Dominant members of cooperatively-breeding groups adjust their behaviour in response to the sexes of their subordinates

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Summary

In cooperatively-breeding species, the sexes of subordinate group members may have important consequences for dominant individuals. We varied subordinates' sexes in aquarium-housed groups of the cooperatively-breeding cichlid fish *Neolamprologus pulcher*, and compared the behaviours of dominant individuals in groups with same- versus opposite-sex subordinates. Dominants tended to be more aggressive towards same-sex subordinates, and dominant males directed more affiliative behaviour towards large female subordinates. These patterns suggest that mixed-sex groups can be viewed as separate male and female dominance hierarchies. Aggressive and affiliative interactions between dominant males and dominant females were more frequent when a large subordinate was female, which indicates that subordinates can be a source of conflict between the members of a breeding pair. Finally, subordinates' sexes affected dominants' locations within aquaria and the performance of territory maintenance behaviour by dominant females. In many cases, the effect of one subordinate's sex depended on a second subordinate's sex or on group members' absolute or relative body sizes. Therefore, predicting effects of subordinates' sexes in larger, more variable groups will be challenging. Our results are the first to experimentally demonstrate the importance of a group's gender composition for the behaviour of dominant individuals in a cooperatively-breeding species.

Keywords: dominance hierarchy, gender composition, group-living, *Neolamprologus pulcher*.

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Introduction

In group-living species, the fitness expectations of dominant group members are typically greater than those of subordinates. Dominant individuals have greater access to resources (e.g., Hannon et al., 1987; Forrester, 1991; Schradin & Lamprecht, 2002), and, in cooperatively-breeding species, have much more opportunity to reproduce (Taborsky, 1994; Emlen, 1997; Solomon & French, 1997; Reeve & Keller, 2001; Koenig & Dickinson, 2004; Heg, 2008; Heg et al., 2008). Because social dominance is so important to an individual's fitness, dominant group members should be sensitive to aspects of group composition that affect their ability to retain their rank. For example, dominants may be more aggressive towards larger, more competitive, or higher-ranked subordinate group members (e.g., Fricke & Fricke, 1977; Reeve & Nonacs, 1997; Hamilton et al., 2005; Cant et al., 2006).

When social dominance is associated with reproductive opportunities, dominants should be very sensitive to the sexes of their subordinates. Same-sex subordinates pose a threat to a dominant individual's future reproductive rank, whereas opposite-sex subordinates do not. Same-sex subordinates may also compete with a dominant individual for current reproduction (Cockburn, 1998; Hughes, 1998; Taborsky, 2001), whereas opposite-sex subordinates pose no direct threat to a dominant's current reproductive success. Differential aggression towards same-sex subordinates has been described in several group-living species (e.g., French, 1997; Schradin & Lamprecht, 2002; Clutton-Brock et al., 2006). However, in many systems, the implications of a group's gender composition for dominant group members have not been fully considered (Cockburn, 2004).

Those implications extend beyond direct dominant-subordinate interactions. In a mixed-sex group, group members of one sex may also influence the structure and stability of the social hierarchy in the other sex. Such effects are expected whenever the status of subordinates is a source of conflict between dominant males and dominant females. For example, in the cichlid fish *Neolamprologus multifasciatus*, dominant males increase their opportunities for polygyny by actively interfering in aggressive interactions between dominant and subordinate females (Schradin & Lamprecht, 2000). In a congener, *N. pulcher*, a previously subordinate male's ability to ascend to the dominant breeding position in its group depends on its size relative to the size of the dominant female (Fitzpatrick et al., 2008). Thus, in addition to interacting differently with same- and opposite-sex subordinates,

conflict over the status of subordinates may result in dominants interacting differently with one another depending on whether subordinates are male or female.

Here, we describe behaviours of dominant individuals in aquarium-housed *N. pulcher* groups that were established specifically to study the consequences of gender composition. Each group consisted of 4 unrelated individuals: a dominant male and female, a large subordinate (LS, male or female), and a small subordinate (SS, male or female). Observations of these groups have allowed us to consider a range of questions, including the effects of gender composition on genetic parentage (Heg et al., 2008) and parasitic spawning (Mitchell et al., in review). In this paper, we considered three aspects of dominant male and female behaviour:

Interactions with same- versus opposite-sex subordinates

If mixed-sex groups function as separate male and female hierarchies then dominants should be more aggressive and direct less affiliative behaviour towards same-sex subordinates. Mitchell et al. (in review) report that, in a subset of the groups used in this study, dominant males were more aggressive towards male subordinates than towards female subordinates. The study reported here used more detailed observations of both dominant males and females under more controlled conditions. These observations were made on the full set of experimental groups, which meant that we were able to control for effects of group members' body sizes by including body size indices as covariates in our analyses. We were also able to test for statistical interactions between the effects of each subordinate's sex. There was no a priori reason to expect such effects. However, we have previously reported that male large subordinates in these groups gained more paternity in groups where the small subordinate was also male (Heg et al., 2008).

Interactions between dominant males and dominant females

We predicted that conflict between dominants would increase when a female subordinate was present, i.e., that aggression would increase and that affiliative behaviours would be less frequent, because the subordinate provides an opportunity for polygyny for the dominant male but poses a threat to the dominant female. We recognize that, conversely, a large male subordinate provides opportunities for female polyandry but threatens the future rank

and current paternity of the dominant male. However, the dominant female is generally subordinate to the dominant male, so her ability to interfere with the male dominance hierarchy is more limited — at least using the direct interactions that we recorded. See Fitzpatrick et al. (2008) for an example of the possible effects when the female is the dominant group member.

Behaviours that do not involve direct social interactions

We looked specifically at the time dominants spent in or near breeding shelters and at their investment in territory maintenance. Viewing groups as separate male and female dominance hierarchies does not lead a priori to directional predictions regarding differences in these behaviours. However, these contrasts illustrate the potential breadth of effects that can be associated with gender composition.

Methods

Study system

Neolamprologus pulcher is a group-territorial, cooperatively-breeding cichlid fish endemic to Lake Tanganyika, Africa (Taborsky & Limberger, 1981; Taborsky, 1984 as *Lamprologus brichardi*; see Duftner et al., 2007 for taxonomic status). A typical group consists of a dominant breeding pair and 5–8 subordinates of both sexes (Taborsky & Limberger, 1981; Balshine et al., 2001). Same-sex group members can be ranked based on size differences (Heg et al., 2004; Hamilton & Heg, 2008). The dominant male is usually the largest fish of either sex. The smallest, youngest group members are usually the offspring of the dominant pair. Larger, sexually mature subordinates (corresponding to those used in this study) are often unrelated to the dominant pair (Stiver et al., 2004, 2006; Dierkes et al., 2005). Group members of both sexes direct aggressive, submissive and affiliative behaviours towards one another (Taborsky, 1985; Taborsky et al., 1986). These interactions are presumed to be important mechanisms through which a group's dominance hierarchy is maintained (Hamilton et al., 2005). On soft substrates, group members excavate and maintain shelters beneath rocks within the group's territory (Taborsky & Limberger, 1981; Taborsky, 1984). The dominant pair spawns within one of these shelters. Spawning cannot be directly observed

in the field, but male parasitic spawning has been recorded in laboratory experiments and inferred from genetic parentage analyses of field samples (Taborsky, 1985; Dierkes et al., 1999, 2008; Heg et al., 2006, 2008). Laboratory observations suggest that female subordinates rarely contribute eggs to dominant females' clutches, but some female subordinates do lay their own clutches, which are fertilized by the dominant male (Heg, 2008; Heg & Hamilton, 2008). Thus, subordinates of both sexes are reproductive competitors of the same-sex dominant, and the possibility of reproduction by female subordinates leads to additional (polygynous) mating opportunities for dominant males.

Experimental design

We established 48 *N. pulcher* groups. Each group consisted of 4 unrelated fish: a dominant breeding male and female, a large subordinate (LS), and a small subordinate (SS). Groups were organized in 12 sets of four. Within each set, like-ranked group members were matched for size (±2 mm) but the sexes of the two subordinates were varied with the intention that all four possible combinations were represented: LS male, SS male (Mm); LS male, SS female (Mf); LS female, SS male (Fm); and LS female, SS female (Ff). Among sets, the lengths of like-ranked fish varied (dominant males 58–77 mm standard length, dominant females 55–68 mm, LS 46–54 mm, SS 38–45 mm), but the size-based ranking within a group was always dominant male > dominant female > LS > SS. Subject to size and gender restrictions, fish were allocated to groups randomly. Some subordinates were initially mis-sexed, and one group was excluded from the study because the dominant male did not accept the intended dominant female, so the sample sizes for the 4 treatments were: 11 Mm, 12 Mf, 11 Fm, 13 Ff. All fish were the laboratory-reared descendents of fish caught at the southern end of Lake Tanganyika, near Mpulungu, Zambia. Prior to this experiment, they had been held in large aggregation aquaria without access to breeding substrate. Under these conditions, *N. pulcher* do not form distinct groups or breed.

Groups were housed in identical 125-l compartments within 1000-l aquaria. Each compartment contained two clay flowerpot halves that were used as shelters and as spawning substrate. A suspended translucent tube and a suspended filter provided subordinates with refugia from dominant aggression. The floor of each compartment was covered with approx. 6 cm of

sand. Compartments were created using alternating clear and opaque partitions across the width of each aquarium, such that each group had visual contact with one neighbouring group. Adjacent groups were from the same set. Among sets, the compositions of groups housed in neighbouring compartments were randomized. Groups were fed daily with pre-weighed commercial flake food (2.5% of the group's combined mass/day).

Each group was observed twice. Thirty min prior to observation, an opaque partition was placed between the focal group's compartment and the adjacent compartment. The partition ensured that behaviours of fish in the focal group were not affected by the adjacent group. The observation then proceeded as 3 sets of 4 consecutive 5 min focal follows of each group member, i.e., the first group member was observed for 5 min, then the second, the third, and the fourth. This sequence was then repeated twice. Thus, over 2 observation periods, each individual was observed for 30 min. During a focal follow, we recorded all behaviours directed by the focal individual towards other group members in successive 30-s intervals. Behaviours were scored as aggressive, affiliative, or submissive (see Taborsky, 1984; Grantner & Taborsky, 1998; Hamilton et al., 2005 for descriptions of specific behaviours and their interpretation). We used the number of 30-s intervals during which behaviours were observed as the response variable in our analyses. We also counted all digging behaviour and, at the end of each 30-s interval, noted whether the focal individual was within 10 cm of the flowerpot shelters. In this paper, we focus on the behaviours of the dominant male and female. We discuss the behaviours of subordinates elsewhere (Mitchell et al., unpubl.).

All observations were made during non-spawning periods, defined as beginning three days post-spawning and ending two days prior to the subsequent spawning. Most groups were observed following the second and third clutches. This delay ensured that relationships between group members had stabilized before observations began. If the dominant pair was not spawning regularly then the first observation was made at least 30 days after the study commenced and the second observation was made at least five days after the first. Spawning dates were identified by inspecting each compartment daily. If a clutch was present, it was removed on the day-of-laying by replacing the flowerpot on which it had been laid. Removing clutches kept group composition unchanged over the course of the study. Because we removed the entire clutch and the laying substrate, it is unlikely that dominants attributed the

disappearance to their subordinates. Any short-term effect of clutch removal should have subsided by the time the group was observed.

Analyses

Statistical analyses were carried out using JMP 3.1.5 (SAS Institute, Cary, NC, USA). Frequencies of most behaviours were analyzed using ANCOVAs, with subordinates' sexes as fixed factors and two group body size composition indices included as covariates (see below). Pairwise interactions between predictors were initially included in these models; higher-order interactions could not be considered. Reduced models were generated through stepwise removal of terms that did not approach statistical significance ($p > 0.1$). Residuals were tested for normality and for variance homogeneity among treatment groups using Shapiro–Wilks and Levene's tests, respectively, and transformed when necessary. Response variable distributions differed, so no single transformation was adequate for all analyses (see Results). Many dominants never directed affiliative behaviour towards their subordinates and/or never dug, so the frequencies of these behaviours could not be transformed for normality. Therefore, we used logistic regressions to test for effects of subordinates' sexes on the likelihoods that these behaviours were observed at least once. Group body size indices could not be included in analyses of affiliative behaviour towards subordinates but were included in analyses of digging behaviour. Patterns of dominant digging were also analyzed using subordinates' observed digging behaviour (dug or not) as predictors. We ran these last analyses because female subordinates were more likely to dig than male subordinates (Mitchell et al., data not shown), and we wished to assess whether dominants were responding directly to subordinates' sexes or were compensating for subordinate behaviour.

To control for possible effects of group members' body sizes, we ran a principal component analysis of group members' body sizes and then used PC1 and PC2 as covariates in our analyses. PC1 (range -2.9 to $+2.9$) explained 66% of body size variation and was positively correlated with the lengths of all four group members ($r = 0.77$–0.85). PC2 (range -2.1 to $+2.4$) explained 27% of body size variation. PC2 was positively correlated with LS and SS lengths ($r = 0.53, 0.54$) and negatively correlated with the lengths of the dominant male and female ($r = -0.50, -0.49$). Thus, PC1 scores >0 indicated groups in which all fish were relatively large, and PC2 scores >0 indicated groups in which length differences between subordinates and dominants were relatively small.

Results

Interactions with same versus opposite-sex subordinates

As expected, dominant males were more aggressive towards male LS than towards female LS (Figure 1a, Table 1) and directed less affiliative behaviour towards male LS than towards female LS (Figure 2a, LR $\chi^2 = 3.8$, $p = 0.05$). Aggression towards the LS was not affected by the sex of the SS. The difference in aggression towards male and female LS was most pronounced when subordinates were small relative to dominants, i.e., PC2 < 0 (Table 1). Patterns of dominant male aggression towards SS were more complex (Figure 1b, Table 1). The sex of the small subordinate had a significant effect, but aggression was also affected by the sex of the LS, by group members' body sizes (PC1), and by interactions between those predictors. On average, dominant males were most aggressive towards SS in Mm groups. Female SS were not more likely than male SS to receive affiliative behaviours from dominant males (Figure 2b, LR $\chi^2 = 2.7$, $p = 0.10$). Dominant females were more aggressive towards female subordinates (Figure 1, Table 1). For LS, this difference was only evident when the SS was male. Dominant female aggression towards SS was independent of LS sex, but was sensitive to length difference between dominants and subordinates (PC2). Dominant females were more sensitive to the SS's sex in groups where subordinates were relatively small.

Most dominants did not direct affiliative behaviours towards subordinates (Figure 2). Dominant males were more likely to do so towards female subordinates than towards male subordinates. This tendency was significant for LS (LR $\chi^2 = 3.8$, $p = 0.05$) but not for SS (LR $\chi^2 = 2.7$, $p = 0.10$). In both cases, the likelihood of affiliative behaviour was independent of the second subordinate's sex (towards LS: LS $\times$ SS: LR $\chi^2 = 0.15$, $p = 0.69$; SS: LR $\chi^2 = 0.15$, $p = 0.70$; towards SS: LS $\times$ SS: could not be evaluated; LS: LR $\chi^2 < 0.1$, $p = 0.93$). Dominant females were equally likely to direct affiliative behaviour towards male and female subordinates (towards LS: LS $\times$ SS: LR $\chi^2 = 0.1$, $p = 0.77$; LS: LR $\chi^2 = 0.1$, $p = 0.78$; SS: LR $\chi^2 = 0.3$, $p = 0.56$; towards SS: LS $\times$ SS: could not be evaluated; LS: LR $\chi^2 = 0.7$, $p = 0.41$; SS: LR $\chi^2 = 1.5$, $p = 0.22$). For SS, the LS $\times$ SS interaction could not be formally evaluated. In groups with a male LS, the likelihoods of affiliative behaviour directed towards male and female SS were similar, whereas in groups with a female LS, affiliative behaviour was never directed towards a male SS.

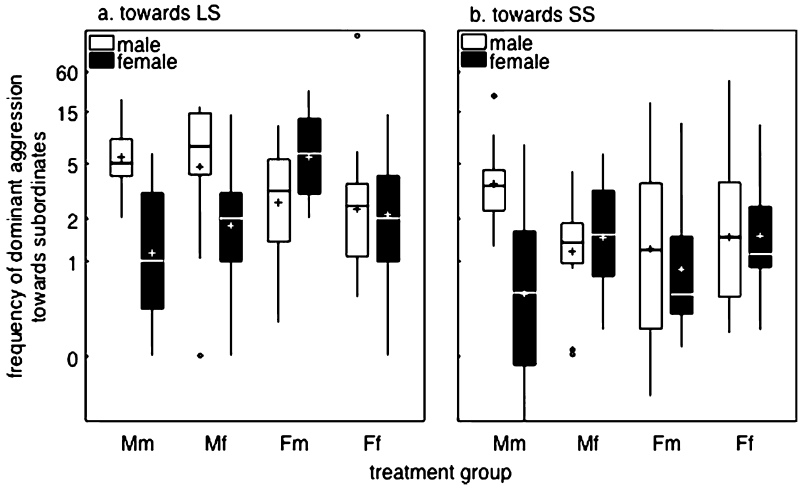

Figure 1. Number of observation intervals (out of 60) during which dominant males (white boxes) and females (black boxes) directed aggression towards large (LS, panel a) and small (SS, panel b) subordinates. Mm, LS male, SS male ($N = 11$); Mf, LS male, SS female ($N = 12$); Fm, LS female, SS male ($N = 11$); Ff, LS female, SS female ($N = 13$). Where significant effects of group body size indices were detected, counts were standardized to control for those effects. For this reason, plotted values can be <0 or >60. Counts are plotted on a negative-reciprocal-square root scale, corresponding to the transformation used in the analyses (Table 1). Boxplots give the medians and interquartile ranges, with whiskers extending to the most extreme values that are within 1.5 × IQR of the quartiles. Outlying values are plotted individually. + symbols denote the back-transformed means.

Interactions between dominant males and dominant females

Both dominant males and dominant females tended to be more aggressive towards one another and to direct more affiliative behaviour towards one another in groups where the LS was female (Figure 3, Table 2). For dominant males, *p*-values associated with effects of LS sex and with the overall ANCOVA models were suggestive but non-significant. For dominant females, the effect of LS sex was more consistent. Effects of LS sex on dominant female aggression towards dominant males and on dominant male affiliative behaviours towards dominant females were independent of group members' sizes. However, effects of LS sex on dominant male aggression towards dominant females, and on dominant female affiliative behaviour towards dominant males, were most pronounced in groups where subordinates were large relative to those in other groups (PC1) or to dominants in their own group (PC2). The frequency with which dominant females directed submissive be-

Table 1. Regression analyses of dominant male and female aggression towards large and small subordinates as a function of subordinates' sexes and group body size composition indices (PC1 and PC2, see text).

Effect	Estimate ± SE	df	F	p
Dominant male aggression towards large subordinates				
Whole model		3, 43	5.5	0.003
Intercept	-0.54 ± 0.04			
LS sex (male = 1)	0.13 ± 0.056	1, 43	5.9	0.02
PC2	0.11 ± 0.03	1, 43	10.6	0.002
LS sex × PC2	-0.10 ± 0.06	1, 43	3.1	0.09
Dominant male aggression towards small subordinates*				
Whole model		5, 41	2.4	0.06
Intercept	-0.64 ± 0.06			
LS sex (male = 1)	-0.05 ± 0.09	1, 41	0.3	0.62
SS sex (male = 1)	-0.04 ± 0.09	1, 41	0.2	0.70
PC1	-0.06 ± 0.04	1, 41	5.0	0.03
LS sex × SS sex	0.24 ± 0.13	1, 41	3.5	0.07
LS sex × PC1	0.08 ± 0.04	1, 41	4.0	0.05
Dominant female aggression towards large subordinates				
Whole model		3, 43	4.7	0.007
Intercept	-0.57 ± 0.05			
LS sex (male = 1)	-0.03 ± 0.08	1, 43	0.2	0.67
SS sex (male = 1)	0.18 ± 0.08	1, 43	5.1	0.03
LS sex × SS sex	-0.26 ± 0.11	1, 43	5.5	0.02
Dominant female aggression towards small subordinates				
Whole model		4, 42	4.2	0.006
Intercept	-0.63 ± 0.04			
SS sex (male = 1)	-0.14 ± 0.03	1, 42	4.9	0.03
PC1	-0.03 ± 0.03	1, 42	1.4	0.24
PC2	-0.06 ± 0.03	1, 42	4.1	0.05
SS sex × PC2	0.11 ± 0.04	1, 42	7.8	0.008

Reduced (reported) models were generated through stepwise removal of effects that did not approach statistical significance ($p > 0.1$). Counts were reciprocal-square root-transformed ($-(\text{count} + 1)^{-0.5}$). Effect estimates refer to the transformed counts.

* Variances associated with dominant male aggression towards small subordinates were not homogenous (Levene's test, $F_{3,43} = 3.1$, $p = 0.03$). However, the qualitative result held under a range of transformations and when outlying response values were excluded.

haviours towards dominant males was not affected by subordinates' sexes or by group body size composition (all interactions and main effects removed at $p > 0.15$).

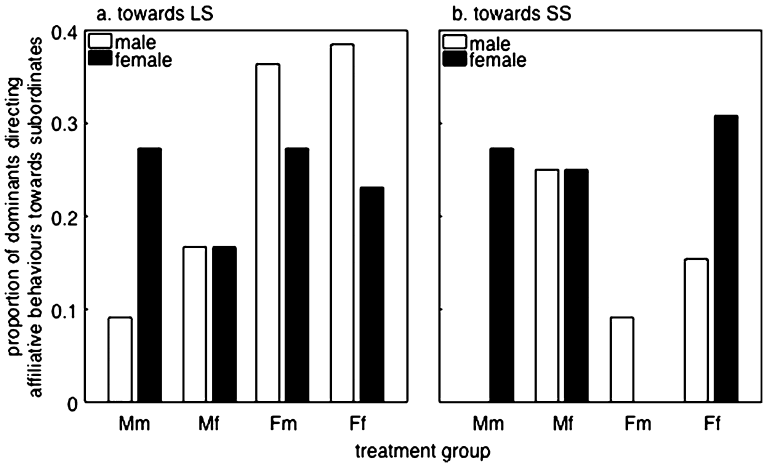

Figure 2. Proportion of groups in which dominant males (white bars) and females (black bars) directed affiliative behaviour towards large (LS, panel a) and small (SS, panel b) subordinates. Mm, LS male, SS male ($N = 11$); Mf, LS male, SS female ($N = 12$); Fm, LS female, SS male ($N = 11$); Ff, LS female, SS female ($N = 13$).

Time spent near breeding shelters

In groups where all individuals were relatively large, i.e., PC1 > 0, dominant males spent less time near breeding shelters if the LS was male (Table 3). In groups where all individuals were relatively small, the effect of the LS's sex was reversed such that, overall, the sex of the LS had no consistent effect (Figure 4). Dominant male space use was also sensitive to the sex of the small subordinate. Again the nature of that effect depended on group members' sizes. Where all individuals were relatively small, i.e., PC1 < 0, dominant males spent less time near breeding shelters if the SS was male, but the effect was reversed when group members were relatively large. For SS, the interaction was such that, overall, dominant males spent less time near shelters where the SS was male. For dominant females, effects of subordinates' sexes were less consistent and the overall ANCOVA model was not statistically significant ($p = 0.09$). Within that model, the sex of the LS had no effect. However, the sex of the SS did have an effect: like dominant males, dominant females spent less time near shelters in groups where the SS was male. Unlike dominant males, this difference became more pronounced, rather than less pronounced, when group members were larger (Table 3).

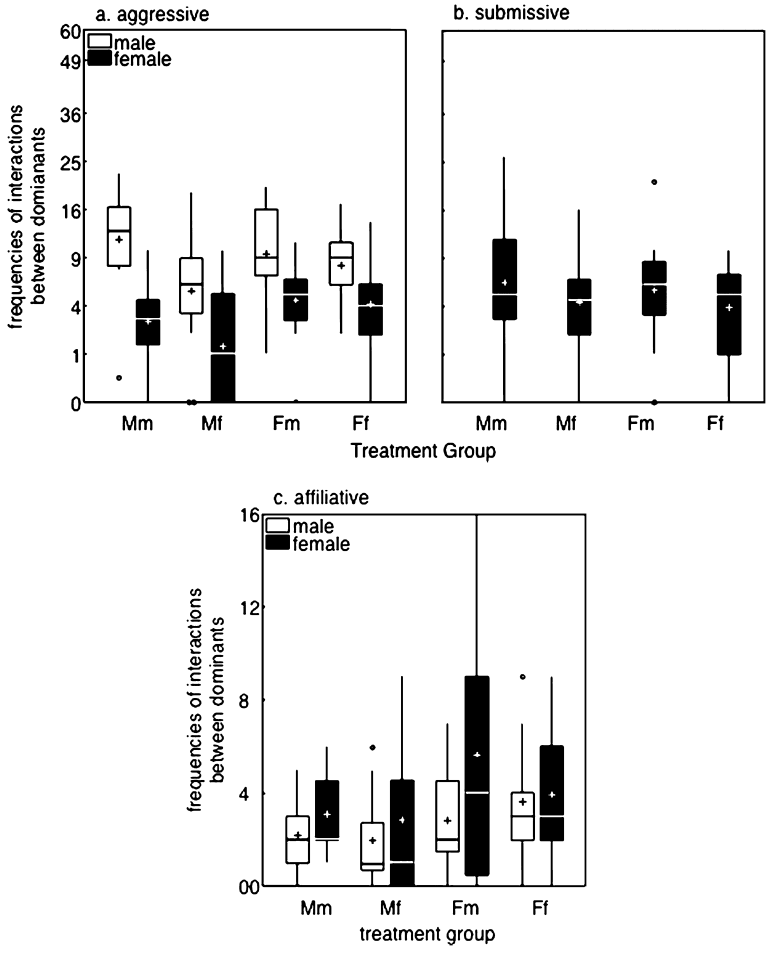

Figure 3. Number of observation intervals (out of 60) during which dominant males (white boxes) and females (black boxes) directed aggressive (a), submissive (b, females only because no submissive behaviour was shown by dominant males) and affiliative (c) behaviours towards one another. Mm, LS male, SS male ($N = 11$); Mf, LS male, SS female ($N = 12$); Fm, LS female, SS male ($N = 11$); Ff, LS female, SS female ($N = 13$). Where significant effects of group body size indices were detected, counts were standardized to control for those effects. Aggressive and submissive behaviours are plotted on a square root scale, corresponding to the transformation used to analyze those behaviours (Table 2). Affiliative behaviour is plotted on a linear scale. Boxplots give the medians and interquartile ranges, with whiskers extending to the most extreme values that are within 1.5 × IQR of the quartiles. Outlying values are plotted individually. + symbols denote the back-transformed means.

Table 2. Regression analyses of dominant male and female aggressive and affiliative behaviours towards one another as a function of subordinates' sexes and group body size composition indices (PC1 and PC2, see text).

Effect	Estimate ± SE	df	*F*	*p*
Dominant male aggression towards dominant females				
Whole model		5, 41	1.9	0.12
Intercept	2.94 ± 0.22			
LS sex (male = 1)	−0.32 ± 0.32	1, 41	1.0	0.33
PC1	0.21 ± 0.14	1, 41	2.2	0.15
PC2	0.04 ± 0.19	1, 41	0.1	0.83
LS sex × PC1	−0.37 ± 0.20	1, 41	3.4	0.07
LS sex × PC2	−0.66 ± 0.33	1, 41	3.8	0.06
Dominant female aggression towards dominant males				
Whole model		1, 45	4.6	0.04
Intercept	2.07 ± 0.22			
LS sex (male = 1)	−0.67 ± 0.31	1,45	4.6	0.04
Dominant male affiliative behaviour towards domimnant females				
Whole model		4, 42	2.4	0.07
Intercept	3.26 ± 0.43			
LS sex (male = 1)	−1.18 ± 0.62	1, 42	3.6	0.06
PC1	−0.01 ± 0.19	1, 42	<0.1	0.97
PC2	−0.29 ± 0.30	1, 42	0.9	0.34
PC1 × PC2	0.58 ± 0.25	1, 42	5.3	0.03
Dominant female affiliative behaviour towards dominant males*				
Whole model		3, 43	3.6	0.02
Intercept	4.70 ± 0.69			
LS sex (male = 1)	−1.75 ± 0.99	1, 43	3.1	0.08
PC2	1.33 ± 0.59	1, 43	5.0	0.03
LS sex × PC2	−2.70 ± 1.04	1, 43	6.7	0.01

Reduced (reported) models were generated through stepwise removal of effects that did not approach statistical significance ($p > 0.1$). Counts for aggressive behaviour were square root-transformed. Effect estimates refer to the transformed counts. Counts of affiliative behaviours were not transformed.

* Variances associated with affiliative behaviours by dominant females towards dominant males were not homogenous (Levene's test, $F_{3,43} = 6.7$, $p = 0.001$). However, the qualitative result held under a range of transformations and when outlying points were excluded.

Territory maintenance (digging)

The likelihood of digging by dominant males was independent of subordinates' genders and of group body size indices (all interactions and main effects removed at $p > 0.15$, Figure 5). However, the likelihood of digging

Table 3. Regression analyses of the time spent by dominant males and females <10 cm from breeding shelters as a function of subordinates' sexes and group body size composition indices (PC1 and PC2, see text).

Effect	Estimate ± SE	df	*F*	*p*
Dominant males				
Whole model		6, 40	4.8	0.009
Intercept	4.60 ± 0.32			
LS sex (male = 1)	0.06 ± 0.39	1, 40	<0.1	0.88
SS sex (male = 1)	−0.91 ± 0.39	1, 40	5.5	0.02
PC1	−0.10 ± 0.21	1, 40	0.2	0.62
PC2	−0.77 ± 0.19	1, 40	15.7	0.0003
LS sex × PC1	−0.42 ± 0.24	1, 40	3.1	0.09
SS sex × PC1	0.66 ± 0.24	1, 40	7.2	0.01
Dominant females				
Whole model		5, 41	2.1	0.09
Intercept	4.33 ± 0.43			
SS sex (male = 1)	−0.34 ± 0.64	1, 41	0.3	0.60
PC1	0.54 ± 0.27	1, 41	3.9	0.05
PC2	−0.34 ± 0.31	1, 41	1.2	0.27
SS sex × PC1	−0.79 ± 0.39	1, 41	4.1	0.05
PC1 × PC2	0.57 ± 0.26	1, 41	4.8	0.04

Reduced (reported) models were generated through stepwise removal of effects that did not approach statistical significance ($p > 0.1$). Counts were square root-transformed. Effect estimates refer to the transformed counts.

by dominant males was affected by the observed digging behaviour of both subordinates (Table 4). Dominant males were more likely to dig if the LS was not digging. On average, dominant males were also more likely to dig if the SS was not digging, but the presence and magnitude of this difference was sensitive to both PC1 and PC2. Dominant females were more likely to dig when their subordinates were female. The magnitude of the SS sex effect was independent of group members' body sizes, but the effect of LS sex effect was sensitive to both PC1 and PC2. The effects of LS and SS sexes were additive, i.e., there was no interaction between the sexes of the subordinates. Thus, overall, dominant females were most likely to dig in Mm groups, were less likely to dig in Mf and Fm groups, and were least likely to dig in Ff groups. The likelihood of digging by dominant females was not related to the observed digging behaviour of subordinates (all interactions and main effects removed at $p > 0.15$).

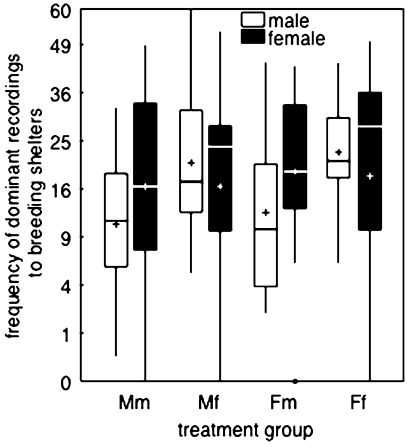

Figure 4. Number of instantaneous observations (out of 60) during which dominant males (white boxes) and females (black boxes) were <10 cm from breeding shelters. Mm, LS male, SS male ($N = 11$); Mf, LS male, SS female ($N = 12$); Fm, LS female, SS male ($N = 11$); Ff, LS female, SS female ($N = 13$). Where significant effects of group body size indices were detected, counts were standardized to control for those effects. Counts are plotted on a square root scale, corresponding to the transformation used in the analyses (Table 3). Boxplots give the medians and interquartile ranges, with whiskers extending to the most extreme values that are within 1.5 × IQR of the quartiles. Outlying values are plotted individually. + symbols denote the back-transformed means.

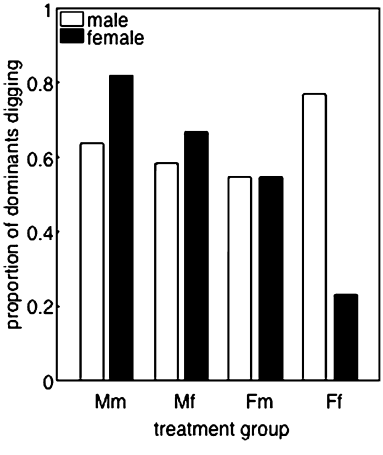

Figure 5. Proportion of dominant males (white bars) and females (black bars) that dug. Mm, LS male, SS male ($N = 11$); Mf, LS male, SS female ($N = 12$); Fm, LS female, SS male ($N = 11$); Ff, LS female, SS female ($N = 13$).

Table 4. Logistic regression analyses of dominant male and female digging behaviour as a function for subordinates' digging behaviour (for dominant males) or sexes (for dominant females) and of group body size composition indices (PC1 and PC2, see text).

Effect	Estimate ± SE	df	LR χ^2	p
Dominant males				
Whole model		6	20.9	0.002
Intercept	0.28 ± 0.56			
LS digging (yes = 1)	−2.36 ± 1.10	1	6.0	0.01
SS digging (yes = 1)	−1.35 ± 1.02	1	2.1	0.15
PC1	−0.15 ± 0.31	1	0.3	0.63
PC2	2.01 ± 0.79	1	12.0	0.0005
SS digging × PC1	1.31 ± 0.62	1	5.9	0.02
SS digging × PC2	−1.95 ± 1.06	1	4.1	0.04
Dominant females				
Whole model		6	19.5	0.04
Intercept	1.59 ± 0.71			
LS sex (male = 1)	−2.16 ± 0.83	1	8.4	0.004
SS sex (male = 1)	−1.81 ± 0.85	1	5.4	0.02
PC1	−0.68 ± 0.35	1	4.7	0.03
PC2	0.55 ± 0.46	1	1.2	0.21
LS sex × PC1	1.10 ± 0.50	1	5.8	0.02
LS sex × PC2	−1.76 ± 0.98	1	4.1	0.04

Reduced (reported) models were generated through stepwise removal of effects that did not approach statistical significance ($p > 0.1$). Effect estimates refer to log-odds.

Discussion

The sexes of subordinate *N. pulcher* group members influenced dominant males' and females' interactions with those subordinates and with one another, and the frequencies of nonsocial behaviours (time spent near shelters and digging activity). These effects demonstrate the importance of considering and accounting for variation in gender composition in studies of mixed sex groups. Differences in social interactions were broadly consistent with our expectations. However, statistical interactions between effects of the two subordinates' sexes and with group body size indices limit our ability to quantify the effect of a single subordinate's sex.

Dominant males and females tended to be more aggressive towards subordinates of their own sex, although the magnitude of these effects depended on the body sizes of group members. In contrast, dominant males

were more likely to direct affiliative behaviour towards female subordinates, and affiliative behaviour by dominant females was independent of subordinate sex. These results confirm and greatly extend our description of differences in dominant male aggression (Mitchell et al., in review), which relied on shorter, less-controlled observations of dominant males in a subset of these groups. Both sets of observations are consistent with our expectation that dominants should invest in maintaining the social hierarchy within their own sex. In cooperatively-breeding cichlids, similar arguments have been used to explain aggressive responses to same-sex conspecific intruders (Schradin & Lamprecht, 2000; Desjardins et al., 2008a). Sex-specific behavioural interactions within groups have been described in cooperatively-breeding meerkats (*Suricata suricatta*) (Clutton-Brock et al., 2006), and Müller & Manser (2008) have argued that scent-marking functions in intrasexual competition within and between banded mongoose (*Mungos mungo*) groups. The important aspect of the latter two studies, and of our own, is the recognition that sex-specific costs and benefits can be associated with subordinate group members, rather than exclusively with intruders. Costs and benefits associated with a subordinate group member's sex are paid and reaped by dominants repeatedly, so differences between male and female subordinates may have appreciable consequences over the course of a dominant's breeding tenure.

Dominants also directed more aggressive and affiliative behaviour towards one another in groups where the LS was female. These differences suggest that the subordinate's sex is a source of conflict between the dominant male and the dominant female. The direction of the difference in affiliative behaviour was opposite to our prediction. However, in hindsight, it is not surprising that an increase in aggression between the two dominants is associated with a compensatory increase in affiliative behaviour. A female subordinate is a source of conflict because her presence leads to opportunities for polygynous mating. Polygynous *N. pulcher* groups are common (Limberger, 1983; Dierkes et al., 2005; Desjardins et al., 2008b; Heg & Hamilton, 2008), and several female subordinates laid clutches over the course of this study (Heg et al., 2008; Mitchell et al., in review). In facultatively polygynous systems, effects of male interference on the settlement and continued residence of secondary females have been well documented (e.g., Walter & Trillmich, 1994; Schradin & Lamprecht, 2000). The resolution of such conflicts depends on the relative competitive abilities of different group members, so statistical

interactions with group body size indices are expected. Conflicts between dominant males and females over the status of a subordinate female highlight a frequently ignored aspect of facultative polygyny: a would-be polygynous male must not only attract an additional mate, but may also need to actively police interactions between his mates.

The effects of subordinates' sexes extended to patterns of space use and territory maintenance (digging) — aspects of dominant behaviour that do not involve direct social interactions. Hamilton et al. (2005) and Mitchell et al. (in review) have used an individual's distance from central shelters as an indirect measure of within-group aggression. That interpretation is not consistent with intrasexual aggression in this study because, overall, both dominant males and dominant females spent more time near their shelters in groups with female SS, although, as with dominant–subordinate interactions, statistical interactions with group body size indices were also important. In an aquarium-based study, evaluating adaptive interpretations of space use patterns is problematic. With respect to our objectives, a specific interpretation is less important than the demonstration that dominants' responses to subordinates' sexes extend beyond direct interactions with those subordinates and with one another.

The variation in digging activity is interesting because the responses of dominant males and females appear to involve different mechanisms. Dominant females were more likely to dig if their subordinates were male, whereas the likelihood of digging by dominant males was not affected by subordinates' sexes. Male subordinates dug less than female subordinates in this study (unpublished data), so one interpretation is that dominant females were compensating for the lower likelihood of digging by male subordinates and that dominant males were not compensating. However, using the observed digging behaviour of subordinates as predictors, rather than subordinates' sexes, leads to a very different interpretation. Dominant males were more likely to dig if their subordinates were not digging (or, equivalently, subordinates were more likely to dig if dominant males were not digging). This result is consistent with compensation. In contrast, digging by dominant females was not well predicted by subordinate's digging behaviour. Instead, dominant females appear to have responded directly to the sexes of their subordinates. Thus, digging activity — a behaviour that does not directly involve interaction with subordinates — was nonetheless influenced by the sexes of those subordinates.

Finally, for most aspects of dominant behaviour, the effect of one subordinate's sex depended on the sex of the second subordinate and on the body sizes of group members. For example, dominant male aggression towards a SS (Table 1) depended not only on the sex of the SS, but also on the sex of the LS and on whether group members were large or small (PC1). Likewise, the locations of dominant males were sensitive to the sexes of both subordinates, to both indices of group members' body sizes, and to interactions between body size and gender composition (Table 3). Effects of two subordinates' sexes can interact if a dominant's responses to one subordinate affect its ability to respond to the other (cf., Heg et al., 2008; Mitchell et al., in review). Effects of body size composition are also expected: Hamilton et al. (2005) reported that conflicts between dominant and subordinate male *N. pulcher* were greatest between fish of similar size, and Cant et al. (2006) found that aggressive interactions were most frequent between female paper wasp *Polistes dominulus* group-mates of adjacent rank. The rationale for expecting effects of gender composition is an extension of these arguments: just as larger or higher-ranked subordinates pose a greater threat to a dominant, so too do same-sex, rather than opposite-sex, subordinates.

Interpreting the range of interactions among components of group composition is beyond the scope of this study. The experimental design ensured that such effects would not obscure direct responses to subordinates' sexes. Thus, we were able to establish that all recorded aspects of dominant male and female behaviour were affected by subordinates' sexes and that effects related to social behaviours were broadly consistent with our expectations. However, with respect to the latter conclusion, statistical interactions between components of group composition do necessitate a caveat: complex statistical interactions limit our ability to quantify the direct effects of a particular subordinate's sex. The average effect of a subordinate's sex in our experimental groups was partly a consequence of the body size compositions that we happened to use when establishing those groups. Also, if interactions among components of group composition are important then the specific consequences of an individual subordinates' sex may be less predictable in the larger, more variable groups that occur in natural populations (e.g., Balshine et al., 2001). This caveat does not weaken our major conclusion: that subordinate's sexes can have far-reaching implications for the behaviour of dominant group members. Those implications have not previously been studied experimentally in any cooperative breeder. We, therefore,

stress the importance of considering gender in future studies of group composition.

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