Pup guarding by greater spear-nosed bats

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Abstract Alloparental care poses an evolutionary dilemma because effort is expended on non-filial offspring. Thus, instances of alloparental care have been attributed to either mistaken identity, (i.e., recognition errors) or active cooperation. In greater spear-nosed bats (*Phyllostomus hastatus*), reproductive females roost together in stable long-term social groups in cave ceilings. Non-volant pups frequently fall from roost sites to the cave floor where they can die unless retrieved by an adult. In this study, we examined the function of adult female visits to non-filial young and tested whether visits were attributable to recognition errors or to cooperation. We found that females visited non-filial pups from their own social group more than expected. Females from different social groups attacked and sometimes killed pups, and male pups were attacked more frequently than female pups. Visits by group mates benefited fallen pups by reducing the likelihood of attack by females from other groups. In contrast to the mistaken identity hypothesis, we found that some females leave their own pups to approach and remain with group mates' pups. We used microsatellite markers to estimate relatedness and test whether kinship could explain this alloparental care and found that females were unrelated to the pups they guard. We conclude that females who reside in highly stable social groups exhibit cooperative behavior that cannot be explained by kinship and is unlikely to be due to direct or generalized reciprocity. Instead, our data suggest that alloparental care likely involves a complex interplay between group membership and cooperative foraging.

Keywords Cooperation · Bat · Parental care · Infanticide

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Introduction

Instances of alloparental care, or care of others' young, attract interest because they appear contradictory to Darwinian natural selection. Adaptive and nonadaptive explanations for the occurrence of such behavior have been reported. The most common nonadaptive explanation is *mistaken identity* where parents confuse their young with others (McCracken 1984; Packer et al. 1992; Murphey et al. 1995; Roulin 2002). Identifying offspring can be difficult in colonial species where the probability of confusing young is high. To counter this problem, young in many colonial vertebrates emit individually distinctive vocalizations that facilitate accurate parent–offspring recognition (Trillmich 1981; Stoddard and Beecher 1983; Gelfand and McCracken 1986). Nevertheless, recognition errors do occur and could result in occasional instances of alloparental care.

Alternatively, individuals may benefit from providing alloparental care to others (Riedman 1982; Packer et al. 1992; Clutton-Brock et al. 2000; Roulin 2002). For such

cooperative behavior to be maintained in a population, the net benefit, in terms of an individual's reproductive success or survival, associated with giving aid must outweigh the alternative of not giving aid. Mechanisms of cooperation are thus defined by how aid givers benefit (Dugatkin 1997; Sachs et al. 2004). The most common explanation for cooperative care is kin selection (Hamilton 1964) where alloparents receive indirect benefits by preferentially providing care to genetic relatives (Emlen and Wrege 1988; Creel et al. 1991; Manning et al. 1992; Pusey and Packer 1994; Gemmell 2003). Alternatively, alloparents could receive direct benefits by caring for others' young. For example, alloparents may benefit from increasing group size if it reduces the risk of predation for their own offspring through selfish herd or predator dilution effects (Wisenden 1999; Lengyel 2007). In other species where young remain in their natal groups through adulthood, long-term group augmentation benefits can also contribute to the maintenance of cooperative care (Rood 1990; Clutton-Brock et al. 2000; Kokko et al. 2000). Finally, instead of deriving benefits from young, caregivers may benefit directly from exchange with other parents. A well-known form of exchange is direct reciprocity (Trivers 1971) where adults reciprocally care for each other's young (Owens and Owens 1984). However, directing cooperative behaviors selectively to previous aid givers may not be required for the evolution and maintenance of cooperation. Recently, support has been found for generalized reciprocity (Rutte and Taborsky 2007, 2008) where individuals base their decision to cooperate on their last encounter regardless of the identity of the participants (Pfeiffer et al. 2005).

In this paper, we describe a previously unknown form of alloparental care, which we refer to as pup guarding, in greater spear-nosed bats (*Phyllostomus hastatus*), a large neotropical omnivorous bat. On the island of Trinidad in the West Indies, unrelated reproductive female *P. hastatus* roost in cave ceilings in groups of eight to 40 individuals that are attended by a single harem male (McCracken and Bradbury 1981; McCracken 1987; Electronic supplementary material, Fig. S1). Social groups are very stable (McCracken and Bradbury 1981), with some females remaining together for 16 years or more (GSW, unpublished data). Cooperative behaviors have been suspected to be important for maintaining these long-term associations (McCracken and Bradbury 1981; Boughman 2006). For example, *P. hastatus* social groups forage together using learned group-specific vocalizations (Boughman 1998; Wilkinson and Boughman 1998). Furthermore, females within groups, but not necessarily across groups, give birth synchronously (Porter and Wilkinson 2001), a trait that is associated with cooperative care in other species (Ims 1990). Nearly all females reproduce only once each year and pup mortality is high, with 25% of pups failing to survive to their first flight at 6 weeks of age (Stern and Kunz 1998). Thus, any behavior that increases the likelihood of pup survival will impact reproductive success. One factor that affects pup survival is pup falling: instances of non-volant pups falling from roost sites in the cave ceiling to the floor where they can easily be captured by predators (GSW, unpublished data) and cannot return to their roost site unless retrieved by an adult.

In this study, we examine how females respond to fallen pups and test alternative possible reasons for their behavior. We divide the paper into two parts. In the first part, we document and quantify pup falling and adult visiting. We then use observations of marked bats to determine if fallen pups are visited non-randomly by adults. In addition, we describe evidence indicating that pup visits influence pup survival and can therefore be considered a form of alloparental care. In the second part, we use experimental and observational data to evaluate alternative, but not mutually exclusive, hypotheses for why females provide alloparental care. The first is the *mistaken identity hypothesis* which states that females visit group mates' pups because they confuse them with their own. We use observations of natural pup falls and staged retrieval events to test whether visits to non-filial pups are consistent with this hypothesis. In contrast, the *cooperation hypothesis* states that females provide care for group mates' young because they receive some benefit. We consider three possible ways females might derive benefits from cooperative care of young: (1) indirect benefits associated with helping close kin, (2) direct benefits from the pups they guard, and (3) direct benefits from exchanging assistance with other group mates.

Materials and methods

Field site

All observations and experiments were conducted at Guanapo Cave, a small limestone cave in the northern range of Trinidad, West Indies (McCracken and Bradbury 1981) between 19 April and 04 May 2001, 12 April and 09 May 2002, and 16 April and 29 May 2004. Guanapo Cave has a single entrance that connects by a short tunnel to a small nearly circular chamber about 10 m in diameter and 2–3 m in height. During this study, approximately 400 reproductive females resided in 20 social groups in this chamber. Each social group occupied a solution cavity in the ceiling of the cave (see Electronic supplementary material, Fig. S1). By labeling each solution cavity with paint and capturing entire groups each year, we confirmed that female fidelity to a social group is very high, as noted previously (McCracken and Bradbury 1981; Porter and

Wilkinson 2001). In a series of studies between 1990 and 2004 (e. g., McCracken and Bradbury 1981; Boughman 1998; Porter and Wilkinson 2001; Stern and Kunz 1998; Boughman 2006), over 2,500 *P. hastatus* were uniquely banded with numbered wing bands and over 4,000 recaptures were recorded in or near this cave. Consequently, we knew the age and reproductive history of many individuals at the time of this study.

Pup falling observations and experiments

By sitting in one location in the cave and using an infrared illuminated digital video camera (Sony CCD-TRV-460), we observed non-volant pups naturally fall to the cave floor on 39 nights between 1900 and 2400 hours over the 3-year study. In 2001, for ten nights, we estimated the fraction of pups in the cave that fell by counting the number of pups on the cave floor between 1900 and 2400 houra and dividing by the total number of pups present. Each night, by 1900 hours, all but a few adults had departed to forage and pups were left behind in their site-specific roosts (Electronic supplementary material, Fig. S2). Due to the low ceiling and small size of the cave, we could easily count the number of pups in each group and locate all fallen pups each night.

We use the following terms to describe pup-related events that we observed in the cave:

Fallen pup—any pup that was on the floor or walls of the cave. Includes both natural and experimental pup falls.
Visit—when an adult landed near a fallen pup and departed without picking the pup up.
Pickup—when an adult landed near a fallen pup and picked the pup up. There were two types of pickups: retrievals and captures.
Retrieval—when an adult landed, allowed the pup to nurse, and departed with the pup in a nursing position (Electronic supplementary material, Video S1).
Capture—when an adult landed, bit the pup, and carried it off in its teeth (Electronic supplementary material, Video S2).
Inspection—when an adult landed, approached a pup, and sniffed it (Electronic supplementary material, Fig. S3).
Fight—when two adults grappled or bit each other while near a fallen pup (Electronic supplementary material, Video S3).
Bite—when an adult bit a pup (Electronic supplementary material, Video S3).

To determine if pup visiting occurs non-randomly among adult bats, we staged pup falls ($n=70$) from seven social groups from which we had individually marked and taken tissue samples from all bats. These experiments enabled us to identify visitors as mothers, group mates, or non-group mates. We captured one, two, and four social groups, which contained 133 adult females and eight adult males in total in 2001, 2002, and 2004, respectively, using a modified bucket (Porter and Wilkinson 2001). We coordinated group captures with parturition times. In this cave, most females give birth in early to mid-April (Porter and Wilkinson 2001). In 2001, we captured groups in early April, which was shortly after parturition for many of the females in the group. In 2002 and in 2004, we captured groups in March before pups were born to reduce disturbance. We sexed, attached permanent numbered bands (females on left wings and males on right wings), took wing membrane tissue samples for subsequent parentage analyses (see below), and made individually distinctive marks on the backs of bats using hair bleach.

To simulate natural pup falls, we removed individual pups from marked groups by hand after adults had departed the cave. We also inspected all roost sites and identified all banded pups in the cave. We then banded, weighed, and measured the forearm of each pup, took a wing tissue sample, and placed it in a cloth bag. In 2001, we staged only one pup retrieval in a night. In 2002 and 2004, we removed pups from more than one group to create instances where females from multiple groups were missing pups (see "Mistaken identity" below). In 2002, we marked two groups and removed two pups from each group on each of five nights. In 2004, we marked four groups, and on each of ten nights, we removed two pups from two of the four marked groups, alternating groups each night. To determine when females initiated searches for missing pups, each night that we staged pup falls, we used infrared illumination to video record (Sony CCD-TRV130) the roost site(s) of the social group(s) where we had removed pups.

We staged pup fall events by placing one pup at a time on a cave wall about 2 h after the initial adult exodus from the cave, by which time most females had returned from foraging. To control for proximity to roost location, we alternated between four cave wall locations and never placed pups further than 5 m from their group's roost site. Pups that fell naturally during retrieval experiments were immediately removed, sampled, measured, banded, and used as additional subjects in experiments. After releasing each pup, we recorded all behaviors around pups with another camcorder (Sony DCR-TRV460) equipped with an infrared light.

For each adult that landed near a fallen pup, we determined the sex and identity, if bleach-marked, and measured the length of the visit (from the moment the bat landed until it flew off) in seconds. We noted whether the visiting bat picked up, inspected, or bit the pup and if it fought with other visiting bats. If pups were

not picked up after 45 min, we returned them to their roost sites.

Estimation of parentage and relatedness

We used microsatellite markers to assign maternity for pups used in retrieval trials and to estimate relatedness between females and pups. Wing membrane samples were removed from 95% ethanol, air-dried, and then DNA was extracted using DNeasy Tissue kits (Qiagen). Using a subset of samples, we tested six *Artibeus jamaicensis* (Ortega et al. 2002) and seven *Lophostoma silvicolum* (Dechmann et al. 2002) microsatellite primer pairs for length variation in *P. hastatus*. To determine the best annealing temperature for the polymerase chain reaction (PCR), we used unlabeled primers and a temperature gradient program on a PTC-200 programmable thermal cycler (MJ Research). The temperature gradient program varied annealing temperatures between 45°C and 65°C. Amplification products were examined using agarose gels.

Of the 13 primer pairs tested, three *A. jamaciensis* and two *L. silvicolum* primer pairs amplified consistently and were polymorphic for length. For these five loci, we performed PCR with one of each primer pair fluorescently labeled (Integrated DNA Technologies) in 10 μl volumes containing 0.5 μl DNA, 2.5 mM MgCl$_2$, 0.5 μM of each primer, 1X PCR Buffer (Invitrogen), and 0.25 U *Taq* polymerase (Invitrogen). The PCR program consisted of 5 min at 95°C, 30 cycles of 45 s at 95°C, 45 s at annealing temperature, 1 min at 72°C, and 5 min of extension at 72°C (Table 1). Fluorescently labeled PCR products were separated on an ABI 3100 DNA Analyzer (Applied Biosystems) and evaluated with Genescan 3.1.2 software (Applied Biosystems). We used Genotyper 2.5 to size and score alleles (Applied Biosystems).

Table 1 Microsatellite loci

Locus	No. of alleles	Temp (deg)[a]	Sizes	Obs. het.[b]
AjA185[c]	2	54	86–88	0.55
AjA74[c]	6	50	145–155	0.77
AjA84[c]	16	50	93–131	0.60
Tsil2Ca1[d]	7	56	111–127	0.68
Tsil3Ca2[d]	8	48	186–204	0.70

The number of alleles, annealing temperature, allele sizes and observed heterozygosity for the five microsatellite loci

[a] Annealing temperature for PCR reaction

[b] Observed heterozygosity. None of the loci showed significant deviation from Hardy–Weinberg expectations in adults

[c] Loci developed for *A. jamaicensis* (Ortega et al. 2002)

[d] Loci developed for *L. silvicolum* (Dechmann et al. 2002)

We first tested if the genotype distribution for each of the five microsatellite loci exhibited deviations from Hardy–Weinberg expectations using GENEPOP (Raymond and Rousset 1995; Table 1). We then used the genotypes to perform maternity exclusions and estimate relatedness using RELATEDNESS 5.0.8 (Queller and Goodnight 1989). We excluded females as mothers if they did not share at least one allele with the pup at each of the five loci. For comparison, we calculated relatedness for 19 female–pup pairs that were captured together while pups were nursing in 2001. We calculated relatedness using RELATEDNESS 5.0.8 (Queller and Goodnight 1989). For Hardy–Weinberg tests and background allele frequencies for relatedness estimates, we used only adult genotypes ($n=140$). We did not use pups because pups from the same social group are often paternal half-siblings.

Testing alternative hypotheses for female visits

Mistaken identity To determine if visits to non-filial pups represent cases of mistaken identity, we first assigned maternity to each experimental pup. We then examined three behavioral variables for each visit that involved a unique marked female–pup combination: (1) the number of return visits to a pup, (2) the number of visits in which a female inspected a pup, and (3) the total time spent visiting a pup. Each time a marked female visited a pup, the event was categorized by social group (*same* or *different*) and pup status (*present* in the roost site or *missing*). Pups that were *missing* had fallen, were removed by us, or were unaccounted for and presumed dead (once unaccounted for, pups never reappeared in the cave again). If fallen pup visits represent cases of mistaken identity, we expected that the number of return visits, the frequency of inspections, and the time spent visiting would all be greater for females missing pups of their own. Although each of the 152 female–pup dyads was unique, they were composed of combinations of 67 different pups and 65 females (32 pups and 45 females were repeated within the dataset). Consequently, we examined the effect of social group and pup status on each response variable using randomization tests with 10,000 permutations (Manly 1991). All randomizations were performed in MATLAB.

Indirect benefits Females could receive indirect benefits if they preferentially visit related pups. We tested this prediction by comparing relatedness between three groups: (1) females and retrieved pups, (2) females and pups visited from the same social group, and (3) females and pups visited from different social groups. To test for significance, we compared each of these estimates with the distribution of means from 10,000 random samples of the same size as the estimate, taken from all female–pup pairs for which we

had genotypes (n=132 females, n=121 pups). We also used a randomization test to determine if the relatedness of females that visited pups from the same social group differed from the relatedness of females that visited pups from different social groups.

Direct benefits We tested three possible ways in which females could benefit directly from visiting group mates' pups. First, we tested whether females benefit directly from the pups they guard. In *P. hastatus*, long-term benefits of group augmentation are unlikely because pups disperse from their natal groups during their first year (McCracken and Bradbury 1981; McCracken 1987). However, guarding other pups could benefit a female's own pup if thermoregulation is enhanced or predation risk is reduced in larger groups. To provide one test of this hypothesis, we regressed pup condition on the number of pups in a group to determine if group size influenced pup survival. Note that because almost all females in social groups reproduce (130 out of 133 females in this study reproduced), the size of the social group (number of adult females) and the number of pups born in a group are nearly identical. Second, we examined direct reciprocity (Trivers 1971). In order to reciprocate, a female would have to observe and identify other females that visited her pup. To assess this possibility, we first determined how often a mother arrived in the presence of a guarder. We then scored reciprocation when a mother visited a guarder's pup at some point in time after the guarding event. Third, we examined generalized reciprocity (Pfeiffer et al. 2005). If this mechanism operates in *P. hastatus*, we expected females whose pups had been visited to be more likely to visit others regardless of the identity of the group mate. For each mother–pup pair and including only same group visits, we tested for an association between (1) whether or not a pup was visited and whether or not a pup's mother visited another pup, (2) the number of group mates that visited a pup and the number of other pups the mother visited, and (3) the total time spent visiting a pup and the total time spent visiting by the mother. All error bars represent the standard error of the mean unless otherwise noted.

Results

Pup fall and visit frequency

Pups fell from the cave ceiling every night during the first week after birth. During 5-h observation periods on each of ten nights in 2001, we observed 85 pups fall to the cave floor, which represents $4 \pm 2\%$ of all non-volant pups (n=77–203). Fallen pups were quite agile. They typically flapped their wings and rapidly moved across the cave floor and then crawled backwards 1–2 m up a wall where they would remain until retrieved. Fallen pups produced audible isolation calls (Bohn et al. 2007) and attracted many adult bats (Electronic supplementary material, Video S4). We observed an average of 17.0 ± 2.6 adults land next to each pup with up to 342 visits to a single pup that was not picked up. Despite the presence of many banded males in the cave, we never observed a banded male land near a pup. Therefore, we assume that all visits by unbanded bats were by adult females. Visits were associated exclusively with pup falls. In over 200 h of cave observations, we never observed adult bats on the cave floor or walls when a pup was not present. We found no difference in the time females spent visiting or the number of female visits between natural and experimental pup falls (Mann–Whitney U tests, P>0.44, n=155), and so below, we consider all visits to all pups together.

Most cases of females landing near fallen pups (2,754 of 2,887) were visits; the females were not the pups' mothers and did not pick up the pups. Short visits (lasting 10 s or less) constituted the majority (60%) of these non-filial visits and could have involved olfactory cue discrimination because females would land briefly (4.2 ± 0.3 s, n=998), inspect the pup, and then depart (Electronic supplementary material, Fig. S3). Next, we examined whether females' visits to pups that were not their own were at random or if they were associated with social group. To obtain random expectations, we assume that any female in the cave is equally likely to detect any fallen pup. We believe that this assumption is warranted because fallen pups produced high-amplitude isolation calls (Bohn et al. 2004). These calls were easily audible to us anywhere in the cave and therefore likely audible to any bat since the frequency of best hearing in *P. hastatus* matches the dominant frequency of pup isolation calls (Bohn et al. 2004). We found that for years in which we had multiple marked groups (2002 and 2004), females visited pups from their own social group more than expected (only marked pups and marked female visits included; Table 2). Females from the single marked group in 2001 also visited pups from their own group more than expected (χ^2=18.3, P<0.0001, df=1). However, because there was only one marked group, we calculated expected values as the proportion of visits by females from all other (unmarked) groups to marked pups (408 of 1,073=38% of unmarked bat visits were to marked pups, while 50 of 83=61% of marked visits were to marked pups). Finally, not only did females tend to visit pups from their own social group more frequently, the average length of visits to pups from the same social group was significantly longer (32.2 ± 5.0 s, n=315) than visits to pups from different social groups (17.5 ± 0.74, n=1448; Mann–Whitney test, Z approximation=24.8, P<0.0001). In some

Table 2 Distribution of visits by females to fallen pups after excluding mother–pup visits

Year	Focal group	No. of focal group pups	A. All visits by focal group females	Observed proportion of (A) to focal group pup	Expected proportion of (A) to focal group pup	χ^2	P
2002[a]						NA	0.0004
	A	9	13	0.64	0.27		
	B	9	35	0.89	0.74		
2004[b]						345.8	<0.0001
	C	14	84	0.53	0.13		
	D	13	112	0.26	0.14		
	E	14	67	0.75	0.24		
	F	12	133	0.77	0.49		

Focal group (A–F) refers to the social groups in which pups and females were recognizable by unique bleach mark patterns each year. We tested for an association between the social group of a fallen pup and the social group of a visiting female with a single contingency table for each year and only including marked bats

[a] Fisher's exact test on 2 × 2 contingency table, $df=1$

[b] 4 × 4 contingency table, $df=9$

cases, females remained near pups for up to 30 min, which was quite distinctive from the short 10-s visits described above. Thus, our results show that females preferentially visit group mates' pups other than their own and spend more time during those visits.

Female behavior to pups

Female behavior towards fallen pups differed depending on whether the female was the pup's mother, another female from the same group, or a female from a different group. After assigning maternity, we inferred that 57 retrieval events involved a mother picking up her pup. Mothers always landed near their pup, lifted a wing, and allowed the pup to attach to a nipple before flying away (Electronic supplementary material, Video S1). In contrast, in each of eight capture events, the pup was bitten in the head and carried away in the mouth of a female who came from a different social group than the pup (Electronic supplementary material, Video S2). We subsequently found five of these captured pups outside of the cave. In addition to captures, we witnessed females bite 27 of 88 marked fallen pups without carrying them away. When bitten, pups often produced distinctive loud vocalizations. We found three dead pups with obvious *P. hastatus* canine punctures in their skulls indicating that captures can be fatal. Neither captures (Fisher's exact test, $P<0.0001$; Fig. 1a) nor bites ($\chi_1^2=8.5$, $P=0.006$, only marked pups used in analysis; Fig. 1b) were independent of social group; nearly all perpetrators were from different social groups than the pups. Interestingly, gender affected the probability of aggression. Male pups were captured (Fisher's exact test, $P=0.006$, eight of eight captured pups male, 32 of 69 retrieved pups male) and bitten ($\chi_1^2=5.3$, $P=0.02$, 20 of 27

bitten pups were male, 29 of 61 unbitten pups were male) more than expected by chance.

Visits to pups by females from the same social group occurred in response to aggression and appeared to decrease the likelihood of capture. We observed fights among adult females at 17 of 88 marked fallen pups (see Electronic supplementary material, Video S3) and participants were from different social groups more often than expected ($\chi_1^2=16.6$, $P<0.0001$; Fig. 1c). In addition, fights were more likely to occur around pups that were bitten ($\chi_1^2=7.85$, $P=0.005$, ten of 17 fights occurred around pups that were bitten). Group mates appeared to respond to pups being bitten; pups that were bitten were more likely to be visited by a group mate ($\chi_1^2=11.3$, $P=0.0005$, $n=88$ pups, 39 visited by group mates, 20 of 27 bitten pups visited), and the number of visits by group mates was higher after a bite than before (Wilcoxon paired-sample test; $S=-46$, $P=0.007$, before median=1, range 0–4, after median=2, range 1–9, $n=31$). Moreover, pups that were visited by group mates were less likely to be captured than pups that were not visited (Fisher's exact test, $P=0.008$, none of eight captured pups were visited, 35 of 80 uncaptured pups were visited). Taken together, these results suggest that group mates respond to pup attacks and their presence protects pups from capture by other bats.
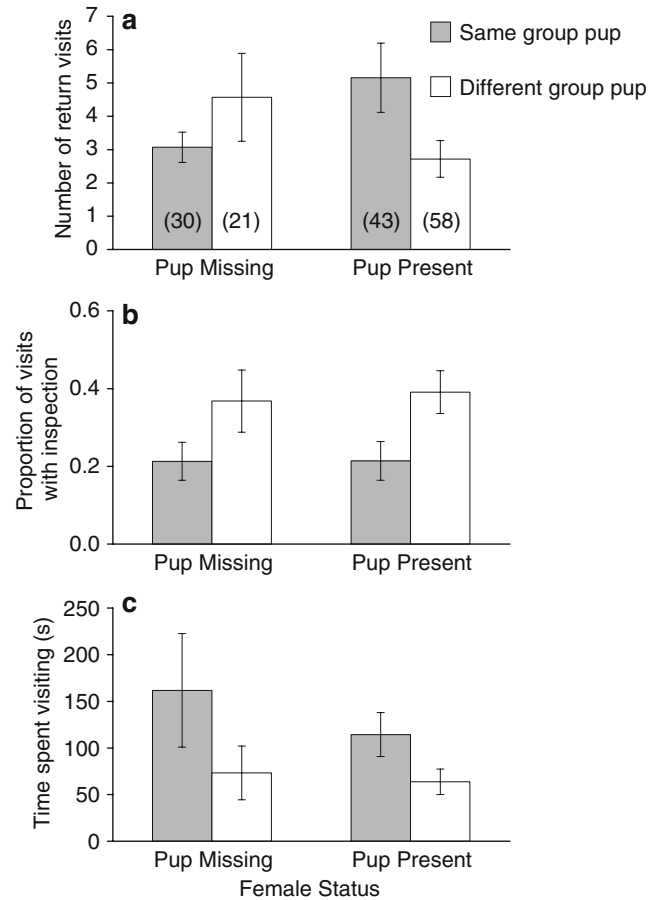
Mistaken identity?

To evaluate this hypothesis, we considered three lines of evidence related to how female visiting behavior varied with pup status (*missing* or *present*) or social group (*same* or *different*). Because females show extreme fidelity to roost locations, we expected females with *missing* pups to
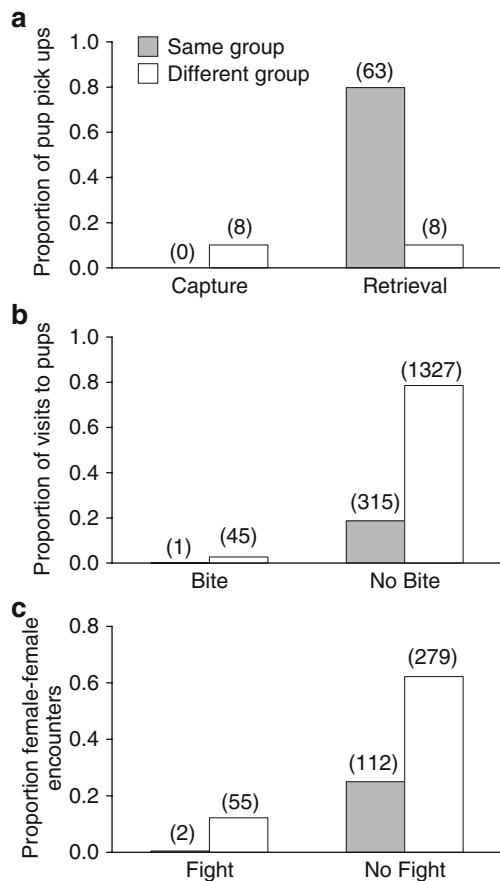
Fig. 1 Aggression occurred more frequently between non-group mates than expected by chance. **a** For marked pup pickups, there was a significant association between whether a female captured or retrieved a pup and whether or not the female was from the same social group as the pup. **b** For all visits to marked pups, there was a significant association between whether or not a female bit a pup during a visit and whether or not a female was from the same social group as the pup. For bite events, all mother–pup visits were excluded. **c** For all female–female encounters that involved at least one marked bat, there was a significant association between whether or not a fight occurred and whether or not females were from the same social group

Fig. 2 The status of a female's pup (*pup missing* or *pup present*) and the social group of the pup she visits affects her behavior. **a** The number of return visits to a pup exhibits an interaction between social group and pup status. **b** Females inspect pups during fewer visits when pups are from the same social group regardless of pup status. **c** Females spend more time visiting pups from the same social group regardless of pup status. *Error bars* are ±SEM. Sample sizes for all tests are shown in **a**. All mother–pup visits were excluded

visit and inspect pups more than females with pups *present*. Instead, the majority (71%) of 313 visits by marked females to group mates' pups occurred when a female's own pup was present in the roost site. Furthermore, although pup status did not affect the amount of time females spent visiting pups ($F=0.8$, $P=0.58$) or frequency of female visits ($F=0.04$, $P=0.52$), there was a significant interaction between pup status and social group for the number of times a female visited a particular pup (Fig. 2). Females whose pups were *present* returned to visit group mates' pups more frequently than females whose pups were *missing* (pup status × social group: $F=4.9$, $P=0.01$; social group: $F=0.28$, $P=0.07$; pup status: $F=0.02$, $P=0.95$; Fig. 2a). These results indicate that guarding behavior is flexible; females visit group mates' pups more often when their own pups are in the roost site.

The second line of evidence is based on the assumption that females use odor to confirm pup identity, as in other bat species (Gustin and McCracken 1987). Under this assumption, females might be expected to visit group mates' pups more frequently to reduce discrimination errors because they are more likely to share a common father (McCracken and Bradbury 1981). Consequently, we would expect females to inspect pups from the same social group by sniffing them (Electronic supplementary material, Fig. S3) more frequently than pups from other groups. However, we found the opposite pattern: Females inspected pups from different social groups more frequently than pups from their own social group ($F=6.9$, $P=0.001$; Fig. 2b) even though they spent more time visiting their group mates' pups ($F=4.5$, $P=0.007$; Fig. 2c).

The third line of evidence is based on retrievals. If offspring identification was difficult, females might be

expected to retrieve non-filial young. Maternity exclusion tests revealed that only six of 63 females retrieved potentially non-filial pups from their social group. In half of those cases, females were missing their own pups, which they picked up later in the evening. In contrast, 44% of 88 fallen pups from marked groups were visited by at least two group members. Thus, these data indicate that females do occasionally make recognition errors, but much less often than they visit and remain near group mates' pups.

Indirect benefits?

Mean relatedness for female/retrieved pup pairs was $r=0.42\pm0.03$ (Fig. 3). This estimate is almost identical to the relatedness estimate we obtained using 19 matched mother–pup pairs (0.45 ± 0.04). Thus, females almost always retrieve their own pups. However, group mates do not selectively visit relatives ($r=-0.02\pm0.03$) and adult females within social groups are unrelated ($r=0.01\pm0.01$; $n=7$ groups, 130 females), consistent with previous reports (McCracken and Bradbury 1981). Interestingly, females appear to attack genetically dissimilar pups; relatedness between females and pups they visited from different social groups was lower ($r=-0.12\pm0.04$) than pups visited from the same social group and lower than expected from a random sample (Fig. 3).

Direct benefits?

We evaluated three ways in which females might receive direct benefits from guarding group mates' pups. First, we examined the group augmentation hypothesis. In contrast to expectations, group size was negatively related to pup condition ($F_{1,6}=27.7$, $P=0.003$, $r^2=0.85$). Next, we
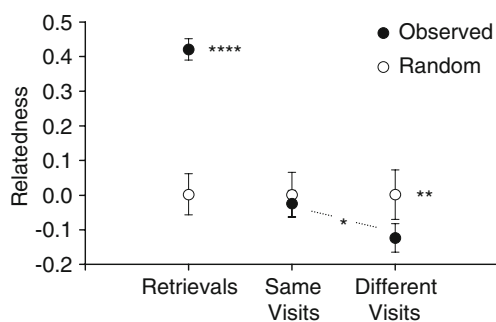
Fig. 3 Relatedness between females and the pups they retrieve or visit compared to random expectations. "Observed" refers to mean ± SEM relatedness between females and pups they retrieved ($n=63$), females and pups they visited that were from the same social group as the female ($n=53$), and females and pups they visited that were from different social groups than the female ($n=43$). "Random" represents the median and 95% upper and lower bounds of 10,000 means of randomly selected samples from all female–pup pairs. *<0.05, **<0.01, ****<0.0001

examined reciprocity among adults. We found that opportunities for direct reciprocity occur, but are limited. Approximately 50% of visits by group mates' overlapped with retrieval by the mother (33 of 68 cases). However, out of 33 opportunities for reciprocal pup guarding, we only observed one case of reciprocation. Generalized reciprocity appears to be equally unlikely. There was no relationship between whether a female visited a pup from her social group and whether her own pup was visited ($\chi_1^2=0.002$, $P=0.99$, 19 of the 33 females whose pups were visited also visited other pups and 18 of the 31 females whose pups were not visited, visited other pups). Furthermore, we found no correlation between the number of visits to a pup and the number of visits the pup's mother made to group mates' pups ($r=0.04$, $P=0.75$, $n=64$) or between the amount of time group mates spent visiting a pup and the time spent by the pup's mother visiting group mates' pups ($r=0.17$, $P=0.18$, $n=64$). These results do not, therefore, provide strong support for either direct or generalized reciprocity.

Discussion

In this paper, we describe how adult female greater spear-nosed bats react to non-volant pups that fall from the roost to the cave floor. Female behavior to fallen pups depends on social group identity. We found that 30% of fallen pups were bitten and 10% were captured and likely killed by females from different social groups. These behaviors likely contribute to the high infant mortality documented in this species (Stern and Kunz 1998). Infanticide has been reported in a wide range of mammals (Ebensperger 1998), but rarely in bats (Kunz and Ebensperger 1999). There are four main explanations for infanticide: adoption avoidance, sexual selection, predation, and resource competition (Hrdy 1979, Ebensperger 1998). While adoption avoidance seems unlikely, our data support the possibility of sexual selection, predation, and/or resource competition being potential benefits of aggression towards pups. Our finding that male pups were attacked more frequently than females is indicative of sexual selection. Indeed, the most well-known cases of infanticide involve males killing other males' offspring to accelerate their own chance of mating (e.g., mice, vom Saal and Howard 1982; lions, Packer and Pusey 1983). Clearly, this is not the case in *P. hastatus*, but given the extreme reproductive skew experienced by males in this species (McCracken and Bradbury 1981), females may reduce future mate competition for their sons by killing other males. Unfortunately, none of the perpetrators were marked bats and so we do not know if they had male or female offspring. Additionally, infanticide may be a form of predation where infants are a source of food (Sherman

1981; Hoogland 1985; Trulio 1996). Although we do not know if captured pups were ever consumed, carnivory in *P. hastatus* has been reported (Dunn 1933). Furthermore, we found that the frequency of captures increased as both female condition and precipitation decreased (for 2002, 2004, and 2001, respectively, as follows: captures: 0% of 26, 11% of 61, and 13% of 33 pups; female condition: 2.9± 0.97, *n*=27; 1.1±0.57, *n*=76; and −5.0±0.95, *n*=25; rainfall: 19.5, 7.2, and 1.1 cm), as would be expected if females supplement their diets by cannibalizing young when other food sources are scarce (Ebensperger 1998). Such attacks might, therefore, also decrease future resource competition among surviving pups.

In contrast to the aggressive behaviors of females from different social groups, females from the same social group appear to protect pups. They visit and spend more time than expected with fallen pups from their own group. Attacks on fallen pups tend to attract adult females from the pup's social group who fight with females from other groups. The presence of visiting group mates decreases the likelihood of an attack. These findings support a protective function for visits by group mates to non-filial young, i.e., females guard pups from the same social group.

While recognition error may explain some pup visits and retrievals, mistaken identity seems inadequate to account for all of our observations. First, females were more likely to visit group mates' pups when their own pups were safe in the roost than when their pups were missing. Indeed, video recordings of group roosting sites showed that females left their own pups behind in the roost in order to visit other pups. Second, we observed females spending long periods of time near group mates' pups without inspecting or attempting to retrieve them. This behavior is quite different from the short visits (<10 s) and rapid inspections (<5 s) that typically occur when females are searching for a missing pup and encounter a non-filial pup. Third, if females visited pups from their own social group more frequently than pups from other groups because group mates' pups were more easily confused with their own pups, then we expected females to also use scent to facilitate recognition and inspect group mates' pups more frequently. Instead, we found that females inspected pups from the same group less frequently. In the context of a dark cave, females first rely on pup vocalizations, termed "isolation calls," to detect and locate fallen pups (Gould et al. 1973). These calls also serve in recognition since they carry individually distinctive frequency spectra, and psychophysical studies have shown that *P. hastatus* can discriminate between isolation calls from different pups in the same social group (Bohn et al. 2007). Although females did occasionally (less than 10% of occurrences) retrieve group mates' pups, in three of the six cases where a female retrieved the wrong pup, her own pup was *missing* and she

correctly retrieved her own pup later that night. Thus, our observations are largely consistent with an effective parent–offspring recognition system in this species. We suspect that alloparental retrievals are rare because they would permit allonursing. The energetic cost associated with losing milk during a retrieval is almost certainly larger than that associated with guarding, since guarding females have just returned from feeding and typically hang passively near fallen pups.

Although pup guarding appears to benefit pups and their mothers, how a female that exhibits this behavior benefits is not as clear. Even if guarding entails little risk of injury or loss of energy, females are leaving their own pups to attend to others. Kin selection is unlikely to be important because females are not related to other group mates or the pups they visit. Therefore, to explain pup guarding as an adaptation, females must benefit in some way from either the pups they guard or from the pups' mothers. The simplest mechanism is that pup guarding helps to maintain group size. Although group augmentation benefits that accrue later in life (Rood 1990; Clutton-Brock et al. 2000; Kokko et al. 2000) are not likely to play a role in this species because pups disperse from their natal groups during their first year (McCracken and Bradbury 1981), prior to dispersal, larger pup groups could be beneficial to the guarding female's pup if they enhance pup survival. In a previous study conducted at the same site (Boughman 2006), group size did not affect pup survival or condition, and in this study, we found a negative relationship between group size and pup condition. Nonetheless, neither of these studies attempted to quantify pup predation or thermoregulation, both of which could influence pup survival. Furthermore, if there is an optimal group size, then no linear relationship would be expected between group size and survival or condition. Greater spear-nosed bats exhibit impressive birth synchrony within social groups that cannot be due solely to environmental factors (Porter and Wilkinson 2001). Our results indicate that at least one advantage of birth synchrony may be communal infant care. In addition to the interactions we observed between adult females and fallen pups, we also observed single adult females remaining in groups of very young pups (GSW, unpublished data) while all other females left the cave to forage. Additional study is needed to determine the identity and behavior of these females, particularly with regard to the behavior we report in this paper.

In addition to benefiting from the pups they guard, adult females could potentially benefit from interactions with other adult females. This scenario is plausible given the long life span and group fidelity exhibited by females (McCracken and Bradbury 1981; Nowak 2006). Females could provide assistance to pups to obtain future benefits from the pups' mothers. However, our study provides no

evidence in support of direct or generalized reciprocity of pup guarding. An alternative possibility is that females could guard pups in exchange for access to roosting or feeding sites much like primates exchange allogrooming for agonistic aid (Schino 2007) or food (de Waal 1997). In both situations, beneficial behaviors can be considered commodities that are traded in a biological market (Noë and Hammerstein 1995; Barrett et al. 1999). In *P. hastatus*, access to foraging sites is a plausible commodity because females from the same social group forage together repeatedly over time on clumped food resources, such as large flowering balsa (*Ochroma lagopus*) trees, while giving group-specific vocalizations (Boughman 1998; Wilkinson and Boughman 1998). Furthermore, group foraging has a positive effect on pup condition and survival (Boughman 2006). Future research into the interplay between group membership, cooperative foraging, and alloparental care is clearly needed to further evaluate this possibility and clarify the relative costs and benefits of pup guarding.

Nonetheless, our results indicate that a more complex model of cooperation may be required to explain pup guarding in this species where cooperators are long-lived females that reside in highly stable social groups and interact repeatedly over long periods. In other bat species that form social groups, genetic relatives commonly occur (cf. Wilkinson 1987; Kerth 2008 for reviews). In those cases, attributing the maintenance of cooperative behavior to direct effects can be problematic because kin selection may also operate. In contrast, cooperative behavior in *P. hastatus* occurs independently of kinship but non-randomly in a socially structured population. Thus, future research on this species may be particularly useful for understanding the evolution of cooperation in cohesive social groups, which lack close kin (Boyd et al. 2003; Gintis et al. 2003; Bowles 2006).

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