Social-bond strength influences vocally-mediated recruitment to mobbing

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Supplementary Material

Material and Methods

Study species

Dwarf mongooses are cooperatively breeding carnivores living in groups of up to 30 individuals [S1]. The dominant pair monopolizes reproduction, with subordinates of both sexes helping to rear offspring [S2]. Group members differ in the strength of their relationships with one another, forming close bonds with certain groupmates, cemented by regular grooming interactions and by foraging in close proximity [S3]. As a result of their small size (<300 g), dwarf mongooses are at risk from a large number of avian and terrestrial predators, including venomous snakes [S4]. Group members alert others to the presence of predators using several alarm calls, of which the best-studied are 'flee' alarm calls, those indicating the approach of aerial and terrestrial threats and triggering escape responses [S5].

Study site and population

This study took place on Sorabi Rock Lodge Reserve, a 4 km^2 private game reserve in Limpopo Province, South Africa (24°11'S, 30°46'E), part of southern Africa's Savanna Biome (see [S6] for full details). All animals in the study groups (mean group size = 8.5; range = 4–15) are individually identifiable either from markings of blonde hair dye (Wella UK Ltd., Surrey, UK) applied with an elongated paintbrush or from natural features such as scars or facial irregularities. The population has been monitored since 2011, thus the age of most individuals is known; individuals can be sexed through observations of ano-genital grooming. Adult group members were classified as either 'dominant' (male and female pair) or 'subordinate' (the remaining individuals) (as in [S7]). The dominant pair could be identified through observations of aggression, feeding displacement, scent marking and greeting behaviour [S1].

Observational data collection

When a mobbing event occurred, the following information was collected where possible: (i) source of the threat; (ii) whether the initiator vocalised; (iii) number of group members that

responded; and (iv) total mobbing duration (time between the first vocalisation being given and the last individual leaving the stimulus).

Assessment of social-bond strength

Experiment 2 required comparison of the responses of subordinate focal foragers to the vocalisations of two subordinate groupmates with whom they were relatively more strongly and more weakly bonded, thus composite sociality indexes (CSI) were only calculated for groups containing a minimum of three subordinate adult group members (N = 4). The use of multiple behavioural indices strengthens the assessment of bond strength [S8, S9]. While some previous primate studies have combined grooming likelihood and sitting proximity (e.g. [S8, S9]), the latter could easily arise in the aftermath of the former at the same times, and thus the measures are not particularly independent. We therefore chose to combine the likelihood of grooming and of foraging in close proximity, two social events that occur at different times. Foraging in close proximity is a likely indicator of affiliation because more strongly bonded groupmates may, for instance, be less likely to steal food from one another, and be more likely to share food and to offer support against other groupmates or in predatory situations. Previous research has established that grooming and foraging associations are strongly correlated within dwarf mongoose groups [S3].

Data were collected during behavioural observation sessions from August 2014 to September 2015. The identity of grooming partners was recorded using all-occurrence sampling from 1240 bouts that lasted longer than 5 s (mean ± SE bouts per group = 310 ± 82; range: 96–466). Once groups had left the overnight refuge to begin foraging, scan samples were carried out every 30 min to record the identity of the nearest foraging neighbour for each group member (N = 2304 scan samples; mean ± SE scans per group = 576 ± 97; range: 294–734). For each group, grooming and foraging data were restricted to the maximum period when all group members present during the relevant playback experiment were present in the group (i.e. beginning from the date of immigration, or when individuals reached 12 months of age and were thus included in grooming and foraging associations). This period covered 4.5–7 months for the 4 groups in question (mean±SE: 5.8±0.5).

CSI scores were calculated using the following formula (as in [S8, S9]):

$$\frac{(\boldsymbol{G}ij \,/\, \boldsymbol{G}) + (\boldsymbol{F}ij \,/\, \boldsymbol{F})}{\mathbf{2}}$$

where G*ij* is the frequency of grooming given and received by members of the dyad *i,j*; G is the mean grooming frequency of all dyads in the group; F*ij* is the frequency of foraging nearest to the members of the dyad *i,j*, and F is the mean frequency of nearest-neighbour foraging for all dyads in the group. This allocates a score to each dyad, denoting the extent to which that dyad differs from the average of all dyads in a group. Dyads with high CSI scores signify group members that have stronger bonds, whereas dyads with low CSI scores signify group members with weaker bonds.

Acoustic recordings

All recordings were made at a sampling rate of 44.1 kHz with a 16-bit resolution onto a SanDisk SD card (SanDisk, Milipitas, California, USA), using a Marantz PMD660 professional solid-state recorder (Marantz America, Mahwah, NJ, USA) and a handheld highly directional Sennheiser ME66 shotgun microphone (Sennheiser UK, High Wycombe, Buckinghamshire, UK) with a Rycote Softie windshield (Rycote Microphone Windshields, Stroud, Gloucestershire, UK). The maximum amplitude of the two call types was measured using a HandyMAN TEK1345 Mini Sound Level Meter (Metrel UK Ltd., Normanton, West Yorkshire, UK). Control close calls were recorded opportunistically from 0.5–5 m during behavioural observations. Putative recruitment calls were recorded during natural mobbing events and induced with rubber-snake presentations.

Twenty snake presentations were conducted to nine groups from July 2014 to May 2015 (mean ± SE presentations per group = 2.5 ± 0.5; range = 1–5). The snake was positioned on the ground, out of sight of the foraging mongooses, and secured by the head to fishing line, which was held by the observer 10 m away. A directional microphone connected to a portable recorder was positioned 1–3 m away, pointing towards the snake. Target individuals (one more strongly bonded and one more weakly bonded subordinate groupmate of each focal subordinate; see above) were enticed towards the snake using a small amount (approx. 10 g) of boiled egg. Once the target was foraging within visible distance of the snake, the fishing line was pulled to raise the snake's head, which typically elicited putative recruitment calls. The snake was removed when all mobbing individuals had lost interest and moved out of sight.

Playback experiments

Calls were played back from an mp3 device (Apple Inc., Cupertino, California, USA) connected to a portable field speaker (Experiment 1: SME-AFS, Saul Mineroff Electronics

Inc., New York, USA; Experiment 2: Rokono, London, UK). Playback amplitude was standardized to the natural range of the relevant vocalisations: recruitment calls 70–75 dB at 1 m from subject, close calls 55 dB at 1 m from the subject. The speaker was positioned on the ground, perpendicular to the direction of group travel (to ensure any approach was intentional), 5–10 m from the target individual, who had no visual access to the caller. The speaker was concealed using vegetation and rocks. Playbacks took place at a similar time of day, when the target individual was foraging at the edge of a group and was the closest group member to the speaker. Following any major disturbances, such as an inter-group encounter or natural mobbing event, a minimum of 30 min was left before playback.

For Experiment 1, nine groups were tested between August 2014 and June 2015. Calls were extracted from original recordings (see above) and pasted into a 10-min recording of ambient noise (similar to the mean duration of observed natural snake mobbing events; see Results in main paper) using Raven Pro 1.5; ambient noise was recorded in the centre of the group's territory. All experimental tracks contained recruitment calls at a rate of 40 calls per minute (cpm), which is comparable to rates during natural snake-mobbing events (JM Kern unpub. data). Calls were inserted into the ambient noise randomly, rather than at regular intervals, to mimic most closely natural bouts of recruitment calling. Call placement differed between groups. All control tracks contained close calls at a rate of 20 cpm, comparable to natural rates during foraging (JM Kern unpub. data), inserted at 3 s intervals into the same ambient-noise tracks as were used for the recruitment calls.

For Experiment 2, more weakly bonded dyads always had CSI scores below the group mean (mean ± SE CSI score 0.44 ± 0.05; range: 0.27–0.62). More strongly bonded dyads had CSI scores greater than the group mean (CSI score = 1.95 ± 0.32; range: 0.58–2.90) in all bar one case, when constraints of group size meant that, though the most closely affiliated subordinate dyad, the relationship was not above the group mean. Some individuals were more strongly/weakly bonded to multiple focal individuals (N = 5) and their recruitment calls were used in multiple playback pairs, though each playback track was discrete, using different calls. There were no sex or age biases in the identity of the more strongly and more weakly bonded group members (McNemar's test, sex: N=8 paired individuals, P=0.248; sex relative to focal: N=8 paired individuals, P=1; age: N=7 paired individuals, P=0.221; age relative to focal: N=6 paired individuals, P=1). N=7 for age as both the more strongly and more weakly bonded individuals were immigrants and age was thus unknown. N=6 for age relative to focal as in one case, the focal, more strongly and more weakly bonded individuals were all the same age.

In both experiments, the behaviour of the focal individual during the 10-min playback period was recorded using a handheld digital camera (Panasonic, Osaka, Japan). The following data were extracted from the videos using Quicktime Player 7.7.9 (Apple Inc.): (i) whether the focal individual looked at the speaker; (ii) duration of looking; (iii) whether the focal individual approached the speaker; and (iv) duration of the physical response (time spent exhibiting typical mobbing behaviours including approaching, searching for the threat, head bobbing, weaving and striking).

Statistical analyses

All analyses were performed using R version 3.2.4. All tests were two-tailed and were considered significant at $P < 0.05$. Parametric tests were conducted where data fitted the relevant assumptions of normality and homogeneity of variance. Transformations were conducted to achieve normality of errors in some cases (details below), otherwise non-parametric tests were used.

In the mixed models used for Experiment 2 data, all likely explanatory terms were included in the maximal model. Model simplification was then conducted using stepwise backward elimination [S10] with terms sequentially removed by order of least significance and models compared using likelihood ratio tests. Removed terms were returned to the minimal model individually to confirm that they were not significant. Presented χ^2 and *P*-values for significant terms were obtained by comparing the minimal model with models in which the term of interest had been removed. Presented χ^2 and *P*-values for non-significant terms were obtained by comparing the minimal model with models in which the term of interest had been added. Presented effect sizes (± SE) were obtained from the minimal model. For categorical terms, differences in average effects are shown relative to one level of the factor, set to zero. Analyses were carried out using the lme4 or glmmADMB packages when data were zero-inflated [S11].

Two GLMMs were conducted to analyse the likelihood of looking and approaching the speaker. These binomial GLMMs suffered from complete separation [S12], as all focal individuals looked at the speaker during the stronger-bond trial, and only one focal individual approached the speaker during the weaker-bond trial. To overcome this problem, the models were fitted using the bglmer function of the "blme" R package [S13]. This type of model uses the addition of a weak prior to correct for bias resulting from complete separation. An LMM

was used to analyse look duration following log 10+1 transformation. A third GLMM was conducted to analyse the duration of physical response. Using the command cbind, the model bound the duration of physical response with the duration of the trial not spent responding.

Results

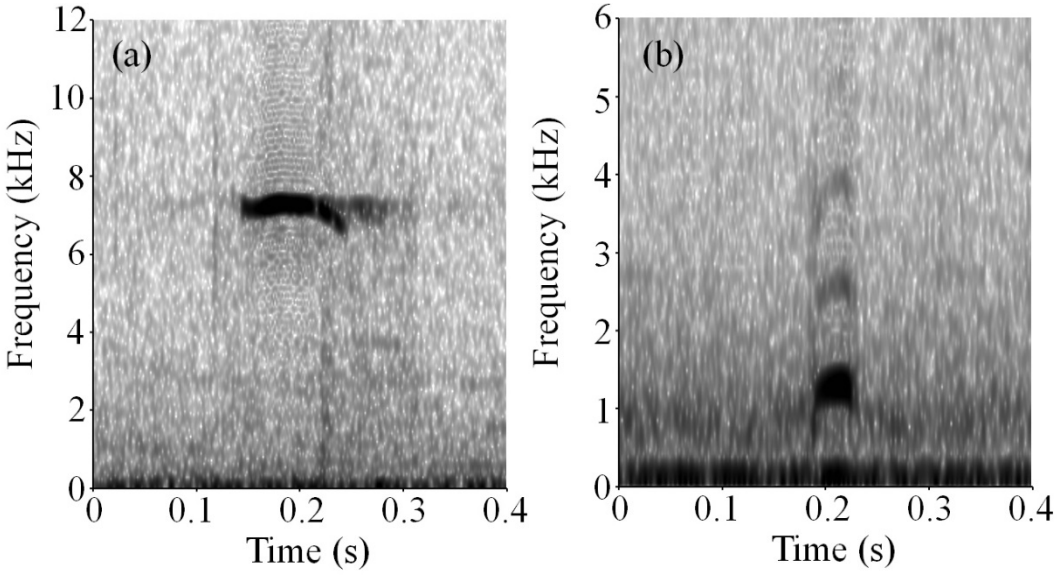

Figure SM1. Spectrograms of (a) a 'recruitment' call (given on detecting a snake), and (b) a close call (given while foraging) from the same adult individual, created in Raven Pro 1.5 using a 1024 point fast Fourier transformation (Hamming window, 75% overlap, 1.45 ms time resolution, 43 Hz frequency resolution).

Table SM1. Output from Linear Mixed Models (LMMs) and Generalised Linear Mixed Models (GLMMs) investigating (a) tendency to look at the speaker (GLMM), (b) total duration of looking (LMM), (c) tendency to approach the speaker (GLMM), and (d) total duration of physical response (GLMM) during playback of recruitment calls by individuals of different social-bond strength. For (a)–(c), *N* = eight individuals, four groups; for (d), *N* = seven individuals, three groups as video camera failed during 8th trial. Significant fixed terms shown in bold; variance ± SE reported for random terms.

	Fixed effect	**Effect ± SE**	***df***	**χ^2**	***P***
(a) Look					
Minimal model	(Intercept)	4.63 ± 1.83			
	Order		**1**	**4.56**	**0.033**
	First trial	0.00 ± 0.00			
	Second trial	-2.43 ± 1.46			
	Bond strength		**1**	**4.56**	**0.033**
	Stronger	0.00 ± 0.00			
	Weaker	-2.43 ± 1.46			
Dropped terms	Group size		1	0.17	0.680
Random terms	Group	0.00 ± 0.00			
	Playback pair in group	0.00 ± 0.00			
(b) Look duration					
Minimal model	(Intercept)	1.14 ± 0.14			
	Order		1	**11.06**	**0.001**
	First trial	0.00 ± 0.00			
	Second trial	-0.46 ± 0.11			
	Bond strength		1	**10.60**	**0.001**
	Stronger	0.00 ± 0.00			
	Weaker	-0.46 ± 0.11			
Dropped terms	Group size		1	0.04	0.850
Random terms	Group	0.00 ± 0.00			
	Playback pair in group	0.04 ± 0.20			
(c) Approach					
Minimal model	(Intercept)	0.32 ± 0.94			
	Bond strength		**1**	**10.62**	**0.001**
	Stronger	0.00 ± 0.00			
	Weaker	-2.30 ± 1.26			
Dropped terms	Group size		1	2.53	0.110
	Order		1	1.32	0.250
Random terms	Group	<0.0001 ± <0.0001			
	Playback pair in group	0.00 ± 0.00			
(d) Response duration					
Minimal model	(Intercept)	-3.73 ± 1.92			
	Bond strength		1	**854.95**	**<0.0001**
	Stronger	0.00 ± 0.00			
	Weaker	-5.87 ± 1.68			
	Order		1	**12.59**	**0.0004**
	First trial	0.00 ± 0.00			
	Second trial	-2.31 ± 1.68			
Dropped terms	Group size		1	0.76	0.380
Random terms	Group	0.00 ± 0.00			
	Playback pair in group	0.02 ± 4.34			

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