Title:

Development of new food-sharing relationships among nonkin vampire bats

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Abstract

Social relationships that involve costly helping occur most often among kin, but in many complex and individualized animal societies, nonkin also demonstrate stable cooperative relationships that share similarities with human friendship. How do such cooperative bonds form between complete strangers? One theory suggests that strangers should 'test the waters' of a new relationship by initially making low-cost cooperative investments and gradually escalating them with good partners. This 'raising-the-stakes' strategy is evident in humans playing short-term economic games, but it remains unclear whether it applies to the natural helping behaviors that underlie a long-term cooperative relationship. Here, we show evidence that unfamiliar nonkin vampire bats (*Desmodus rotundus*) selectively escalate low-cost investments in allogrooming before developing higher-cost food-sharing relationships. By introducing females from geographically distant sites in pairs or groups and fasting them repeatedly over 15 months, we observed that bats first established new reciprocal grooming relationships, and that increasing grooming rates predicted the occurrence of first food donations, at which point grooming rates no longer increased. New food-sharing relationships emerged reciprocally in 14.5% of 608 female pairs and formed faster when strangers lacked alternative familiar partners. Our results suggest that 'raising-the-stakes' might be easier to detect when tracking multiple types of behavior during relationship formation, rather than measuring a single behavior within an established relationship. The general principle of 'testing the waters' might play an underappreciated role across many other social decisions with long-term consequences, such as joining a new social group or choosing a long-term mate.

Keywords: cooperation, social relationships, vampire bats

Significance statement

Vampire bats form long-term cooperative social bonds that involve reciprocal regurgitated blood sharing. But how do two individuals go from complete strangers to reciprocal food donors? By introducing unfamiliar bats, we found evidence that low-cost grooming paves the way for higher-cost food donations. Food sharing emerged in a reciprocal fashion and it emerged faster when two strangers could not access their original groupmates. Bats that did form new food-sharing relationships had a history of escalating reciprocal grooming up until the food sharing began. The finding that unfamiliar nonkin vampire bats appeared to gradually and selectively transition from low-cost to high-cost cooperative behaviors is the first evidence that nonhuman animals 'raise the stakes' when forming new cooperative relationships.

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Animal societies are fundamentally shaped by repeated interactions among individuals over time. Repeated interactions allow individuals to choose to cooperate based on their past experience across different partners (1-3). Organisms as diverse as animals, plants, and fungi have demonstrated partner choice: individuals prevent exploitation by shifting their cooperative investments towards partners that provide better reciprocal returns (3-7). Across several nonhuman species, repeated cooperative interactions can lead to adaptive and enduring social bonds that share similarities with human friendship (8-12), though it remains unclear how these initially form. A significant challenge has been testing how individuals prevent exploitation while forming these stable bonds. How do complete strangers develop a long-term cooperative relationship?

68 A key idea is that individuals should reduce the risk of exploitation by initially spreading
69 out smaller cooperative investments across time ('parceling' (13)) or across different partners
70 ('social bet-hedging' (14)), and then gradually escalating investments in the most cooperative
71 partnerships ('raising the stakes' (15)). For example, one might first assess a potential partner's
72 tolerance by clustering for warmth, then gain feedback by grooming the partner, and then use the
73 partner's response to decide whether to provide higher-cost food donations or coalitionary
74 support (16). Despite its intuitive appeal for explaining how new cooperative relationships
75 develop, evidence supporting the 20-year-old 'raising-the-stakes' model (15) is surprisingly
76 scarce. An early test using the cleaner and client fish mutualism suggested that the model does
77 not apply well to situations with severe asymmetries in partner payoffs or options (17). Studies
78 with nonhuman primates (18-21) have tested only snapshots of established relationships rather
79 than the formation of new ones. Human strangers 'raise the stakes' when making monetary bids
80 in cooperation games (e.g. 22, 23), but the strategy has never been properly tested in a more
81 ecologically-relevant context of relationship formation. Doing so requires measuring the
82 emergence of natural helping behaviors between randomly introduced strangers.

83 We tracked the development of cooperative relationships between previously unfamiliar
84 wild-caught vampire bats (*Desmodus rotundus*) over 15 months. Cooperative relationships in this
85 species involve low-cost social grooming (hereafter *grooming*) and higher-cost regurgitations of
86 ingested blood (*food sharing*), which are provided to close kin and frequent associates (14, 24-
87 28). Here, we found evidence that vampire bats use reciprocal grooming to gradually establish
88 new bonds that entail food donations. Our data supported four key predictions: (i) new food-
89 sharing relationships should form gradually but faster when bats have fewer alternative partners,
90 (ii) grooming rates should predict the probability that the grooming recipient later donates food

91 to the groomer, (iii) low initial grooming rates should increase over time but only up until the
92 first reciprocal food donation, and (iv) the emergence of new food sharing should be reciprocal.

93 Female vampire bats demonstrate kin-biased fission-fusion social dynamics (24, 27, 28).
94 New nonkin social bonds can form when an unrelated female joins a social network about once
95 every two years (24, 28), with individual bats living for up to 16 years in the wild (29). To
96 observe how new food-sharing relationships form between adults, we captured adult females
97 from two distant sites in Panamá, Tolé (n=19) and Las Pavas (n=8), and we then ran 638 fasting
98 trials in which an overnight-fasted subject could be fed by a previously unfamiliar bat from
99 another site. To test the prediction that new sharing relationships would form faster when
100 strangers have fewer options of alternative partners, we compared the occurrence of new sharing
101 when wild-caught strangers were introduced in isolated pairs (one Las Pavas and one Tolé bat),
102 in small groups (one Las Pavas and three Tolé bats), or in one large mixed group (all bats
103 together; see Methods, Supplementary Information (SI) Appendix, Fig. S1). New bonds can also
104 form when individuals are born into a group, and these relationships might form differently. We
105 therefore also measured the development of non-maternal cooperative relationships between 26
106 female adults and 13 younger captive-born bats (6 males and 7 females, 11 to 21 months old) in
107 the large mixed group.

108 To test our hypotheses, we compared the observed coefficients from general and
109 generalized linear models (slopes β, and odds ratios OR, respectively) to expected distributions
110 of coefficient values expected under the null hypotheses using permutations of the network or the
111 event data (see Methods). We use the term 'potential relationship' for a pair of bats that could
112 have groomed or shared food, 'relationship' for an *observed* network edge (directed), and 'bond'

113 to discuss the underlying construct that we *inferred* from the observed relationship (see glossary,
114 SI Appendix, Table S1).

115 Over 424 days and 12,012 opportunities for new food donations, new food sharing
116 developed in 10.8% of the 996 potential relationships among all bats, 14.5% of 608 potential
117 relationships among females, and 15.6% of 243 potential relationships among wild-caught adult
118 females (SI Appendix). All bats had at least one donor (range=1-16, mean=6.6). The average
119 number of new food donors per adult female bat was 2.7 (range=0-7) and the average per
120 captive-born bat was 2.6 (range=0-6). New grooming relationships developed much more
121 frequently (all bats=51.9% of 1008; females=58.9% of 618; wild-caught adult females=78.2% of
122 248; SI Appendix). The average number of new groomers was 7.2 (range=0-16) for adult
123 females and 14.4 (range=1-23) for captive-born bats. New food sharing emerged after grooming
124 observations more than expected based on the relative frequency of grooming and sharing (see
125 supplement).

126 If social experience and partner availability influence the development of new food-
127 sharing bonds, then first food donations should occur gradually but faster when bats have fewer
128 alternative partners. As expected, when strangers from Las Pavas and Tolé were introduced and
129 housed as isolated pairs, we observed higher rates of new food sharing (β=1.14, p=0.004; effect
130 on new grooming: β=1.09, p=0.04), compared to when one Las Pavas bat was introduced to three
131 Tolé bats, despite there being much fewer potential new bonds available to form. When we
132 aggregated bats from the controlled introduction trials into a large mixed group, bats
133 preferentially fed and groomed their original familiar groupmates, and new sharing emerged
134 even more gradually than in the isolated pairs or in small groups (SI Appendix, Fig. S2-S3).

135 According to the ‘raising-the-stakes’ hypothesis, strangers should make low-cost
136 investments and use the return rates to inform future investments. We therefore predicted that if
137 grooming helps to build sharing bonds, then the grooming rate should predict the probability of
138 the first food donation in the opposite direction, initial grooming rates should start low and then
139 increase over time but only up until the first reciprocal food donation, and the emergence of new
140 food-sharing should be reciprocal. As expected, the grooming rate given by actor A to recipient
141 B predicted the later occurrence of new food sharing from B back to A (OR=2.15, $p<0.0002$,
142 n=897). The trajectory of grooming rates over time clearly differed between pairs that developed
143 new food-sharing relationships versus pairs that did not (interaction: OR=1.60, $p<0.0002$, Fig.
144 1). The slope of this increase in grooming was also greater *before* the new food-sharing
145 relationship formed than it was after the relationship formed (Fig. 2). New food sharing was
146 more reciprocal than expected if the new donations were random, even when controlling for
147 kinship (estimated via maternal pedigree and microsatellite genotyping, see Methods; MRQAP-
148 DSP; reciprocal sharing: $\beta=0.40$, $p<0.0002$, kinship: $\beta=0.07$, $p=0.10$; SI Appendix). Grooming
149 rates were highest between bats that formed two-way food-sharing relationships, intermediate in
150 relationships where we observed sharing in only one direction, and lowest in pairs where we
151 never saw food sharing (SI Appendix, Fig. S4).

152 The rarity of new food-sharing relationships corroborates past evidence that food
153 regurgitations are energetically costly and that food-sharing bonds require investments of time
154 and energy (14, 24, 30, 31). Our finding that the emergence of new food sharing between wild-
155 caught adults is influenced by partner behavior and availability provides the clearest evidence to
156 date that nonkin food sharing in vampire bats is not a mere byproduct of kin selection (26).
157 Before this study, one hypothesis was that food sharing decisions could be based entirely on

158 phenotypic similarity, which could result in a spurious pattern mimicking reciprocal sharing
159 among nonkin (32, 33). For instance, nonkin pairs with scents or calls that are, by chance, similar
160 would feed each other in both directions (33). However, this hypothesis incorrectly predicts that
161 food-sharing relationships should form immediately, and not vary dramatically with partner
162 availability and past social experience. Under the phenotypic similarity hypothesis, we would
163 expect to have observed more food-sharing relationships in larger groups simply because there is
164 a substantially greater opportunity to have pairs of individuals with matching phenotypes.

165 Previous evidence for 'raising-the-stakes' in nonhuman social relationships came from
166 observations of grooming among familiar male chimpanzees after the death of an alpha male
167 (18). The authors suggested that, during this period of social instability, these groupmates may
168 have needed to re-establish their relationships, and that a diminishing threat of violence led to the
169 increasing rates of grooming (18). Although the increase in grooming rates is consistent with
170 each male 'raising the stakes' to assess the risk of aggression from their grooming partner, it
171 might have also resulted from a general decline in vigilance against possible aggression from any
172 other groupmate.

173 Past studies on 'raising the stakes' during relationship development have also focused on
174 single types of cooperative investments (18-23). However, individuals can raise the stakes either
175 by increasing the magnitude of one kind of cooperative investment or by adding new higher-cost
176 forms of cooperative investment. In our study, we show that female vampire bats appear to do
177 both, first by increasing grooming rates and then by transitioning from low-cost grooming to
178 high-cost food-sharing.

179 Past evidence for the 'raise-the-stakes' model (15) has been scarce in part because it is a
180 variation on the classic 'tit-for-tat' strategy in the iterated prisoner's dilemma (1), a model which

181 is difficult to test unambiguously using natural forms of cooperation (4, 32, 34). 'Tit-for-tat'
182 forms of reciprocity have been demonstrated using experiments with trained instrumental tasks
183 (e.g. pulling a lever to deliver food) and payoffs accruing in distinct rounds (4-6). However, the
184 'tit-for-tat' model of reciprocity should not be operationalized too literally, because it excludes
185 many factors crucial to real-world cooperation, such as partner choice, social bonding, different
186 service types, and the cost-benefit asymmetries resulting from demography, market effects, or
187 social dominance (4, 7, 34). Also, long-term cooperative social bonds do not produce strict 'tit-
188 for-tat' patterns of helping. Instead, primates with stronger bonds show *less* evidence for short-
189 term contingencies in grooming (9), which makes sense if social bonding involves integrating
190 many different kinds of social interactions into a single positive association.

191 The relevance of our findings extends beyond high-cost cooperative behaviors. For
192 example, in some species, courtship behaviors could be seen as a short-term investment in the
193 formation of longer-term pair bonds with substantial fitness consequences (35). Similarly, the
194 role of mere physical contact as a low-cost method for building tolerance and trust might be
195 more general than currently recognized. The key role of grooming for relationship maintenance
196 in primates is well established, but growing evidence suggests that similar tactile behaviors can
197 reduce fear and encourage tolerance and cooperation in many other species of mammals, birds,
198 and fish (e.g. 5, 36-42). Recently developed methods for tracking formation of social bonds at
199 fine temporal scales (43, 44) could provide new opportunities to test whether gradual escalation
200 of proximity and body contact is a widespread mechanism for 'testing the water' of a potential
201 social relationship.

202

203

204

205 **Methods**

206 *Animals*

207 We used 41 common vampire bats (*Desmodus rotundus*) as subjects, including 19 female
208 bats captured exiting a roost in Tolé, Panamá; 8 female bats captured foraging at a cattle pasture
209 in Las Pavas, Panamá about ~215 km from Tolé; and 14 captive-born bats (8 females, 6 males).
210 We studied adult females and their young, because these individuals form the basis of food-
211 sharing networks in the wild, whereas adult males compete for access to territories and females
212 and do not form stable bonds as often (24-28). To ensure familiarity within groups and
213 unfamiliarity between groups, we housed the groups separately (Tolé bats for 6 months and Las
214 Pavas bats for 2 weeks) before the study began. Bats were marked with subcutaneous passive
215 integrated transponders (Trovan Ltd. USA) and a visually unique combination of forearm bands
216 (Porzana, National Tag, and birdbands.com). To feed bats, we provided refrigerated or thawed
217 cattle or pig blood defibrinated with sodium citrate and citric acid.

218 We used a 3-4 mm biopsy punch to collect tissue samples in 80% or 95% ethanol, then used
219 a salt–chloroform procedure for DNA isolation, and a LI–COR Biosciences® DNA Analyser
220 4300 and the SAGA GT allele scoring software to genotype individuals at 17 polymorphic
221 microsatellite loci. Allele frequencies were based on 100 bats from Tolé and 9 bats from Las
222 Pavas, respectively. Genotypes were 99.9% complete. To estimate genetic relatedness, we used
223 the Wang estimator in the R package 'related'. To estimate kinship, we assigned a zero kinship
224 to known unrelated individuals from different sites and to individuals with negative pairwise
225 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 allogrooming 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 hour 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 seconds long. Bats were weighed before and after trials, and total observed mouth-licking durations predicted weight gain (SI Appendix).

We conducted fasting trials in each group during three experimental phases (SI Appendix, Fig. S1). First, we conducted 57 'baseline' trials to assess preliminary sharing rates between the 19 Tolé bats housed in a 1.7 x 2.1 x 2.3 m outdoor flight cage in Gamboa, Panama (1 group, 3420 possible sharing interactions). 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 x 28 x 40 cm clear plastic observation cage (10 pairs and 8 quartets). These controlled introductions provided for 162 opportunities for new food sharing between previous strangers (SI Appendix, Table S2). 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

249 described above (19 Tolé, 7 Las Pavas, and 14 captive-born bats). The introductions in this
250 combined group provided 11,823 more opportunities for new sharing.
251
252 *Statistical analyses*
253 During the baseline and mixed-group trials, we estimated food donation size as the number
254 of seconds that a fasted subject spent mouth-licking a fed groupmate. During the controlled
255 introduction trials, however, when bats were forced in close proximity, we saw a greater
256 frequency of begging, defined as mouth-licking that is clearly not food-sharing because the
257 partner is turning away from the mouth-licking bat and the mouth-licking bat does not gain the
258 weight that would be expected from food-sharing. To be conservative when measuring sharing,
259 we therefore did not count mouth-licking as food sharing during the controlled introduction trials
260 unless the subject weighed more than expected based on the average weight change for bats that
261 did not perform any mouth-licking.
262 Durations of sharing and grooming were lognormal. To create a standard index of grooming
263 rates, we therefore transformed the total duration of directed dyadic interactions in each trial
264 using natural log (x+1). We call these measures of the log duration per hour 'rates'. When
265 interaction bout duration and probability had different meanings, we decomposed rates into two
266 separate response variables: amounts (the magnitude of nonzero rates in a trial) and probabilities
267 (the presence or absence of a nonzero rate in a trial). We used permutation tests with 5,000
268 permutations for all hypothesis testing (p-values) and bootstrapping for all 95% confidence
269 intervals. To approximate two-sided p-values, we doubled any one-sided permutation p-values
270 that were below 0.05. Null distributions were not always centered on zero due to structure in the
271 data, so caution must be taken when considering the observed coefficients.

272 Grooming could occur before sharing simply because it is more frequent. To test whether
273 grooming preceded new sharing more than expected by chance, we therefore compared the
274 observed probability and latency of grooming before sharing to the values expected based on
275 randomizing the days on which grooming and sharing interactions occurred. To test for ingroup-
276 outgroup biases in sharing for each site, we calculated observed coefficients for the effect of
277 being from the same capture site on actor grooming rates, then we calculated expected
278 coefficients by permuting the grooming rates within each actor. To test the effects of kinship and
279 reciprocal grooming on the formation of new food-sharing relationships in the mixed-group
280 trials, we used multiple regression quadratic assignment procedure with double semi-partialing
281 (MRQAP-DSP) via the netlogit function in the sna R package. We also used this method to test
282 the effect of grooming on occurrence of new sharing only within the controlled introduction
283 trials. This procedure uses generalized linear models via the glm function in lme4 package to
284 calculate the observed coefficients and uses network-level permutations to get expected
285 coefficients. Since MRQAP-DSP cannot test interaction effects, we compared observed and
286 expected interaction coefficients using permutations in which we shuffled trial rates given by the
287 actor among different possible receivers and then shuffled the trial rates received by the receiver
288 among different possible actors. If the interaction coefficients were significant ($p<0.05$), we
289 conducted separate MRQAP-DSP tests within each group.
290 To test whether interaction rates changed over time, we generated expected coefficients for
291 general or generalized linear models by permuting the order of interactions within each potential
292 relationship. One captive-born bat died for unknown reasons during the mixed-group trials, so
293 we removed it from all temporal analyses. To test for evidence of reciprocal sharing, we used
294 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 availability

Behavioral data, genotypes, and R code are available as supplementary information.

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Author contributions

Conceptualization, GC; Methodology, GC; Investigation, GC, RC, JV; Genotyping, SR; Statistical analysis, GC; Original draft, GC; Review & Editing, GC, RC, JV, SR, DF, RP; Funding acquisition, GC, SR, RP; Resources, RP; Supervision, GC, DF, RP;

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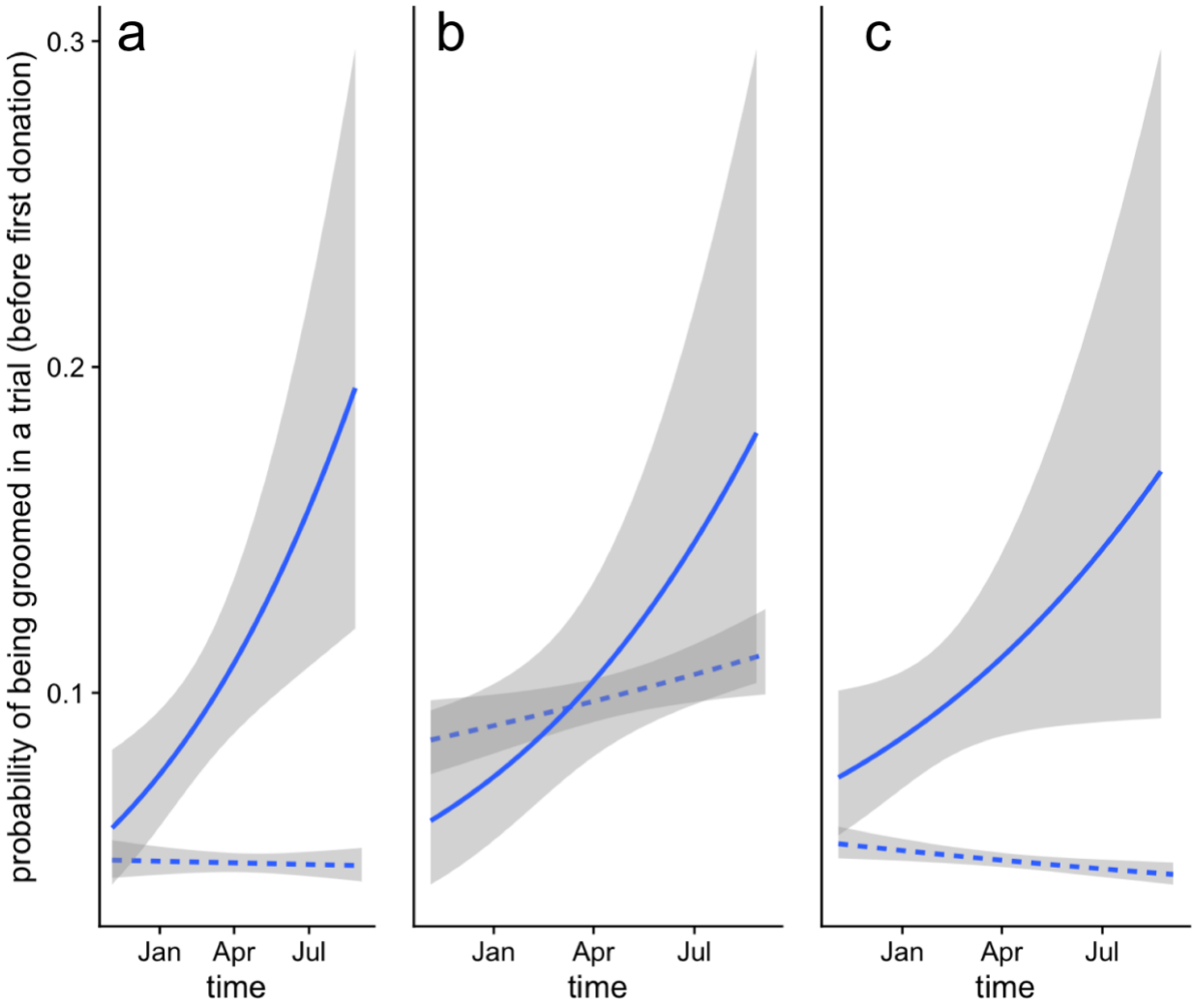
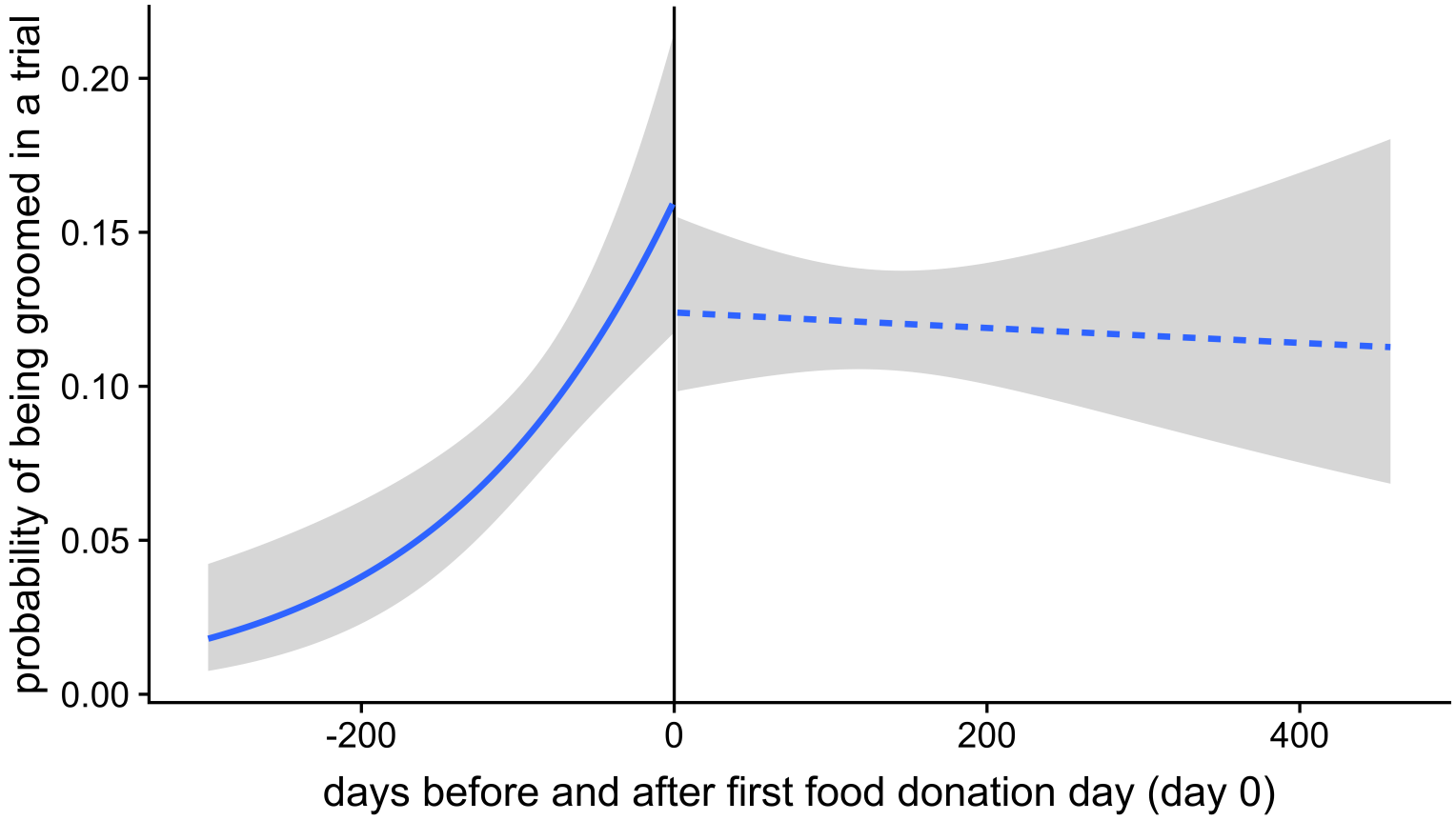
Figure Legends

Fig. 1. Increasing A-to-B grooming led to new B-to-A food-sharing relationships. In cases where a new food-sharing relationship formed (solid line), the grooming rate towards the future donor increased over time before the first donation occurred (OR=1.40, n=33, p<0.0002), but the grooming rate towards a potential donor remained low in cases where no food-sharing relationship formed (dashed line; OR=1.00, n=420, p=0.37). This divergence existed for all potential new relationships (panel a), for previously unfamiliar adults (panel b), and for relationships with captive-born bats (panel c; no interaction effect: OR=0.152, p=0.34). Relationships with captive-born bats had more divergent grooming trajectories (SI Appendix). Shading shows the 95% CI for the fitted model's predictions.

Fig. 2. Grooming rates increased before, but not after, new food-sharing occurred. The probability of a focal bat grooming the new donor in a 1-h trial (y-axis) increased before the first day that the donor fed the focal bat (i.e. 'day zero'; OR=1.4, p=0.0004), but not after this day zero (OR=0.946, p=0.61; interaction: OR=1.69, p<0.0002). This effect was seen in new food-sharing relationships with or without captive-born bats (SI Appendix). Shading shows the 95% CI for the fitted model's predictions.


a
b
c
probability of being groomed in a trial (before first donation)
0.3
0.2
0.1
Jan
Apr
Jul
time


probability of being groomed in a trial
0.20
0.15
0.10
0.05
0.00
-200
0
200
400
days before and after first food donation day (day 0)

Supplementary Information Appendix
For the paper, Carter et al. *Development of new food-sharing relationships among nonkin vampire bats*.

Evidence of food sharing

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 [1-3]).

Rates of food sharing

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 roughly 95% success rate observed in the previous long-term captive colony (95% CI = 92 to 98%, 29 bats, 183 trials; described in [1-3]). Assuming that mouth-licking events over 5 seconds 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 only two trials had five donors.

Development of new food-sharing relationships

We induced 12 of the 38 new food-sharing relationships between wild-caught adult females during the 106 'controlled introduction' fasting trials (Table S2), in which a single stranger Las Pavas bat was introduced to either one unfamiliar Tolé bat (forming an isolated pair) or to three Tolé bats (forming a quartet). The 26 other new food-sharing relationships between wild-caught adult females developed gradually during the 532 'mixed-group' fasting trials in the subsequent period when all the bats from both groups could freely interact (see Figures S1, S2, S3).

During the mixed-group period, there was a 10% chance that a new food-sharing relationship would develop between an adult and a captive-born bat (7 females, 6 males, 3-19 months old; 68 of 748 potential relationships) and 3.9% chance of a new food-sharing relationship between two captive-born bats. Captive-born bats groomed each other less than they groomed adult females, even when excluding the mother ($\beta = -0.10$, n = 460, $p = 0.012$).

During the mixed-group trials, the Las Pavas bats were biased towards feeding and grooming other Las Pavas bats (sharing: $\beta = 0.28$, n = 158, $p < 0.0002$, grooming: $\beta = 0.53$, n = 161, $p < 0.0002$). The same 'within-group bias' was seen in the Tolé bats (sharing: $\beta = 0.11$, n = 342, $p = 0.0008$; grooming: $\beta = 0.14$, n = 460, $p = 0.028$). New sharing relationships with captive-born bats were also more likely among bats from the same source population (OR = 1.73, $p = 0.035$). After controlling for this within-group bias, we found no evidence for a kinship bias (MRQAP-DSP, $\beta = 0.21$, $p = 0.13$). We lacked the statistical power to test for increases in food donation sizes over time within new dyads, but when pooling donations across all dyads, new donations that occurred later in time were not significantly larger ($\beta = 5.6$, n = 37, $p = 0.95$).

Relationships appeared to develop faster during controlled introductions

During the mixed-group trials, the appearance of first donations became more probable over time (OR = 1.56, n = 3072, $p = 0.0188$), so new sharing relationships appeared to form gradually (Figures S2, S3). During the mixed-group trials, first donations in new dyads were observed on average 247 days after their introduction (95% CI = 227 to 267, range = 66 to 556 days, n = 83 dyads, Figure S2), and the first evidence of new grooming was seen on average 198 days after

their introduction (95% CI = 186 to 209, range = 7 to 546, n = 351 dyads, Figure S2). During the controlled introductions trials, first donations were observed on average 33 days post-introduction (95% CI = 1 to 56, range = 1 to 193, n = 12 dyads) and first grooming was observed on average 24 days post-introduction (95% CI = 0 to 43, range = 1 to 205, n = 23 dyads).

Food sharing relationships emerged faster in isolated pairs than in quartets

The seven food donations in new relationships in isolated pairs also tended to occur sooner on average (mean latency = 3.6 days, 95% CI = 1.9 to 5, range = 1-8 days) than the three donations that occurred in quartets during the same time period (latency = 6, 32, and 34 days). During controlled introduction trials, food sharing occurred in 6 of 11 possible cases between familiar bats in the quartets but only in 2 of 20 possible cases between unfamiliar bats in those same quartets (OR = 0.09, df = 1, p = 0.012).

New grooming preceded new food sharing more than expected by chance

It is important to note that our tests of whether new grooming occurs before new food sharing are highly conservative (i.e. biased away from detecting new grooming before new food sharing), because the actual first grooming events in a new pair almost certainly occurred before our first observations of it, whereas the first food donations we observed were very likely to be the actual first donations. Bats were only focal sampled when in need of food, and they were only in need during the fasting trial, because we isolated and fed them immediately after. Food donations were therefore only necessary and likely during the 1-hour trial when we observed them. In contrast, grooming in the same pair could occur in any hour during the several days between the trials where the same dyad was sampled again (median = 8 days, inter-quartile range = 5 to 14 days). We sampled close to 100% of the time when food sharing was actually necessary, but less than 2% of the time when grooming could have occurred. Moreover, fasting trials increase the probability the subject will receive food, they also decrease the probability it will groom others (see 'Grooming Symmetry' below). In summary, our approach was very conservative: even if 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, making any evidence for raising-the-stakes hard to detect.

Despite this conservative bias, we still observed new grooming events before new sharing more than expected based on their relative frequencies. We observed new food sharing in 87 new pairs (30 adult past stranger pairs and 57 pairs with a captive-born bat). In most of these new sharing pairs (50 of 87), we observed new grooming on previous days before the fasting trial with the first food donation. In 28 of 87 pairs, we observed the first grooming during the same trial as the first sharing event. In the remaining 9 pairs (2 adult female pairs and 7 pairs with captive-born bats), we observed the first new donation in a trial without grooming. Note that this does not mean that absolutely no grooming occurred in these trials: food-sharing interactions almost always begin with sniffing and social grooming immediately prior to mouth-licking, but we only counted grooming bouts that were 5 seconds or longer, and the initial interactions were missed by the observer. Regardless, we observed grooming before or during the first trial with food sharing in 90% of the new sharing pairs, which is more than three times higher than expected when the dates of trial observations were shuffled within each new pair and type of behavior ($p < 0.0002$, expected frequency = 27%, 95% CI = 18% to 37%). To test whether the duration of the time lag between new grooming and sharing observations was larger than expected, we also applied the same permutation procedure to test the significance of the mean

latency between new grooming and sharing observed on different days. We found that the mean latency was also greater than expected based on the relative frequency of new grooming and new sharing (observed = 85 days, expected = 58 days, n = 59, p = 0.013).

Grooming trajectories over time predicted new sharing

The age composition of new potential relationships affected the pre-donation grooming rate trajectories. For adult past strangers, the grooming probabilities increased for all recipients, including those that never donated (OR = 1.12, p = 0.004), and they increased significantly faster for grooming recipients that later donated (OR = 1.49, p < 0.0002; interaction: OR = 1.45, p = 0.032, Figure 1b). For new potential relationships with captive-born bats, however, the grooming probabilities actually decreased for grooming recipients that never donated (OR = 0.9, p = 0.01), but tended to increase for recipients that did later donate (OR = 1.32, p = 0.051; interaction: OR = 1.70, p < 0.0002), Figure 1c).

Grooming before versus after new sharing

Grooming increased before but not after first donations in new relationships. The same pattern (Fig. 2) was found in new relationships between adults (interaction: OR = 1.60, p = 0.033; before: OR = 1.49, p = 0.024; after: OR = 1.01, p = 0.88) and in new relationships with captive-born bats (interaction: OR = 1.7, p = 0.0004; before: OR = 1.32, p = 0.010; after: OR = 0.92, p = 0.6).

Reciprocal development of food sharing

New food sharing rates were more symmetrical across dyads than expected by chance (Mantel test: r = 0.54, p = 0.0002). 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 = 0.017). 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 < 0.0002, observed bidirectional pairs = 13, expected = 4.6, expected 95% CI = 1 to 9). The probability of reciprocation is low because new food sharing rates were overall low, all bats had access to multiple donors, and most sharing occurred among familiar bats with established relationships.

Grooming symmetry

Previous studies of raising-the-stakes have focused on grooming symmetry within short time periods [4-9], but our experimental design did not allow for this kind of analysis. Grooming rates in new relationships increased over time, predicted reciprocal food-sharing rates, and were symmetrical across dyads over the study period (mantel test: r = 0.77, p < 0.0002), but we could not reliably test whether grooming symmetry increased over time within each dyad, for three main reasons. First, fasting trials reduced grooming symmetry, because the fasted bat was often initially greeted by many groupmates and it also spent less time grooming and more time begging (trying to lick the mouth of a potential donor). Consequently, a fasted subject was twice as likely to be groomed by a groupmate (13% probability) than to groom a groupmate (6% probability). Second, observed grooming rates that are undersampled are expected to become more balanced over time merely as a byproduct of grooming rates increasing over time, and we know that grooming rates increased over time. Third, increases in grooming symmetry over time

could be driven by age effects, because mutual grooming (and hence grooming symmetry) is much lower when one bat is not yet an adult [10].

Evidence that new grooming and sharing are not both caused by proximity

One null hypothesis is that bats initiate new grooming and sharing based entirely on proximity, and the correlation between new grooming and new sharing is spurious. In this case, grooming rates should correlate with new sharing among strangers that are able to freely associate during the mixed-group period, but when strangers are forced into close proximity as in the controlled introduction trials, then the apparent grooming effect should disappear or be much smaller. In other words, if proximity is actually driving the correlation between grooming and sharing, then removing variation in proximity (forced close contact) should reveal the lack of an association between grooming and sharing. In sharp contrast to this prediction, the effect of grooming given on new food received for the strangers held in constant close proximity during the controlled introduction periods was even *greater* than the effect during the mixed-group trials where proximity was allowed to vary greatly across dyads and over time (forced close proximity: OR = 5.44, p = 0.036; variable proximity: OR = 1.63, p = 0.037; network logistic regression in the sna R package).

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SI Figures and Tables

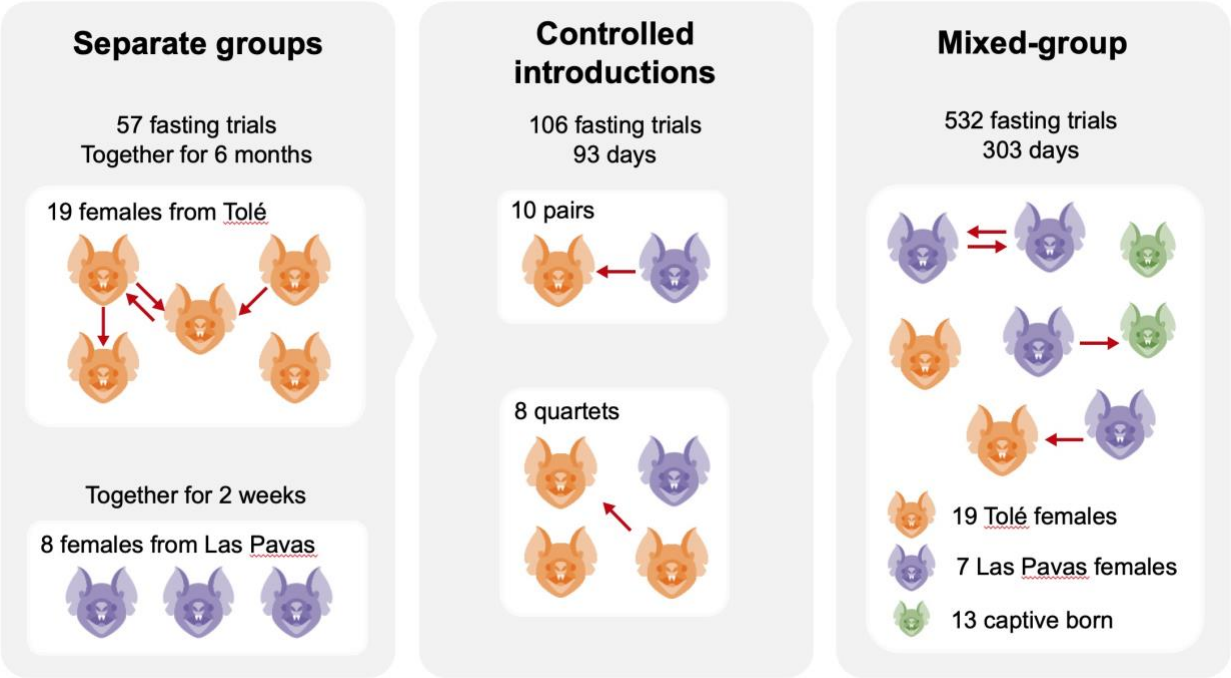

Figure S1 | Experiment overview
To see how vampire bats form new social bonds, we created groups of bats from two different sites (colors), then we induced and sampled food sharing and grooming events between bats that are either previously familiar or unfamiliar. Red arrows depict food sharing events during repeated fasting trials. For details of controlled introductions, see SI Table 2. Icons from icons8.com used under a Linkware license.

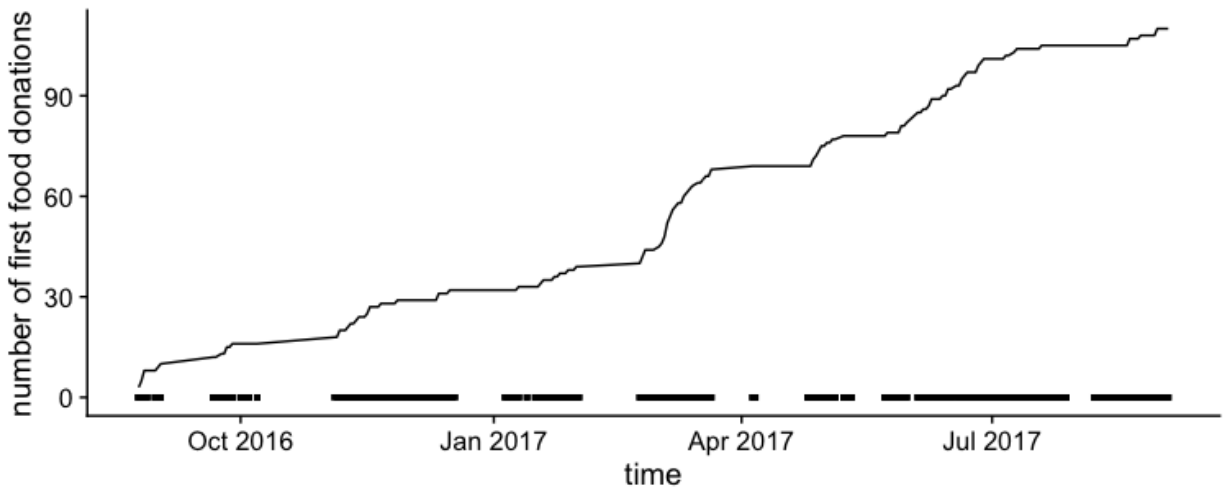

Figure S2 | First food donations over time
New food-sharing relationships accumulated gradually over time. Black rectangles above X-axis show the occurrence of fasting trials.

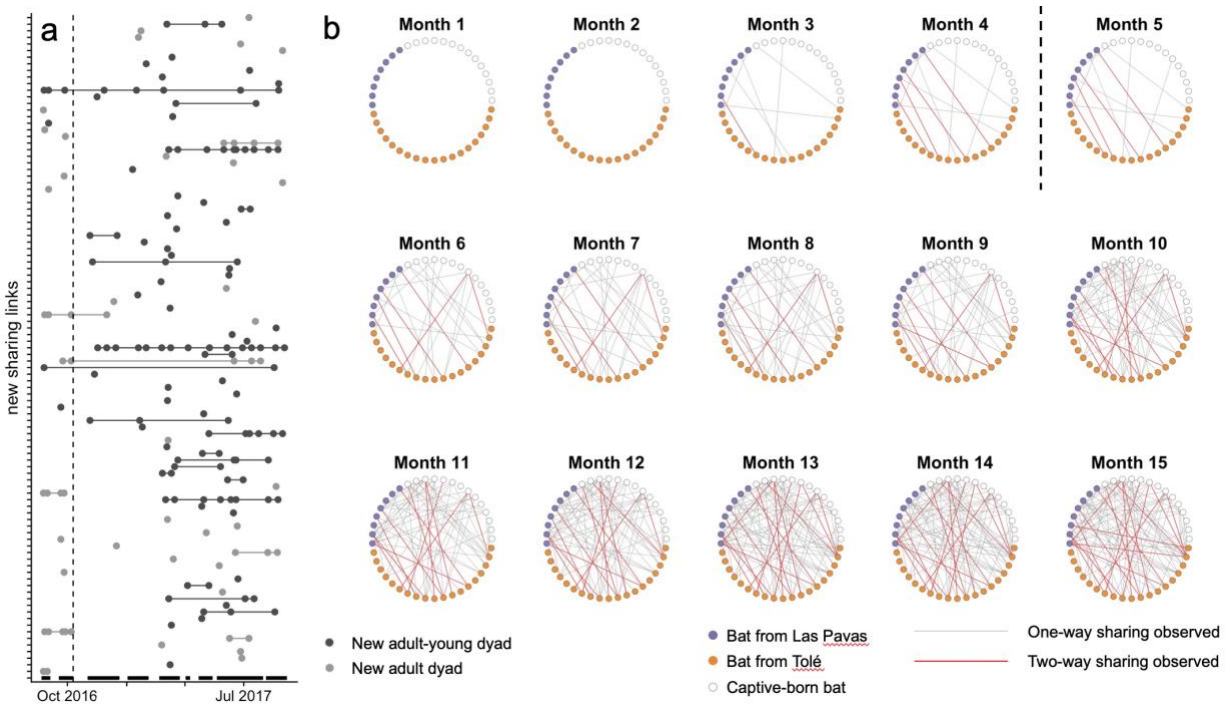

Figure S3 | Gradual development of new food-sharing relationships
Panel A shows food donations (points) over time (x-axis) within new actor-receiver relationships (y-axis) between two adult females (grey points) or with a captive-born bat (black points). Repeated dyadic donations are connected by horizontal lines. The end of the controlled introduction period, after which all bats could interact freely (months 1-4), is shown by the vertical dotted line. Black rectangles above the x-axis show the fasting trials, when new donations could be observed. Panel B shows the monthly formation of the food-sharing network between Las Pavas bats (orange), Tolé bats (purple), and captive-born bats (white). Grey edges show one-way sharing and red edges show two-way sharing. Two-way sharing occurred more often than expected by chance (see results).

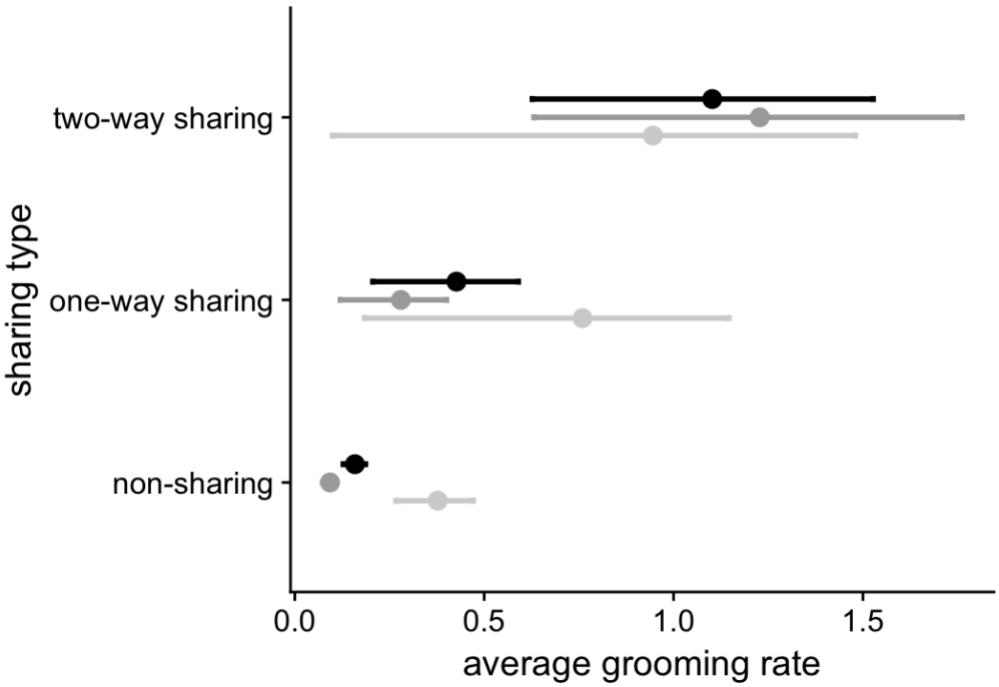

Figure S4 | Dyadic grooming rates predict new food-sharing relationships.
Mean within-dyad grooming rates, with bootstrapped 95% confidence intervals, are shown for three possible outcomes (y-axis) and for all potential relationships (black), potential relationships with captive-born bats (grey), and potential relationships between adult strangers (light grey).

Table S1 | Glossary.
Definition of terms used in the text.

Term	Definition
Dyad	An undirected pair of bats (e.g. AB, BC, AC)
Potential relationship	A directed pair of actor and receiver bats (e.g. AB, BA, AC)
Relationship	A directed actor-receiver pair that is observed to groom or share food during fasting trials.
New relationship	Relationship between bats that first met during the experiment, excluding mother-offspring dyads.
Social bond	The unobserved underlying social relationship (as experienced by the animal) that we infer from observations.

Table S2 | Controlled introductions

The same bats were used in multiple introductions. Bats were moved to and from groups to make new combinations or because of health issues (pregnancy, weight loss). Bats not in a small cage group during controlled introduction trials were kept with familiar individuals in a flight cage.

No.	Group type	No. trials (range of days together)	Adult female bats (*Las Pavas stranger)	Opportunities for new sharing	Introduction date
1	quartet	1 (1 day)	scs, hilga, rc, eve*	3	2016.07.06
2	quartet	1 (1 day)	ccs, sss, sc, una*	3	2016.07.06
3	quartet	1 (1 day)	scc, sd, c, dos*	3	2016.07.06
4	quartet	1 (1 day)	csc, ss (w/pup), s, tes*	3	2016.07.06
5	pair	1 (1 day)	ccc, cat*	1	2016.07.06
6	pair	1 (1 day)	dcd, ivy*	1	2016.07.06
7	pair	1 (1 day)	dd, six*	1	2016.07.06
8	pair	1 (4 days)	d (w/pup), ola* (w/pup)	1	2016.07.02
9	quartet	17 (1–44 days)	sd, scs, d (w/pup), una*	32	2016.08.24
10a	quartet	5 (1–9 days)	s, rc, hilga, dos*	9	2016.08.24
10b	quartet	12 (1–44 days)	s, rc, ccc (w/pup), dos*	21	2016.09.21
11	quartet	17 (1–44 days)	ccs, sc, sss, tes*	27	2016.08.24
12	pair	10 (1–44 days)	dd, cat*	10	2016.08.24
13	pair	10 (1–44 days)	c, ivy*	10	2016.08.24
14	pair	5 (1–10 days)	csc, six*	5	2016.08.24
15	pair	9 (1–44 days)	dcd, eve	9	2016.08.24
16	pair	9 (1–97 days)	ss (w/pup), ola* (w/pup)	19	2016.08.24
17	pair	4 (1–7 days)	cd, six*	4	2016.09.21

Summary of supplied data

Dataset S1. genotypes.csv

Microsatellite genotypes used to assess relatedness.

Dataset S2. vampire_maternal_kinship.csv

Maternal pedigree data

Dataset S3. new_bonds_data.Rdata

Food sharing and allogrooming data

Dataset S4. new_bonds_analysis20.txt

R script for analyzing data