Behavioral Ecology
doi:10.1093/beheco/arn062
Advance Access publication 12 June 2008

Helping as a signal: does removal of potential audiences alter helper behavior in the bell miner?

Paul G. McDonald,[a,b] **Anahita J.N. Kazem,**[c] **Michael F. Clarke,**[b] **and Jonathan Wright**[c]
[a]School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK, [b]Department of Zoology, La Trobe University, Bundoora 3086, Australia, and [c]Institute of Biology, Norwegian Institute of Science and Technology, Trondheim N-7491, Norway

Despite many studies on the evolution of cooperative breeding and helping at the nest, relatively few have explored the possibility that helping functions as a signal to gain social advantage within groups (the "pay to stay" and "social prestige" hypotheses). One of the most promising candidates for such a signaling system is the cooperatively breeding bell miner, *Manorina melanophrys.* Large numbers of unrelated helpers attend multiple nests while giving individually identifiable vocalizations, breeding females usually remain within monitoring distance of the nest area, and females often re-pair with the hardest working male helper after the death/removal of their breeding partner. We examined the possibility that helping operates as a signal by temporarily removing the potential audience: the breeding male or the breeding female. However, there was no discernable change in provisioning behavior of helpers, relative to control periods. We also simulated the presence of the removed birds through playbacks of their individual-specific calls and again found no effect on others' visit rates, prey types, load size, and a variety of other behaviors at the nest. If either signaling hypothesis explained helping in this system, we might have expected facultative decreases in conspicuous provisioning behaviors when one or other potential audience was absent. Thus, despite possessing many of the prerequisites of a signal-based helping system, there is no evidence for such a phenomenon in bell miners. Cooperation in these groups of mixed relatedness may instead be driven by a combination of kin selection and direct benefits via group augmentation and/or pseudoreciprocity. *Key words:* nestling provisioning, cooperative breeding, pay to stay, signaling hypotheses, social prestige. *[Behav Ecol 19:1047–1055 (2008)]*

INTRODUCTION

The evolution of cooperative breeding has attracted a large amount of research interest (for reviews, see Brown 1987; Emlen 1991; Cockburn 1998, 2006; Russell 2004). Cooperation in these systems is provided by "helpers," individuals which care for offspring that are not their own. The type of care provided varies across taxa but typically involves parent-like behaviors such as assisting in the provisioning and defense of offspring. Kin-biased aid and subsequent indirect fitness benefits have long been held to be the main factor driving cooperative breeding. Although indirect benefits are clearly important in some systems (Komdeur 1994; Russell and Hatchwell 2001), many others are characterized by substantial aid from helpers that are unrelated to the young they assist. Thus, recent evidence suggests that direct benefits associated with helping may be more important than has previously been realized (Cockburn 1998; Clutton-Brock et al. 2000).

The multitude of hypotheses proposed to account for helping behavior can be simplified into 2 broad types (Wright 1997, 1999). Under "investment" hypotheses, helpers derive benefits due to the positive effect that their assistance has upon the "condition" of the young aided, being contingent upon the existence or actions of these recipients upon reaching maturity. Hypotheses in this category not only include kin selection (Hamilton 1964; Maynard Smith 1964) but also the direct mutualistic benefits of enhancing the number and/or quality of group members raised, thereby producing future increases in the survival and/or reproduction of helpers (group augmentation, pseudoreciprocity: Woolfenden and Fitzpatrick 1978; Ligon 1981; Connor 1986, 1995; Brown 1987; Kokko et al. 2001). Less often studied are the "signaling" hypotheses, which can be distinguished by the fact that benefits accrue to a helper only if it is "known" to have provided care, rather than due to any beneficial consequences their help has for the welfare of the young (Clarke 1989; Wright 1999). For example, under the "pay to stay" hypothesis (Gaston 1978; Kokko et al. 2002), dominant breeders tolerate helpers near a nest or on a territory only if their assistance (rent) is both required and provided. Apparently noncooperative helpers may ultimately face expulsion and thus exclusion from the benefits associated with group membership. The other main signaling hypothesis is "social prestige" (Zahavi 1977, 1995; Clarke 1989; Wright 1999), in which helping is regarded as a reliable signal of an individual's quality or cooperative propensity. Individuals that are able to display a high level of altruism, and thus gain high levels of "prestige" in the eyes of other group members, are predicted to be favored during subsequent mate choice and/or social alliance formation.

Investment and signaling hypotheses are obviously not mutually exclusive. However, the different pathway through which signaling benefits accrue to helpers provides an important distinction as there is a clear requirement for helping behavior to operate like any other form of signaling. A signal of effort that is typically not witnessed by other adults (e.g., because it occurs out of sight and/or earshot) and contains the potential for cheating (the helper may itself consume the food brought

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Address correspondence to P.G. McDonald, Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney 2109, Australia. E-mail: paul@galliform.bhs.mq.edu.au.

Received 20 June 2007; revised 9 May 2008; accepted 15 May 2008.

to young) cannot provide reliable information and thus should not be attended to. This is particularly the case for hypotheses such as social prestige, in which helping effort is regarded as conveying individual quality. In addition, helping behavior involves considerable costs (e.g., Russell et al. 2003; Canestrari et al. 2007); thus under signaling hypotheses, one might expect individuals to reserve effort for situations where their help is likely to be witnessed by others and conversely to reduce their work rate under conditions where appropriate signal receivers appear to be absent. Given this, when examining the potential for helping to be acting as a signal, the mechanics of and possibilities for reliable information transfer in any given system need to be considered (Wright 1997, 2007).

Numerous studies have focused upon the traditional investment hypotheses, quantifying potential direct and indirect fitness interests that helpers might have in the offspring they aid (see reviews above). Although coercion of other's effort appears common in some taxa (e.g., eusocial insects: Monnin and Ratnieks 2001), few studies have examined predictions of the pay to stay hypothesis. Under this hypothesis, the assessing individual, typically the breeding male in male-biased helper systems, harasses helpers into increasing their effort when conditions are harsh or if they do not appear to be providing sufficient "rent." Support for this study has been found in 2 avian (Reyer 1990; Mulder and Langmore 1993) and fish empirical studies (Balshine-Earn et al. 1998; Bergmüller and Taborsky 2005). However, critical tests of the social prestige hypothesis remain to be undertaken. Observational data on Arabian babblers (*Turdoides squamiceps*; Zahavi 1977, 1995; Carlisle and Zahavi 1986) suggesting that young helpers "interfere" with food delivery by other subordinates, as if competing to help, has been taken as circumstantial support. However, more detailed experimental work on provisioning behavior in the same study population failed to find any evidence consistent with social prestige (Wright 1997, 1998). Similarly, reports that young white-winged chough (*Corcorax melanorhamphos*) helpers "false feed" in an apparently deceptive manner (Boland et al. 1997) have been suggested as indicating a role for social prestige, but in other species, closer examination suggests that such apparently atypical behavior toward the young is instead fully consistent with normal provisioning effort (Wright 1997; Canestrari et al. 2004; Clutton-Brock et al. 2005; McDonald et al. 2007b). Given these often conflicting lines of evidence, an experimental test of helping as signal in a suitable study system seems long overdue.

The bell miner *Manorina melanophrys* offers a promising model system for several reasons. Colonies are comprised of a series of coteries, a group of individuals that provision broods at the same subset of nests within a colony. Unlike many other cooperative species, bell miners do not forage communally, rather each individual has a small (ca. 25 m diameter), nonexclusive activity space where food is obtained (Clarke and Fitz-Gerald 1994). Individuals then cooperate to provision broods and mob-potential predators and food competitors throughout the coterie and colony outside of their activity space. Nests are attended by numerous helpers, many of whom are unrelated to the brood assisted (Table 1; Conrad et al. 1998; Painter et al. 2000; McDonald et al. 2008), and even males with a breeding position help at multiple nests within a colony (Clarke 1984). This precludes indirect benefits as the sole explanation for helping behavior in this species. Furthermore, when naturally or experimentally widowed, breeding females often re-pair with the unrelated male helper that exhibited the greatest provisioning effort during her previous nesting attempt (Clarke 1989; Jones 1998). Thus helping by unrelated males might constitute a prestige-like form of self-advertisement, directed mainly toward the dominant female. Bell miners are also unusual in having ample opportunity for both visual and acoustic monitoring of conspecifics' activity at nests. This is particularly true of the breeding female, whose small activity space approximately centered on the nest area may allow females to often observe others' activity at the nest. Moreover, adults often produce individually distinctive "mew" calls, not only as they feed nestlings but also as they leave the nest area after a provisioning visit (Heathcote 1989; McDonald et al. 2007a), which could enable others to monitor their visit rate even from a distance. Provisioners do appear to alter some aspects of behavior at the nest depending on the presence of other individuals nearby as helpers produce more mew calls during visits when they coincide at the nest with another helper or the breeding male (McDonald et al. 2008). Finally, bell miner broods normally contain only 2 nestlings that, remarkably, are often satiated (Clarke 1984) by the high levels of care they receive from so many helpers (8–10 per nest on average, McDonald PG, unpublished data). Such satiation is

Table 1

Mean coefficients of relatedness (*r*) ± SE and sample sizes for the different social classes (i.e., breeding female, breeding male, and the 3 classes of male helper, based upon relatedness—see Materials and Methods)

	Relatedness to female			Relatedness to male		
	r	SE	*n*	*r*	SE	*n*
(a) Male removals						
Breeding females	—	—	10	0.2993	0.0671	10
Breeding males	0.2993	0.0671	10	—	—	10
Unrelated male helpers	−0.0331	0.0261	49	−0.0161	0.0417	29
Unresolved male helpers	0.2215	0.0318	25	0.0985	0.0465	25
Related male helpers	0.4994	0.0362	5	0.1767	0.0413	25
(b) Female removals						
Breeding females	—	—	10	0.1568	0.0823	10
Breeding males	0.1568	0.0823	10	—	—	10
Unrelated male helpers	−0.0428	0.0373	30	0.1018	0.0436	20
Unresolved male helpers	0.2326	0.0316	22	0.0324	0.0603	21
Related male helpers	0.4242	0.0488	8	0.2374	0.0525	19

Results are presented for individual relatedness to the breeding female and the breeding male for groups during experiments involving either (a) removal of the breeding male or (b) removal of the breeding female. Relatedness values of interest are underlined and exhibit values close to those expected for the different classes of male helpers, particularly in the case of relatedness to the breeding female.

extremely unusual (McDonald PG, Wright J, Kazem AJN, personal observations on begging effort in this vs. other passerine species) and warrants further examination.

To test whether interactions at bell miner nests involve social exchanges beyond straightforward nestling provisioning, we carried out temporary experimental removals of either the breeding male or breeding female (and incorporated periods of concurrent playback of the removed individual's calls in order to simulate their presence at the nest). By comparing the normal provisioning effort of "related" versus "unrelated" helpers with their behavior when specific group members are absent, we hope to provide one of the few experimental tests of hypotheses concerning helping as a signal.

MATERIALS AND METHODS

Study populations

The study was conducted between June 2004 and December 2005 on 2 bell miner colonies located northeast of Melbourne, Australia. The first consisted of 40–45 individuals at the La Trobe University Wildlife Reserve, 20 km northeast of Melbourne (37°42′8″S, 145°03′20″E), whereas the second colony of 120–135 birds was situated near Saint Andrews, 50 km northeast of Melbourne (37°35′09″S, 145°15′41″E). This research was approved by the La Trobe University Animal Ethics Committee (license AEC01/19(L)/V2) and the Department of Sustainability and Environment (license 10002082).

Molecular analyses

Individuals within colonies were captured with mist nets, color banded, and a 70-μL blood sample collected from the alar vein for analysis. This sample was stored in 70% ethanol and then transported to The Australian National University, Canberra, Australia where birds were sexed and 6 loci genotyped according to the protocols outlined in Fridolfsson and Ellegren (1999) and Painter et al. (1997), respectively. Relatedness between individuals was assessed using KINSHIP v1.2, which calculated the likelihood of male helpers being either related (primary hypothesis $r = 0.5$, null hypothesis $r = 0$) or unrelated (primary hypothesis $r = 0$, null hypothesis of $r = 0.5$) to the breeding female, based on the ratio required to exclude 95% of 1000 simulated pairwise comparisons (Goodnight and Queller 1999). Values of coefficients of pairwise relatedness (r) can range between -1 and $+1$, with negative values indicating that individuals share fewer alleles than average for the population (Queller and Goodnight 1989). In a randomly mating population, r should approximate 0.5 for full siblings and 0.25 for half-sibs and so forth. Helpers were divided into 3 groups based on the outcome of these tests and their relatedness to the breeding female, being either significantly related, significantly unrelated, or, if both these tests were nonsignificant ($P > 0.05$), placed in an intermediate category we have referred to as "unresolved." The latter group is comprised of both individuals that are either truly $r = 0.25$ or those that, by chance, share some alleles due to their abundance in a focal population rather than any shared pedigree. Statistically, this category does not differ significantly from unrelated individuals ($r = 0$), nor does it differ significantly from related ($r = 0.5$) individuals. Logically these individuals are likely to be of intermediate relatedness, and by assuming this, we have adopted a conservative approach.

Molecular results were also produced for helper relatedness to the breeding male, although in this case the data for the unresolved category were less clear than for relatedness to the breeding female (Table 1). Due to apparent inbreeding avoidance in this system (Conrad et al. 1998; Painter et al. 2000), only males unrelated to the female are likely to pair with her in the future and thus are the primary candidates for any prestige signaling in this system. Therefore, for simplicity of interpretation, results have been presented throughout using relatedness calculated relative to the breeding female. It is important to note however that the results presented do not differ substantially if relatedness is instead calculated relative to the breeding male or to a mean of the breeding pair (results not presented for reasons of brevity). Finally, although female helpers do occur in this system, they are comparatively rare, and insufficient sample sizes during the experimental manipulations necessitated omitting this class of individuals from statistical comparisons.

Monitoring of nesting attempts

Nesting activity at each colony was monitored throughout the year as breeding in this species can occur in all months. Once found, nest contents were monitored every second day to determine hatching date (termed day 0). At each nest, only one female participated in nest construction, incubation, and brooding, allowing the breeding female to be identified in this manner. To identify breeding males, nests were observed remotely via a video camera 4 m from the nest and/or from a hide placed at 20 m, for a 2-h period within 48 h of nestlings hatching. Helper males rarely feed during this initial period, allowing the individual provisioning at highest rates to be identified as the putative breeding male (Poiani 1993). Putative parentage assigned in this manner closely matches genetic parentage in this species (Conrad et al. 1998), with extrapair offspring comparatively rare (4%).

Monitoring provisioning behavior

Nests were watched by the first author from a hide placed 16 m ± 0.8 standard error (SE) ($n = 20$) from the nest using a Kowa TS662 spotting scope with a 20–60× zoom eyepiece (Tokyo, Japan) and simultaneously videotaped using either an analogue (CCD-TR1100E, Sony, Tokyo, Japan) or digital Hi8 Camcorder (DCR-TRY265E, Sony) placed on a tripod. The time-stamped videos were later burnt onto DVDs using a DVD recorder (Pioneer DVR-310, Tokyo, Japan) and reviewed using Power DVD v4.0 (LG Electronics, Sydney, Australia) on a laptop computer (Higrade Notino C7000, Essex, UK). Using a combination of dictation recorded in the field and perusal of videotapes, the number and duration of all individuals' visits to the nest were recorded (to the nearest second). The size (relative to bill volume) and prey composition (proportion of "lerp"—a white carbohydrate-rich sugar excretion from phytophagus psyllids; Psyllidae) of each load brought to the nest area were also noted, as well as the number of mew calls given by the attendant during the visit. The distance individuals traveled after leaving the nest area (less than or further than 10 m) was also noted. Finally, the identity of all other birds at the nest (within 2 m radius of the nest cup) while the focal individual was present was recorded. The dense vegetation around most nests meant that while a larger radius was also monitored, we could not be sure that we had recorded every conspecific present on every occasion; by the same token, though, birds present at these greater distances themselves would not necessarily be able to monitor one another's behavior at the nest.

Acoustic begging levels were recorded using a small tie clip microphone (Sony ECM77B, Sony), placed 20 cm below each nest and connected to a Marantz PMD670 solid-state recorder (Tokyo, Japan). Calls were recorded at 48 kHz in uncompressed pulse code modulation format. These files were subsequently loaded into Raven 1.2.1 (Cornell Lab of Ornithology, Ithaca, NY) and spectrograms constructed with a 512-point fast Fourier transform length (3-dB bandwidth 124 Hz with smoothing function enabled), with a Hanning window function and overlap set at 90% (grid resolution 1.16 mS, grid spacing 86.1 Hz). Calls were then high-pass filtered to remove background noise at frequencies lower than the begging vocalizations. Amplitude of the first bout of begging given by nestlings following arrival of an individual at the nest was then measured using the root-mean-square algorithm within Raven (Charif et al. 2004).

Experimental protocols

The number of helpers present at bell miner nests reaches its maximum when nestlings are 5–6 days old, with attendant number, visit rates, and load compositions remaining more or less constant thereafter (te Marvelde L, McDonald PG, Kazem AJN, and Wright J, in preparation). Therefore, removal experiments ($n = 10$ for each sex of breeder) were carried out when nestlings were at least 6 days old (male removal: mean nestling age 7.7 days ± 0.26 SE; female removal: 7.7 days ± 0.4 SE). All removals were carried out during periods of fine weather, although environmental variables other than rain and time of day do not appear to influence bell miner provisioning effort markedly (te Marvelde L, McDonald PG, Kazem AJN, and Wright J, in preparation).

Hides were placed in the observation position (15.9 m ± 0.8 SE from the nest, $n = 20$), a distance known to cause no disturbance to normal provisioning behavior (McDonald et al. 2007b), for at least 24 h before the observations began. In all observations, data collection did not commence until 10 min after the observer had entered the hide. This allowed any disturbance associated with observer presence to dissipate, and in most cases, all birds resumed provisioning within 2–3 min of an observer entering the hide.

Nest visit data were initially collected for all birds during a 2-h period (pre-control observation). Mist nets were then erected around the nest area and the randomly predetermined focal bird (either breeding female or breeding male) was captured. Removed birds were placed, out of visual and acoustic contact with the rest of the colony, in a temporary cage with sugar water available ad libitum. Mist nets were then removed and the nest area left for at least 40 min to minimize any effects of disturbance prior to observations resuming. Nest visit data were then collected for all birds during a 2-h "removal" observation period.

In addition, a 1-h "playback" observation period was also carried out while the removed bird was still absent, with the order of the removal and playback periods being counterbalanced across nests. Each time a visiting individual arrived in the nest area, one mew call from the removed breeder was played at natural volume from a Sony D-EJ100 player through a speaker (Sony SRS-A27) located 4 m from the nest. This playback was intended to simulate the removed bird being in the vicinity of the nest area but out of visual contact, that is, an experimental period where this audience was potentially present in contrast to the removal periods. Over the hour of observation, responses to the playback (measured as degree of head movement toward the speaker) did not diminish for individuals, indicating that habituation did not occur over this period (data not presented). Calls used in the playback were recorded during normal nest observations on the day prior to the removal, with 20 different exemplars from 20 different visits to the nest recorded for each focal breeder. Calls were recorded using the same equipment used to measure nestling begging (see above), high-pass filtered in Raven to remove background noise, and then burnt onto CDs in an uncompressed format for later playback.

Following completion of the removal and playback periods, the removed individual was released within 50 m of the nest area in a "soft" manner: the cage door was opened remotely from a distance of 10 m and the bird allowed to leave the cage in its own time. All removed individuals went back to regularly provisioning nestlings within a relatively short period, for example, most within 30 min. To allow for any possible disturbance following release of the individual, a final 2-h "post-control" observation began at least 40 min after the release. Nest visit data were therefore collected for all birds during 2-h control periods both before and after the experimental removal and playback treatments. This allowed us to test for any longer term effects of the temporary removal and made it possible to sample normal provisioning behavior while controlling for variation due to differences in time of day between experimental and control periods.

Statistical analyses

Prior to carrying out the main statistical analysis comparing behavior before, during, and after the experimental period, we first tested for any change in the few individuals per nest that were inadvertently captured (and immediately released) during attempts to capture the focal bird ($n = 11$ birds; mean 1.4 per nest ± 0.4 SE). There was no significant influence of these incidental captures on any of the variables measured (all P values >0.05). Likewise, 6 removals involved both members of a breeding pair being removed, either in consecutive nesting attempts or on consecutive days for the same nest ($n = 3$ for both). Whether a removal was the first or second a group was exposed to did not influence individual behavior in any of the variables measured (all P values >0.05). Such incidental aspects of the experimental protocol are therefore omitted from the following analyses and discussion.

As both members of the breeding pair were not removed in all nests, the results for breeding male and breeding female removals are not directly comparable and were therefore analyzed separately. For each removal, nest visits were aggregated into a mean value per individual per observation type (pre-control, removal, playback, or post-control). Our focus is the effect of "social class" (i.e., breeding female, breeding male, or helper, with the latter category further divided into individuals significantly related, unresolved, or unrelated to the breeding female) upon response to the manipulation. We therefore used repeated measures analyses of variance (RM-ANOVAs) to examine provisioning behavior according to observation type (within-subjects effect) and social class (between-subjects effect). Helmert contrasts were used to determine significant differences between specific social classes within RM-ANOVA models: breeding females were compared against the mean for all remaining individuals, breeding males against the mean for all helpers, unrelated male helpers against the mean for related/unresolved helpers, and finally unresolved helpers compared with related helpers. Provisioning variables examined included visit rate per hour, load size per visit, prey type (proportion of lerp within each load), duration of visit to nest (seconds), proportion of visits where individual remained within 10 m of the nest area after visiting, and the number of mew calls given per visit. Tests for an effect of treatment order within removal periods (removal or playback first) did not yield significant differences in any of the variables measured (all P values > 0.05); for simplicity, order is therefore omitted from the results presented below. Variables were arcsine- or log-transformed as appropriate to normalize prior to use in RM-ANOVA. Mauchly's test for sphericity was used, and where sphericity could not be assumed, we adjusted the degrees of freedom according to the Greenhouse–Geisser method. Data are presented as means ± 1 SE, and 2-tailed tests and a critical P value of

0.05 are applied throughout. All statistical analyses were carried out using SPSS v12.0.2.

RESULTS

Comparison of pre- and post-control periods

Initial RM-ANOVA analyses compared all variables in pre- versus post-control observation periods, including social class as a factor. There were no significant differences between the 2 control periods, and no interactions between control period and social class, during either breeding male or female removals, for all dependent variables assessed in this study (all P values >0.05; results not presented for reasons of brevity). Therefore, a mean value was calculated from the 2 control periods, and all further analyses compare an individual's "mean" control value against those obtained in the removal and playback observations.

Breeding male removals

(i) Visit rate and load composition

There were no significant changes in provisioning behavior, in terms of visit rate, load size, or the proportion of lerp within loads (load quality) according to experimental treatment and no interaction between social class and experimental treatment when breeding males were temporarily removed (Table 2a(i); Fig. 1). There was a significant overall effect of social class upon visit rate, with Helmert contrasts revealing that breeding females visited nests more frequently than all other classes of bird (mean 9.8 visits/h ± 1.4SE, $n = 10$; vs. 2.7 visits/h ± 0.3 SE, $n = 79$; $P < 0.001$), regardless of experimental treatment.

(ii) Conspicuous behaviors at the nest

Given the absence of significant differences in the frequency or quality of nest visits when a potential audience was present versus absent, we further examined conspicuous behavior around the nest area as a potential mechanism for individuals to modify their signaling effort. Birds might remain for longer visit durations, pause in the vicinity of the nest area following a feeding event, or produce more mew calls during their visit, when the breeding male was sometimes present (mean control) or apparently always present (playback). However, none of these variables were significantly influenced by experimental treatment or an interaction between treatment and social class (Table 2a(ii)). A significant overall influence of social class was discernible for visit duration; however, Helmert contrasts revealed that this simply indicated that breeding females visited the nest for longer periods than all other classes of individual (mean: 66.7 s ± 19.1 SE, $n = 10$; vs. 33.0 s ± 4.1 SE, $n = 79$; $P = 0.024$).

Breeding female removals

(i) Visit rate and load composition

Identical analyses were carried out on data relating to the temporary removal of breeding females. Once again, feeding rate, load size, and proportion of lerp within loads did not differ according to experimental treatment, and there was no interaction between social class and experimental treatment (Table 2b(i); Fig. 1). A significant overall effect of social class was present, and within this subset of the data, Helmert contrasts revealed that breeding males (mean 7.7 visits/h ± 1.3 SE; $n = 10$) visited nests more than all other helpers ($P = 0.002$), unrelated helpers (2.5 visits/h ± 0.4 SE; $n = 30$) visited less often than the mean of the unresolved/related helper classes ($P = 0.044$), and related helpers (5.8 visits/h ± 1.6 SE; $n = 22$) visited more often than unresolved helpers (3.3 visits/h ± 0.6 SE; $n = 8$; $P = 0.009$).

(ii) Conspicuous behavior at the nest

None of the variables considered to increase a provisioner's conspicuousness during nest visits (see above) exhibited significant differences during female removal and playback treatments (Table 2b(ii)) nor were there any significant interactions between social class and experimental treatment.

In all analyses presented above, excluding the unresolved relatedness category of helpers did not alter any of the main

Table 2

Results of repeated measures ANOVAs assessing changes in parameters between different experimental treatments (mean control, removal only, and removal with playback) and social class of group members relative to the breeding female

		(a) Breeding male removals			(b) Breeding female removals		
Variable	Factor	F ratio	Degrees of freedom	P value	F ratio	Degrees of freedom	P value
Visit rate per hour[a]	Treatment	1.215	1.76, 149.23	0.296	0.054	1.61, 106.44	0.916
	Social class	**8.863**	**3, 85**	**<0.001**	**8.000**	**3, 66**	**<0.001**
	Treat × class	0.434	5.27, 149.23	0.834	1.061	4.84, 106.44	0.386
Load size per visit[a]	Treatment	0.375	1.61, 80.68	0.643	0.131	1.75, 71.55	0.851
	Social class	**3.814**	**3, 50**	**0.015**	1.227	3, 41	0.312
	Treat × class	0.222	4.84, 80.68	0.949	1.002	5.24, 71.55	0.425
Proportion of lerp in load[b]	Treatment	1.452	2, 100	0.239	0.774	1.72, 70.55	0.447
	Social class	0.689	3, 50	0.563	0.350	3, 41	0.789
	Treat × class	0.505	6, 100	0.803	0.951	5.16, 70.55	0.456
Duration of nest visit	Treatment	0.049	2, 104	0.953	3.046	2, 86	0.053
	Social class	**4.139**	**3, 52**	**0.010**	0.905	3, 43	0.446
	Treat × class	0.851	6, 104	0.534	1.319	6, 86	0.257
Proportion of visits >10 m traveled from nest following feed[b]	Treatment	0.327	2, 98	0.722	1.022	1.58, 61.67	0.350
	Social class	1.478	3, 49	0.232	0.751	3, 39	0.529
	Treat × class	0.411	6, 98	0.870	2.002	4.74, 61.67	0.094
Number of mew calls given at nest[b]	Treatment	2.557	2, 104	0.082	0.459	1.75, 75.01	0.607
	Social class	0.535	3, 52	0.660	0.991	3, 43	0.406
	Treat × class	0.761	6, 104	0.602	0.341	5.23, 75.01	0.893

Results are shown separately for (a) breeding male removals and (b) breeding female removals. Significant results are emboldened.

[a] Greenhouse–Geisser method used for both male and female removal analyses.

[b] Greenhouse–Geisser method used for breeding female removal analyses.

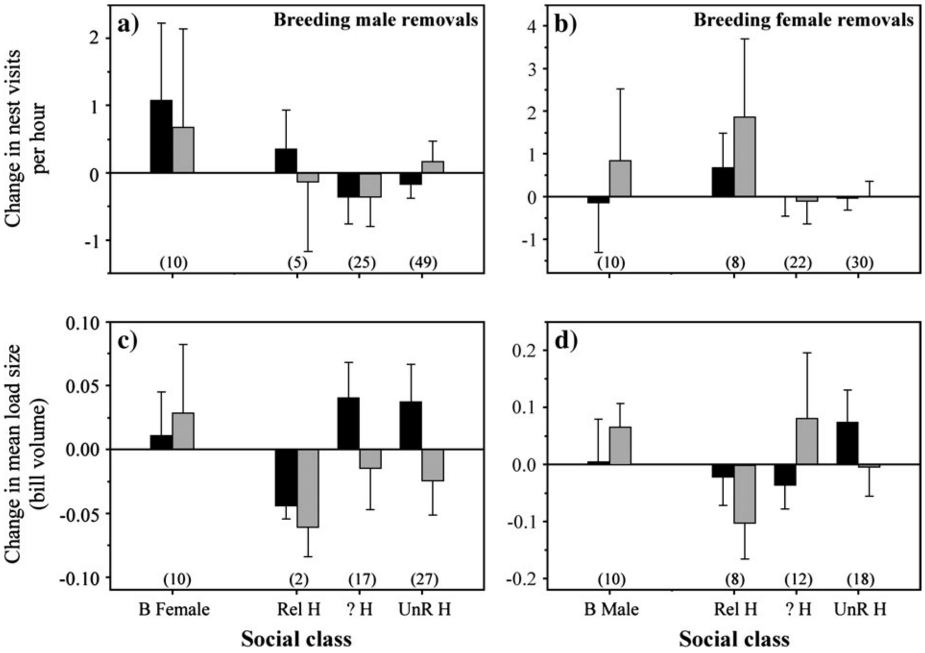

Figure 1
Influence of temporary removal of breeding male on (a) mean (± SE) nest visit rate per hour and (c) mean (± SE) load size (per bill volume) delivered or temporary removal of breeding female on (b) mean (± SE) visit rate per hour and (d) mean (± SE) load size (per bill volume) delivered. Filled bars show the difference in value between experimental removal (without playback) and control periods (mean of pre-control and post-control), and shaded bars show the difference between playback periods and mean control, where positive values represent an increase relative to mean control levels. Data are presented according to social class: breeding female (B Female), breeding male (B Male), related male helper (Rel H), unresolved male helper (? H), or unrelated male helper (UnR H). Sample sizes given in parentheses; values for load size are lower than visit rate as individuals failing to visit do not contribute to the latter data set.

results. Indeed as social class (relatedness) was entered as a factor (and not a covariate) in ANOVAs, had the 2 extreme relatedness categories ($r = 0$ vs. $r = 0.5$) differed significantly in their responses to the experimental treatments, this would have been detected—irrespective of whether or not the unresolved helpers were included in the analysis. Yet, there was not only no significant effect of experimental treatment but also no significant interaction between relatedness and experimental treatment, in any of the dependent measures.

Overall provisioning effort and nestling hunger

Lastly, we examined the biomass delivered to nests, in terms of bill loads per hour, and begging effort as a surrogate of nestling hunger levels. The later was measured using residuals from an ANOVA comparing brood size with root-mean-square amplitude of the first begging bout given by nestlings during visits. No significant differences were apparent in biomass delivered or nestling begging levels during either the breeding male or breeding female removals (Fig. 2), although sample size for related helpers was low in certain analyses. This indicates that—despite the temporary removal of a member of the breeding pair—nestlings nevertheless received similar levels of total biomass and displayed similar hunger levels throughout the experimental (removal and playback) period as during the control observations.

DISCUSSION

Our analyses examined, a broad array of provisioning behaviors that could potentially have been used as signals of individual helping effort within this social system. However, none of these behaviors changed in individual helpers (or either member of the breeding pair) in response to the experimental removal of socially important potential audiences (i.e., the breeding pair). There was also no evidence of differential responses to the experimental removals by nest attendants according to their social class (related vs. unrelated to the breeding female).

Following removal of breeding males and possible experimental creation of information indicating a breeding vacancy, we might have expected an “increase” in the rate of conspicuous provisioning behavior and thus “showing off” by unrelated male helpers if, for example, relative helper effort is used by breeding females in subsequent mate choice. Alternatively, under a pay to stay system, the removal of the breeding male might have been predicted to lead to a “decrease” in male helper effort (especially by unrelated male helpers) due to the absence of the likely enforcer of any male helper rent payments. Removed breeding males could also have been expected to show an increase in aggression upon their release if they needed to advertise their return and coerce helpers into a renewal of rent payment. Conversely, following the removal of breeding females, we might have expected a decrease in the rate of conspicuous provisioning by unrelated male helpers as they have little to gain from provisioning in her absence if effort facilitates future mating opportunities.

None of these possible scenarios are apparent in the results presented here. It should also be noted that this conspicuous lack of any effect held regardless of whether the social class of helpers was calculated relative to the breeding female (the results presented here), the breeding male, or a mean value for both combined. All our results are clearly contrary to any and all predictions concerning facultative responses made by either signaling hypothesis, which together with other observational evidence from this system (see McDonald et al. 2008)

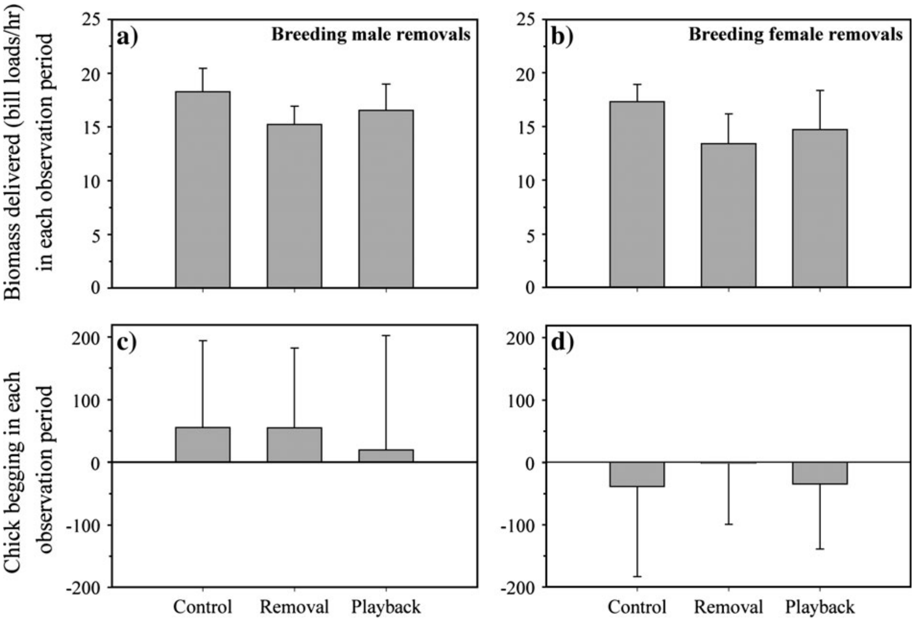

Figure 2
Effect of temporary removal of breeding male on (a) mean (± SE) total biomass delivered per brood (bill loads per hour) and (c) mean (± SE) begging amplitude per visit (presented as residuals after controlling for brood size) or temporary removal of breeding female on (b) mean (± SE) total biomass delivered per brood (bill loads per hour) and (d) mean (± SE) begging amplitude per visit (presented as residuals after controlling for brood size). Data are divided into the 3 experimental treatments: mean control, removal, and playback observations ($n = 10$ nests for each removal type; see text for details).

leads us to reject the hypothesis that helping is operating as a signal in bell miner colonies either for social prestige or as a form of pay to stay.

Compensatory provisioning in response to removals

The experimental removal of one member of the breeding pair may have been expected to lead to a reduction in the total amount of food delivered to nestlings and a concurrent increase in both begging and thus provisioning levels of the remaining birds during removal observations (e.g., Bart and Tornes 1989; Wright and Cuthill 1990; Wright and Dingemanse 1999). However, this was not the case, despite removal of one of the breeding pair—that is, the group members that were responsible for the largest individual provisioning effort during control periods.

It is possible that any experimental effect on overall feeding rates was too small to be detected, which is likely due to the combination of small brood sizes and large numbers of nest attendants and thus the near overfeeding of broods in this species. Hence, any shortfalls in total food delivery caused by the temporary removal of one bird were small relative to the total amount of food being delivered by the whole group to a well-fed brood of nestlings. Any subsequent increases in brood demand would therefore have been rather small (and undetected by our methods) and could be met by relatively small (and again undetected) individual increases in effort by each of the large number of provisioners.

The experimental removals protocol

Finally, it is possible that the experimental removal was not of sufficient duration to prompt the expected changes in helper provisioning behavior, perhaps because the absence of the removed bird was not immediately noticed by all group members. However, all available evidence suggests that this was not the case. First, following capture of the removed birds, most other group members routinely vigorously mobbed researchers in a manner analogous to a predation event (McDonald PG, personal observation). Mobbing in bell miners involves interaction of the whole social group in quite close proximity, meaning individuals are likely to be aware of the presence (or absence in this case) of other group members (e.g., Griesser and Ekman 2005). Secondly, social interactions are very common between members of the breeding pair and other coterie members, and as such information concerning the absence of birds either around the nest area (breeding females) or during mobbing and provisioning events at other nests (breeding males) is highly likely to have been available to attendants within the experimental time frame.

The possibility remains that helping does function as a signal to conspecifics, but that any "audience effect" has been fixed over evolutionary time. However, a fixed high level of signaling is likely to evolve only if the relevant audience type is nearly always present in the environment of the signaler (Matos and Schlupp 2005). This argument therefore appears plausible only when the audience invoked is the breeding female and even they are absent from the nest area for significant periods (McDonald et al. 2008). Moreover, provisioning is likely to be costly to perform, and as such, it is probable that there is "always" a benefit to be gained by saving on helping effort—provided such "cheating" is unlikely to be detected by others, which is a likely scenario in a system where nests are placed in the undergrowth. On balance then, we feel that a facultative response to conspecific presence/absence would be likely to evolve if the type of signaling system envisaged under social prestige or pay to stay was in fact in operation.

CONCLUSIONS

Despite testing a large array of behavioral measures in one of the most likely cooperative systems, we found no evidence that bell miners adjust their individual helping effort in response to the presence of potential audiences for such a signal. Furthermore, the negative results presented here concur with other studies examining other aspects of helping as a potential signal in bell miners (McDonald et al. 2008; Pacheco et al., forthcoming). Therefore, previous findings that female bell miners prefer to re-pair with the male exhibiting the highest provisioning rate during her previous nesting attempt (Clarke 1989; Jones 1998) may instead reflect the process of females selecting mates on the basis of age and/or quality, rather than on helping effort per se, because these attributes are likely to be correlated in this system (Clarke 1989).

The results presented here cast doubt upon the broad applicability of helping as a signal in explaining cooperative acts by unrelated helpers, at least in avian systems. Instead, indirect benefits associated with kin selection may explain a certain proportion of helping in these systems, including bell miners. Future research effort should therefore focus upon experimental assessments of the other pathways via which helpers may accrue direct benefits, such as group augmentation (Wright 2007).

FUNDING

Biotechnology and Biological Sciences Research Council grant (5/S19268) to J.W.; the University of Wales, Bangor.

Nick Hoogenraad and Joan Hoogenraad and the La Trobe University Wildlife Reserve kindly allowed fieldwork to be undertaken on their land. We thank Maria Pacheco, Luc te Marvelde, James O'Connor, and Amanda Dare for assistance with fieldwork. Anna Lashko, Mike Double, and Andrew Cockburn provided facilities for and carried out the molecular analyses. Leg bands were provided by the Australian Bird and Band Banding Service. Mark Elgar and 2 anonymous reviewers provided helpful comments on the manuscript.

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