation (Taborsky and Limberger, 1981).

From laboratory observations we know that *N. pulcher* may start to breed from a size of about 3.5 cm standard length (SL). A fish of this size is approximately 8–9 months old. In the field, most fish up to 4.0 cm and some even up to 5.5 cm act as helpers in a family territory owned by a pair of breeders. Do helpers parasitize the reproduction of breeders in their natal territory? In *N. pulcher,* we should expect that reproductive parasitism becomes more beneficial with increasing helper size. Apart from the sperm production potential, which is probably size dependent, this is mainly due to the natural replacement of breeders. When one or both breeders are exchanged by conspecifics due to mortality or other reasons, the helpers usually stay in the territory and continue to help the new owners. There is no indication that helpers behave differently with new or unrelated breeders (Taborsky, 1984). The older helpers get, the more likely one or both breeders have

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been exchanged already. Therefore, the expected degree of relatedness between helpers and beneficiaries declines with a helper's age. In the northern population the average relatedness (r) between breeders and helpers at the onset of maturity (3.5 cm SL) is approximately 0.36 (Taborsky and Limberger, 1981).

Attempted SPS of helpers has been observed by Taborsky (1985), but it was unclear whether offspring are successfully sired by male helpers. Successful SPS of fish of unknown social status was demonstrated in this species by hybridization events with *Julidochromis ornatus* (Taborsky, 1994). In this study, we used multilocus DNA fingerprinting to investigate whether and to what extent male helpers of *N. pulcher* parasitize the breeding pairs' reproduction.

METHODS

The study species

We present in our study data from *N. pulcher* from the south of Lake Tanganyika and from its northern variant *N. brichardi*. In the past these were considered two subspecies (Trewavas and Poll, 1952), and later based on the examination of only one specimen, they were split in two separate species (Poll, 1974). We believe that the validity of this division is doubtful. Our field and laboratory observations on the social system and behavior of *N. brichardi* from Burundi (Taborsky 1982, 1984, 1985; Taborsky and Limberger, 1981) and *N. pulcher* from Zambia (Balshine et al., 1998; Dierkes et al., personal observations) indicate the division is invalid. A systematic study in which 13 morphological characters were measured and statistically compared between these species did not find strong support for the separation into two species (Balshine-Earn et al., unpublished data).

Test fish and housing conditions

N. pulcher were imported from Mpulungu, Zambia, from the southern tip of Lake Tanganyika, near Mbita Island. We used these fish and their first-generation progeny for the experiments, supplemented by fish from two aquarium stocks from a northern population. The fish were kept in 160-l tanks with 2–3 cm of quartz-sand (average grain size 1 mm), and flowerpot halves were provided as breeding shelters. Water quality was maintained according to Taborsky (1982) and with help of air filters. Fish were fed once daily either with commercial dry food, *Artemia salina* nauplia, or chironomid larvae. Day length was held constant at 13 h light.

Experimental design

Three fish of about 2.0 cm SL were introduced into an empty tank (80 × 50 × 40 cm) to serve as potential helpers. After 1 h, a pair was added. For the next 2–3 h we observed whether the pair tolerated the potential helpers in their new territory. If the helpers were expelled, the trial was terminated and a new trial was started. When the helpers reached an SL of about 3.0 cm, the genital papilla of males and females differed in size and shape, so we could determine the sexes. Only families with male helpers were used for experimental trials. In total, we established six families with one male helper each and one family with two male helpers. Four of these families were from the southern population of Lake Tanganyika and three from the north. We intended to combine experimental "families" from nonrelatives to allow for unequivocal interpretations of the DNA data. This is not an unusual situation in nature, as families may also exist of pair members and unrelated helpers due to the exchange of pair members due to natural mortality (Taborsky and Limberger, 1981). Helpers behave similarly with their own or with foster parents (Dierkes et al., personal observations). A helper was regarded as being fully accepted when it was observed cleaning eggs. Helpers were measured monthly (weight and SL). After the helpers reached an SL of 3.5 cm, all subsequent broods produced in the respective tanks were collected and raised separately. In each clutch the number of eggs was recorded.

DNA preparation

Members of experimental broods were collected after they had reached 15 mm SL, killed with help of the anesthetic MS 222, and cut into small pieces. The tissue was digested in 3 ml buffer B (25 mM EDTA, 75 mM NaCl, 10 mM Tris) and 0.3 ml sodium dodecyl sulfate (10%) and 3 mg proteinase K for 2 h at 56°C, and overnight at 37°C in a waterbath. The DNA was then purifed by three extractions, first with an equal volume of phenol, second with phenol:chloroform:isoamyl alcohol (25:24:1), and finally washed with chloroform (Sambrock et al., 1989). DNA was precipitated with ethanol, washed in 70% ethanol, and redissolved in 300–500 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). For DNA from the breeding pair and helper, we collected fin clips from caudal and dorsal fins. (The removal of the fin tissue had no apparent effect on behavior, survival, or subsequent reproduction. Fins were completely regrown within 9 weeks.) The fin tissue was digested in 0.5 ml buffer B, 0.5 mg proteinase K, and 50 μl sodium dodecyl sulfate. After extraction the DNA was redissolved in 100–150 μl TE buffer. We obtained approximately 10–40 μg DNA from each fin-clip sample. We assessed concentration and purity by spectrophotometry.

DNA fingerprinting

Each fingerprint gel contained 18 individuals, the breeding pair, helper, and 15 offspring. On each gel we placed four digoxigenin-labeled DNA-molecular weight markers (Boehringer). Approximately 10 μg DNA of each sample was digested with *Hinf* I and electrophoresed through 20 cm 0.7% agarose gels at 42 V for 30 h in TBE buffer (0.09 M Tris HCl, 0.09 M boric acid, 0.02 M EDTA, pH 8.0). The DNA was transferred to a nylon membrane by Southern blotting and hybridized with a dig-11-uridine triphosphate-labeled oligonucleotide $(GATA)_4$ probe (for details on methods, see Epplen and Zischler, 1990).

Scoring fingerprints

We scored all fingerprints by marking the position of bands in the 3.5–23 kb size range onto acetate sheet overlays, using differently colored permanent markers to differentiate between maternally derived bands in fry, and those derived either from a pair male or a helper (Lifjeld et al., 1993; Westneat, 1990). The DNA markers were used as reference to ensure that the same bands in different individuals were not scored as different because of gel distortions. We assumed that bands of different individuals in the same gel were identical if their centers were <0.5 mm apart and did not differ greatly in intensity. Each scored band of a young matched a band of either the pair male, the pair female, or the helper.

Parentage analysis

For parentage analyses we used two methods to quantify the band sharing between possible parent–offspring pairs. First, we performed conventional band-sharing analysis, following Wetton et al. (1987). The band-sharing coefficients (BSCs)

Table 1
Clutch sizes, numbers of analyzed young (i.e., all that survived to 15 mm standard length) of 19 broods, and numbers of helper offspring in seven families

Family, brood	Clutch size	No. of analyzed offspring	No. of offspring sired by helpers	Mean (SD) Bsf of		
				Pair female with all offspring/clutch	Pair male with all offspring/clutch	Helper with all offspring/clutch
1(1)	175	2	0	—	—	—
1(2)	180	53	19 (35.8%)	0.78 (0.2)	0.78 (0.21)	0.25 (0.31)
2(1)	134	27	0	0.6 (0.09)	0.44 (0.13)	0.37 (0.15)
2(2)	70	7	0	0.64 (0.09)	0.46 (0.10)	0.41 (0.08)
3(1)	65	4	0	—	—	—
3(2)	?	7	0	0.44 (0.06)	0.45 (0.09)	0.08 (0.08)
3(3)	30	8	0	0.41 (0.08)	0.57 (0.13)	0.03 (0.06)
3(4)	24	4	0	—	—	—
3(5)	?	32	4 (12.5%)	0.45 (0.11)	0.48 (0.22)	0.04 (0.13)
3(6)	?	16	0	0.48 (0.14)	0.52 (0.13)	0.09 (0.06)
4(1)	110	18	0	0.52 (0.12)	0.52 (0.09)	0.17 (0.15)
4(2)	86	1	0	—	—	—
4(3)	?	3	0	—	—	—
4(4)	32 larvae	16	0	0.55 (0.12)	0.48 (0.09)	0.16 (0.13)
5(1)	114	3	0	—	—	—
5(2)	75	56	7 (12.5%)	0.65 (0.08)	0.49 (0.15)	0.06 (0.10) lH; 0.06 (0.09) sH
6(1)	?	12	0	0.68 (0.08)	0.48 (0.12)	0 (0)
6(2)	240	22	0	0.65 (0.11)	0.49 (0.14)	0 (0)
7(1)	129	3	0	—	—	—

Band-sharing frequencies (Bsf) are given for pair female, pair male, and male helper with all offspring of each family. lH, large helper; sH, small helper; ?, unknown clutch size.

were calculated as BSC = $(N_{ab})/N_a + N_b$, where N_{ab} equals the number of bands shared between *a* and *b*, and N_a and N_b represent the total number of bands in the fingerprints of individuals *a* and *b*. By this analysis, the total number of fragments in the fingerprints of two individuals is compared with each other. Second, we used the distribution of unique fragments or bands in the profiles of offspring that are present in a single adult member of a family (Gibbs et al., 1994). This method is based on the existence of fragments that are present only in a single putative parent, and at the same time also in one or more offspring.

In a segregation analysis of 14 offspring of family 3, 23 out of 24 resolved fragments >2 kb were heterozygous. There were three pairs of allelic bands and one case of consistent cosegregation.

RESULTS

Clutch sizes and offspring survival rates

Clutch sizes in 13 broods varied widely (mean±SD, 110.2±62, range 24–240 eggs; Table 1). Survival rates of offspring varied widely (1.1%–73.3%) between families and between different broods of a family. The median offspring survival rate was 10 young per brood (quartiles 2.6 and 20.1, $n = 13$).

Tab1e 2
Band-sharing frequencies between pair females (F), pair males (M), and helpers (H)

Family	F and M	M and H	F and H
1	0.47	0	0
2	0.05	0.35	0.1
3	0	0	0.08
4	0	0.1	0.1
5[a]	0.1 (sH)	0.04 (sH)	0 (sH)
	0.18 (lH)	0.07 (lH)	0 (lH)
6	0	0	0
7	0	0	0

[a] Family with two helpers; sH, small helper, lH, large helper.

Mutation rate

We estimated the mutation rate by counting the unattributable bands and dividing this number by the total number of 2435 bands scored in 292 young. Two bands that did not match with either female or both putative fathers were assumed to be the result of mutation. Thus, the mutation rate was calculated as 8.2×10^{-4}. All other bands were attributable to at least one of the putative parents.

Paternity

Band-sharing frequencies were used to estimate the relatedness between pair male, pair female, and helper (see Table 2), and the offspring (Table 1). Due to some degree of inbreeding in the laboratory population, in some families, band-sharing frequencies were not suitable for identifying parent–offspring relationships; however, the distribution of unique fragments was suitable (see appendix).

In total, the paternity analyses of 19 broods of 7 families showed that 30 out of 292 offspring (10.3%; Table 3) had a fingerprint profile that made a complete match to the pair female and the male helper, revealing that these offspring were fathered by the helper and not by the pair male. All helper offspring had a minimum of two unique helper fragments (i.e., bands that were not represented in pair male and pair female profiles). The remaining 262 offspring had a fingerprint profile that made a complete match to the female and the breeding male, suggesting that these offspring had been sired by the pair male.

Helper size when performing reproductive parasitism

The mean sizes and weights of helpers at the start of the experiment were 3.8 cm SL (SD = 0.47) and 1.4 g (SD = 0.6),

Table 3
Rate of reproductive parasitism by male helpers in families of *N. pulcher*

	Total no.	Parasitized	%
Families	7	3	42.8
Broods	19	3	15.8
Offspring	292	30	10.3

respectively. At the end of the experiment, helper sizes and weights were on average 4.7 cm SL (SD = 0.6) and 2.6 g (SD = 1.1). When the helpers of families 1, 3, and 5 performed reproductive parasitism, their sizes were 4.7 cm, 5.2 cm, and 4.75 cm, respectively (weights 2.3 g, 3.5 g, and 2.9 g). Figure 1 shows the change of helpers' sizes during the experiment. The helper of family 1 (4.7 cm SL) was found killed by the breeding pair in the morning of spawning, when he had performed SPS as revealed by the parentage analyses. In family 3, helper size was 5.2 cm SL when he performed SPS successfully. The number of attacks on this helper increased during the day after spawning. The helper was not allowed to approach the eggs and had to stay at the edge of the territory. When the breeding pair spawned the next time, this helper was again not allowed access to the eggs, and the experiment was stopped. The helper of family 5 was 4.75 cm SL when he performed SPS successfully. He was found killed by the breeding pair in the morning he performed SPS.

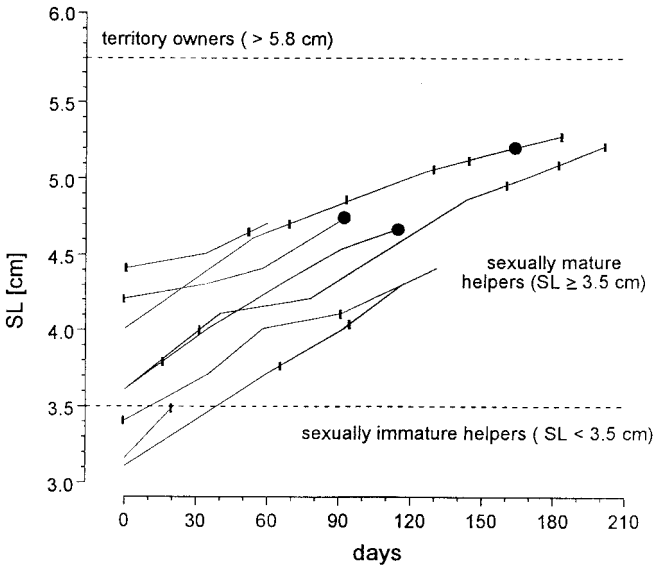

Figure 1
Sizes (SL) of the helpers in seven families during the experimental period (abscissa: number of days since start of experiment). Vertical ticks mark a spawning event, and filled circles indicate a spawning at which the helper performed reproductive parasitism successfully. All lines start at the sizes when helpers were investigated in the experiment and end when helpers were expelled from the territory.

DISCUSSION

Our results demonstrate that male helpers in families of *N. pulcher* sire offspring by performing simultaneous parasitic spawning when the owners of their residential territories reproduce. This confirms expectations based on behavioral observations that sometimes male helpers perform spawning movements when the territory owners spawn (Taborsky, 1985). Using our experimental procedure, each offspring could be assigned to one of the putative fathers. Three of 19 broods were parasitized, and the reproductive success of helpers in these parasitized broods varied between 12.5% and 35.%.

The rates of reproductive parasitism found in this study correspond with rates shown in other fish species with males performing alternative mating tactics. In the Atlantic salmon, mature male parr successfully fertilized up to 23% of the eggs of anadromous females (Hutchings and Myers, 1988). In this study a single parasitic male fertilized on average 5% of the eggs, but the proportion declined to 1% if up to 20 parasitic males were present at the same spawning event. Other studies of Atlantic salmon found 0.9–27.7% (mean 10.8%; Jordan and Youngson, 1992) and 26–40% (Thomaz et al., 1997) eggs fertilized by parasitic parr males. In three-spined sticklebacks (*Gasterosteus aculeatus*), 3.5% of eggs were sired by parasitic males (Rico et al., 1992), and in bluegill sunfish (*Lepomis macrochirus*) the rates of reproductive parasitism varied widely (0–59%) among different colonies, depending on the density of parasitic males (Philipp and Gross, 1994). However, none of these fish species is a cooperative breeder, and the reproductive parasites are not members of a social unit.

An example where reproductive parasitism occurs in a social unit was found in the West African cichlid *Pelvicachromis pulcher*. In this species, satellite males who help in territory defense, but not in direct broodcare, sire offspring produced in the territories where they are tolerated (Martin and Taborsky, 1997). Males of a yellow color morph either reproduce monogamously or associate with males of a red morph which defend harem territories containing up to three females. The dominant male of the yellow satellites achieves on average as much reproductive success as monogamously reproducing yellow males.

How does *N. pulcher* compare with other cooperatively breeding species?

This study allows for the first time a quantitative estimate of the success of reproductively parasitic helpers in a cooperatively breeding fish. In cooperative breeders, family groups are usually composed of a reproductive pair and their offspring from previous broods. To date, the reproductive success of parasitic helpers has been studied only in a few cooperatively breeding birds. In stripe-backed wrens, 6 of 69 investigated young were fathered by helpers (8.7%). The four reproductive helpers were found in four groups in which they helped a brother or father and an unrelated pair female who had immigrated during the helper's lifetime (Rabenold et al., 1990). In splendid fairy wrens (*Malurus splendens*), 35 offspring (38.5%) of 91 were fathered by the dominant group male, 7 offspring (7.7%) were fathered by male helpers, and 59 (65%) were not fathered by any of the males of the group (Brooker et al., 1990). In a DNA fingerprinting study of superb fairy wrens (*Malurus cyaneus*), 76% out of 181 analyzed young were sired by extragroup males, 39 young (21.5%) were fathered by the dominant male, and 4 (2.2%) by helpers. In latter cases, helpers gained paternity only when they were the sole helpers (Mulder et al., 1994). In bicolored wrens (*Campylorhynchus griseus*), Haydock et al. (1996) found that 8.6% of 222 investigated juveniles were not fathered by the pair male: 2.3% were fathered by helper males, which happened only when the female breeder had been replaced. No matings occurred between close relatives (e.g., between mother and son). Studies of cooperatively breeding white-fronted bee-eaters and red-cockaded woodpeckers found no evidence at all for a helper participation in reproduction (Haig et al., 1994; Wrege and Emlen, 1987).

In the above studies on reproductive parasitism in cooperatively breeding bird species, it is male helpers that performed the reproductive parasitism. Although in most cooperatively breeding mammals, helpers are reproductively suppressed by the dominant breeders, in dwarf mongooses (*Helogale parvula*) it has been shown that subordinates of both sexes sire offspring: 24% of young had subordinate fathers, and 15% had subordinate mothers (Keane et al., 1994). The alpine marmot is another example where only subordinate males obtain reproductive success (Bruns U and Arnold W, personal communication).

Do female helpers in *N. pulcher* also parasitize the reproduction of breeders?

For female helpers, SPS is much more difficult to perform because of the required synchronization of egg production with the breeding female. Additionally, egg laying needs much more time than releasing sperm and is therefore more conspicuous, which raises the risk of discovery by pair members. We never observed egg dumping of female helpers in *N. pulcher*. However, female helpers may engage in reproductive competition with pair females, by usurping parts of the territory or even the male breeder (see Limberger, 1982; Taborsky, 1985).

Brood size and estimates of parasitism rates of helpers

Clutch sizes and survival rates of eggs and young varied greatly among different broods and families. The three broods in which helpers fathered parts of the brood were also the largest broods. It might be possible that helper participation in reproduction caused a higher survival rate of broods. However, a more parsimonious explanation for this coincidence may be that it is easier to detect rare events in large numbers. Due to the high mortality of offspring during early ontogeny (see Table 1), some of the brood sizes we analyzed were quite small. For example, in family 7 the helper was observed passing through the breeding shelter during spawning of the dominant territory owners. The clutch had 129 eggs, of which only 3 young survived, which were fathered by the breeding male. In such a case it is likely that parasitism could remain undetected even if it occured. Therefore, our estimate of reproductive parasitism by male helpers (3 of 11 clutches and 10.3% of young) may be regarded as a minimum estimate.

Helper size and reproductive parasitism

For fish of helper size, the only possibility to reproduce is to engage in reproductive parasitism within their natal territories. However, this bears a risk of punishment. All three helpers that parasitized in our experiments were evicted. Helpers may need to reach a critical size at which they can survive outside the family territory. The risk of punishment by eviction may explain why male helpers start so late to parasitize the reproduction of breeders. In our experiments, the helpers expelled from the territory were on average 4.9 cm SL in size (weight 2.9 g). In a northern population studied near Magara, Burundi, the helpers did not exceed 5.4 cm SL (Taborsky, 1985), and the sex ratio of helpers was female biased (2:1), while that of same-size, family-independent fish in aggregations was male biased (1:2; Taborsky, 1984). We think that this was probably due to evictions of helpers that started to parasitize the reproduction of territory owners.

There are two possible reasons that it may not pay small helpers to parasitize. First, the increased probability of expulsion from the territory when caught performing SPS will inflict higher costs to small helpers than to large ones, due to the mortality risk outside of territories (Taborsky, 1985). Second, the probability of helpers being related to breeders is higher for small helpers (Taborsky and Limberger, 1981); therefore, they can obtain fitness benefits via kin selection. Which of these two possibilities are more important will be discussed in a separate paper.

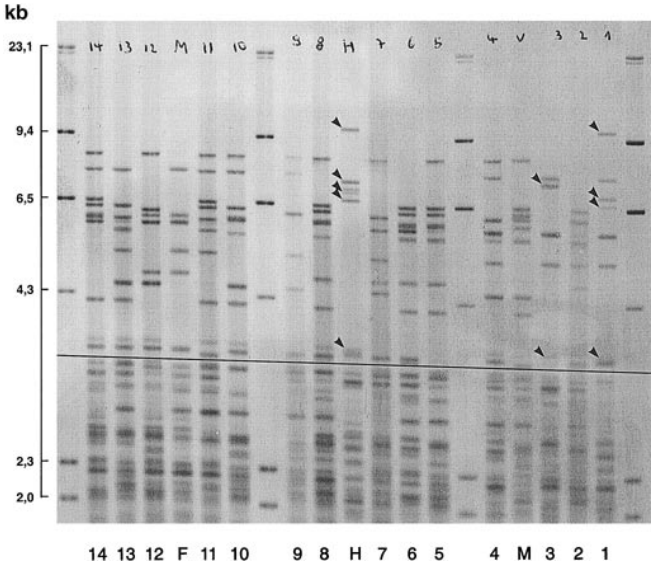

Figure 2
Multilocus DNA fingerprint of a part of the fifth brood of family 3. F = pair female, H = helper, M = pair male. Bands were scored from the horizontal line upward. Offspring 2 and 4–14 have been fathered by the pair male as supported by the common bands (see text), offspring 1 and 3 have been fathered by the helper as derived by the marked bands in the helper lane which are unique fragments not shared with the pair male or pair female. These two helper offspring share bands with the pair female and the helper, but none with the pair male.

APPENDIX

Relatedness patterns and parentage of fry within the experimental families

Family 1: The band-sharing frequency of pair 1 was $r = 0.47$, which suggests that male and female were related; probably they were siblings. Only the female showed one unique fragment. In the second brood ($n = 53$) of this family, 19 offspring (35.8%) were fathered by the helper as revealed by the unique fragment method.

Family 2: Pair male and helper shared four bands ($r = 0.35$), which were also found in the offspring. These bands did not reveal any information about paternity. But both pair male and helper had two unique fragments each. Twenty-three offspring out of 34 (both broods) shared the unique pair male fragments; 11 offspring did not, but did not show the unique helper fragments either. In this case, there is no indication for helper participation in reproduction.

Family 3: Figure 2 shows a DNA fingerprint including 14 offspring of brood 5 from family 3. The pair male is rejected as a father of offspring 1 and 3; these young clearly shared all bands with the pair female and the helper. Brood 5 ($n = 32$ offspring) of family 3 included four (12.5%) offspring fathered by the helper, each showing two to four unique fragments derived from the helper, and no unique pair male fragment. Helper and pair male did not show common bands. All other offspring of this family were unequivocally fathered by the pair male.

Family 4: All offspring of both broods showed unique pair male fragments, which proves that they had been sired by the pair male.

Family 5: The second brood had 55 offspring and contained 7 (12.7%) young fathered by the helper, as revealed by the unique fragment method. This family was the only family with two helpers. These differently sized helpers had an r of 0.12 (i.e., they were not related). The seven young that were not fathered by the pair male shared two unique fragments with the large helper, which identified them as offspring of this helper.

Families 6 and 7: All bands of the offspring were explained by pair male and female bands. In these families the helper did not sire offspring.

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