Common vampire bat contact calls attract past food-sharing partners

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Several social birds and mammals, including some corvids, primates, carnivores, cetaceans and bats, form individualized and enduring social relationships that involve multiple forms of cooperation (Brent, Chang, Gariépy, & Platt, 2014; Carter, 2014; Seyfarth & Cheney, 2012). These cooperative social bonds can provide indirect and direct fitness benefits, so animals should invest in them based on kinship cues (Hamilton, 1964), past experiences of reciprocal cooperation (Trivers, 1971), or both (Wilkinson, 1984). However, kinship and reciprocal help are often correlated and confounded. An understanding of why animals invest in cooperative relationships can therefore require teasing apart their relative importance (Carter & Wilkinson, 2013a; Schino & Aureli, 2010).

Among adult common vampire bats, Desmodus rotundus, enduring reciprocal relationships appear to stabilize regurgitated food sharing (Carter & Wilkinson, 2013a, 2013b; Wilkinson, 1984, 1985a, 1986). Food sharing has inclusive fitness consequences because vampire bats often fail to feed and can starve to death after missing only two to three nightly meals of blood (Wilkinson, 1984, 1988). Food-sharing rates among dyads in a social network are predicted by several correlated factors: kinship, association, allo-grooming and reciprocal sharing. The relative roles of kinship versus social experience have been assessed by Monte Carlo simulation (Wilkinson, 1988) and by testing predictors of food sharing among bats observed under natural conditions (Wilkinson, 1984), among unrelated bats with different levels of association (Wilkinson, 1984) and among familiar bats with different levels of relatedness (Carter & Wilkinson, 2013a). Food donation sizes in the wild and in captive experiments are similar (Wilkinson, Carter, Bohn, & Adams, 2016), and together these tests have shown that past experiences of food sharing or social grooming can overshadow kinship for predicting food donation rates among adults (Carter & Wilkinson, 2013b). The hypothesis that food sharing provides reciprocal benefits is further supported by the observation that vampire bats that feed more nonkin maintain a larger and more robust network of donors (Carter & Wilkinson, 2015). However, the most common and crucial context for food sharing involves mothers feeding their dependent offspring, suggesting that regurgitated food sharing may have evolutionary origins in maternal care.

Another possibly neotenous social behaviour in vampire bats is contact calling. In all bat species, pups produce isolation calls that allow mothers to recognize and retrieve them (Balcombe, 1990;
In all three vampire bat species, contact calls are also produced by isolated adults (Carter, Logsdon, Arnold, Menchaca, & Medellin, 2012). These calls are longer and of lower frequency than echolocation pulses and so travel farther distances. They also vary substantially in acoustic structure both among and within individuals (Fig. 1). Contact calls appear to allow bats to find and recognize familiar group-mates. In playback studies, white-winged vampire bats, Diaemus youngi, showed vocal discrimination: vocal responses occurred more often when playbacks of different contact calls shifted to a novel caller (Carter, Skowronsksi, Faure, & Fenton, 2008) and also when two or three isolated group-mates called from swapped spatial positions rather than from each of their original locations (Carter, Fenton, & Faure, 2009). Vocal discrimination has not yet been experimentally demonstrated among adult common vampire bats.

To determine the social factors that influence responses to contact calls in common vampire bats, we conducted two kinds of playback trials. In ‘past donor trials’, we compared the attraction of subjects to calls recorded from two individuals matched by kinship but differing in their history of donating food to the subject. We predicted that responses during kinship trials would show a stronger influence on responses to calls from the more frequent donor.

In ‘kinship trials’, we compared the attraction of subjects to calls recorded from bats matched in food-sharing history but contrasting in kinship with the subject. Since we previously found that reciprocal food sharing is eight times more important than kinship for predicting donation rates (Carter & Wilkinson, 2013a), we predicted that responses during kinship trials would show a weaker effect size compared with past donor trials. In summary, we predicted that isolated vampire bats would seek out past food donors more strongly than relatives.

**METHODS**

**Subjects**

Subjects were 31 common vampire bats from five matrilines and three zoo populations, housed and cared for during the study by the Organization of Bat Conservation at the Cranbrook Institute of Science (Bloomfield Hills, MI, U.S.A.) under permits from the U.S. Department of Agriculture (34-C-0117) and the U.S. Fish and Wildlife Service (MB003342-0). Unrelated bats from the different populations were introduced as adults. Bats were housed at 25–28 °C with >33% humidity on a 12:12 h light:dim light cycle in a 3 x 1.5 x 2 m cage with a soft paper bedding floor that allowed flight and free association among cage-mates. Bats were fed on physically or chemically defibrinated bovine blood that was available ad libitum and changed twice daily. All procedures with animals adhered to the ASAB/ABS Guidelines for the Use of Animals in Research and were approved by the University of Maryland Institutional Animal Care and Use Committee (Protocol R-10-63).

**Social Data**

Measures of food-sharing history were derived from a food-sharing network involving 355 trials—donations and more than 1250 regurgitation observations made during 2010–2014. To quantify food sharing history we used a food-sharing index from individual A to individual B, which we calculated as the ln(x + 1), where x is the food-sharing rate from A to B (seconds of mouth licking per h per experimental trial). To calculate relatedness, we genotyped 19 microsatellite markers (Carter & Wilkinson, 2015) and used Wang’s (2002) estimator in the R package ‘related’ (Pew, Muir, Wang, & Frasier, 2015). We defined kinship by substituting zeros for all negative relatedness values and replacing relatedness values of known maternal kin with the appropriate pedigree kinship values (e.g. mother = 0.5, grandmother = 0.25). Findings remained the same whether we used kinship or relatedness.

**Playback Stimuli**

Isolated vampire bats produce multiharmonic contact calls that are variable between and within individual bats (Fig. 1; Carter et al., 2012). We recorded contact calls by placing an adult individual in a small soft-mesh butterfly cage at a distance of 10–30 cm from a CM16 ultrasound condenser microphone (frequency range 10–200 kH, Avisoft Bioacoustics, Berlin, Germany) inside an acoustic recording booth (plastic bin lined with acoustic foam) for 1–24 h. Due to these ideal recording conditions (e.g. ultrasonic microphone close to the bat’s mouth, low gain, semi-anechoic

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**Figure 1.** Example contact calls from common vampire bats. Three calls recorded from an 8-year-old female (top left), a 10-year-old female (top right), a 6-year-old male (bottom left) and a 2.5-year-old female (bottom right). Darkness in spectrogram shows relative intensity. All bats were individually isolated during recording. See Carter et al. (2012) for descriptions of call structure variation.
Playback Experiment

Trials took place in darkness and were filmed with an infrared spotlight (IRlamp6, Wildlife Engineering, La Crosse, WI, U.S.A.) and a Sony Nightshot DCR-SR85 camcorder. In each trial, a subject bat was placed in a 76 × 76 cm plastic mesh experimental ‘maze’ with five arms that allowed the subject bat in the centre of the maze to move forwards, backwards, left, right, or upwards (Fig. 2). The bat was placed in the arm farthest from the camera, equidistant to the two speakers. After moving forwards, it could enter the arms to the left or right, with each option leading towards a speaker simultaneously playing calls from either a control bat or a test bat. The bat could also move straight backwards or forwards away from either speaker, or climb upwards, which bats typically do as an escape response. These options allowed us to remove cases where bats were not motivated to move towards either speaker.

Playback trials were of one of two types: ‘past food donor trials’ and ‘kinship trials’. In past food donor trials, a subject with a history of food sharing (N = 12 females, 3 males) was presented with a choice between approaching contact calls from a frequent food donor (test bat) and an infrequent donor or nondonor (control bat). Test and control bats differed in their food donation rates to the subject, but were matched by age and kinship (Table 1).

In kinship trials, a subject (N = 12 females, 19 males) was presented with a choice between contact calls from a close relative (test bat) and a distant or nonrelative (control bat). Test and control bats differed in their pairwise kinship with the subject, but they were closely matched wherever possible by age and past food-sharing experience (Table 1).

Each trial lasted 10 min. If the bat did not make a choice within the first trial, the trial was discarded and a new trial began. Test stimuli were presented on the left or right randomly. The maze was rotated 90° clockwise between each trial. We presented the two trial types on a random schedule to remove order effects. Each subject experienced a unique playback combination of test and control bat except for one test subject experienced a unique playback combination of test and control bats together with a close relative (test bat) and a distant or nonrelative (control bat). Test and control bats differed in their pairwise kinship with the subject, but they were closely matched wherever possible by age and past food-sharing experience (Table 1).

Playback Response Analysis

Responses were measured from videos by observers that were blind to the playback treatments and design. The blind observers recorded (1) the number of seconds that bats spent in either the left or right arm of the maze and (2) the latency in seconds from the start of a trial until the bat entered either the right or left arm. If the bat never entered the arm, latency was scored as 600 s. Response data were non-normal, so we calculated P values using bootstrapping. Using the R package ‘boot’, we resampled the mean...
Food sharing and kinship of callers with subject bat

<table>
<thead>
<tr>
<th>Playback treatment</th>
<th>Food-sharing index</th>
<th>Kinship</th>
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<tr>
<td></td>
<td>Median</td>
<td>Range</td>
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<td>Past donor</td>
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<tr>
<td>Test bat</td>
<td>4.9</td>
<td>2.5–6.2</td>
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<td>Control bat</td>
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<td>0–5.0</td>
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<tr>
<td>Test–Control</td>
<td>4.8</td>
<td>0.8–6.2</td>
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<tr>
<td>Kinship</td>
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<tr>
<td>Test bat</td>
<td>5.5</td>
<td>0–7.4</td>
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<tr>
<td>Control bat</td>
<td>0</td>
<td>0–4.9</td>
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<tr>
<td>Test–Control</td>
<td>0</td>
<td>0–7.3</td>
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* Food-sharing records were not available for all 31 bats used for playbacks because some males were not fasted.

Acoustic Analysis of Playback Stimuli

We performed acoustic analyses of the playback stimuli to help interpret our behavioural results. A bias towards frequent donors or kin could indicate vocal recognition. Alternatively, without vocal recognition, an attraction towards kin or frequent donors could occur if kin or frequent donors sound more alike and subjects were attracted to more similar-sounding group-mates. We therefore tested whether the pairwise relatedness or food-sharing index differences (test–control) for both the subject’s time in each arm and the subject’s latency to enter each arm, and calculated the one-sided P value as the proportion of the 5000 sample means that exceeded the observed mean. We calculated 95% confidence intervals (CI) using the BCa method (Puth, Neuhäuser, & Ruxton, 2015). Like all inferences, resampling tests can be problematic when a sample is too small to properly capture the variation of the population. For inferences based on small sample sizes (<20), we therefore also present more conservative P values calculated using a nonparametric Wilcoxon signed-ranks test. All response data are available on FigShare (https://dx.doi.org/10.6084/m9.figshare.1613493).

RESULTS

Vampire bats placed in the test arena typically walked and echolocated towards one speaker and then the other, alternating between them. In past donor trials, subjects were more attracted to calls of more frequent donors compared to less frequent donors. They spent on average 51 s longer in the test arm than in the control arm (95% CI = 12–91 s; permutation: P = 0.0014; Wilcoxon signed-ranks test: S = 44, N = 15, one-sided P = 0.005; Fig. 3a). Attraction to the frequent donor was still evident even when only using the nine trials where the test bat was unrelated to the subject (test kinship < 0.02; mean time in test arm = 57 s, 95% CI = 16–151 s;
males are attracted to group-specific calls, as is true for decisions about responding to contact calls. In contrast, they were not attracted to contact calls of frequent donors, even when controlling for kinship. We also did not find evidence indicating that playbacks from related bats or frequent donors sounded more similar to each other, suggesting that responses were not based on self-phenotype matching of calls.

Measures of only a few acoustic parameters (duration, distance to maximum amplitude and peak frequency at three points) provided better than chance classification of call to individual, and bats probably use a much richer set of acoustic information in these richly harmonic calls, such as formant-like spectral features shaped by the vocal tract (Yovel, Melcon, Franz, Denzinger, & Schnitzler, 2009). Rather than merely being attracted to certain call characteristics, the acoustic and behavioural results suggest that bats were matching calls to a specific group-mate, and basing their decisions on caller identity. This ability would not be surprising given the experimental evidence that white-winged vampire bats can discriminate among familiar conspecifics using similar contact calls (Carter et al., 2008, 2009), greater spear-nosed bats, _P. hastatus_, can recognize individual pups using isolation calls (Bohn, Wilkinson, & Moss, 2007), and greater mouse-eared bats, _Myotis myotis_, can recognize individuals using voice characteristics of echolocation calls (Yovel et al., 2009), which function primarily for biosonar.

With regard to kin discrimination, the results here are consistent with two interpretations. Either the bats were not able to use calls to recognize kinship, or any perceived kinship was overshadowed by social experience. This latter hypothesis is consistent with prior evidence on social decision making by vampire bats. Free-ranging female vampire bats both positively assort and share food with close kin, and this pattern is unlikely to result from simple mechanisms for kin-biased association, such as spatial location or familiarity (Penn & Frommen, 2010), because female common vampire bats develop multイヤear stable associations with kin and nonkin even when frequently switching roosts (Tschapka & Wilkinson, 1999; Wilkinson, 1984, 1985a, 1985b). The mechanisms of kin discrimination in vampire bats remain unclear. Given the results presented here, a parsimonious explanation is that vampire bats learn the calls of maternal kin based on early life experiences (prior association) and do not recognize or favour unfamiliar kin (phenotype matching). However, there is growing recognition that social vertebrates may have multiple forms of kin discrimination, including both prior association and phenotype matching (Breed, 2014; Cornell, West, & Griffin, 2009; Green et al., 2015; Mateo, 2010; Mateo & Hauber, 2015; Mcdonald & Wright, 2011; Pfefferle, Kazem, Brockhausen, Ruiz-Lambides, & Widdig, 2014). Different kin recognition mechanisms may be used in different contexts (e.g. inbreeding avoidance versus nepotism) and might interact with past experience to influence social decisions (Breed, 2014; Daniel & Ridd, 2015; Delton, Krasnow, Cosmedes, & Tooby, 2011; Mateo, 2010; Mateo & Hauber, 2015; Mcdonald & Wright, 2011; Penn & Frommen, 2010; Zöttl, Heg, Chervert, & Taborsky, 2013). Like most mammals, bats appear to use olfaction for social recognition at close range (Bloss, Acree, Bloss, Hood, & Kunz, 2002; De Fanis & Jones, 1995; Engelt & Greene, 2009; Gustin & McCracken, 1987; Safi & Kersh, 2003), but a system of kin recognition based on acoustic phenotype matching (Kessler, Scheumann, Nash, &
Zimmermann, 2012; Levrero et al., 2015; Pfefferle, Ruiz-Lambides, & Widdig, 2014) would allow for recognition of unfamiliar or paternal relatives at a distance, which would be necessary for co-roosting preferentially with unfamiliar kin over unfamiliar non-kin. Analysis of call variation using better feature extraction across a larger sample of calls and bats is therefore needed to determine whether there are subtle kinship cues that can be used to discriminate unfamiliar (paternal) kin.

Common vampire bats are likely to use contact calls in at least two cooperative contexts. First, common vampires, like many forest-dwelling bats, possess ‘fission–fusion’ social dynamics where stable relationships are maintained despite frequent regrouping and roost switching (Aureli et al., 2008; Kerth, Perony, & Schweitzer, 2011; Popa-Lisseau, Bontadina, Mora, & Ibáñez, 2008; Willis & Brigham, 2004). In Costa Rica, particular females often end up in the same roost despite frequently moving among as many as six hollow tree roosts (Wilkinson, 1985a). Given the importance of acoustic communication for bats, and the time and energy it would take to visit multiple trees to smell roost-mates in each, it seems most likely that vampire bats use vocalizations to coordinate these co-roosting associations, as has been found in other bat species (Arnold & Wilkinson, 2011; Chaverri, Gillam, & Kunz, 2012; Kerth & Reckardt, 2003). Second, contact calls might be used within the roost. Food donors often initiate and approach hungry bats to feed them (Carter & Wilkinson, 2013b). In fact, they will sometimes fly to trapped hungry partners, and even feed them through cage bars (Carter, 2016), suggesting that vampire bats can recognize a needy partner from a distance.

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