

Cichlids do not adjust reproductive skew to the availability of independent breeding options

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Helpers in cooperatively breeding species forego all or part of their reproduction when remaining at home and assisting breeders to raise offspring. Different models of reproductive skew generate alternative predictions about the share of reproduction unrelated subordinates will get depending on the degree of ecological constraints. Concession models predict a larger share when independent breeding options are good, whereas restraint and tug-of-war models predict no effects on reproductive skew. We tested these predictions by determining the share of reproduction by unrelated male and female helpers in the Lake Tanganyika cichlid *Neolamprologus pulcher* depending on experimentally manipulated possibilities for helper dispersal and independent breeding and depending on helper size and sex. We created 32 breeding groups in the laboratory, consisting of two breeders and two helpers each, where only the helpers had access to a nearby dispersal compartment with (treatment) or without (control) breeding substrate, using a repeated measures design. We determined the paternity and maternity of 1185 offspring from 47 broods using five to nine DNA microsatellite loci and found that: (1) helpers participated in reproduction equally across the treatments, (2) large male helpers were significantly more likely to reproduce than small helpers, and (3) male helpers engaged in significantly more reproduction than female helpers. Interestingly, in four broods, extragroup helper males had fertilized part of the brood. No helper evictions from the group after helper reproduction were observed. Our results suggest that tug-of-war models based on competition over reproduction within groups describe best the reproductive skew observed in our study system. Female breeders produced larger clutches in the treatment compared to the control situation when the large helpers were males. This suggests that male breeder-male helper reproductive conflicts may be alleviated by females producing larger clutches with helpers around. *Key words*: Cichlidae, clutch size adjustment, cooperative breeding, ecological constraints, reproductive partitioning. [*Behav Ecol* 17:419–429 (2006)]

It has become increasingly clear that subordinates in group-living animals may not only be helpful to the breeders but may also engage in reproduction (e.g., Macedo et al., 2004; Nonacs et al., 2004; Reeve and Keller, 1995; Richardson et al., 2001; Rusu and Krackow, 2004; Sumner et al., 2002; Widdig et al., 2004). Subordinate reproduction is often assumed to reduce the fitness of the same-sex dominant breeders in the group (e.g., Dierkes et al., 1999; Griffin et al., 2003). The question arises under which conditions subordinates should engage in reproduction and whether breeders should retaliate by punishing these subordinates for doing so (e.g., Hamilton, 2004; Hamilton and Taborsky, 2005; Reeve et al., 1998). Breeders may expel subordinates from the group after they have engaged in reproduction (Johnstone and Cant, 1999). Eviction may have considerable costs for the subordinates, for example, when emigration to another group is risky and unlikely to succeed or when living independently from a group decreases subordinate survival (e.g., due to “ecological constraints,” Emlen, 1982). On the other hand, breeders might allow some degree of subordinate reproduction, if prolonged subordinate group membership is expected to increase the fitness of the breeders despite engagement of subordinates in reproduction (so-called “concessions,” Clutton-Brock, 1998; Emlen et al., 1998). The dynamics of subordinate-dominant share in reproduction and its consequences for group mem-

bership have been extensively modeled under the “reproductive skew theory” framework (see review of the models in Johnstone, 2000; Kokko, 2003; Magrath et al., 2004). This has become the major framework to study and understand the reproductive ecology of social systems, including participation in reproduction, dispersal, eviction, and the individual benefits and costs associated with group living.

Reproductive skew models originally assumed that dominants had complete control over reproduction (complete control models, also referred to as concessions, incentives, or optimal skew models, Johnstone, 2000; Reeve and Ratnieks, 1993; Vehrencamp, 1983). To keep helpful subordinates in the group, dominants may give staying or peace incentives (concessions) by sharing reproduction with the subordinate group members (Emlen et al., 1998). In contrast, restraint models assume that dominants cannot prevent the subordinates from reproducing but they can “punish” subordinates for doing so, for example, by inflicting wounds or evicting them from the group (Johnstone and Cant, 1999). Due to this “threat of punishment,” subordinates may refrain from reproduction voluntarily (restraint) to be able to stay in the dominants territory (Hamilton, 2004; Johnstone and Cant, 1999), if the benefits from doing so (e.g., due to enhanced survival and queuing for the breeding position) outweigh the net benefits from immediate reproduction followed by punishment. Complete dominant control seems unlikely to apply in all cases or all time periods when dominants and subordinates share in reproduction (e.g., Faulkes and Bennett, 2001). Finally, tug-of-war models assume that dominants have incomplete control over the subordinate’s share in reproduction, resulting in dominants and subordinates competing for

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Table 1
Expected changes in unrelated subordinate reproduction and eviction rate, under varying models, subordinate size, and ecological constraints

Model	Dominant control ^a	Small subordinates		Large subordinates	
		Reproduction	Evictions	Reproduction	Evictions
Concessions	Complete	+	0	++	0
Restraint	None	0	0 or + ^b	0	0 or + ^b
Tug-of-war	Incomplete	0	0 ^c	0	0 ^c

It is assumed that large subordinates can compete better for within-group reproduction and independent breeding than small subordinates. The predictions assume dominants have complete control over group membership by evictions. 0 Denotes no change – or + denotes a decrease or increase from the control treatment (ecological constraints) to the breeding option (no ecological constraints).

^a Dominant control over reproduction, due to preventing, for example, subordinates acquiring the necessary resources to reproduce (e.g., food, breeding locations), subordinate access to breeding opportunities (e.g., Werner et al., 2003), and subordinate reproduction by direct (Clutton-Brock et al., 1998; Creel and Waser, 1997; Macedo and Melo, 1999; Malcolm and Marten, 1982) or indirect suppression (e.g., pheromonal control; threat of punishment, e.g., Bergmüller et al., 2005; Bergmüller and Taborsky, 2005).

^b Evictions may increase if breeders make assessment errors.

^c Groups assumed stable in original tug-of-war models, but if this assumption is relaxed, groups may dissolve, for example, due to high fighting costs over a share in reproduction, or, for example, helpers may be evicted after severely competing for a large share in reproduction, which may be more worthwhile when breeding options are available to disperse to after eviction.

their shares at the expense of total group productivity (Reeve et al., 1998).

Despite the great interest in reproductive skew concepts and the question whether reproductive sharing is mediated by dominant control or the lack of control, the number of experimental studies is small (see for review of experiments done in insects: Reeve and Keller, 2001). In some experimental studies, the perceived level of male reproduction was manipulated by restricting male access to the breeder females during their fertile period. This may lead to, for example, dominant breeder male acorn woodpeckers *Melanerpes formicivorus* destroying clutches on return (Koenig, 1990) and male dunnocks *Prunella modularis* adjusting their level of chick feeding to their presumed paternity in the brood (Burke et al., 1989; Davies et al., 1992). Other experimental studies have focused on testing effects of relatedness and inbreeding avoidance on skew (e.g., Greeff and Bennett, 2000; Reeve et al., 2000; Rusu and Krackow, 2004). The only study that addressed experimentally the effects of ecological constraints on skew found no effect of these constraints on reproductive sharing in the allodapine bee *Exoneura nigrescens* (Langer et al., 2004). The purpose of this paper is to test reproductive skew theory under experimentally varied ecological constraints with unrelated group members, using the cooperatively breeding fish *Neolamprologus pulcher* (Taborsky and Limberger, 1981).

The ecology of cooperative breeding of this Lake Tanganyika cichlid *N. pulcher* has been studied extensively, using a combination of field and laboratory experiments. Recent studies have focused on testing the “pay-to-stay” hypothesis as a mechanism, whereby helpers remain tolerated by the breeders inside the group (Balshine et al., 1998; Bergmüller and Taborsky, 2005; Bergmüller et al., 2005; Taborsky, 1985), which is mediated by status dependent and strategic adjustments in growth (Heg et al., 2004b; Taborsky, 1984). Other studies have focused on the benefits of group living and whether ecological constraints might make it worthwhile for helpers to delay dispersal, for example, due to protection against predators (Heg et al., 2004a), workload reduction (Balshine et al., 2001; Brouwer et al., 2005), group augmentation benefits of living in groups (Heg et al., 2004a, 2005), and the limiting availability of independent breeding options (Bergmüller et al., 2005). Breeding females lay eggs on the surface of rocks within protected crevices and males externally

fertilize these eggs, which makes it physically difficult for the breeders to gain complete control over reproduction and prevent simultaneous spawning. Indeed, Dierkes et al. (1999) found that male helpers in *N. pulcher* achieved 10.3% of all fertilizations. The probability of eviction is highest during reproduction (Taborsky, 1985), and there is some, although anecdotal, evidence that eviction of male helpers is related to attempts to fertilize broods of the breeders (Dierkes et al., 1999). Thus, restraint and tug-of-war models appear to describe this system more adequately than concession models.

In the experiment described in this paper, we tested for the combined effects of ecological constraints, helper size and helper sex on reproductive skew, that is, helper engagement in reproduction. We created 32 artificial groups of one breeder pair with two helpers (one small plus one large), with either access to a dispersal compartment with or without breeding substrate. In a parallel paper, we described the effects of these treatments on helper dispersal and helping behavior (Bergmüller et al., 2005). We showed that helpers readily dispersed and bred independently in the breeding option treatment and appeared to pay-to-stay for group membership otherwise. In this paper, we tested three additional predictions related to reproductive skew. Given the strong effects of the breeding option treatment on helper dispersal and behavior reported previously (Bergmüller et al., 2005), we also expected a strong effect, if any, on reproductive skew.

First, we compared the treatments to assess the importance of ecological constraints on reproductive skew (Langer et al., 2004). In their most “simplified” versions, concession, restraint, and tug-of-war models make a number of qualitatively different predictions when dominants are assisted by “unrelated” subordinates of different sizes (Table 1; Johnstone, 2000; Magrath et al., 2004). When independent breeding options are available, concession models predict that dominants will share reproduction with the subordinates as an incentive to keep them in the territory as helpers, and no subordinate evictions from the group should be observed. In contrast, restraint models predict that reproductive skew will not change in this situation (Table 1). However, if breeders make errors in deciding when to evict subordinates (Hamilton and Taborsky, 2005) and in their judgment of relatedness to helpers, evictions might increase in the independent breeding option treatment because the benefits of having extra helpers will

only be marginally higher than the indirect fitness benefits of evicting related helpers who have a high chance to breed by themselves. Finally, classical tug-of-war models predict no change in reproductive skew with the availability of independent breeding options and assume that no evictions occur from the group (Table 1). Alternatively, in extensions of both the restraint and tug-of-war models, subordinates might claim a larger share in reproduction than dominants would be willing to give, because subordinates can risk expulsion by doing so, if independent breeding options are available. Therefore, under this scenario, breeders should evict helpers immediately after these helpers have engaged in reproduction (see Balshine et al., 1998; Dierkes et al., 1999), and this should occur more often in the breeding option treatment. Helpers not involved or participating only little in reproduction should not be evicted. We stress that the predictions of the various models may change and may become similar when one or several of the assumptions are changed or are not being met (see Johnstone, 2000).

Second, we predicted that overall the large helpers should engage more often in reproduction than the small helpers, both in the control and in the breeding option treatment (Table 1), based on previous findings (Dierkes et al., 1999). A helper size effect on reproductive sharing can be accommodated by all three types of models. Also, large helpers were more likely to leave the territory and breed independently in the breeding option treatment (Bergmüller et al., 2005) and therefore should show a stronger difference in the amount of reproduction conceded or taken, comparing the control with the breeding option treatment in all three types of models (Table 1).

Third, we predicted that male helpers should reproduce more than female helpers, if breeders have some control over subordinate reproduction. This is expected because egg laying is time consuming and conspicuous (females swim upside down or sideways to attach the eggs to the substrate), whereas male helpers may quickly dart to the entrance of the shelter and release the sperm there (comparable to parasitic spawning in nongroup living fish, Taborsky, 1994). We assumed breeders had at least some control over subordinate reproduction because breeders chased helpers approaching too closely the breeding shelters during spawning (Heg D and Bergmüller R, personal observations). Combining these arguments, we predicted that female helper spawning would be more easily detected and prevented by the breeders (more control) than male helper spawning (less control), resulting in higher relative levels of male helper reproduction.

Finally, Cant and Reeve (2002) modeled sharing of reproduction by males if paternity is controlled by the female breeder and if paternity determines how much males are willing to invest in care. Their predictions concerning the effect of ecological constraints on reproductive skew are largely the same as for the concessions model (Table 1). However, females may not only control paternity (e.g., Williams, 2004) but may also increase their clutch size, depending on whether they are assisted by subordinates (Taborsky, 1984). Preliminary theoretical modeling results suggest that such an increase in clutch size under helper assistance may alleviate male-male conflict over reproduction, for example, breeder males will be more willing to concede reproduction to subordinate males (Hamilton IM and Heg D, in preparation). Therefore, we predicted the female breeders in our experiment to lay larger clutches when assisted by large male helpers but not when assisted by large female helpers. However, because large female fish are in general more capable of producing large clutches (e.g., Sargent and Gross, 1993; Wootton, 1990), this effect might only come about in the large breeder females.

METHODS

Study species

N. pulcher occurs along the shores of Lake Tanganyika at depths of approximately 2–45 m and has an almost continuous distribution from Zambia northward (although in the north *N. pulcher* is replaced by its sister taxon or subspecies *Neolamprologus brichardi*, formerly known as *Lamprologus brichardi*, Konings, 1998). Breeding pairs defend long-term territories assisted by subordinates mainly produced from previous broods (Taborsky, 1984, 1985), but frequent breeder exchange also creates groups of (partly or completely) unrelated group members (Dierkes et al., 2005). This opens the possibility of creating artificial groups in the laboratory, simulating such “breeder exchange,” where group composition can be altered to fit the experiment (Heg et al., 2004b; Taborsky, 1984).

Experimental setup

For the general experimental setup see Bergmüller et al. (2005). The experiment was conducted with fish caught in 1996–1997 at the southern end of Lake Tanganyika at Mpulungu (Zambia), and their laboratory reared offspring. The fish were kept in a 8000-l ring tank, which was partitioned into smaller compartments. The bottom of the tank was covered with sand (30 mm, 1-mm grain size), and water quality was kept constant. Water temperature was $27 \pm 1^\circ\text{C}$, and the light regime was 13:11 h light:dark with a twilight period of 15 min in between. The food consisted of commercial dry food (Tetramin) and frozen fish food (a mixture of *Daphnia* spp., nauplia *Artemia salina*, and chironomid larvae) and was provided once a day after observational recordings.

The ring tank was divided into eight experimental sections. Each of the sections contained four compartments with one breeding group in each (i.e., 32 groups in total) plus one dispersal compartment in between, using transparent plexiglass partitions. Breeding compartments contained two pot halves that served as breeding substrate. Each group consisted of one male breeder, one female breeder, a large male or female helper, and a small helper of the opposite sex (in total 128 fish). The fish were randomly chosen from different tanks lacking breeding shelters (i.e., these fish were not reproducing). Before the fish were introduced to the experimental tank, body measurements of all fish were taken (mass in mg and standard length [SL] in mm), and the fish were familiarized within the group compartment for a period of 26 days. Helpers were trained for an additional period of 48 days to swim through dispersal slots connecting the group and dispersal compartments before the experiment was started. Body measurements of all fish were taken 37 days after introduction to the tank and again before the treatment phase I started, that is, after another 37 days. This preexperimental period of 74 days is called the pretesting phase throughout.

At the start of the experiment, breeders were >60-mm SL, and male breeders were larger than female breeders as generally observed in the wild (Balshine et al., 2001). Small helpers were 35- to 42-mm SL, and large helpers were 42- to 51-mm SL. Breeding groups were created artificially, that is, by putting the helpers in the compartments first and the breeders one day later, with the breeders immediately taking the dominant position within the group due to their size advantage (see also Taborsky, 1984, 1985). The target was to have helpers of the opposite sex in each group, such that in each section two groups had a large helper female with a small helper male and the other two groups had a large helper male with a small helper female. However, sexing of the helpers, particularly the small helpers, could not be done without

Table 2

Body measurements of the group members for which parentage of their broods were assessed for the two experimental treatments: body size in SL (mm) and body mass (g)

Group member	Treatment			
	No breeding option		Breeding option	
	SL	Mass	SL	Mass
Groups with large helper females	<i>(n</i> = 8)		<i>(n</i> = 11)	
Breeder male	69.6 ± 5.0 (61–77)	10.19 ± 1.59 (7.51–11.90)	69.6 ± 2.6 (66–73)	9.73 ± 1.12 (7.53–11.53)
Breeder female	66.8 ± 3.8 (61–73)	8.14 ± 1.77 (5.00–10.97)	65.4 ± 2.2 (62–69)	7.86 ± 1.09 (6.32–9.96)
Large helper female	52.4 ± 4.1 (47–57)	4.20 ± 0.95 (2.94–5.09)	50.7 ± 2.5 (48–56)	3.57 ± 0.51 (2.91–4.52)
Small helper male	46.1 ± 3.2 (39–49)	2.77 ± 0.54 (1.82–3.44)	44.8 ± 2.1 (41–47)	2.57 ± 0.39 (1.88–3.05)
Groups with large helper males	<i>(n</i> = 9)		<i>(n</i> = 11)	
Breeder male	68.4 ± 2.3 (66–72)	9.27 ± 0.87 (7.87–10.27)	68.2 ± 4.1 (63–75)	9.29 ± 1.45 (6.99–11.72)
Breeder female	64.2 ± 3.1 (58–70)	7.37 ± 1.03 (5.97–9.06)	65.3 ± 3.4 (61–70)	7.64 ± 1.23 (6.12–9.87)
Large helper male	50.8 ± 2.8 (46–55)	3.62 ± 0.75 (2.64–4.91)	51.2 ± 3.8 (46–57)	3.81 ± 0.84 (2.53–5.10)
Small helper female	44.3 ± 1.3 (42–46)	2.39 ± 0.23 (2.11–2.75)	45.2 ± 3.3 (41–53)	2.39 ± 0.33 (1.80–2.84)
Groups with only helper females	<i>(n</i> = 2)		<i>(n</i> = 0)	
Breeder male	74.5 ± 0.7 (74–75)	10.00 ± 1.33 (9.06–10.94)		
Breeder female	65.5 ± 0.7 (65–66)	7.93 ± 0.63 (7.48–8.37)		
Large helper female	52.5 ± 5.0 (49–56)	4.02 ± 1.15 (3.20–4.83)		
Small helper female	45.5 ± 0.7 (45–46)	2.58 ± 0.23 (2.41–2.74)		
Groups with only helper males	<i>(n</i> = 1)		<i>(n</i> = 2)	
Breeder male	68	9.13	70.5 ± 2.1 (69–72)	8.50 ± 0.57 (8.10–8.90)
Breeder female	62	7.28	63.0 ± 0.0 (63–63)	6.98 ± 0.45 (6.66–7.29)
Large helper male	48	2.83	49.5 ± 0.7 (49–50)	2.92 ± 0.42 (2.62–3.21)
Small helper male	45	2.34	44.3 ± 2.1 (42–46)	2.43 ± 0.44 (2.04–2.90)

Depicted are means ± SD with ranges in parentheses and sample sizes (*n* denotes the number of broods and thus individuals assessed, groups with multiple broods only counted once).

failures at the start of the experiment, so it was checked at the end of the experiment, when all helpers had grown above 42-mm SL. It turned out that some groups had helpers of the same sex. The body sizes and sexes of the fish for which broods were analyzed for parentage are given in Table 2. Fish were marked individually by clipping the dorsal fin. Five days after creation, the first group produced a clutch.

Experimental procedure

The experiment comprised two phases of 23 days each. In phase 1, half of the eight dispersal compartments contained pot halves. Sections with pot halves alternated with sections without. In phase 2, the pot halves were moved to the sections that had not had breeding options before. After phase 1, all fish were moved back from the dispersal compartment to their groups, body measurements were taken, and the fish were allowed to accommodate to the new situation for 8 days before start of phase 2. The location of each helper was recorded once per day on 21 of 23 days within each phase. It was recorded whether the helpers were (1) in their group compartment, (2) in another groups' compartment, or (3) in the dispersal compartment. Note that these cichlids need breeding substrate to reproduce (in our experiment: pot halves), so only in the breeding option treatment some helpers dispersed, formed new pairs, and bred independently within the dispersal compartments, whereas in the control treatment, helpers visited the dispersal compartments but did not breed (Bergmüller et al., 2005).

To assess reproduction, we checked for new eggs in the pot halves every second day during pretesting and phases 1 and 2. Eggs were counted and removed to avoid changes in helping and agonistic behaviors caused by the presence of new offspring (*n* = 257 broods in total). In 31 of 32 groups, the

breeders produced at least one clutch (range 1–4) during the experimental period (and up to 12 broods including the pretesting phase). Broods produced in the dispersal compartments by former helpers were also counted and removed afterward. Each removed clutch still attached to the pot half was transferred within a separate brooding net to a separate 400-l tank, and offspring were raised artificially within these nets. Oxygen flow through the pot halves was created by water filters and air stones, but without brood care by fish, eggs frequently failed to hatch due to, for example, fungi. Fry of hatched broods surviving to 2 weeks of age (8- to 12-mm total length) were stored in 90% ethanol for future microsatellite DNA analyses.

Parentage analysis

We aimed to select from a subsample of 20 groups one brood each produced during each treatment. However, due to the frequent hatching failures and low offspring numbers in some broods this was not possible, so we had two broods available only from 15 groups. Therefore, additional 17 broods were selected for parentage analyses giving a total of 47 broods analyzed, and results were analyzed accordingly using generalized linear mixed-effects models (GLMMs) (see Data Analysis). The mean number of offspring (±SD, range in brackets) per brood where DNA was successfully extracted, amplified, typed, and assigned to the parents was 25.2 ± 7.3 offspring (range 6–38, *n* = 47; control treatment: 25.2 ± 7.6, range 9–38, *n* = 20; breeding option treatment: 25.3 ± 7.3, range 6–30, *n* = 27). Five to nine polymorphic microsatellite loci (see the Appendix; loci NP007, NP773, ULI2: Schliewen et al., 2001; Pzeb4: van Oppen et al., 1997; TmoM11, TmoM25: Zardoya et al., 1996; UME003: Parker and Kornfield, 1996; NP101: Brandtman et al., 1999; and UNH154: Lee and

Kocher, 1996) were used to determine the parentage of these 47 broods. All the loci had at least five alleles per 128 unrelated individuals (combined breeders and helpers in the experiment) and showed independent segregation in all tested groups (see Appendix).

Genomic DNA was extracted from ethanol-preserved finclip samples from the breeders and helpers (ca. 1–2mm² in size each) or from whole offspring using the Wizard Genomic DNA Isolation Kit (Catalys Promega AG, Wallisellen, Switzerland) according to the manufacturer's protocol for the treatment of animal tissue with a few small modifications. Genomic DNA was dissolved in 50 µl of DNA Rehydration Solution (Catalys Promega) and stored at –20°C until further analysis. For polymerase chain reaction (PCR) amplification up to seven microsatellite primer pairs were multiplexed in one PCR using the QIAGEN® Multiplex PCR Kit (Qiagen AG, Basel, Switzerland). PCRs were carried out in a 10-µl volume containing 10 ng of genomic DNA, 1× QIAGEN Multiplex PCR Master Mix (consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM MgCl₂, dNTP mix, and HotStar *Taq* DNA polymerase), 2 µM of locus-specific fluorescent-labeled forward primer (fluorescent dyes were 6-FAM, HEX, and NED) and nonlabeled reverse primer. Amplification was achieved in a GeneAmp 9700 Thermocycler (Applied Biosystems, Rotkreuz, Switzerland) by using the following sequence of cycling parameters: 15 min at 95°C; 33 cycles at 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s, followed by a final step of 72°C for 10 min. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI3100 Genetic Analyser and analyzed by the GeneScan software version 2.1 (Applied Biosystems).

Parentage assignment

Potential parents for each offspring were the group member breeder male, breeder female, and the two helpers plus all the six other helpers from the same section. Helpers from the same section could potentially enter all compartments and thus engage in reproduction, via the dispersal compartment. To minimize costs, parentage was initially assigned on the basis of five loci only (NP007, NP773, Pzeb4, UNH154, TmoM25). Based on a previous publication, suggesting low levels of extrapair maternity (Dierkes et al., 1999), maternity was assigned first and paternity was assigned after mothers had been assigned, using the CERVUS 2.0 software and the resulting number of mismatching alleles for the different parent combinations. All offspring had zero mismatching alleles with their putative parents. If parentage was ambiguous, that is, more than one parent combination resulted in zero mismatches, the offspring were analyzed for the remainder of the loci (total 9 loci) and parentage could then be successfully assigned in all cases. Note that we used unrelated individuals to create breeding groups in all cases (see Bergmüller et al., 2005).

Data analysis

Allele frequencies, observed and expected heterozygosities, and exclusion probabilities were determined using the CERVUS 2.0 software package (Marshall et al., 1998, see Appendix). Parentage was analyzed for 47 different broods. From two groups multiple broods were sampled during the breeding option treatment (two and three broods, respectively). The data gathered from these broods could not be treated as independent events and thus were lumped in all analyses, giving a total sample size of 44 broods. The proportion of offspring sired by helper males or helper females were analyzed with GLMMs, using a weighted logit link, with a random effect of group identity and with the fixed effect of treatment, using the shareware statistical software R1.0.8 (Crawley, 2002). We

additionally tested whether outliers severely affected the results. To assess whether the level of reproduction was influenced by helper size, the body size (SL, mm) of each helper measured at the start of each phase was entered into the GLMMs. Helpers visited the dispersal compartments, and some helpers, particularly the large helpers in the breeding option treatment, formed breeding pairs and produced clutches in the dispersal compartment. To assess whether dispersal behavior might have reduced and increased the likelihood to engage in reproduction in the home group and other groups, respectively, the proportion of days each helper was inside the home compartment (of the total number of days each helper was observed) was calculated per treatment and also entered into the above GLMMs. Clutch sizes were normally distributed and related to the fixed effects treatment (pretesting, no breeding option, breeding option) and large helper sex (female helper or male helper), with covariate breeder female body mass using a repeated measures GLMM in SPSS 11.0 (with 31 breeder females as “subject” effects and up to 12 broods per female as “repeated measures” effects). Note that the female body mass was determined before each phase of the experiment and entered accordingly into the GLMM. All other statistical analyses were performed with SPSS 11.0 with α set at 0.05 and two-tailed testing throughout.

RESULTS

We determined the parentage of 1185 offspring from 44 different broods (Table 3). As expected, the majority of offspring (91.4%) were assigned to the breeder male and female (i.e., within-pair parentage, Table 3). However, extrapair paternity and maternity were not rare, accounting for 0.0–7.8% of the total number of offspring (Table 3). Extrapair paternity was due to male helpers participating in reproduction in their own group (57 or 4.8% of all offspring, Table 3), but surprisingly also due to male helpers engaging in sneaky sperm release in other groups (15 or 1.3% of all offspring, Table 3). Only one brood appeared to be completely sired by an extrapair male, that is, a large male helper in the breeding option treatment. Extrapair maternity was detected in only one brood of the control treatment (Table 3). In this case, the large female helper of the group produced the whole clutch together with the breeder male, whereas none of the offspring were assigned to the breeder female.

Group membership and helper male reproduction

We detected extragroup paternity in four out of the 44 broods (Table 3). In one brood, a neighboring large helper male was involved (two of 30 offspring sired); in two broods, a neighboring small helper male participated (one of 26 and three of 75 offspring sired); and in one brood, a small helper male from the group directly across the dispersal compartment parasitized (nine of 17 offspring sired). Note that in all these cases the parasitic males had to enter the group's territory via the dispersal compartment. All extragroup parasitic helpers were also checked for paternity in broods produced by their own group, and the last helper mentioned above also fathered 19 out of 75 offspring in his own group. Group member and nongroup member males were similarly likely to reproduce, both in small helpers (2 of 24 versus 3 of 44 broods, likelihood ratio $\chi^2_1 = 0.05$, $p = .82$) and in large helpers (3 of 25 versus 1 of 44 broods, likelihood ratio $\chi^2_1 = 2.65$, $p = .10$). The mean overall proportion of offspring sired by male helpers varied between 0 and 1 (for actual values see subheader of Table 3) and was not significantly related to their group membership ($n = 42$, i.e., excluding all-female helper groups, the median was 0.0% for both types): 4.0% for the group member helpers

Table 3
Total numbers of offspring assigned to the different parents in the two experimental treatments

Assigned parents		Treatment	
Father	Mother	No breeding option	Breeding option
Broods without extrapair offspring		(<i>n</i> = 13 broods)	(<i>n</i> = 21 broods)
Breeder male	Breeder female	344	551
Broods with extrapair paternity		(<i>n</i> = 6 broods)	(<i>n</i> = 3 broods)
By male group members			
Breeder male	Breeder female	110	78 ^a
Large helper	Breeder female	6 ^b	30 ^c
Small helper	Breeder female	2 ^c	19 ^{c,a}
By male nongroup members			
Large helper	Breeder female	2 ^c	0
Small helper	Breeder female	9 ^c	4 ^{d,a}
Broods with extrapair maternity		(<i>n</i> = 1 brood)	(<i>n</i> = 0 brood)
By female group members			
Breeder male	Breeder female	0	0
Breeder male	Large helper	30	0
Breeder male	Small helper	0	0
Total number of offspring		503	682
% Extrapair paternity ^c		3.8%	7.8%
% Extrapair maternity		6.0%	0.0%

Note that no broods were discovered where both helper males and helper females participated simultaneously in reproduction (group members or nongroup members) and that at least one of the breeders was the parent of each offspring. Also note that all offspring were assigned to group member females. Multiple broods of the same group within the same treatment were treated as one brood.

^a Includes one brood where two helper males were parasitizing simultaneously: 53 offspring fathered by the breeder male, 19 by the small helper male, and 3 by the small helper from the adjacent group.

^b In three different broods.

^c In one brood.

^d In two different broods.

^e The percentages of male helper reproduction per brood were as follows (in parentheses are the number of broods). No breeding option, group member males: 0.0% (14), 7.4% (1), 10.0% (1), 11.1% (1), 12.5% (1); nongroup member males: 0.0% (18), 6.7% (1), 52.9% (1). Breeding option, group member males: 0.0% (22), 25.3% (1), 100.0% (1); nongroup member males: 0.0% (22), 3.9% (1), 4% (1).

and 1.6% for the nongroup member helpers (Wilcoxon signed-ranked test, $Z = -1.36$, $p = .17$).

Treatment and extrapair maternity

Female helpers never dumped eggs in other group's shelters. Extrapair maternity occurred in only one control brood ($n = 19$ broods, excluding the one group with only male helpers), whereas it was not detected in the breeding option treatment ($n = 22$ broods, excluding the two all-male helper groups, likelihood ratio $\chi^2_1 = 1.57$, $p = .21$). The proportion of offspring produced by female helpers did not increase in the breeding option compared to the control treatment (GLMM with weighted logit link: effect of treatment, $z_1 = -0.02$, $p = .98$). The only brood with extrapair maternity was produced during the second phase and was completely sired by the helper female. The breeding female (SL 73 mm) seemed not to interfere with the large helper female (SL 54 mm) laying this clutch, and the breeder female was seen to retain her dominant position in the group. This reproducing helper female was well above the size where females are expected to be reproductively mature, and she was the sixth largest helper female in the experiment during this phase (mean SL \pm SD of all helper females during this phase: 49.4 ± 4.3 mm, range: 42–57 mm).

Treatment and extrapair paternity

Extrapair paternity occurred in a total of nine broods (Table 3). We discovered one brood where three males had apparently

participated in spawning: the breeding male and his small helper male plus a small helper male from the adjacent group (Table 3). The overall proportion of helper male reproduction was not related to the treatment (GLMM with weighted logit link, effect of treatment: $t_1 = 0.73$, $n = 44$, $p = .48$). These results did not change when only reproduction by group member or nongroup member helper males were considered (Table 3, GLMM with weighted logit link, effect of treatment: $t_1 = 0.96$, $n = 42$, $p = .36$ and $t_1 = -0.87$, $n = 44$, $p = .39$, respectively). Again, these results did not change when the outlying brood completely sired by a large helper male was omitted from all analyses (similar three GLMMs as above, $t_1 = -1.09$, -1.28 , and -0.83 , respectively, with $n = 43$, 41 , and 43 , respectively, and $p = .29$, $.23$, and $.41$, respectively).

In 15 groups, broods were sampled during both treatments ($n = 30$ broods in total). Again, helper male reproduction was not related to the treatment within this subsample of broods (similar three GLMMs as above, all $n = 30$, $t_1 = 0.45$, 0.81 , and -1.10 , respectively, with $p = .66$, $.43$, and $.29$, respectively).

Contrary to expectation, large male helpers were similarly likely to engage in reproduction than small male helpers (Table 3). Group member large male helpers engaged in reproduction in three out of 25 broods, whereas group member small male helpers did so in two out of 24 broods (likelihood ratio $\chi^2_1 = 0.18$, $p = .67$). Also, parasitic spawning by nongroup member helper males was not related to their size (Table 3, by small helpers in three out of 44 broods and by large helpers in one out of 44 broods, likelihood ratio $\chi^2_1 = 1.09$, $p = .30$). However, there was overlap in the sizes of the small and large

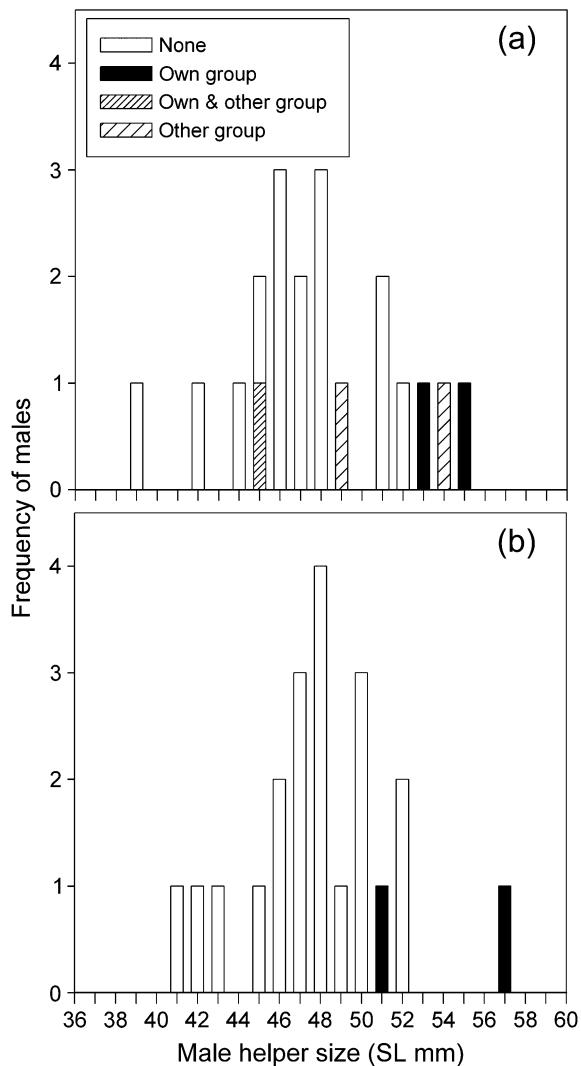


Figure 1
The likelihood of male helpers engaging in reproduction in the own group increased with helper body size, both in the (a) first and (b) second phase of the experiment. Note that for the sake of completeness, also reproductive parasitism of these males in other groups is indicated. White bars: male helpers not reproducing; black bars: male helpers reproducing in own group; fine-hatched bars: male helpers reproducing in own group as well as neighboring group; and coarse-hatched bars: male helpers parasitic only in neighboring group.

helpers (Table 2), partly due to individual variation in growth rates, and small helpers growing faster than large helpers. If helper reproduction was plotted against their actual size at the beginning of the phase wherein the broods were sampled, large male helpers were significantly more likely to engage in reproduction within their group than small male helpers (Figure 1, logistic regression, $n = 43$ males, effect of phase: Wald = 1.14, $p = .23$, effect of body size SL: Wald = 1.45, $p = .041$, with coefficient \pm SE for SL of 0.262 ± 0.128).

The design of the experiment allowed helpers to leave the group voluntarily for forays into other groups and into the dispersal compartment, and some helpers (particularly the large ones) formed pairs and were eventually breeding inside these dispersal compartments (see Bergmüller et al., 2005). None of the reproducing group member helpers (including the one helper female) were evicted from the group after engaging in reproduction, and these helpers were not found

outside their group's compartment more often after they reproduced ($n = 6$ males and 1 female, median % of days in the group before helper reproduction [25 and 75 percentiles]: 95% [84–100] and after reproduction: 100% [92–100], Wilcoxon signed-rank test, $Z = -0.31$, $p = .75$). However, the dispersal opportunities may have reduced the likelihood of male helpers to engage in reproduction at home, and conversely, it may have increased the likelihood of male helpers to visit neighboring groups and parasitize their reproduction.

To assess the confounding effect of dispersal behavior on the likelihood to engage in reproduction, the proportion of days each male helper was observed to be in the group was calculated (henceforth called “proportion days in the group”) and added with helper size (SL) in the GLMMs above. The proportion of helper male reproduction within the own group tended to increase with male helper size (GLMM with weighted logit link: $t_1 = 1.67$, $p = .12$) but was neither related to the treatment ($t_1 = 1.07$, $p = .31$) nor to the proportion days in the group ($t_1 = 0.15$, $p = .89$, all interactions were nonsignificant $p > .23$). Also, the proportion of helper male parasitism in neighboring groups was not related to helper size (GLMM with weighted logit link: $t_1 = -1.20$, $p = .24$), treatment ($t_1 = -0.72$, $p = .48$), and proportion days in the home group ($t_1 = 0.98$, $p = .33$, and again all interactions were nonsignificant $p > .21$). Thus, the failure to find a difference in helper reproductive rate between the two treatments could not be attributed to more helpers dispersing in the breeding option treatment.

Helper sex and reproduction

Group member helper males were more likely to engage in reproduction (six out of 42 broods) than group member helper females (one out of 41 broods, likelihood ratio $\chi^2_1 = 4.16$, $p = .041$). Similarly, nongroup member helper males were more likely to engage in reproductive parasitism (four out of 44 broods) than nongroup member helper females (zero out of 44 broods, likelihood ratio $\chi^2_1 = 5.74$, $p = .017$). However, because in the one case where the helper female participated in reproduction she produced the whole brood, the mean overall proportion of offspring produced by all helpers ($n = 44$), only group members helpers ($n = 39$, selecting only those broods with both sexes of helpers present) or only nongroup member helpers ($n = 44$) was not significantly related to helper sex (note that the median proportion was 0.0% in all cases): 5.3, 3.9, and 1.5% for the males and 2.3, 2.6, and 0.0% for the females, respectively (Wilcoxon signed-rank tests, $Z = -1.84$, -1.05 , and -1.83 with $p = .066$, $.29$ and $.068$, respectively).

Clutch size, treatment, and large helper sex

Clutch size did not depend on the treatment (Figure 2, pre-testing, no breeding option or breeding option treatment, repeated measures GLMM, $n = 257$ broods of 31 female breeders, $F_{2,115.8} = 1.32$, $p = .27$) but depended on breeder female body mass ($F_{1,212.6} = 17.81$, $p < .001$), large helper sex ($F_{1,203.8} = 16.37$, $p < .001$), and the interactions treatment \times large helper sex ($F_{2,115.8} = 5.10$, $p = .008$) and breeder female body mass \times large helper sex ($F_{1,212.6} = 15.52$, $p < .001$). All other interactions, sex of the small helper, and body masses of other group members were nonsignificant when entered into the model (results not shown). Female breeders increased their clutch size depending on their body mass only with large male helpers in the group, and clutches were on average “larger” in the breeding option treatment compared to the control treatment (Figure 2b). In contrast, female breeders showed no such effects on clutch size when assisted

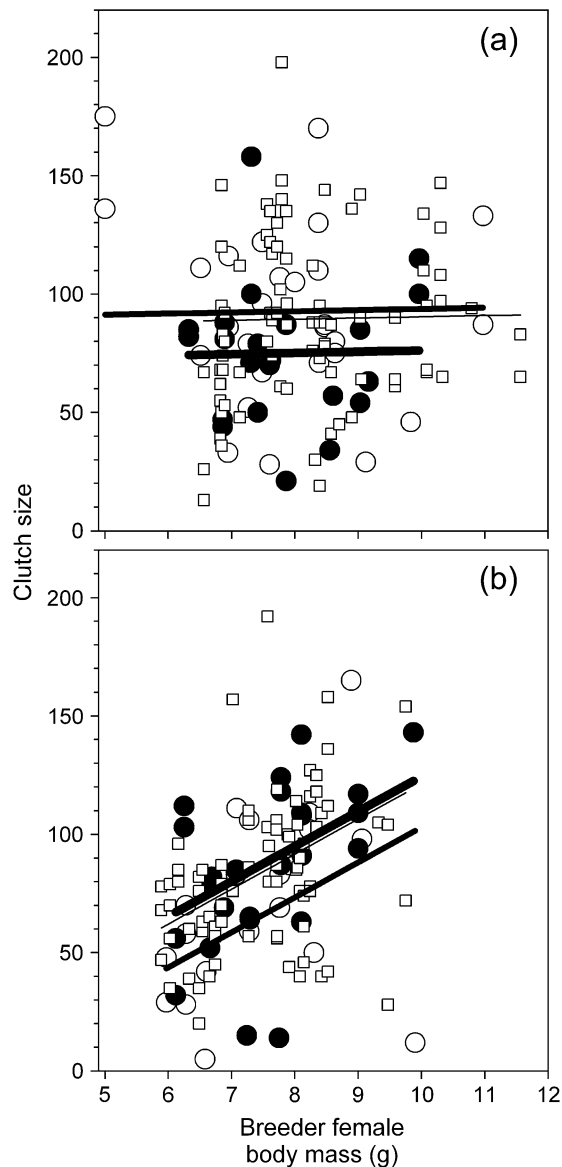


Figure 2

Clutch size depending on breeder female body mass and the sex of the largest helper in the group: (a) with large female helpers and (b) with large male helpers, during the pretesting phase (squares, thin lines) and the no-breeding option (white circles, medium lines) and breeding option treatments (black circles, bold lines). Regression lines from repeated measures GLMM (see text).

by large female helpers (Figure 2a) and now clutch sizes were on average “smaller” in the breeding option treatment compared to the control treatment. Testing for these effects on helper paternity was not attempted due to the low resulting sample sizes.

DISCUSSION

In line with the restraint and tug-of-war models (Table 1), we found no effect of our experimental treatment on reproductive skew, both in males and females. A similar lack of effect of experimentally manipulated ecological constraints on reproductive skew has been found in a social bee by Langer et al. (2004) in the only other experimental study to date. The facts that in our study large helpers engaged more in reproduction than small helpers and that male helpers engaged

more in reproduction than female helpers are also consistent with the tug-of-war model. This was expected, first, because large helpers seem in general more competitive than small helpers (Werner et al., 2003), which is also apparent from helper size-dependent success in territory takeovers (Balshine et al., 1998) and independent breeding (Bergmüller et al., 2005). Second, as argued in the introduction, breeders are expected to have less control over helper male spawning than over helper female egg laying. The results suggest that helpers participate in reproduction based on their reproductive competitive abilities when breeders have incomplete control over reproduction, particularly over helper male reproduction. A theoretical model predicted high levels of reproductive sharing in very small and large but not medium sized helpers (Skubic et al., 2004), but the smallest helpers used in our experiment were similar to the medium helpers modeled by Skubic et al. (2004). No evictions after helper reproduction were observed, although some evictions were expected to occur if helpers take a large share in reproduction that does not outweigh the helper’s positive effect on the fitness of the breeders, and although Dierkes et al. (1999) observed evictions in a previous study where dispersal was not possible.

The three major types of reproductive skew models make partly similar predictions regarding the effects of ecological constraints on reproductive sharing when group members are unrelated (Table 1), and in the end, testing the assumptions of the models may be critical to assess which type of model applies to *N. pulcher* reproductive behavior. Behavioral observations suggest that helpers and breeders compete for the dominant breeding positions (Balshine et al., 1998), helpers compete with the breeders for access to breeding shelters during spawning (Dierkes et al., 1999), and helpers compete among each other for proximity and access to the breeding shelters (Werner et al., 2003). Also, the level of conflict and the resulting changes in male helper growth rate and hence relative breeder and helper reproductive capacity is depending on the difference in body size between breeders and male helpers (Hamilton et al., 2005; Heg et al., 2004b). These facts all suggests that tug-of-war models are more applicable to the *N. pulcher* cooperative breeding system than restraint or concession models, although the possibility of evictions occurring should be incorporated in extended versions of the tug-of-war model to derive explicit predictions for these cichlids under, for example, varying degrees of relatedness and ecological constraints.

We propose that male-male conflict over reproduction may be alleviated by females increasing their clutch size when assisted by male helpers (see also Taborsky, 1984). Preliminary theoretical models of concession, restraint, and tug-of-war reproductive skew models indicate such an increase, for example, may make it more worthwhile for the dominant male to concede reproduction to the subordinate males or may reduce conflicts over reproduction, which may lead to no change in reproductive skew depending on, for example, ecological constraints (Hamilton IM and Heg D, in preparation). Female adjustments in clutch size may affect the predictions of all three types of models on the absolute and relative number of offspring sired by the different group member males, ideas that are currently explored in more advanced reproductive skew models (Hamilton IM and Heg D, in preparation). Consistent with this proposition was our finding of an increase in clutch size in the breeding option treatment compared to the control treatment when females were assisted by large male helpers and vice versa when assisted by large female helpers. Interestingly, clutch size only increased with female body mass if these females were assisted by large male helpers but not when assisted by large female helpers.

Despite both the small helpers and large helpers being able to reproduce in our study (Bergmüller et al., 2005), we found that large helpers participated more in reproduction than smaller helpers did. Such an effect might be typical for societies where dominance status and thus the ability to engage in reproduction is age or body size dependent (e.g., Altmann et al., 1996; Tibbets and Dale, 2004; Widdig et al., 2004; Yuan et al., 2004; but see Engh et al., 2002), possibly mediated by breeding status-dependent adjustments in growth rate (Heg et al., 2004b; Russell et al., 2004). Hitherto, only few studies addressed directly the effects of (differences in) body size on reproductive skew. Reproductive skew was not related to body size in social *Polistes* wasps (Field et al., 1998; Seppa et al., 2002) and the beetle *Parastizopus armaticeps* (Heg et al., in press); and only weakly related to body size but not to the difference in body size in female communally breeding burying beetles (Scott, 1997). Alternatively, large subordinates may be more valuable to the dominant breeders than small helpers are, and therefore, dominants concede more reproduction to large subordinates than to the small subordinates to ensure that valuable helpers remain in their group.

Clearly, more experimental studies are needed to test whether some subordinates increase the fitness of dominants more than others do, depending on, for example, subordinate's phenotype, sex, and status and whether this affects the amount of reproductive skew conceded or taken, the likelihood of eviction from the group and the willingness of the subordinates to help dominants to raise offspring. In our study species, most helpers assist the breeders in raising offspring, but helpers engage in various tasks depending on their body size (Taborsky et al., 1986; von Siemens, 1990). Small helpers primarily provide direct brood care and territory defense against small intruders including egg stealers, whereas large helpers primarily defend the territory against larger conspecifics and piscivores. Although, in general, helpers increase the fitness of the breeders in *N. pulcher* (Brouwer et al., 2005; Heg et al., 2004a; Taborsky, 1984), it is as yet not known whether these benefits depend on helper size, sex, or relatedness (see Stiver et al., 2005).

We found that helper males engaged more in reproduction than helper females. Nevertheless, the overall level of skew was very high, typical for so-called "nuclear families," that is, a dominant breeding pair assisted by retained offspring from previous broods (e.g., Gibbs et al., 1994; Girman et al., 1997; Griffin et al., 2003; Haig et al., 1994; Quinn et al., 1999). High reproductive skew in these societies can be explained, at least partly, by inbreeding avoidance, that is, retained offspring avoid mating with their parents (Koenig and Pitelka, 1979). Individuals may leave territories to avoid inbreeding in these societies (e.g., Cockburn et al., 2003; Daniels and Walters, 2000; but see Keane et al., 1996), and subordinates engage in reproduction when given access to unrelated mates (Cooney and Bennett, 2000; Saltzman et al., 2004). In *N. pulcher*, however, groups do not reflect classical nuclear families but are a mixture of related and unrelated individuals due to frequent breeder exchange (Dierkes et al., 2005; Taborsky and Limberger, 1981). Moreover, by design, all individuals within the groups in our experiment were unrelated, so inbreeding avoidance cannot explain the high level of skew in our experiment.

We conclude that none of the theoretical skew models fully explained our results. Most of our results were consistent with a tug-of-war model over reproduction between the dominant breeders and their helpers. However, the overall high level of reproductive skew and the absence of evictions after helpers engaged in reproduction both suggests that breeders may concede some reproduction to helpers or helpers may restrain themselves from participating in reproduction, regardless of our experimental treatment, despite the fact that these helpers engaged in costly helping behaviors during the experiment (Bergmüller et al., 2005). More importantly, our results on changes in clutch size depending on the experimental treatment and helper sex suggest that the failure to find an effect of our treatment on reproductive skew may be due to alleviating effects of dominant female adjustments in clutch size. This idea awaits further theoretical modeling and experimental testing.

APPENDIX

Number of adults successfully typed (*n*), number of different alleles (Alleles), number of heterozygotic (Het) and homozygotic individuals (Hom), observed (H_{obs}) and expected homozygosities (H_{exp}), the polymorphic information content (PIC), exclusionary power of the first (Excl₁) and second parent (Excl₂), and estimated null allele frequencies (Null), respectively, per locus

Locus	Sequence (5'-3')	<i>n</i>	Alleles	Het	Hom	H_{obs}	H_{exp}	PIC	Excl ₁	Excl ₂	Null
UNH154	F': ACGGAAACAGAAGTTACTT R': TTCCTACTTGTCCACCT	125	7	78	47	0.624	0.61	0.561	0.204	0.372	-0.0224
NP773 (US-758/773)	F': ATCAGCAGGTCATCTGCATGAG R': GCAAAGCAAAGCTGAGAAACAA	128	11	92	36	0.719	0.81	0.782	0.448	0.623	+0.0624
NP007 (UME002)	F': TCAGAGTGCAATGAGACATGA R': AATTTAGAAGCAGAAAATTAGAG	128	6	79	49	0.617	0.62	0.565	0.206	0.365	-0.0022
Pzeb4	F': GCTTGTTTTGGGTTGGTTTTG R': ATGGACACGTGGACTCAAAGAC	127	5	95	32	0.748	0.78	0.742	0.383	0.562	+0.0174
TmoM25	F': CTGCAGTGGCACATCAAGAATGAGCAGCGGT R': CAAGAACCTTTCAAGTCATTTTTG	124	15	92	32	0.742	0.8	0.774	0.449	0.625	+0.0363
TmoM11	F': ATTCAGGTAGAGACGAAATATTA R': TAGTCACAGTTTACACACAAC	125	16	103	22	0.824	0.84	0.819	0.523	0.691	+0.0062
UME003 (NP780/783)	F': GCCACATGTAATCATCTAACTGC R': GAGATTTTTTTGGTTCCGTTG	128	18	101	27	0.789	0.84	0.825	0.536	0.701	+0.0310
NP101 (LOC101)	F': TTCACACTCCAAATGCATG R': TCTGGCACTCTTACACACCC	88	19	66	22	0.750	0.9	0.883	0.647	0.786	+0.0923
ULI2	F': TAAGTTCCATGCACCGAGATA R': TATGGGAACCTGTGAATGTGAG	128	17	111	17	0.867	0.87	0.858	0.591	0.744	-0.0031

Based on the CERVUS analysis of 88 to 128 unrelated, adult individuals (breeders and helpers in the experiment). Total exclusionary power (first parent): 0.996196. Total exclusionary power (second parent): 0.999874. Note that none of genotype frequencies per locus differed significantly from the expectation based on the Hardy-Weinberg equilibrium.

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