



Experimental manipulation of helping in a cooperative breeder: helpers ‘pay to stay’ by pre-emptive appeasement

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The ‘pay-to-stay hypothesis’ proposes that subordinate group members help dominants in order to be tolerated in the territory. Accordingly, helpers should be punished if they are not helping sufficiently and should increase helping behaviour thereafter. We tested whether helping and social behaviours of group members of the cooperatively breeding cichlid *Neolamprologus pulcher* change according to these predictions. A focal helper was experimentally prevented from helping to defend the territory against a conspecific intruder by depriving it of the information that an intruder was present. At the same time the other group members witnessed both the intruder and the ‘passive’ focal helper. When a helper was prevented from providing help, the other group members compensated by increasing defence of the territory, which suggests that the contribution of the passive helper was beneficial. As predicted by the pay-to-stay hypothesis, helpers increased helping behaviour after being prevented from helping. However, we found no indications that dominants punished the focal helper for not having helped before. Punishment may not be measurable, though, because of an appeasement function of helping behaviour. In accordance with this hypothesis, agonistic interactions between focal helpers and dominants were reduced when helpers helped. Apparently, helpers prevent punishment by increasing helping and submissive behaviours. Our data support the pay-to-stay hypothesis and suggest a new mechanism for the regulation of cooperative investment by subordinates: pre-emptive appeasement of dominants through helping and submissive behaviour.

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The apparent altruism of helping behaviour has inspired many theoretical (e.g. Hamilton 1964; Trivers 1971; Pen & Weissing 2000; Kokko et al. 2002) and empirical studies (reviewed in Emlen 1997; Cockburn 1998; Hatchwell & Komdeur 2000). Delayed dispersal is considered to be a prerequisite for helping in most species. However, as delayed dispersal also occurs without offspring helping their parents (Ekman et al. 1994), the questions ‘why do helpers stay?’ and ‘why do they help?’ should be treated separately. Ecological constraints have been widely assumed to be responsible for delayed dispersal and reproduction (Koenig et al. 1992; Emlen 1997; Hatchwell & Komdeur 2000; but see Pen & Weissing 2000; Kokko & Ekman 2002). The ‘benefits of philopatry hypothesis’ suggests subordinates obtain benefits from staying, for

example increasing the production of kin, inheriting the territory or improving survival (Woolfenden & Fitzpatrick 1984; Taborsky 1985; Stacey & Ligon 1991). Both hypotheses are closely linked, as they differ mainly in the emphasis set on the costs or benefits of staying versus leaving (e.g. Emlen 1997).

Helping is often associated with direct brood care, which has been studied in great detail in many bird and mammal species, but it may also involve other parental duties such as assistance in territory maintenance or defence against conspecific intruders or predators (e.g. Taborsky & Limberger 1981; Clutton-Brock et al. 2003). Several hypotheses have been proposed to explain helping behaviour by subordinates that benefits offspring of others. Kin selection theory (Hamilton 1964) provides an explanation for altruistic behaviour between relatives via indirect fitness benefits, but not for the behaviour of unrelated helpers. Thus, helpers may also receive direct benefits from delaying dispersal such as (1) protection in the dominants’ territory (Taborsky 1984,

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1985), (2) parental experience (Brown 1987; Komdeur 1996), (3) group augmentation benefits (Taborsky 1984; Brown 1987; Kokko et al. 2001), (4) territory inheritance (Hamilton 1963; Woolfenden & Fitzpatrick 1984), (5) participation in reproduction (Dierkes et al. 1999) and (6) reciprocated benefits in the future (Trivers 1971; Ligon & Ligon 1983). However, why individuals delaying dispersal help in their home territory is much less clear and has been ardently discussed (Heinsohn et al. 1990; Emlen 1994).

In accordance with the concept of reciprocal altruism (Trivers 1971), Gaston (1978) proposed that help might be a payment by helpers for being tolerated in the dominants' territory. This principle could be a general mechanism to regulate group cohesion and cooperation whenever fitness interests of dominants and subordinates are in conflict. For example, in reproductive skew theory, restraint models propose that subordinates may reduce or cease participation in reproduction to avoid expulsion by dominants (Johnstone & Cant 1999), which may be one way subordinates can 'pay' to remain tolerated in the territory. With regard to the regulation of cooperative behaviour, dominants might use punishment to enforce helping behaviour by subordinates, particularly in groups with asymmetric dominance relationships (Clutton-Brock & Parker 1995). Although solutions to the question of when punishment should occur have been modelled recently (Kokko et al. 2002; Hamilton 2004), empirical studies addressing this issue in animal societies are scarce (Clutton-Brock & Parker 1995; but see Fehr & Gächter 2002 for an example in humans).

In cooperative breeders, dominants may adjust the level of aggression towards subordinates, according to the help the latter provided. In *Neolamprologus pulcher* breeders reaccepted expelled helpers when competitors for space were experimentally added, as a result of which these reaccepted helpers participated in territory defence against the competitors (Taborsky 1985). In some primitively eusocial *Polistes* wasps, queens increased the activity of workers: queens acted aggressively towards 'lazy' workers and experimental cooling of queens quickly decreased workers' activity (Reeve & Gamboa 1983, 1987). Similarly, in naked mole-rats, *Heterocephalus glaber*, queens increased aggressive behaviour towards lazy workers that were otherwise less active (Reeve 1992). In superb fairy-wrens, *Malurus cyaneus*, temporarily removed helpers received increased levels of aggression from the dominant male during the nestling stage (i.e. when help was needed) but not during the nonbreeding season (Mulder & Langmore 1993). In the extreme, refusal of help may even lead to the expulsion of a subordinate. By increasing their helping activities, helpers may thus avoid increased levels of aggression and prevent expulsion. Consequently, punishment may provide an effective and simple mechanism to maintain cooperation by subordinates.

Although punishment is probably a common means of enforcing cooperative behaviour in animal societies (Clutton-Brock & Parker 1995), it has only rarely been demonstrated. A possible reason for this is that aggressive behaviour might often be regarded as an obstacle rather than a possible cause of cooperation. In addition, the

behaviour that elicits punishment might often be difficult to manipulate experimentally. Finally, pre-emptive cooperation might often be shown before actual punishment occurs, thereby stabilizing the level of tolerance of dominants. In the latter, punishment might not be observed easily, but may still influence the degree of cooperation. Punishment may be shown only when cooperative behaviour needs to be reinforced. As punishment presumably involves costs for both the actor and the receiver (functionally it is defined as inflicting costs on the punished at one's own expense (Clutton-Brock & Parker 1995, page 209), pre-emptive helping would eventually reduce these costs for both parties (Boyd et al. 2003). Pre-emptive appeasement could work through helping and submissive behaviour when it serves to reduce the dominants' aggression. Whether submissive or helping behaviour is more appropriate to appease dominants might depend on the context.

Neolamprologus pulcher is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa. Groups consist of a breeding pair and several helpers. The social system is similar to that of cooperatively breeding birds and mammals, as helpers participate in brood care (cleaning and fanning eggs and larvae), territory maintenance (digging sand away from the breeding shelter) and defence against competitors for space, predators and conspecifics (Taborsky & Limberger 1981; Taborsky 1984). Large helpers are usually not kin of the broods they help to rear, mainly because breeders are regularly replaced (Taborsky & Limberger 1981; P. Dierkes, D. Heg, M. Taborsky, E. Skubic & R. Achmann, unpublished data). Kin benefits are thus not sufficient to explain cooperative behaviour in this species. Several studies have shown that helpers gain direct fitness benefits from staying in a group's territory, such as protection (Taborsky 1984; Balshine et al. 2001; R. Bergmüller, D. Heg & M. Taborsky, unpublished data), the opportunity to participate in reproduction (Dierkes et al. 1999) and the opportunity to inherit a breeding position (Balshine-Earn et al. 1998). Some evidence suggests that helpers in *N. pulcher* pay to be allowed to stay in the group. Helpers remain in the territory and continue to help dominants, even if one or both breeders are replaced (Taborsky & Limberger 1981). Above a certain size, helpers are tolerated in the group territory only when they are needed (Taborsky 1985). Temporarily removed helpers assisted more in territory maintenance and defence and visited the brood chamber more often after they were returned (Balshine-Earn et al. 1998).

By directly manipulating the amount of help they contributed, we tested experimentally whether helpers in *N. pulcher* pay to stay. We prevented a target helper from helping (in territory defence against a conspecific intruder) by limiting its information about the presence of an intruder, while the other group members could observe both the threat and the passive helper. We predicted that if help is needed, other group members would compensate for the passive helper. As the other group members could watch the inactive target helper, we predicted that they would punish it after not cooperating. As a consequence, we predicted that helpers should increase their helping behaviour after not having helped.

METHODS

Experimental Groups

The experiment was conducted from 5 August 2001 to 8 January 2002 at the Ethologische Station Hasli, Institute of Zoology in Berne. We used fish that had been purchased by the Konrad Lorenz Institute für Vergleichende Verhaltensforschung (KLIVV) in Vienna from official dealers in 1996, and their laboratory-reared offspring. The fish had been caught at the southern end of Lake Tanganyika at Mpulungu, Zambia. In April 2001 the fish had been taken to Berne and were kept in 200-litre tanks, each containing one group. Water temperature was held at $27 \pm 1^\circ\text{C}$ and water quality was kept constant, close to the values found in Lake Tanganyika (Taborsky 1984, 1985). The fish were fed once a day with commercial dry food or frozen fish food (a mixture of daphnia, *Artemia salina* nauplia and chironomid larvae). The experimental groups consisted of two breeders and two sexually mature helpers (one large and one small, size difference > 5 mm standard length, SL). We formed standardized groups by either temporarily removing surplus helpers from existing groups ($N = 6$) or creating new groups ($N = 7$). Groups consisting of unrelated helpers are common in nature, mainly as a result of regular replacement of breeders (P. Dierkes, D. Heg, M. Taborsky, E. Skubic & R. Achmann, unpublished data) and differences in behaviour between related and unrelated groups have not been observed (Taborsky 1984, 1985). We established new groups according to standard procedures (Taborsky 1984) by introducing two sexually mature fish of helper size (between 35 and 60 mm SL) into a tank containing two clay flowerpot halves. After 1–2 days, when these fish had become accustomed to the new tank, we added one male and one female of breeder size (> 60 mm SL).

Experimental Set-up

The experimental set-up (Fig. 1) was established in the tanks where the groups were kept, to minimize disturbance of the groups. Before the experiment, the fish had at least 4 days to become used to the set-up. When spawning, the fish attached the eggs to clay flowerpot halves, which were used as breeding substrate. Before the test we checked whether the group had fry or eggs. If this was the case, we removed them from the tank (eggs by exchanging flowerpot halves, fry by sucking them off with rubber tubes) and the experiment with this group was delayed for at least 3 days, because breeders are significantly more aggressive against helpers when a new brood is present (Taborsky 1985). Eggs on removed flowerpot halves were hatched in 50-litre tanks, by providing an oxygen supply within the flowerpots. Removed and reared fry were kept in these rearing tanks until they were large enough (> 30 mm SL) to be added to aggregations (i.e. groups of fish in large 400-litre tanks without breeding shelters). Aggregations are social groups, which occur naturally in the north of Lake Tanganyika and consist of fish that are sexually mature but nonreproducing, as they do not occupy shelter sites of their own (Taborsky 1984). Because

the fish dug out ditches and piled up sand on the tank floor, we spread out the sand in the test tank 1 h before the experiment to establish optimal conditions for observation. Thirty minutes before the experiments, we introduced a conspecific intruder matching the size of the focal helper into the intruder compartment 3 (Fig. 1), which was visually separated by an opaque partition from the experimental group. For this purpose the intruder was caught from the intruder tank (10 fish of intruder size were kept together as an aggregation), and transported in a box filled with water to the experimental tank.

Before and between experiments, the group could move freely between compartments 1 and 2 (Fig. 1). Two pot halves, one in each group compartment, provided shelter. At the start of the experiment we gently moved all fish that happened to be in compartment 2 to compartment 1. This could be done with a minimum of disturbance by gently guiding the fish with a transparent board made of Plexiglas. After that we closed a transparent gate between compartments 1 and 2.

Behavioural Observations

After 3 min of acclimatization, the social interactions between the focal helper and all other group members were recorded for 10 min (pretest phase, Fig. 1) with the software package The Observer 3.0 (Noldus, Wageningen, the Netherlands). Behaviours recorded (described in Taborsky 1984, 1985) included overt attacks (ramming, biting, mouth fight), restrained aggressive displays (fast frontal approach, head-down display, S-shaped bending, head jerking, opercula spreading, raising dorsal fin) and submissive behaviour (tail quivering, hook display and escape). In the treatment phase, we recorded the defence of the territory (overt attacks and aggressive displays towards the intruder) of all group members.

Experimental Procedure

The experimental procedure involved four treatments (Fig. 1): NH: the helper was prevented from helping; H: the helper contributed help; C1: the focal helper was not translocated; and C2: the intruder was not presented. Each treatment involved either three or four phases (pretest phase, treatment phase, test phase and, after treatment NH, post-test phase). We observed the social interactions between the focal helper and the other group members in each phase and the defence of the territory of all group members in all treatment phases and the post-test phase of NH.

We prevented the focal helper from seeing the intruder by placing an opaque partition between compartments 2 and 4. For the test, the intruder was moved into compartment 4 and thus presented to the group. For this purpose, we removed the opaque partition between compartments 3 and 4, guided the intruder fish into compartment 4 and re-established the partition between compartments 3 and 4.

Between treatments, we moved the focal helpers between the two group compartments. As described above,

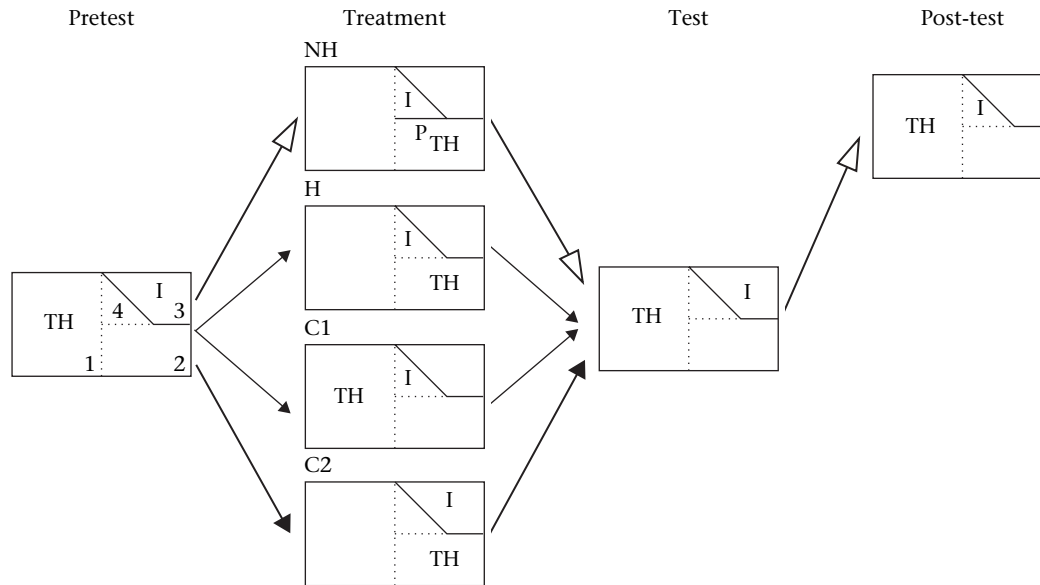


Figure 1. Experimental set-up in a 200-litre tank, viewed from above. Dotted lines indicate transparent Plexiglas partitions and solid lines are opaque partitions. For compartment numbers see the tank in the pretest phase. When no experiment was conducted, the group members could move freely between compartments 1 and 2. At the onset of the experiments, all fish were moved to compartment 1 and a gate was closed between compartments 1 and 2. A conspecific intruder (I) was introduced into compartment 3 before the experiment and could be presented in compartment 4. When in compartment 2, the focal helper (TH) could be prevented from seeing the intruder by placing an opaque partition (P) between compartments 2 and 4. The experimental procedure involved four treatments and three or four phases (each of 10 min), respectively, that followed one another in immediate succession: during the pretest phase, all group members including the focal helper were in compartment 1 and the intruder was in compartment 3; during the treatment phase (four treatments) the focal helper was either in compartment 1 or 2 and the intruder was either exposed (compartment 4) or not (compartment 3), according to the treatment. The test phase, which was identical to the pretest phase, followed the treatment phase. During the experiment, all other group members (second helper and the breeders) stayed in compartment 1. The four treatments applied during the treatment phase were: NH (no help): helper was prevented from helping by an opaque partition; H (help): helper defended; C1 (control 1): no relocation of the focal helper; C2 (control 2): no intruder presentation. Only treatment NH (sequence indicated by open arrows) was followed by a post-test phase: a second intruder presentation during which the focal helper could defend.

this was done with little disturbance to the fish. When helpers were not relocated between different treatment steps (i.e. in treatment C2), we moved the helper with the board within group compartment 1 in a similar way as in the other treatments to provide comparable levels of disturbance between treatments.

All four treatments were conducted with small and large helpers of all 13 groups, in total 26 helpers. Between successive treatments (consisting of three or four phases) there was a break of 1 h to minimize effects of previous treatments. The treatments were randomized to avoid order effects. The entire test was completed in 2 days for each family group, to minimize variation caused by possible time effects.

Ethical Note

As territory defence and aggressive behaviour may be stressful, we kept sample sizes small and observed the fish throughout the experiments. As the groups and the intruders were separated by partitions, the fish could not injure each other. Aggressive behaviours included overt attacks such as ramming (rapid approach with contact), biting and mouth fights between opponents. When attempting to attack the intruder, the group members

could bump into the clear Plexiglas partitions. However, this was usually moderate, and no noticeable harm (no injuries or obvious signs of disturbance) was caused by this behaviour. Intruders might have been subjected to stress from the attacks of the defending group members. However, they were active and engaged in aggressive displays, which suggests that stress levels were comparable to those experienced in natural environments. To minimize the potential stressful situation, we restricted the treatment phase to only 10 min. In family groups, group members can be subjected to increased aggression from dominants, which can result in injuries and eviction. To avoid escalated aggression among group members between the experiments (i.e. when the fish were not continuously observed), we provided opaque plastic tubes near the water surface as shelter. During experimental group formation, two groups were not stable initially. In one group a breeder female was not accepted by the breeder male; in a second group, the breeders did not accept a large helper. Both individuals remained hidden in the plastic tubes to avoid aggression by other group members and were replaced after acceptance was no longer likely (after about 2 days). During the experiment, no fish was injured or died. After the experiment, the reduced groups were re-established (i.e. the removed helpers were reintegrated into their groups) and the newly

created groups were kept for further experiments. The experiment was approved by the Swiss Federal Veterinary Office (Department of Economic affairs).

Experimental Analysis

To explore whether other group members would compensate when one helper was prevented from helping, we compared the defence of the territory by the other group members in the treatment phases of treatments NH and H. We investigated whether the experimental treatment caused changes in social interactions between group members by comparing the change in behaviour between the treatments. To test whether helpers increased help after being prevented from helping, we presented the intruder again after the test phase in treatment NH. We compared territory defence and social behaviour of helpers in this post-test phase with that in the treatment phase in treatment C1 (no prior prevention of helping).

Data Analyses

Territory defence and social behaviours within treatments were analysed as frequencies using the SPSS package, Release 11 (SPSS Inc., Chicago, U.S.A.). We analysed the changes in behaviour between all four treatments (calculated as the frequency of behaviour after the treatment minus that before the treatment) with repeated measures ANOVAs using treatment H as the reference treatment. To check for assumptions about normality, we tested the data with the one-sample Kolmogorov–Smirnov test for goodness of fit against a normal distribution and with the Levenes' test to check for equality of variances. When the data deviated significantly from normality, we used nonparametric statistics as indicated in the results. We analysed the behaviour of large and small helpers separately and all statistical tests are two tailed.

RESULTS

Compensation for Passive Helper

When the focal helper did not participate in territory defence, because it was prevented from seeing the intruder (treatment NH), the remaining group members increased their defence compared to when the focal helper was able to see the intruder and participated in defence (treatment H; small helpers: $F_{1,12} = 7.47$, $P = 0.018$; large helpers: $F_{1,12} = 5.21$, $P = 0.042$; Fig. 2). Defence by other group members (breeders and second helper) was higher when large intruders were presented than when small intruders were presented (intruder size: $F_{1,24} = 6.137$, $P = 0.017$; treatment: $F_{1,24} = 5.883$, $P = 0.019$).

Punishment of Helpers

The frequency of aggression of other group members towards the focal helper did not increase after the latter had been prevented from helping (small helpers:

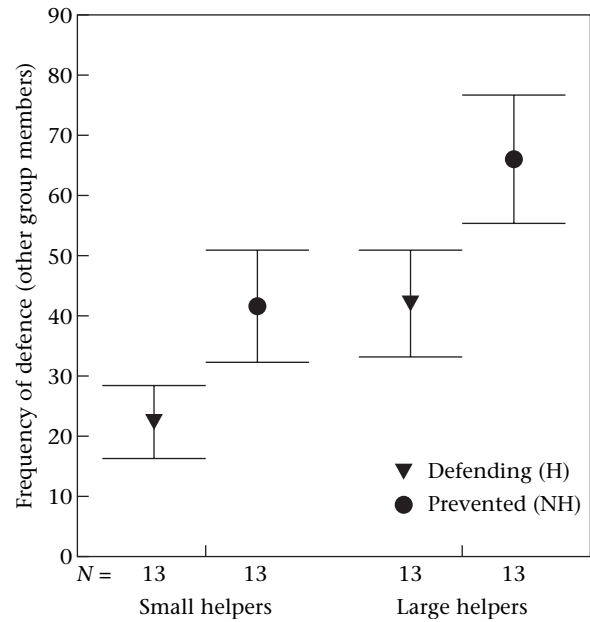


Figure 2. Frequency (behaviour/10 min) of territorial defence ($\bar{X} \pm SE$) by other group members (breeders and the second helper) when the focal helper was prevented from helping (treatment NH) compared to when the latter participated in defence (treatment H). See Fig. 1 legend for an explanation of treatments.

$F_{3,12} = 2.01$, $P = 0.182$; large helpers: $F_{3,12} = 3.10$, $P = 0.104$) nor did it increase in control treatments (no relocation of focal helper, C1; no intruder, C2) compared to when the focal helper helped (H). The submissive behaviour of focal helpers did not increase after they had been prevented from helping (NH) compared to that of the control (H; small helpers: $F_{3,12} = 0.15$, $P = 0.703$; large helpers: $F_{3,12} = 0.001$, $P = 0.977$). However, small helpers were less submissive when they had remained in group compartment 1 (C1) than when they had been relocated into compartment 2 for the treatment (H) and then back to 1 ($F_{3,12} = 5.42$, $P = 0.038$), whereas in larger helpers no significant effect of relocation was detected ($F_{3,12} = 0.66$, $P = 0.434$; Fig. 3).

Post-test Help

Small helpers defended more after they had been prevented from helping (post-test phase of treatment NH) than in the control (C1; Wilcoxon signed-ranks test: $T = 7.50$, $N = 13$, $P = 0.041$; Fig. 4). They showed more aggressive displays towards intruders ($T = 4.50$, $N = 13$, $P = 0.019$) but did not attack them more often ($T = 16.00$, $N = 13$, $P = 0.128$). Large helpers did not increase their overall frequencies of defence after being prevented from helping ($T = 10.50$, $N = 13$, $P = 0.155$; Fig. 4). However, their aggressive displays towards intruders tended to increase, although not significantly so, after the treatment ($T = 6.00$, $N = 13$, $P = 0.093$), whereas attacks did not ($T = 14.00$, $N = 13$, $P = 0.311$).

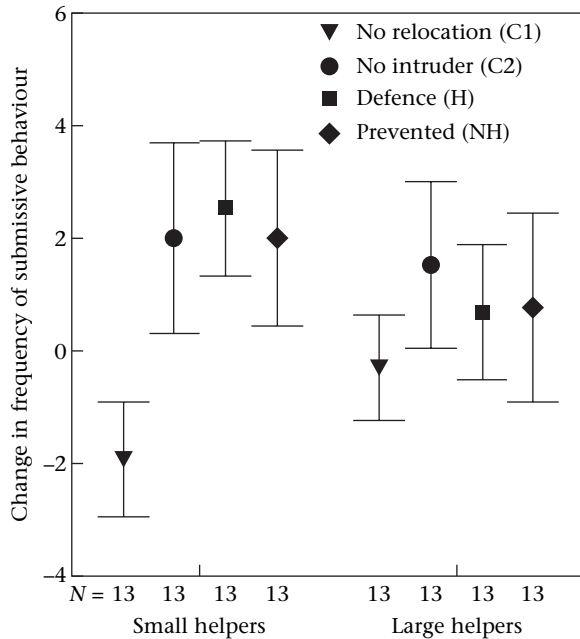


Figure 3. Change in frequency of submissive behaviour ($\bar{X} \pm SE$) of focal helper towards other group members according to the treatments. See Fig. 1 legend for an explanation of treatments.

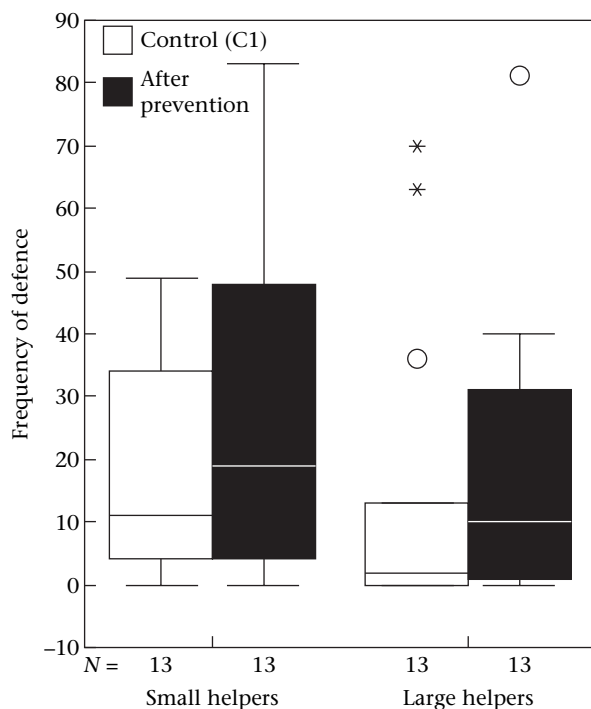


Figure 4. Frequency of territorial defence (behaviour/10 min) of helpers after being prevented from helping and in the control treatment. Horizontal lines are medians; boxes are upper and lower quartiles, whiskers indicate the highest and the lowest values excluding extreme values and outliers, circles are extreme values and asterisks are outliers.

Post-test Defence by Other Group Members

Territory defence by the other group members was not reduced when the group defended for the second time in succession (post-test of NH) compared to the control (treatment C1; paired t test: small intruders: $t_{12} = -0.321$, $P = 0.753$; large intruders: $t_{12} = -0.172$, $P = 0.867$).

Helping as Appeasement

Did helpers receive more aggression while not helping?

Small helpers did not receive more aggression from the group while they were prevented from defending (treatment NH) than during defence (treatment H; Wilcoxon signed-ranks test: $T = 17.00$, $N = 13$, $P = 0.514$), nor did their submissive behaviour towards the other group members differ between the treatments ($T = 12.00$, $N = 13$, $P = 0.113$). However, small helpers were less submissive towards large helpers while defending than while being prevented from helping ($T = 1.50$, $N = 13$, $P = 0.019$), whereas the aggressive behaviour of large helpers towards small focal helpers did not differ ($T = 6.00$, $N = 13$, $P = 0.680$).

Did helpers reduce submissive behaviour while helping?

We analysed whether the relation between received aggression and submissive behaviour was influenced by the treatment (treatment H (defence)/treatment NH (no defence)). An ANCOVA with submissive behaviour as response variable and received aggression as a covariate showed no significant difference between the treatments (defence/no defence, treatment: $F_{1,10} = 0.454$, $P = 0.516$), but a significant difference between the family groups (group: $F_{1,10} = 3.328$, $P = 0.033$). Received aggression predicted the amount of submissive behaviour of focal helpers ($F_{1,10} = 6.717$, $P = 0.027$). The interaction treatment*received aggression was significant ($F_{1,10} = 5.273$, $P = 0.045$) showing that the relation between submissive behaviour and received aggression was influenced by the treatment. Small helpers showed less submissive behaviour per received act of aggression while helping than while prevented from helping (Fig. 5). When we removed the two outliers with extreme aggression rates from the analysis, this did not alter these results substantially (treatment: $F_{1,8} = 0.011$, $P = 0.920$; group: $F_{1,8} = 1.198$, $P = 0.399$; received aggression: $F_{1,8} = 5.256$, $P = 0.048$). However, the interaction treatment*received aggressive behaviour was no longer significant ($F_{1,8} = 2.150$, $P = 0.177$).

Were breeders less aggressive when helpers helped more?

Overt attacks of breeders towards small helpers tended to be reduced while they helped more (comparison of treatment C1 (less help) with post-test NH (more help), paired t test: $t_{12} = 1.801$, $P = 0.097$). However, aggressive displays of breeders towards small helpers ($t_{12} = 0.833$, $P = 0.421$) and submissive behaviour of small helpers towards breeders ($t_{12} = 0.964$, $P = 0.354$) did not differ between the treatments.

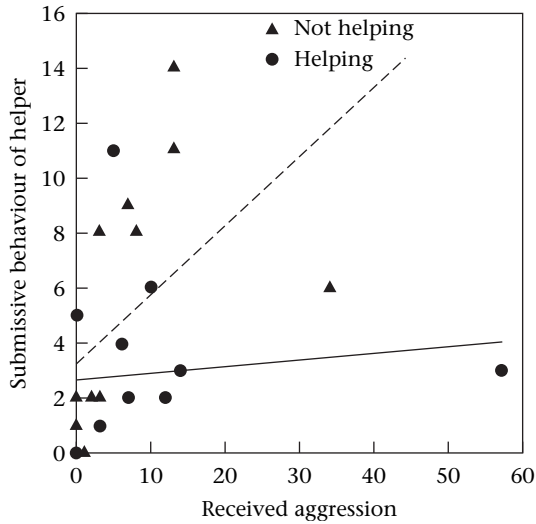


Figure 5. Submissive behaviour (behaviour/10 min) of small helpers in relation to the amount of aggression received while helping and while prevented from helping.

Were helping and submissive behaviour correlated?

Small helpers were less submissive towards large helpers when they helped more. The increase in defence from the control (C1) to the post-test (NH) correlates negatively with the change in submissive behaviour between both situations (Spearman rank correlation: $r_s = -0.558$, $N = 13$, $P = 0.048$; Fig. 6).

Helping versus Submission as Appeasement

We tested whether helping is more efficient appeasement than submission by analysing whether the relation of total appeasement behaviour of small helpers (combined submissive and helping behaviour) and received aggression was influenced by the treatment, that is when helpers helped more (post-test NH) or less (treatment C1).

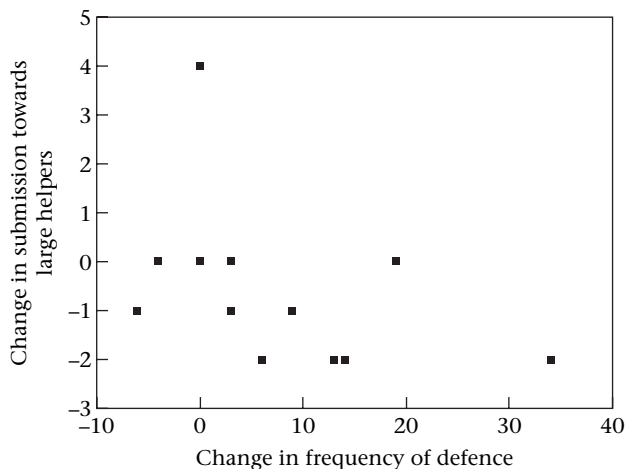


Figure 6. The change in submission of small helpers towards large helpers between the control treatment (C1) and the post-test (NH) after helpers were prevented from helping in relation to the change in frequency of defence by small helpers.

An ANCOVA with received aggression as the response variable and total appeasement behaviour as a covariate showed no significant difference between the treatments (less help/more help, treatment: $F_{1,10} = 1.008$, $P = 0.339$), or between the family groups (group: $F_{1,10} = 2.027$, $P = 0.136$). Appeasement behaviour predicted the amount of received aggression of focal helpers ($F_{1,10} = 5.396$, $P = 0.043$). The interaction treatment*appeasement behaviour was significant ($F_{1,10} = 9.907$, $P = 0.010$) showing that the relation between appeasement behaviour and received aggression was influenced by the treatment. Small helpers showed less total appeasement behaviour per act of aggression received when helping more (post-test NH) than when helping less (treatment C1; Fig. 7).

DISCUSSION

Our study shows that focal helpers helped more after they had been prevented from doing so, which was predicted by the pay-to-stay hypothesis. However, contrary to our predictions, there was no indication that dominants punished the focal helper after the latter had not helped, which would have explained how helping behaviour is regulated between the group members. Punishment may not be detectable, however, if helping behaviour serves an appeasement function. We showed that helping behaviour reduced the amount of aggression from dominant individuals and found that helping and submissive behaviours were negatively correlated. These results suggest that helpers are able to prevent punishment by pre-emptive appeasement.

Other group members compensated for the lack of helping behaviour of the 'lazy' focal helper by increasing defence against the intruder, suggesting that the helpers' help is indeed beneficial. This result accords with previous findings that the workload is partitioned among group members (Taborsky 1987), helper acceptance in a group depends on the group's present requirements for help (Taborsky 1985), and breeder workload decreases with increasing group size (Balshine et al. 2001). Furthermore, large groups are more stable between years (D. Heg, L. Bower, Z. Bachar & M. Taborsky, unpublished data), which suggests that they might be more capable of sustaining a territory, which could, at least in part, be the result of higher capacities for defence of the territory against predators and competitors.

Why Did 'Lazy' Helpers Help More?

Our results do not suggest that lazy helpers were punished as they neither received more aggression from other group members, nor displayed more submissive behaviour. Why then did small helpers increase their territory defence after being prevented from doing so? We propose four hypotheses to explain our results.

(1) Helpers may manipulate the aggressive behaviour of breeders by pre-emptive cooperation, thereby stabilizing the aggression of dominants to standard levels. In this case, punishment might not easily be observed, but still influence cooperative behaviour. Experiments by Hert

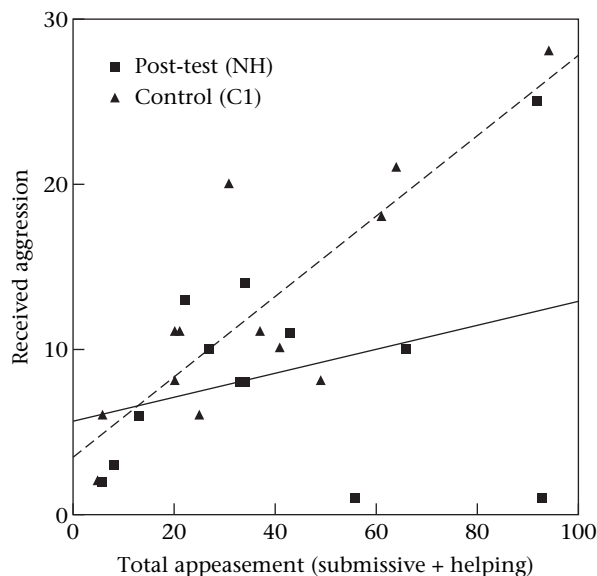


Figure 7. Relation between the frequency of total appeasement behaviour (combined submissive and helping (defence) behaviour) of helpers and received aggression in the control treatment (C1) and the post-test (NH) after helpers were prevented from helping.

(1985) showed that helpers are indeed able to influence attack rates of breeders. When breeders and focal fish could see each other through a clear partition, strange helpers (i.e. helpers of other families) were more submissive towards breeders than were unfamiliar nonhelpers and were therefore attacked at lower rates. In a second test the focal fish could not see the breeders and thus did not display submissively. Here, no differences between attack rates of breeders towards strange helpers and controls were found. Helpers are thus able to reduce aggression of breeders by reacting appropriately, that is, by showing more or fewer submissive displays. We propose that helping may serve a function that is similar to submissive behaviour. Hence, helping more should reduce (a) attack rates received by dominant group members and (b) submissive displays required to appease dominants.

Our results are in accordance with both predictions. Small helpers tended to be attacked at lower rates from breeders when they defended more, and they were less submissive towards larger helpers when defending. Small helpers showed less submissive behaviour per act of aggression received from dominant group members when defending than when prevented from defending, and helping levels of small helpers correlated negatively with submissive behaviour towards large helpers. At the level of behavioural causation, defence of the territory and submissive behaviour may be antagonistic because of common regulatory mechanisms (e.g. Oliveira et al. 2001). However, this cannot be the only underlying mechanism, because in some parts of our experiment, these two behaviours varied independently from each other (e.g. submission of small helpers towards breeders did not differ while helpers helped more (post-test NH) or less (treatment C1)). The result that small helpers showed less appeasement behaviour (the combined total frequency of

submissive and helping behaviour) when they helped more, while receiving comparable amounts of aggression as in the control, suggests that helping might be a more efficient means of appeasing dominants than submissive behaviour. But how did helpers essentially realize that they should help, as they were not able to see the intruder during the treatment phase? One possibility is that they sensed the intruder's presence through other cues, for example by olfaction. More likely, social cues by the group members could have provided the required information. For example, focal helpers could watch the defence of the territory by other group members during the treatment phase. In addition, after joining the group again, even only a few aggressive displays by dominants may have sufficed to reinforce cooperation, which could not be detected in the behavioural analyses. Whether submissive or helping behaviour is more appropriate for appeasing dominants might depend on the context. An acute threat to the family or territory may be the appropriate situation to demonstrate commitment to the group by helping in the form of increased levels of territory defence.

(2) Helpers could observe other group members defending; this may have raised their aggressive state by social facilitation, which was retained until the intruder was presented once again. However, this explanation seems unlikely, as the aggressive interactions between focal helpers and dominants did not differ between the treatments 'helper helped' (H) and 'helper did not help' (NH). Furthermore, it assumes that helpers retain their aggressiveness, acquired by mere observation, without showing it over a period of more than 10 min, after which the intruder was presented again.

(3) Helpers might have compensated if other group members had reduced their defence, for example because of exhaustion or habituation. However, as defence of the territory of group members did not differ between the control (treatment C1) and when helpers defended after being prevented from helping (post-test NH), this explanation does not hold.

(4) Increased defence could have been the result of a relocation effect, although helpers were relocated only within their home territory and into an area they frequently used on their own accord before and after the manipulation. Nevertheless, this manipulation resulted in increased submissive behaviour towards other group members after the helpers returned to compartment 1 in the test phase. In a field study, helpers that had been completely removed from the territory for an extended period (4–6 h) performed more submissive behaviour and received more attacks from resident helpers, but not from the breeders (Balshine-Earn et al. 1998). In the present study, the reaction was more moderate when helpers again joined the group. As helpers remained in the territory and within sight of the group, it is unlikely that, in our experiment, relocation alone caused defence of the territory to rise in the post-test phase. Furthermore, large helpers also tended to increase their defence whereas they were not more submissive after relocation.

The first of these hypotheses appears to be the most likely in explaining our results. Pre-emptive appeasement may reduce the costs of within-group regulation of

cooperative behaviour considerably (Boyd et al. 2003), because punishment presumably involves costs for both the actor and the receiver (Clutton-Brock & Parker 1995). Pre-emptive appeasement is fully compatible with the concept of negative reciprocity (Clutton-Brock & Parker 1995), because, ultimately, cooperation is still maintained through reinforcement, as defecting group members should be punished when they fail to prove their commitment to cooperate. The idea of pre-emptive appeasement shows obvious parallels to reproductive skew theory. Restraint models propose that subordinates may refrain 'pre-emptively' from participating in reproduction to avoid expulsion by dominants (Johnstone & Cant 1999). The pay-to-stay principle could thus be regarded as a more general underlying mechanism to regulate the amount of cooperative behaviour when fitness interests of dominants and subordinates are in conflict.

Why Did Only Small Helpers Pay?

Large helpers might be less willing to pay for being tolerated, because their survival prospects when expelled are higher than for small helpers. However, if helping serves an appeasement function, large helpers should be expected to invest more in territory defence than smaller helpers to be tolerated by the dominants. This expectation is based on three observations: (1) large helpers are closer in size to breeders and pose a possible threat for them with regard to territory ownership (Taborsky 1987); (2) they are more likely to parasitize reproduction (Dierkes et al. 1999); and (3) in general they invest more in territorial defence, than small helpers do (Taborsky 1987). However, only small helpers significantly increased their defence of the territory after being prevented from doing so. Perhaps an effect could be detected only for small helpers because the response intensity depends on the actual probability of being punished. Small helpers had to appease three dominants, whereas large helpers needed to appease only the breeders. Conflicting interests exist not only between breeders and helpers but also between helpers in *N. pulcher* (Balshine-Earn et al. 1998; Werner et al. 2003). This is because a territory apparently supports only a limited number of helpers (Balshine et al. 2001; R. Bergmüller, D. Heg & M. Taborsky, unpublished data). Therefore, competition between helpers is expected to be high, and small helpers might need to appease dominant helpers to be able to stay in the territory. As helping effort is partitioned among group members, dominant helpers may force subordinates to help, thereby reducing their own workload.

Conclusions

Our results support the hypothesis that helpers pay to stay in the dominants' territory by signalling their readiness to cooperate. Helpers appear to be able to use helping and submissive behaviour to manipulate the amount of aggression received from dominants. Thus, actual punishment is difficult to detect. The ability to reduce aggression of dominants pre-emptively could be a key quality characterizing a socially competent helper.

Helpers gain direct fitness benefits when staying in a group's territory, such as protection from predators. Socially competent helpers able to anticipate when to engage in submissive behaviour and helping tasks may have key selective advantages, as these helpers should have better chances of being tolerated for a prolonged time in a group's territory as a subordinate.

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