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Cite this article: Carter GG, Wilkinson GS. 2015 Social benefits of non-kin food sharing by female vampire bats. *Proc. R. Soc. B* **282**: 20152524.
http://dx.doi.org/10.1098/rspb.2015.2524

Received: 20 October 2015
Accepted: 26 October 2015

Subject Areas:
behaviour

Keywords:
bet-hedging, cooperation, *Desmodus rotundus*, reciprocity, social bonds, social networks

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.2524 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY
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Social benefits of non-kin food sharing by female vampire bats

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Regurgitations of blood among vampire bats appear to benefit both direct and indirect fitness. To maximize inclusive fitness, reciprocal food sharing should occur among close kin. Why then do females with kin roost-mates help non-kin? We tested the hypothesis that helping non-kin increases a bat's success at obtaining future donations by expanding its network of potential donors. On six occasions, we individually fasted 14 adult females and measured donations from 28 possible donors. Each female was fasted before, during and after a treatment period, when we prevented donations from past donors (including 10 close relatives) by simultaneously fasting or removing them. This experiment was designed to detect partner switching and yielded three main results. First, females received less food when we prevented donations from a past donor versus a control bat. Donors within a group are therefore not interchangeable. Second, the treatment increased the variance in donors' contributions to food received by subjects, suggesting the possibility of alternative responses to a partner's inability to reciprocate. Finally, bats that fed more non-kin in previous years had more donors and received more food during the treatment. These results indicate that a bat can expand its network of possible donors by helping non-kin.

1. Introduction

Cooperating organisms face the challenge of investing preferentially in partners that provide the best inclusive fitness returns. Indirect fitness benefits can be ensured through kin discrimination [1,2], while direct fitness returns can be enforced through some combination of partner choice and partner control ('reciprocity' in the broad sense [3]). However, direct and indirect fitness benefits are often complimentary and their relative importance can shift over evolutionary time. Cooperation might first arise through kin selection, but later become stabilized by direct fitness benefits [4,5]. We hypothesize that this scenario explains regurgitated food sharing in the common vampire bat (*Desmodus rotundus*) [6].

Vampire bats are obligate blood-feeders on a tight energy budget. Stable social networks of eight to 12 adult females arise despite frequent switching among two to five roosts [6]. Frequent roost-mates regurgitate blood to kin and non-kin in dire need due to failed foraging (mean kinship per group: 0.03–0.11) [6]. Past work on predictors of food sharing [6–8] suggest that these donations may have originated as maternal care and were co-opted for helping adult kin, and then co-opted again for promoting reciprocal donations. If vampire bat food donations do indeed provide both direct and indirect benefits, then the largest possible inclusive fitness benefits should accrue from reciprocal sharing with close kin. Why then do females with kin roost-mates help non-kin?

Non-kin donations may allow bats to create and maintain a wider network of potential donors than would be available if food sharing was limited to close kin. This could occur with or without direct reciprocity. In the absence of reciprocity and assuming that bats exhibit no dyadic preferences when aiding non-kin groupmates, non-kin sharing might simply help sustain a larger group of possible donors by increasing survival of roost-mates (via passive group augmentation [9–11]). Alternatively, cooperation might be enforced by partner choice, partner control or both [3,12–16]. This scenario assumes that bats invest in specific social bonds based on the fitness-relevant returns, and it predicts that bats which make better social investments will be more

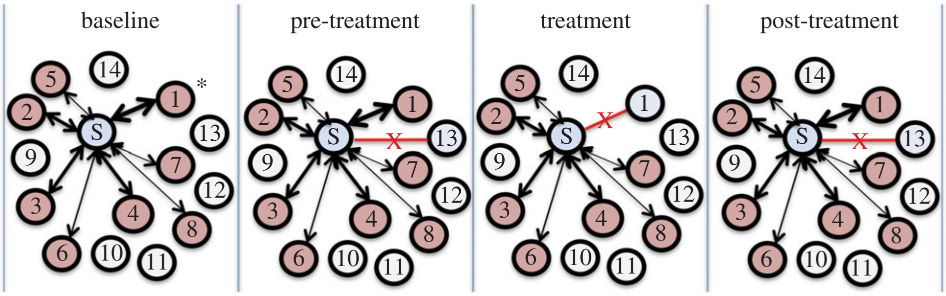

Figure 1. Illustration of how the partner-switching experiment was conducted for a representative individual. Circles are female bats. Arrows show food given. Fasted bats are labelled blue. Each of 14 subjects (S) started the experiment with a baseline history of sharing with several prior donors (red) ranked by donation rate (number). For each subject, we targeted a unique donor of highest possible rank (*). In treatment trials, we prevented donations from this targeted donor. In pre- and post-treatment trials, we prevented donations from a control bat. We measured food donations in each trial. (Online version in colour.)

successful even within a group of constant size. In other words, non-kin sharing widens social network size not merely by increasing groupmate survival, but by creating or strengthening social ties that yield reciprocal returns. As a consequence, individuals that feed more non-kin should have more donors when the need arises.

We tested this prediction in a captive group of vampire bats. We experimentally prevented food sharing by simultaneous fasting or temporarily removing a partner with a prior history of food sharing. We tested three hypotheses. If donors within the group are not interchangeable, then preventing donations from past donors, rather than control bats, would lead to an immediate decline in total food received. If non-kin investments provide long-term cooperative returns, then bats that fed more non-kin in previous years would have more donors and be fed more when targeted kin donors could not feed them. Finally, if an inability to reciprocate leads to rapid partner switching, then after a subject repeatedly fails to feed a past donor, a lower proportion of a subject's received food would come from that donor.

2. Material and methods

(a) General methods

Animal care and methods for inducing food regurgitations are described elsewhere [7]. We housed 24–39 captive-born *D. rotundus*, sourced from three different zoos, which led to multiple matrilines and unrelated bats, as found in wild colonies [6]. Durations of regurgitations in these captive bats are similar to those observed in the wild [17]. To induce and quantify food sharing, we removed and fasted two subjects for 24 h, returned the first subject to the group for 1 h of video focal sampling, and then repeated this with the second subject. To measure food sharing, we scored seconds of time that the subject licked the mouth of a donor. Mouth-licking is a good measure of food sharing because it is highly correlated with trial weight gain, but it is not affected by urination and defaecation (see the electronic supplementary material, figure S1).

We used data on food sharing spanning the years 2010–2014 (approx. 1250 regurgitation observations, 355 trial-donations, 442 directed links between dyads, from [7] and afterwards). We measured two basic forms of social network centrality: the number of bats that have fed a subject ('indegree') and the number of bats that a subject has fed ('outdegree'). We estimated relatedness using the R package 'related' [18] from genotypes of 19 polymorphic microsatellite markers (see the electronic supplementary material for details). We define 'non-kin' as partners with a pairwise relatedness estimate of zero or less.

(b) Partner-switching experiment

In this experiment, we quantified food sharing before, during and after a treatment period. During this treatment, we inhibited food sharing between members of seven targeted dyads, then tested for subsequent partner switching, i.e. that a targeted past donor contributed significantly less than before to feeding the fasted subject (figure 1). The targeted dyads were 14 female subjects paired according to their history of food sharing (electronic supplementary material, table S3). We paired as many females as possible with their most frequent donor, which resulted in two pairs of non-kin, four mother–adult daughter pairs and one other kin pair (electronic supplementary material, table S3). We focused on testing females because they perform about 80% of food donations [7], and we targeted frequent donors to maximize our ability to detect a decrease in donation rate.

We tested each subject six times for a total of 84 fasting trials. We conducted two trials each trial day; one round of testing all 14 subjects lasted one week. For each trial, we fasted the subject, then measured any donations from bats in the group. A subject could potentially be fed by 13 females and 11–15 males, but one female (the other subject that day) was fasted on the same night and was therefore either missing or unfed. We grouped these 'missing' and 'unfed' conditions together because we did not detect any difference in how bats responded to these two ways of preventing donations (see the electronic supplementary material). During the first two-week *pre-treatment* period, we prevented donations from a control bat that had not previously fed the subject much or at all. During the next three-week *treatment* period, we prevented donations from the targeted donor. In the final *post-treatment* week, we again prevented donations from a control bat. We predicted that the treatment would: (i) reduce the amount of food received by subjects, (ii) reveal that bats that fed more non-kin in previous years would have more donors and receive more food when their primary donors could not feed them, and (iii) reduce food sharing with targeted donors relative to other donors.

(c) Statistical analyses

Before conducting any parametric test, we tested for deviations from normality using a goodness of fit test and assumed unequal variances for all *t*-tests. Whenever parametric assumptions were violated, we used permutation tests (lmPerm package in R). To help interpret null results, we provide 95% confidence intervals. We accounted for repeated measures by testing before versus after contrasts by bat.

To examine the effect of prior non-kin donations on sharing success in the experiment, we first used permuted simple linear regressions to test whether the number of male or female non-kin recipients in prior years predicted: (i) number of donors in prior years, (ii) number of donors during the experiment, or

(iii) total food received across all treatment trials. To select the best multivariate model for predicting total food received during treatment, we entered six predictors into backwards and forwards stepwise regression and evaluated model fit using minimum Akaike and Bayesian information criterion (AIC and BIC). These predictors were age, the number of times the bat was fasted (which is proportional to the number of times they could have donated), and the numbers of previously fed male kin, male non-kin, female kin and female non-kin. We used Spearman's correlation to confirm that females with more donors per trial received more food.

To determine if targeted donor contributions were replaceable, we used a paired *t*-test to determine if a subject's total food received declined during the first treatment trial, specifically when donations from the targeted donor were first prevented. We also tested if a decline in food received was detectable during any other trial rounds. We repeated this analysis for the number of donors per trial. Next, to determine if the bats showed a response to a past donor's inability to reciprocate, we first compared the targeted donor's contribution to food received in the pre-treatment and post-treatment periods. We tested both absolute values of donation sizes and arcsine-transformed proportions of subjects' total food received. Three subjects (from two dyads) were not fed by their targeted donors during pre-treatment and were therefore excluded from this analysis (electronic supplementary material, table S3); however, the main result was consistent whether we included these cases or not.

Finally, we tested if the targeted donor's contribution to a subject's total food received became more variable after the treatment period. We conducted Levene's test and O-Brien's test on the arcsine-transformed percentages, as well as a permutation test (see the electronic supplementary material). Results were consistent across all tests, so we present only the results of the first test we conducted.

3. Results

Females that previously fed more non-kin had more donors and they received more food. The number of non-kin fed by a female in previous years predicted the average number of donors per experimental trial (rank-transformed response: $R^2 = 0.43$, $p = 0.008$). More donors per trial led to more food received overall (Spearman's rank correlation: $r = 0.59$, $p = 0.025$). The total food received by a female during treatment trials was predicted by its prior propensity to feed non-kin females ($R^2 = 0.48$, $p = 0.006$), but not non-kin males ($R^2 = 0.12$, $p = 0.23$), related males ($R^2 = 0.02$, $p = 0.60$) or related females ($R^2 = 0.07$, $p = 0.38$). Stepwise regression chose 'number of female non-kin previously fed' as the chief determinant of sharing success while excluding other numbers of previously fed bats. Minimum BIC also selected 'times tested', so we included it in the model (adj. $R^2 = 0.60$, $F_{1,1} = 11.8$, $p = 0.0064$; figure 2). The model confirmed that sharing success was predicted by the number of previously fed female non-kin ($p = 0.002$) but not the number of times the bat was tested in prior years ($p = 0.09$).

Targeted donors were not immediately replaced by other groupmates in a trial. Subjects received less food when their targeted donor, rather than a control bat, was removed from the donor pool (paired $t_{13} = -2.7$, one-sided $p = 0.0089$). A decline in food received (mean = 272 s, 95% confidence interval (CI) = 55–489 s) was detectable only when the targeted donor was first removed and not during other fasting rounds. During this same time, we did not detect a change in the number of donors (mean = −0.36, 95% CI = −0.84 to 0.13 bats). Across all trials, bats were fed by an average of 2.6 donors (95% CI = 2.3–2.9).

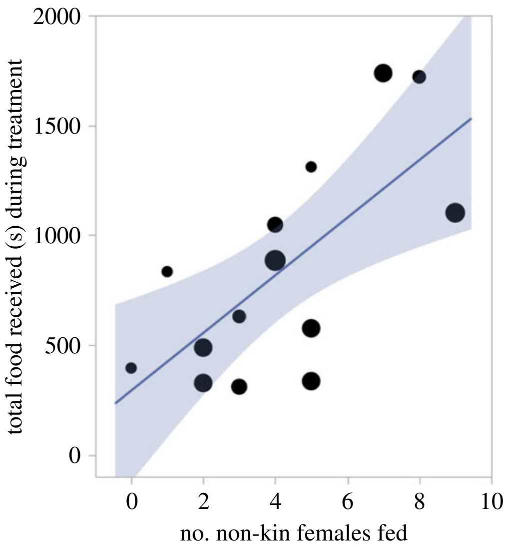

Figure 2. Donations to unrelated females predict success when primary donors are removed. The number of non-kin females previously fed by a subject during 2010–2014 predicts the total food received during the experimental treatment period. Model fit is improved by controlling for the number of times the subject was tested (circle size, see the electronic supplementary material). (Online version in colour.)

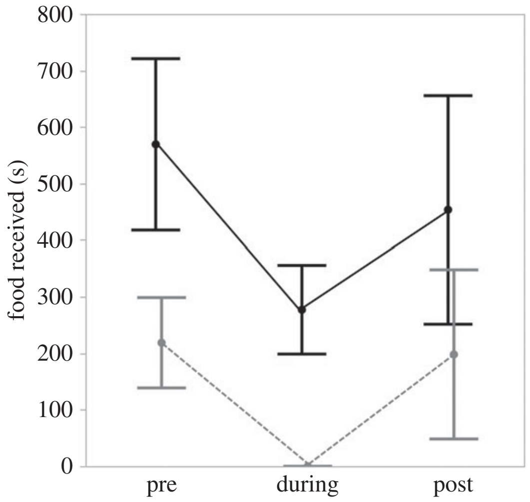

Figure 3. Mean food received during experiment. Mean and 95% CIs for seconds of food received in total (black) and from targeted donors (grey) during the pre-treatment, treatment and post-treatment periods.

After three weeks with no sharing between targeted partners, the mean food given by targeted donors did not decrease (figure 3; mean = −20.68 s, 95% CI = −177 to +136 s, $t_{10} = -0.29$, $p = 0.77$) nor did the donor's contribution to the subject's total food received (mean = +18%, 95% CI = −34% to +70%, paired *t*-test with arcsine-transformed proportions: $t_{10} = 0.78$, $p = 0.45$); however, this lack of a difference was not due to sharing rebounding to near its original level across all donors. Rather, after the treatment donors tended to provide either a much higher or lower percentage of their partner's total food gain, i.e. a bimodal response, with a higher variance in donor contributions to total food received (figure 4; Levene's test: $F_{1,31} = 17.4$, $p = 0.0002$). The donor's contributions to food

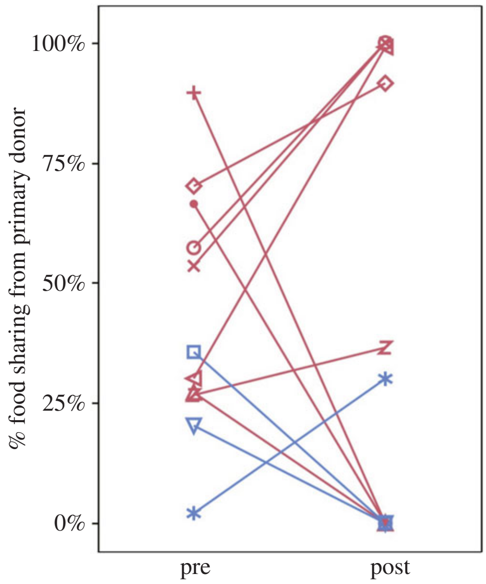

Figure 4. Effect of treatment on proportions of food received from targeted donor. Changes in the targeted donor's contribution to total food received from the pre-treatment period to the post-treatment period. Red lines show bats paired with maternal kin targeted donors. Blue lines show bats paired with non-kin targeted donors. Electronic supplementary material, figure S3, shows both rounds of the pre-treatment. (Online version in colour.)

received decreased to 0% in five of 11 cases and increased to near 100% in four cases (figure 4; electronic supplementary material, figure S3).

4. Discussion

Females that had previously fed more non-kin females were subsequently more successful at obtaining food when we prevented sharing from a primary donor (such as a mother or adult daughter). Prior donations to female non-kin, a specific form of network centrality, predicted the ability of a female bat to obtain later donations. Food-sharing bonds are individualized, stable [7,8] and non-interchangeable, such that food from a primary donor cannot be quickly replaced by food from other group members when that donor is absent or unable to help. Consequently, the quantity of these food-sharing bonds can influence sharing success on both short and long timescales even within a group of stable size.

The social rewards of food sharing are evident even before taking into account any potential for passive group augmentation through increased group mate survivorship. However, this experiment failed to demonstrate a consistent contingent response within highly bonded dyads. Not all targeted donors reduced their investments to zero after the treatment period. Instead, some donors increased their contributions leading to a bimodal distribution of outcomes (figure 4). This pattern is consistent with the possibility of divergent strategies for responding to an inability to reciprocate. A significant decrease in a donor's contribution could indicate partner switching, whereas a significant increase in a donor's contribution could indicate a form of relationship repair. More experimental trials with kin and non-kin are needed to test this possibility.

Food sharing in vampire bats is commonly cited as an example of reciprocity because non-kin dyads appeared to exchange blood in a simple tit-for-tat like pattern [6]. However, the results from this experiment show that some food-sharing bonds are not based on strict contingent turn-taking and persist beyond three weeks without reciprocal exchanges. Conclusions regarding contingent reciprocity should be drawn carefully with several factors in mind. First, close kinship may reduce contingency. Second, if contingency is subtle or gradual, then more dyads and more measures over time are needed to achieve the necessary statistical power. We have observed that previously stable food-sharing relationships changed when three bats were moved into a larger colony with more potential partners (G. G. Carter 2014, unpublished data). Hence, partner switching occurs with dramatic changes in group composition. However, partner switching based on food-sharing experiences probably require more time, given that new food-sharing bonds appear to develop gradually [8]. Third, subjects may respond less severely to non-reciprocating partners that are absent or unfed, such as in this experiment, compared to cases where partners have food but still reject solicitations (see further discussion of this issue in the electronic supplementary material). This 'excuse principle' has been demonstrated in cooperatively mobbing pied flycatchers [19] and cooperatively breeding cichlid fish [20]. Finally, allogrooming in vampire bats is more frequent than in other bats [21] and linked to food sharing both by occurrence [7] and a common neuroendocrine mechanism [22]. Allogrooming may therefore compensate for imbalances in food sharing, as shown in primates [23].

Vampire bat food sharing deviates from many assumptions of simple cooperation models based on the iterated Prisoner's Dilemma [4,5]. These models have had a major impact on subsequent social evolution theory, but literal translations overemphasize short-term contingent exchanges of a single service with a single partner, and this view constrains how researchers have tested cooperation [3]. Increasing evidence suggests that mechanisms for maintaining cooperation depend on factors missing from the iterated Prisoner's Dilemma model, such as partner choice, continuous (rather than binary) investments and multiple cooperative services (e.g. grooming, sharing or alliances [3]). A stable social bond with diverse benefits might require only a single cognitive index of relationship quality rather than separate accounting of all prior cooperative investments and returns, allowing for more subtle contingencies [3,23–25]. For example, short-term contingency is weakest in strongly bonded primates and more evident in weakly bonded partners [24]. In vampire bats, each dyadic relationship might involve multiple cooperative returns, including indirect fitness benefits [6], allogrooming [7] and social thermoregulation [26]. Testing alternative mechanisms for maintaining cooperation will therefore require carefully manipulating the cooperative value of specific partners while measuring cooperative investments across different partners (e.g. [21]).

Reciprocity is most clear in non-kin without social bonds or partner choice [3], but in nature, reciprocity might often occur among kin with multiple social bonds. In our experiments, the total cost of feeding each hungry bat was often divided among several donors. Even bats with a mother or adult daughter present still received about half of their food from other donors (electronic supplementary material, figure S2). By increasing their sharing networks, bats can also reduce the costs incurred by each of their donors. This has interesting potential consequences for the cost-benefit ratios of helping. For example, if bats with more sharing partners require less help from each donor, they might be preferred social partners. This might help explain why bats sometimes strongly prefer particular non-kin partners to the point of rejecting initial donation offers from others [7].

Ethics. All procedures were approved by the University of Maryland Institutional Animal Care and Use Committee (protocol R-10–63).

Data accessibility. The data supporting this article have been posted to Figshare.com or uploaded as part of the electronic supplementary material. DNA sequences: GenBank accession no. PRJNA279293.

Authors' contributions. G.G.C. conceived of and designed the study, carried out the molecular laboratory work, data collection and statistical analysis, and drafted the manuscript; G.S.W. advised the study design, data analysis and writing. Both authors gave final approval for publication.

Competing interests. We have no competing interests.

Funding. This work was supported by a Ford Predoctoral Fellowship, a Dissertation Improvement Grant from the National Science Foundation and grants from the American Society of Mammalogists and Animal Behavior Society.

Acknowledgements. We thank the Organization for Bat Conservation for their extraordinary support. K. Carleton, G. Borgia, K. Thompson, M. Taborksy and three anonymous reviewers provided feedback that improved the manuscript.

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