

Current Biology

Development of New Food-Sharing Relationships in Vampire Bats

Highlights

- We observed the formation of new social bonds among introduced nonkin vampire bats
- Bats appeared to use low-cost grooming to “test the waters” before sharing food
- First food donations were preceded by an increasing rate of reciprocal grooming
- New food-sharing relationships were rare and formed in a reciprocal pattern

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In Brief

By introducing unfamiliar female vampire bats and measuring the emergence of social grooming and regurgitated food donations over 15 months, Carter et al. show evidence that vampire bats selectively escalate low-cost grooming to develop higher-cost food-sharing relationships. This finding supports a “raising-the-stakes” model of bond formation.



Development of New Food-Sharing Relationships in Vampire Bats

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<https://doi.org/10.1016/j.cub.2020.01.055>

SUMMARY

Some nonhuman animals form adaptive long-term cooperative relationships with nonkin that seem analogous in form and function to human friendship [1–4]. However, it remains unclear how these bonds initially form, especially when they entail investments of time and energy. Theory suggests individuals can reduce the risk of exploitation by initially spreading out smaller cooperative investments across time [e.g., 5] or partners [6], then gradually escalating investments in more cooperative partnerships [7]. Despite its intuitive appeal, this raising-the-stakes model [7] has gained surprisingly scarce empirical support. Although human strangers do “raise the stakes” when making bids in cooperation games [8], there has been no clear evidence for raising the stakes during formation of social bonds in nature. Existing studies are limited to cooperative interactions with severe power asymmetries (e.g., the cleaner-client fish mutualism [9]) or snapshots of a single behavior within established relationships (grooming in primates [10–13]). Raising the stakes during relationship formation might involve escalating to more costly behaviors. For example, individuals could “test the waters” by first clustering for warmth (no cost), then conditionally grooming (low cost), and eventually providing coalitionary support (high cost). Detecting such a pattern requires introducing random strangers and measuring the emergence of natural helping behaviors that vary in costs. We performed this test by tracking the emergence of social grooming and regurgitated food donations among previously unfamiliar captive vampire bats (*Desmodus rotundus*) over 15 months. We found compelling evidence that vampire bats selectively escalate low-cost grooming before developing higher-cost food-sharing relationships.

RESULTS AND DISCUSSION

New Food-Sharing Relationships Were More Likely When Introduced Bats Lacked Familiar Partners

To observe how new food-sharing relationships form between adults, we captured adult females from two distant sites in Panamá: Tolé (n = 19) and Las Pavas (n = 8). First, we initially housed them in two *separate groups* (Figure 1). Then, during the *controlled introductions*, we housed them in either isolated pairs (one Las Pavas and one Tolé bat) or small groups (one Las Pavas and three Tolé bats), where either one or both introduced bats lacked familiar partners. Next, we merged them into one large *mixed group* (Figure 1; Table S1). We repeatedly fasted individuals to induce food-sharing (638 fasting trials) and compared the occurrence of new food sharing during the controlled introductions and the mixed-group period (Figure 1). Such captive manipulations are ecologically relevant because new adult bonds form when a new unrelated female joins a social network (on average every two years [14–16]; wild vampire bats can live for up to 16 years [17]). Bonds also form when bats are born into a group. As it is unknown if these latter relationships form differently, we also measured the development of non-maternal cooperative relationships between 26 female adults and 13 younger captive-born bats (6 males and 7 females, 11 to 21 months old) in the large mixed group.

Food donations to new partners depended on the availability of familiar partners. New relationships formed faster when the introduced bats had no familiar partners available. Both food sharing and grooming emerged faster in isolated pairs than in quartets (sharing: $\beta = 1.14$, $p = 0.002$; grooming: $\beta = 1.09$, $p = 0.02$), despite there being fewer potential new bonds available to form (Table S1). When we aggregated bats from the controlled introduction trials into a large mixed group, new donations between previously unfamiliar adults emerged even more gradually than in pairs or small groups (latency from introduction to first donations in the free-association mixed group: mean = 232 days [95% CI = 199 to 263, range = 67–372, 28 relationships] versus in forced-association controlled introductions: mean = 18 days [95% CI = 5–29, range = 2–63, 12 relationships], Table S1). The same pattern was found in new



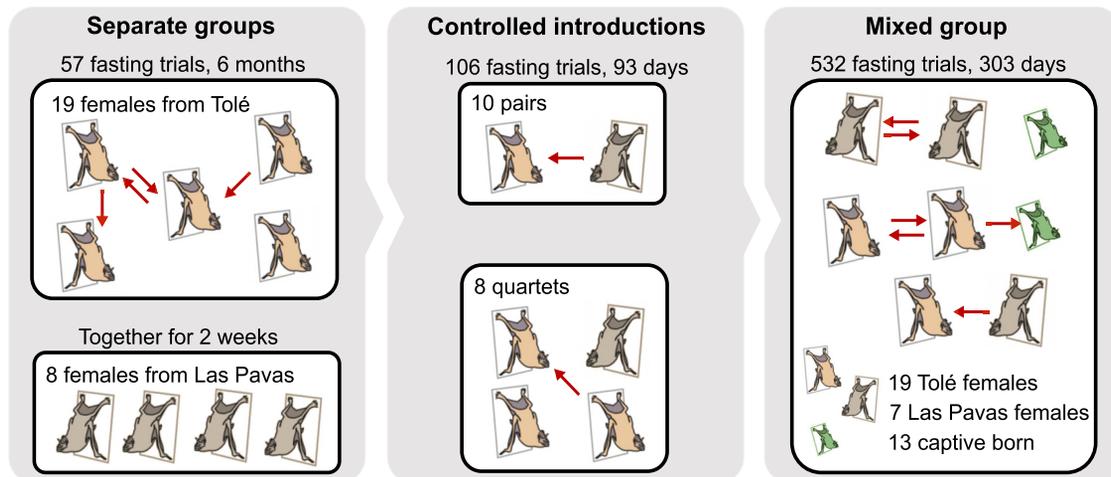


Figure 1. Experiment Overview

To see how vampire bats form new social bonds, we created groups of bats from two different sites (colors), then we induced and sampled food-sharing and grooming events between bats that were either previously familiar (same site) or unfamiliar (different sites). Red arrows depict hypothetical food donations during repeated fasting trials. See also [Table S1](#) and [Figure S1](#).

grooming among previously unfamiliar adults (latency from introduction to first grooming observation in the mixed group: mean = 147 days [95% CI = 134–159, range = 30–378, 187 relationships] versus in controlled introductions: mean = 10 days [95% CI = 4–15, range = 2–57, 30 relationships]).

When given the opportunity, bats preferentially fed and groomed their original familiar groupmates. During the controlled introduction trials, food sharing in the quartets was more common among familiar bats (6 of 11 possible cases) than unfamiliar bats (2 of 20 possible cases, OR = 0.09, $df = 1$, $p = 0.012$). During the mixed-group trials, Las Pavas bats preferentially fed and groomed each other (sharing: $\beta = 0.28$, $n = 160$, $p < 0.0001$, grooming: $\beta = 0.53$, $n = 160$, $p < 0.0001$), and Tolé bats showed the same within-group bias for sharing ($\beta = 0.09$, $n = 390$, $p = 0.003$) but not grooming ($\beta = 0.10$, $n = 390$, $p = 0.12$). When controlling for these within-group biases during the formation of new relationships with captive-born bats, we did not detect evidence for a kinship bias in grooming (MRQAP-DSP, $\beta = 0.12$, $p = 0.57$) or sharing (MRQAP-DSP, $\beta = 0.21$, $p = 0.15$). As expected, if sharing increases with familiarity, the appearance of first donations became more probable over time (OR = 1.56, $n = 3072$, $p = 0.01$).

New Food Sharing Was Rare and Emerged after Social Grooming

New food-sharing relationships were rare compared to new social-grooming relationships. Over 424 days, new food sharing developed in 10.8% of the 996 potential relationships among all bats ([Figure S1](#)), 14.5% of 608 potential relationships among females, 15.6% of 243 potential relationships among wild-caught adult females, and 9.1% of 748 potential relationships between an adult female and a captive-born bat (7 females, 6 males, 3–19 months old). New grooming relationships developed far more often (all bats = 51.9% of 1,008 potential relationships; females = 58.9% of 618; wild-caught adult females = 78.2% of 248). We observed that adult bats gained on average 2.7 new food donors (range = 0–7) and 7.2 new

groomers (0–16), while captive-born bats gained 2.6 donors (range = 0–6) and 14.4 groomers (range = 1–23). The relative rarity of new food-sharing relationships corroborates past evidence that food regurgitations are energetically costly investments [6, 14, 18, 19].

Grooming was a precursor to food sharing. We were very unlikely to observe the first grooming events, but the first food donations we observed were likely to be the actual first donations (see [STAR Methods](#)). Despite this conservative bias, we still observed mutual grooming before the first trial with food sharing more than twice as often as expected based on their relative frequencies (null model: $p < 0.0001$, observed frequency = 40% of the new sharing pairs, expected frequency = 12% [95% CI = 9%–15%]).

Grooming Dynamics Predicted Subsequent Food Donations in New Pairs

If vampire bats use low-cost grooming to build higher-cost sharing bonds, then the grooming rate from actor A to recipient B within a dyad should predict the probability of the first food donation from B to A. As expected, the rate of grooming given by actor A to recipient B predicted the later occurrence of new food sharing from B back to A (OR = 2.15, $p = 0.0002$, $n = 897$). The trajectory of grooming rates over time clearly differed between pairs that developed new food-sharing relationships versus pairs that did not (interaction: OR = 1.60, $p < 0.0001$, [Figure 2](#)). The slope of this increase in grooming was also greater *before* the first reciprocal food donation than after. Initial grooming rates started low, then increased over time up until the new food-sharing relationship formed ([Figure 3](#)). Grooming increased before but not after first donations in new relationships.

First Food Donations Occurred in a Reciprocal Pattern

Emergence of new food sharing was more reciprocal than expected by chance, even when controlling for kinship (MRQAP-DSP; reciprocal sharing: $\beta = 0.33$, $p < 0.0002$, kinship:

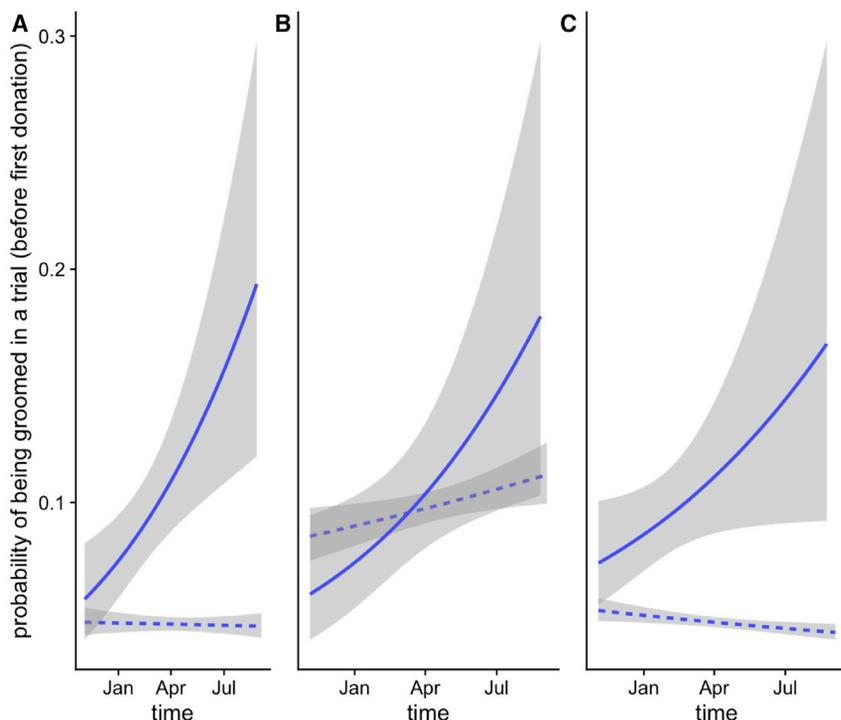


Figure 2. Increasing Grooming from Bat A to B Led to New Food Sharing from Bat B to A

In cases where a new food-sharing relationship formed, the grooming rate toward the future donor increased over time before the first donation occurred (solid line; OR = 1.40, $n = 33$, $p < 0.0001$), but the grooming rate toward a potential donor remained low in cases where no food-sharing relationship formed (dashed line; OR = 0.99, $n = 420$, $p = 0.58$).

(A–C) This divergence occurred in all potential new relationships (A) and was also detected in previously unfamiliar adults (B) and in relationships with captive-born bats (C), which had more divergent grooming trajectories. For previously unfamiliar adults, the grooming probabilities increased for all recipients, including those that never donated (OR = 1.12, $p = 0.004$), and they increased faster for grooming recipients that later donated (OR = 1.49, $p < 0.0002$; interaction: OR = 1.45, $p = 0.017$). For relationships with captive-born bats, the grooming probabilities decreased for grooming recipients that never donated (OR = 0.90, $p = 0.01$), and they tended to increase for recipients that did later donate (OR = 1.33, $p = 0.04$; interaction: OR = 1.72, $p < 0.0001$). Shading shows the 95% CI for the fitted model's predictions. See also Figure S2.

$\beta = 0.02$, $p = 0.65$). Among adult past strangers, the proportion of previous trials in which bat A fed B predicted the occurrence of the first new reciprocal donation from bat B to A (OR = 6.00, $n = 235$, $p = 0.016$), and the number of previously unfamiliar pairs that donated food in both directions during the study period was greater than expected if new donations were random ($p = 0.0001$, observed bidirectional pairs = 13, expected = 4.6, expected 95% CI = 1 to 9). Grooming rates in new relationships were also symmetrical across dyads (reciprocal grooming: $\beta = 0.64$, $p < 0.0002$, kinship: $\beta = -0.04$, $p = 0.11$) and were highest between bats that formed two-way food-sharing relationships, intermediate in relationships where we observed sharing in only one direction, and lowest in pairs where we never saw food sharing (Figure S2). Before this study, one plausible argument was that reciprocal food sharing among nonkin could depend entirely on heuristics based on phenotypic similarity, resulting in a spurious pattern of symmetrical helping that looks like reciprocity [20–22]; however, this phenotypic-similarity hypothesis predicts that food-sharing relationships should form immediately and occur most frequently in larger groups where there are more opportunities for similar phenotypes. Therefore, our findings did not support this hypothesis.

Evidence for the Raising-the-Stakes Model

New food-sharing relationships were rare, formed in a reciprocal pattern, were preceded by mutual grooming, developed more often when strangers lacked alternative familiar partners, and were predicted by an increasing grooming rate on previous days toward the food donor up until the first donation day. Taken together, these findings are the strongest evidence to date that relationship formation occurs through some form of raising the stakes [7]. Previous evidence for raising the stakes in nonhuman

social relationships came from observations of grooming among familiar male chimpanzees after the death of an alpha male [11]. The authors suggested that groupmates may have needed to re-establish their relationships during this period of social instability, and that a diminishing threat of violence led to the increasing rates of grooming. Although the increase in grooming rates is consistent with each male raising the stakes to assess the risk of aggression from their grooming partner, it might have also resulted from a general decline in vigilance against possible aggression from any groupmate.

Here, reciprocal food sharing bonds almost certainly did not arise as a byproduct of other factors such as phenotypic similarity or proximity. If bats choose new partners based on their phenotype alone, then relationships should form more often when bats have more alternative partners. Instead, we observed the opposite: food-sharing relationships formed more often when bats had fewer alternative partners, consistent with a raising-the-stakes model of bats choosing partners based on both the availability and the past positive experiences with different partners. The relationship between new grooming and new food sharing was unlikely to be caused by mere proximity because the effect of new grooming on new food sharing remained evident regardless of whether or not bats were forced into close proximity. If bats initiated new grooming and sharing based entirely on proximity (i.e., if there was a spurious correlation between grooming and sharing), then new grooming rates should correlate with new sharing when strangers were able to freely associate during the mixed-group period, but this correlation should be much smaller or disappear entirely during the controlled introduction trials. This is because when strangers were forced into close proximity during controlled introductions, most of the variation in proximity was removed (i.e., proximity was roughly equal

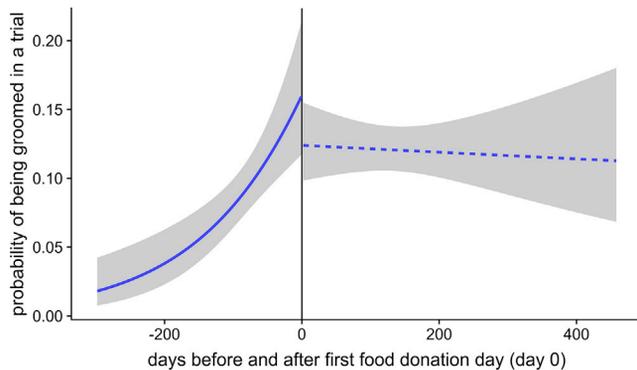


Figure 3. Grooming Rates Increased before, but Not after, New Food Sharing Occurred

The probability of a focal bat grooming the new donor in a one-h trial (y axis) increased before the first day that the donor fed the focal bat (i.e., “day zero”; OR = 1.4, $p = 0.0005$), but not after day zero (OR = 1.01, $p = 0.47$; interaction: OR = 1.57, $p = 0.0003$). This effect was seen in new food-sharing relationships with or without captive-born bats (three-way interaction: $p = 0.55$). The same pattern was found in new relationships between adults (interaction: OR = 1.60, $p = 0.013$; before: OR = 1.49, $p = 0.012$; after: OR = 1.01, $p = 0.45$) and in new relationships with captive-born bats (interaction: OR = 1.45, $p = 0.009$; before: OR = 1.33, $p = 0.014$; after: OR = 1.06, $p = 0.34$). Shading shows the 95% CI for the fitted model’s predictions.

between all the bats in the small cage). Put differently, if variation in proximity is actually driving the correlation between grooming and sharing, then removing this variation with forced close contact should reveal the lack of an association between grooming and sharing. In sharp contrast to this prediction, the estimated effect of new grooming given on new food received was greater during the controlled introduction periods compared to the same effect during the mixed-group trials where proximity was allowed to vary (forced close proximity: OR = 5.44, $p = 0.037$; variable proximity: OR = 1.63, $p = 0.033$; network logistic regression in the *sna* R package).

All our findings are consistent with the raising-the-stakes hypothesis, but to clearly demonstrate this strategy as a mechanism for enforcing cooperation, future experiments should prevent reciprocation in specific dyads to test for a subsequent decrease in the actor’s cooperative investment. Lab experiments demonstrate such contingent reciprocity in the absence of individualized long-term social bonds [22–25], and field experiments have shown a preference to groom or help partners that are more likely to provide a cooperative public good such as opening a food cache [26] or vigilance against predators [22, 27–31], suggesting in some cases that individuals attempt to form stronger bonds with more helpful individuals [e.g., 29]. However, helping that is conditional on past reciprocal help has not been unambiguously demonstrated within the context of a stable long-term social bond, probably because strongly bonded partners show less evidence for short-term contingencies in cooperation [1]. Stable bonds are, by definition, difficult to perturb. Instead of focusing on clearly alternating exchanges of help with increasing bout durations, our findings suggest that tests for conditional escalating investment in long-term bonds would be most effective if researchers target newly developing relationships rather than established ones,

and if they track (and possibly manipulate) multiple forms of cooperative behavior.

The relevance of our findings extends beyond costly helping behaviors. For example, many courtship behaviors could be seen as a short-term investment in the formation of longer-term pair bonds with substantial fitness consequences [32]. Similarly, the role of mere physical contact as a low-cost method for building tolerance and trust might be more general than currently recognized. The importance of grooming for relationship maintenance in primates is well established, but growing evidence suggests that similar tactile behaviors can reduce fear and encourage tolerance and cooperation in many other mammals, birds, and fish [29, 33–38]. Recently developed methods for tracking bond formation and dynamics at fine temporal scales [4, 40] provide exciting new opportunities to test whether gradual escalation of proximity and body contact is a widespread mechanism for socially “testing the water.”

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.01.055>.

ACKNOWLEDGMENTS

We thank the Smithsonian Tropical Research Institute for logistical support. Isabelle Waurick conducted the molecular lab work. Frieder Mayer enabled the molecular lab work. G.G.C. was supported by a Smithsonian Postdoctoral Fellowship and a Humboldt Research Fellowship. D.R.F. received funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy—EXC 2117—422037984 and a DFG grant (1420/4-1). This work was also supported by a Smithsonian Institution Scholarly Studies Grant and a grant from the National Geographic Society Committee for Research and Exploration (WW-057R-17). Bat drawings in Figure 1 by Elke Siebert, Museum für Naturkunde, Berlin. We thank Gloriana Chaverri and two anonymous reviewers for comments that improved the manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization, G.G.C.; Methodology, G.G.C.; Investigation, G.G.C., R.J.C., and J.K.V.; Formal Analysis, G.G.C.; Original Draft, G.G.C.; Review & Editing, G.G.C., R.J.C., J.K.V., S.P.R., D.R.F., and R.A.P.; Funding Acquisition,

G.G.C., S.P.R., and R.A.P.; Resources, S.P.R. and R.A.P.; Supervision, G.G.C., D.R.F., and R.A.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: November 16, 2019

Revised: January 2, 2020

Accepted: January 15, 2020

Published: March 19, 2020

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Data and Code for reproducing all analyses	Figshare	https://doi.org/10.6084/m9.figshare.11369268.v1
Experimental Models: Organisms/Strains		
Common vampire bat (<i>Desmodus rotundus</i>)	Wild	N/A
Oligonucleotides		
Microsatellite primers for estimating relatedness	[4]	GenBank: EF591569–EF591580; PRJNA279293 AL2_27850
Software and Algorithms		
R 3.5	R Core Team 2013	https://cran.r-project.org/

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gerald Carter (gcarter1640@gmail.com). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

We conducted experiments at the Smithsonian Tropical Research Institute in Gamboa, Panama. We used 41 common vampire bats (*Desmodus rotundus*) as subjects, including 19 female bats captured exiting a roost in Tolé, Panamá; 8 female bats captured foraging at a cattle pasture in Las Pavas, Panamá about ~215 km from Tolé; and 14 captive-born bats (8 females, 6 males). We studied adult females and their young, because these individuals form the basis of food-sharing networks in the wild, whereas adult males compete for access to territories and females and do not form stable bonds as often [14–16]. To ensure familiarity within groups and unfamiliarity between groups, we housed the groups separately (Tolé bats for 6 months and Las Pavas bats for 2 weeks) before the study began. Bats were marked with subcutaneous passive integrated transponders (Trovan USA) and a visually unique combination of forearm bands (Porzana, National Tag, and birdbands.com). To feed bats, we provided refrigerated or thawed cattle or pig blood defibrinated with 44 g sodium citrate and 16 g citric acid per 19-L container.

Research permits

All experiments were approved by the Smithsonian Tropical Research Institute Animal Care and Use Committee (#2015-0915-2018-A9 and #2017-0102-2020) and by the Panamanian Ministry of the Environment (#SE/A-76-16).

METHOD DETAILS

Terminology

We use the term ‘potential relationship’ for a directed actor–receiver pair of bats that could have groomed or shared food. We use the word ‘relationship’ for a directed actor–receiver pair that we observed to groom or share food during fasting trials (i.e., an *observed* network edge). ‘New relationships’ are those between bats that first met during the experiment, excluding mother–offspring dyads. We use the word ‘bond’ for the unobserved underlying social relationship (as experienced by the animal) that we infer from observations (i.e., the construct that we *inferred* from the observed relationship).

Genetic relatedness

We used a 3–4 mm biopsy punch to collect tissue samples in 80% or 95% ethanol, then used a salt–chloroform procedure for DNA isolation, and a LI–COR Biosciences DNA Analyzer 4300 and the SAGA GT allele scoring software to genotype individuals at 17 polymorphic microsatellite loci. Allele frequencies were based on 100 bats from Tolé and 9 bats from Las Pavas, respectively. Genotypes were 99.9% complete. To estimate genetic relatedness, we used the Wang estimator in the R package ‘related’. To estimate kinship,

we assigned a zero kinship to known unrelated individuals from different sites and to individuals with negative pairwise relatedness, and we assigned a kinship of 0.5 for known mother–offspring pairs or pairs with genetic relatedness estimates greater than 0.5. For all other pairs, we used genetic relatedness as the estimate for kinship.

Experimental design

We induced social grooming and regurgitated food sharing using a fasting trial, in which a focal subject was isolated from the group without food for a night and a day, then released back to the group of fed bats for 1 h the following night. During the hour, all grooming or food-sharing interactions with the subject were recorded using an infrared (IR) light and an IR-sensitive video camera. Each food sharing bout was estimated by the number of seconds that the unfed subject spent licking the mouth of a particular groupmate. Grooming was defined as chewing or licking the fur or wings of another bat. The dyadic sharing or grooming for a trial was estimated as the sum of all bouts that were at least 5 s long. We weighed bats before and after trials to measure the mass increase from food sharing.

We conducted fasting trials in each group during three experimental phases (Figure 1). There is only one fasted bat per fasting trial, so the number of sharing opportunities is the number of possible donors for the fasted bat per fasting trial. A new donation means that the actor has never before fed the recipient. First, we conducted 57 ‘baseline’ trials to assess preliminary sharing rates between the 19 Tolé bats housed in a 1.7 × 2.1 × 2.3 m outdoor flight cage (3,420 possible donations in one group but none of them are considered new). Second, we conducted 106 ‘controlled introduction’ trials to assess possible formation of new food-sharing bonds between bats introduced as either an isolated pair (one Las Pavas bat and one Tolé bat) or a quartet (one Las Pavas bat and three Tolé bats), housed in a 28 × 28 × 40 cm clear plastic observation cage (10 pairs and 8 quartets). These controlled introductions provided for 162 opportunities for new donations between previous strangers (SI, Table S1). Finally, we conducted 532 ‘mixed-group’ trials to assess the formation of new sharing relationships when all bats were housed together in the flight cage described above (19 Tolé, 7 Las Pavas, and 14 captive-born bats). The introductions in this combined group provided 11,823 more opportunities for a new donation.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral measures

Food sharing was evident from mouth-licking because fasted subjects gained an average of 51 mg of mass per minute of mouth-licking ($R^2 = 0.75$, $p < 0.001$, 95% CI: 45 to 57 mg/min, $n = 619$ trials without missing data), which is comparable to previous estimates from another captive colony (38 mg of mass per minute of mouth-licking, $R^2 = 0.67$, 95% CI: 33 to 46 mg/min, $n = 121$ trials, colony described in [18, 39]). Across all trials, the probability that a given bat received food from any groupmate was 61% (95% CI = 57 to 64%, 41 bats, 693 trials), which is much lower than the 95% success rate observed in the previous long-term captive colony (95% CI = 92 to 98%, 29 bats, 183 trials). Assuming that mouth-licking events over 5 s were food donations, 64% of the 340 mixed-group trials with food sharing involved one donor, 24% had two donors, 9% had three donors, 2% had four donors, and two trials had up to five donors.

We estimated food donation size as the number of seconds that a fasted subject spent mouth-licking a fed groupmate during the baseline and mixed-group trials. However, during the controlled introduction trials, when bats were forced in close proximity, we saw a greater frequency of begging, which we defined as mouth-licking that is clearly not food-sharing because the partner is turning away from the mouth-licking bat and the mouth-licking bat does not gain the weight that would be expected from food-sharing. To be conservative when measuring sharing, we therefore did not count mouth-licking as food sharing during the controlled introduction trials unless the subject weighed more than expected based on the average weight change for bats that did not perform any mouth-licking.

Previous studies of raising-the-stakes have focused on grooming symmetry within short time periods [8, 11–13], but our experimental design did not allow us to clearly test for increases in grooming symmetry within each dyad, for three main reasons. First, grooming symmetry was actually reduced during the fasting trials when we sampled grooming. Fasted subjects were twice as likely to be groomed by a groupmate (13% probability) than to groom a groupmate (6% probability), because they were typically ‘greeted’ by many groupmates at the start of the trial, which involves receiving simultaneous one-way grooming from several bats, and because they typically spent less time grooming and more time trying to lick the mouth of a potential donor (begging). Second, due to the rarity of grooming in new dyads, we did not sufficiently sample dyadic grooming rates to accurately estimate the one-way grooming rate within each new dyad. Given that actor-receiver grooming rate estimates were under-sampled, increasing over time, and symmetrical across dyads (mantel test: $r = 0.77$, $p < 0.0002$), they should largely converge over time and hence appear more symmetrical merely because greater sample sizes lead to more precise estimates of the two grooming rates. Third, any observed increase in grooming symmetry over time could be driven by age effects, because mutual grooming (and hence grooming symmetry) is lower when one bat is not yet an adult. Finally, grooming symmetry in shorter time windows might decrease even as grooming becomes more balanced on longer timescales, as seen in primates [1]. We therefore focused on whether increases in reciprocal grooming predicted subsequent food sharing.

Statistical analyses

To test our hypotheses, we compared the observed coefficients from general and generalized linear models (slopes β , and odds ratios OR, respectively) to expected distributions of coefficient values expected under the null hypotheses using permutations of the

network or the event data. Durations of sharing and grooming were lognormal. To create a standard index of grooming rates, we therefore transformed the total duration of directed dyadic interactions in each trial using natural log ($x+1$). We call these measures of the log duration per h 'rates'. When interaction bout duration and probability had different meanings, we decomposed rates into two separate response variables: amounts (the magnitude of nonzero rates in a trial) and probabilities (the presence or absence of a nonzero rate in a trial). We used permutation tests with 10,000 permutations for p values and bootstrapping for all 95% confidence intervals. Null distributions were not always centered on zero due to structure in the data, so caution must be taken when considering the observed coefficients.

To test for ingroup-outgroup biases in sharing for each site, we calculated observed coefficients for the effect of the actor and receiver being from the same capture site on actor grooming rates, then we calculated expected coefficients by permuting the grooming rates within each actor to different possible recipients.

Grooming could occur before sharing simply because it is more frequent. To test whether mutual grooming preceded new donations more than expected by chance, we compared the observed probability of observing mutual grooming before new donations to the values expected from a null model based on randomly swapping the label of interactions (grooming versus sharing) within each dyad. This permutation test controls for the relative frequency and timing of grooming and sharing events in new dyads. We analyzed if sharing was preceded by mutual grooming, i.e., both bats grooming each other in a trial, because mutual grooming is a better indication than one-way grooming of relationship development. Our tests of whether new grooming occurred before new food sharing are highly conservative (i.e., biased away from detecting new grooming before new food sharing) for several reasons. Food donations were only necessary during the 1 h trial when we observed them. Bats were only focal sampled during fasting trials, and they were only in need during the fasting trials, because we isolated and fed them immediately after every trial. In contrast, grooming between the same bats could occur at any time during the days before the same dyad was sampled again (median gap period = 8 days, interquartile range = 5 to 14 days). In sum, we sampled close to 100% of the time when food sharing was necessary, but less than 2% of the time when grooming could have occurred. Additionally, although fasting trials increase the probability the subject will receive food, they also decrease the probability the subject will groom others. Therefore, when we observed the first grooming and sharing events during the same fasting trial, it is very likely that the first grooming actually occurred in the days before this trial.

To test the effects of kinship and reciprocal grooming on the formation of new food-sharing relationships in the mixed-group trials, we used multiple regression quadratic assignment procedure with double semi-partialing (MRQAP-DSP) via the `netlogit` function in the `sna` R package. We also used this method to test the effect of grooming on occurrence of new donations only within the controlled introduction trials. This procedure uses generalized linear models via the `glm` function in the `lme4` package to calculate the observed coefficients and uses network-level permutations to get expected coefficients. Since MRQAP-DSP cannot test interaction effects, we compared observed and expected interaction coefficients using permutations in which we shuffled trial rates given by the actor among different possible receivers and then shuffled the trial rates received by the receiver among different possible actors. If the interaction coefficients were significant ($p < 0.05$), we conducted separate MRQAP-DSP tests within each group.

To test whether interaction rates changed over time, we generated expected coefficients for general or generalized linear models by permuting the order of interactions within each potential relationship. One captive-born bat died for unknown reasons during the mixed-group trials, so we removed it from all temporal analyses. To test for evidence of reciprocal sharing, we used MRQAP-DSP to test if the matrix of new sharing in the mixed-group trials was predicted by reciprocal sharing when controlling for kinship. As an additional test, we also counted the occurrence of both novel sharing and reciprocal sharing for all new potential relationships, then counted the same number after randomizing the presence of sharing across potential relationships.

DATA AND CODE AVAILABILITY

All behavioral data, genotypes, and R code for reproducing all analyses are available on Figshare (<https://doi.org/10.6084/m9.figshare.11369268.v1>).