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2 **Title:**

3 Development of new food-sharing relationships among nonkin vampire bats

4

5 **Authors and affiliation:**

6 Gerald G. Carter<sup>1,2</sup>, Damien R. Farine<sup>3,4,5</sup>, Rachel J. Crisp<sup>2,6</sup>, Julia K. Vrtillek<sup>2,7</sup>, Simon P.

7 Ripperger<sup>2,8</sup>, Rachel A. Page<sup>2</sup>

8 <sup>1</sup>Department of Ecology, Evolution, and Organismal Biology; The Ohio State University, Columbus, OH, USA

9 <sup>2</sup>Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá

10 <sup>3</sup>Edward Grey Institute of Field Ornithology, Department of Zoology, University of Oxford, Oxford, UK

11 <sup>4</sup>Department of Collective Behaviour, Max Planck Institute for Ornithology, Konstanz, Germany

12 <sup>5</sup>Chair of Biodiversity and Collective Behaviour, Department of Biology, University of Konstanz, Konstanz,

13 Germany

14 <sup>6</sup>Centre for Research in Animal Behaviour, University of Exeter, Exeter, UK

15 <sup>7</sup>Department of Biology, Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland

16 <sup>8</sup>Museum für Naturkunde, Leibniz-Institute for Evolution and Biodiversity Science, Berlin, Germany

17

18 **Corresponding author:**

19 Gerald Carter, 318 W. 12th Avenue, 300 Aronoff Laboratory, Columbus, OH 43210,

20 carter.1640@osu.edu

21 **Abstract**

22           In an individualized animal society, social bonds can foster cooperation and enhance  
23 survival and reproduction. Cooperative bonds often exist among kin, but nonkin can also develop  
24 high-investment cooperative bonds that share similarities with human friendship. How do such  
25 bonds form? One theory suggests that strangers should ‘test the waters’ of a new relationship by  
26 making small initial cooperative investments and gradually escalating them with good partners.  
27 This ‘raising-the-stakes’ strategy is demonstrated by human strangers in short-term economic  
28 games, but it remains unclear whether it applies to helping in a natural long-term social bond.  
29 Here we show evidence that unfamiliar vampire bats (*Desmodus rotundus*) selectively escalate  
30 low-cost investments in allogrooming before developing higher-cost food-sharing relationships.  
31 We introduced females from geographically distant sites in pairs or groups and observed that  
32 bats established new reciprocal grooming relationships, and that increasing grooming rates  
33 predicted the occurrence of first food donations, at which point grooming rates no longer  
34 increased. New food-sharing relationships emerged reciprocally in 14% of female pairs, typically  
35 over 10-15 months, and developed faster when strangers lacked alternative familiar partners. A  
36 gradual grooming-to-sharing transition among past strangers suggests that ‘raising the stakes’  
37 might be more evident when tracking multiple cooperative behaviours as new relationships form,  
38 rather than measuring a single behavior in an established relationship. ‘Raising the stakes’ could  
39 play a similar underappreciated role across a broader spectrum of social decisions with long-term  
40 consequences, such as joining a new social group or forming a long-term pair-bond.

41

42 **Keywords:** cooperation, social relationships, vampire bats

43

44 **Significance statement**

45 Vampire bats form long-term cooperative social bonds that involve reciprocal food  
46 sharing. How do two unrelated bats go from being strangers to having a high-investment food-  
47 sharing relationship? We introduced unfamiliar bats and found evidence that low-cost grooming  
48 paves the way for higher-cost food donations. Food sharing emerged in a reciprocal fashion and  
49 it emerged faster when two strangers did not have access to their original groupmates. The bats  
50 that formed new food-sharing relationships had a history of escalating reciprocal grooming up  
51 until the food sharing began. Our finding that unfamiliar nonkin vampire bats appear to gradually  
52 and selectively transition from low-cost to high-cost cooperative behaviors is the first evidence  
53 that nonhuman individuals ‘raise the stakes’ when forming new cooperative relationships.

54

55 **Text**

56 Animal societies are fundamentally shaped by repeated interactions among individuals  
57 over time, and repeated interactions allow for individuals to choose to cooperate based on their  
58 social history across different partners (1-3). Experiments have shown that organisms as diverse  
59 as animals, plants, and fungi can prevent exploitation by shifting cooperative investments  
60 towards partners that provide better reciprocal returns (4-7). However, a greater challenge has  
61 been testing if and how individuals prevent exploitation while forming stable social bonds.

62 Although long-term bonds are known to enhance survival and reproduction (8-12), it remains  
63 unclear how they form. How do complete strangers form a long-term cooperative relationship?

64 A key idea is that individuals can reduce the costs of defection by first spreading out  
65 smaller cooperative investments across time (‘parceling’ (13)) or across different partners  
66 (‘social bet-hedging’ (14)), and then gradually increasing investments in the most cooperative

67 partnerships ('raising the stakes' (15)). For example, one might first assess a potential partner's  
68 tolerance by clustering for warmth, then gain feedback by investing in low-cost social grooming,  
69 and then use the partner's response to make higher-cost decisions about sharing food or  
70 providing coalitionary support (16). Despite its intuitive appeal for explaining how new  
71 cooperative relationships develop, evidence supporting the 20-year-old 'raising-the-stakes'  
72 model (15) is surprisingly scarce. An early test using the cleaner and client fish mutualism  
73 suggested that the model does not apply well to situations with severe asymmetries in partner  
74 payoffs or options (17). Studies with nonhuman primates have only tested snapshots of  
75 established relationships (18-21), rather than the formation of new ones. Human strangers do  
76 'raise the stakes' when playing economic cooperation games (e.g. 22, 23), but the strategy has  
77 never been properly tested in the more ecologically-relevant context of long-term relationship  
78 formation. Doing so requires measuring the emergence of natural helping behaviors between  
79 randomly introduced strangers.

80         We tracked the development of cooperative relationships between previously unfamiliar  
81 wild-caught vampire bats (*Desmodus rotundus*). Cooperative relationships in vampire bats  
82 involve low-cost social grooming (hereafter *grooming*) and higher-cost regurgitations of ingested  
83 blood (*food sharing*)(14, 24, 25), and we show evidence that vampire bats use reciprocal  
84 grooming to gradually establish bonds that entail food donations. Our data support four key  
85 predictions: (i) new food-sharing relationships should form faster when bats have fewer  
86 alternative partners, (ii) grooming rates should predict the probability that the grooming recipient  
87 later donates food to the groomer, (iii) low initial grooming rates should increase over time only  
88 up until this first reciprocal food donation, and (iv) the emergence of new food-sharing should be  
89 reciprocal.

90 Female vampire bats can live for 16 years in the wild (26), and new social bonds form  
91 when an unrelated female joins a social network about once every two years (25, 27). To observe  
92 how new adult food-sharing relationships form, we captured adult females from two distant sites,  
93 Tolé (n=19) and Las Pavas (n=8), in Panamá, and we then ran 638 fasting trials in which an  
94 overnight-fasted subject could be fed by a past stranger. To test the prediction that new sharing  
95 relationships would form faster when strangers have fewer options of alternative partners (2), we  
96 compared the occurrence of new sharing when wild-caught strangers were introduced in isolated  
97 pairs (one Las Pavas and one Tolé bat), in small groups (one Las Pavas and three Tolé bats), or  
98 in one large group (see Methods, Supplementary Information (SI) Figure S1). New bonds can  
99 also form when individuals are born into a group, and these relationships might form differently.  
100 In the large mixed group, we therefore also measured the development of non-maternal  
101 cooperative relationships between 26 female adults and 13 younger captive-born bats (6 males, 7  
102 females, 11 to 21 months old). To analyze non-independent social network data, we compared  
103 observed coefficients from general and generalized linear models (slopes  $\beta$ , and odds ratios OR,  
104 respectively) to expected distributions of null coefficient values generated using network and  
105 data permutations (see Methods). For clarity, we use the term ‘relationships’ for the *observed*  
106 network edges (directed), and the term ‘bonds’ to discuss the underlying construct that we  
107 *inferred* from the measured relationships (see glossary, SI Table S1).

108 Over 424 days and 17,621 donation opportunities, there was an 11% probability that two  
109 bats would form a new food-sharing relationship (all bats: 111 of 1036 potential relationships;  
110 females: 14% of 648 potential relationships; wild-caught adult females: 16% of 243 potential  
111 relationships; see SI). The probability of new relationships depended on availability of  
112 alternative familiar partners. Per prediction (i), when strangers were introduced as isolated pairs,

113 new relationships emerged much faster (see SI), and we observed higher rates of grooming ( $\beta =$   
114 1.09,  $p = 0.04$ ) and food sharing ( $\beta = 1.14$ ,  $p = 0.004$ ), compared to when one Las Pavas bat was  
115 introduced to three Tolé bats. In the large mixed group, bats preferentially fed and groomed their  
116 original familiar groupmates, and new sharing emerged even more gradually than in isolated  
117 pairs or small groups (SI, Fig. S2-S3).

118 Under the ‘raising-the-stakes’ hypothesis, strangers should make low-cost investments  
119 and use the return rates to inform future investments. Per prediction (ii), the grooming rate given  
120 by actor A to recipient B predicted the later formation of new food sharing from B back to A  
121 (OR = 2.24,  $p < 0.0002$ ,  $n = 960$ ). The trajectory of grooming rates over time clearly differed  
122 between pairs that developed new food-sharing relationships versus pairs that did not  
123 (interaction: OR = 1.57,  $p < 0.0002$ , Fig. 1).

124 If reciprocal grooming serves to ‘test the waters’ for more costly cooperative  
125 investments, then the slope of the increase in grooming given should be greater *before* the food-  
126 sharing relationship begins. Per prediction (iii), grooming rates from bat A to B increased only  
127 before the first day that B fed A (Fig. 2). As expected, grooming rates were highest between bats  
128 that formed reciprocal food-sharing relationships, intermediate in one-way sharing relationships,  
129 and lowest among bats that never shared food (SI Fig. S4), and grooming rates towards  
130 previously familiar donors were greater than towards either previously unfamiliar donors or  
131 previously familiar non-donors (SI Fig. S5).

132 Finally, per prediction (iv), the formation of new food-sharing relationships was  
133 reciprocal. Directed food-sharing networks were more symmetrical than expected if new  
134 donations were random, even when controlling for kinship (estimated via maternal pedigree and

135 microsatellite genotyping, see Methods; MRQAP-DSP; reciprocal sharing:  $\beta = 0.40$ ,  $p < 0.0002$ ,  
136 kinship:  $\beta = 0.07$ ,  $p = 0.10$ ; SI).

137       Individuals can ‘raise the stakes’ quantitatively, by increasing the magnitude of  
138 cooperative investments, and qualitatively by performing higher-cost cooperative behaviors.  
139 Female vampire bats appeared to both increase grooming rates and transition from low-cost  
140 grooming to high-cost sharing. The rarity of new food-sharing relationships corroborates past  
141 evidence that food regurgitations are energetically costly and that social bonds require  
142 investments of time and energy (14, 25, 28-30). Our finding that the development of new food-  
143 sharing relationships between wild-caught adults is influenced by partner behavior and  
144 availability provides the clearest evidence to date that nonkin food sharing in vampire bats is not  
145 a mere byproduct of kin selection (30, 31).

146       Prior to this study, some previous evidence for ‘raising-the-stakes’ in nonhuman social  
147 relationships came from observations of grooming among familiar male chimpanzees after the  
148 death of an alpha male (18). The authors suggested that, during this period of social instability,  
149 these groupmates may have needed to re-establish their relationships, and that a diminishing  
150 threat of violence led to the increasing rates of grooming (18). Although the increase in  
151 grooming rates is consistent with each male ‘raising the stakes’ to assess the risk of aggression  
152 from their grooming partner, it might have also resulted from a general decline in vigilance  
153 against possible aggression from any other groupmate.

154       The empirical evidence for the ‘raise-the-stakes’ model (15) has been scarce in part  
155 because it is a variation on the classic ‘tit-for-tat’ strategy in the iterated prisoner’s dilemma (1),  
156 a model which is difficult to test unambiguously using natural forms of cooperation (4, 32).  
157 Contingently alternating forms of reciprocity similar to ‘tit-for-tat’ have been demonstrated using

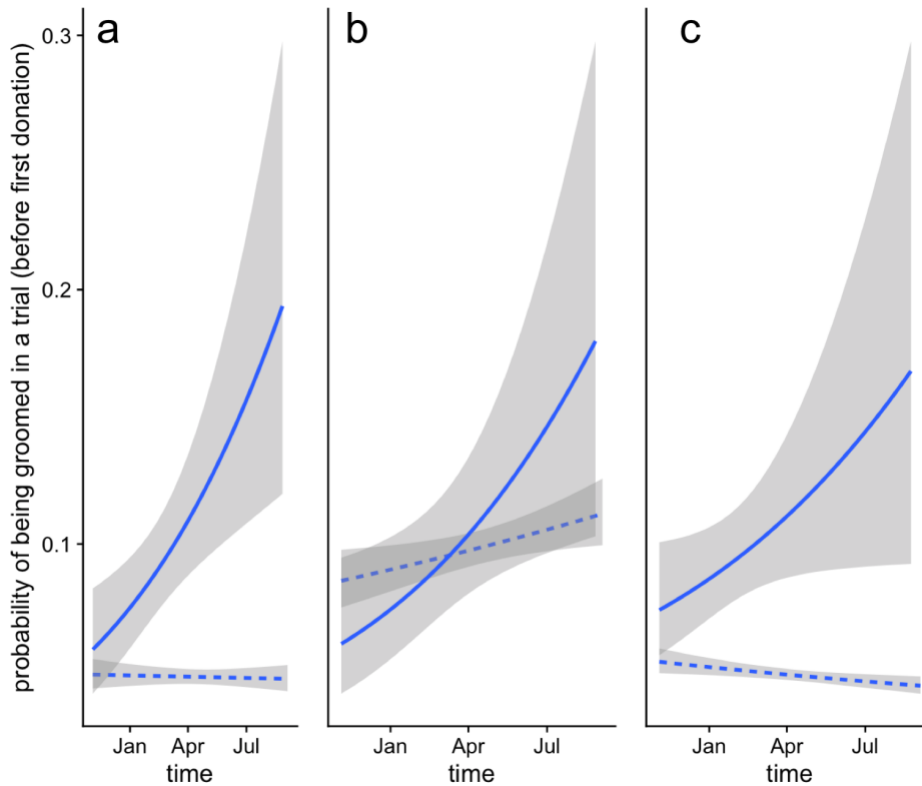
158 experiments with trained instrumental tasks (e.g. pulling a lever to deliver food) and payoffs  
159 accruing in distinct rounds (4-6). However, the ‘tit-for-tat’ model of reciprocity must not be  
160 operationalized too literally. It excludes many factors crucial to real-world cooperation, such as  
161 partner choice, social bonding, different service types, and the cost-benefit asymmetries resulting  
162 from demography, market effects, or social dominance (4, 7, 32). Moreover, long-term  
163 cooperative relationships do not produce strict ‘tit-for-tat’ helping. For example, primates with  
164 stronger bonds show *less* evidence for short-term contingencies in grooming (9). Models of  
165 cooperative relationships as a ‘social exchange’ must therefore consider the cognition  
166 underpinning the helping decisions. Social bonds and ‘trust’ result from heuristics that reduce  
167 many cooperative interactions to a single positive association, just as similar generalizations in  
168 economic choices lead to ‘brand loyalty’ (33). Even in humans, which possess the most  
169 extensive suite of cognitive specializations for social interactions (34), many of the neural  
170 processes that underlie simple operant conditioning also underlie learning from people (35) or  
171 about them (36).

172         The relevance of our findings extends beyond high-cost cooperative behaviors. For  
173 example, in some species, courtship behaviors could be seen as a short-term investment in the  
174 formation of longer-term pair bonds with substantial fitness consequences (e.g. 37). Similarly,  
175 the role of mere physical contact as a low-cost method for building tolerance and trust might be  
176 more general than currently recognized. The key role of grooming for relationship maintenance  
177 in primates is well established, but growing evidence suggests that tactile behaviors can reduce  
178 fear and encourage cooperation in many other species of mammals, birds, and fish (e.g. 5, 38-  
179 44). New methods for tracking formation of social bonds at fine temporal scales (45) will



180 provide new opportunities to test whether gradual escalation of proximity and body contact is a  
181 widespread mechanism for ‘testing the water’ of a potential relationship.

182



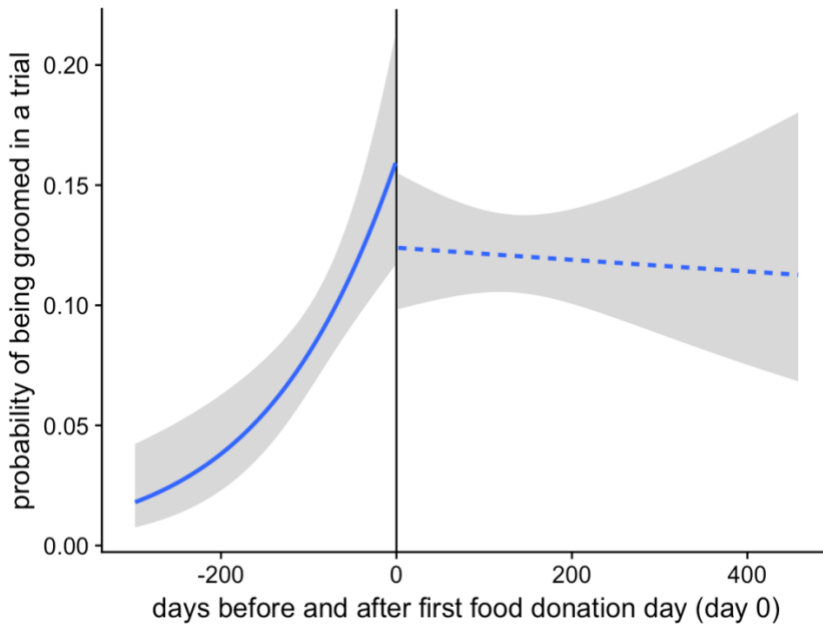
183

184 **Fig. 1. Increasing A-to-B grooming led to new B-to-A food-sharing relationships.** In cases  
185 where a new food-sharing relationship formed (solid line), the grooming rate towards the future  
186 donor increased over time before the first donation occurred (OR = 1.40, n = 33,  $p < 0.0002$ ), but  
187 the grooming rate towards a potential donor remained low in cases where no food-sharing  
188 relationship formed (dashed line; OR = 1.00, n = 420,  $p = 0.37$ ). This divergence existed for all  
189 potential new relationships (panel a), for previously unfamiliar adults (panel b), and for

190 relationships with captive-born bats (panel c; no interaction effect: OR = 0.152,  $p = 0.34$ ).

191 Relationships with captive-born bats had more divergent grooming trajectories (SI).

192



193

194 **Fig. 2. Grooming rates increased before, but not after, new food-sharing occurred.** The

195 probability of a focal bat grooming a new future donor in a 1-h trial (y-axis) increased before the

196 first day that the donor fed the focal bat (i.e. 'day zero'; OR = 1.4,  $p = 0.0004$ ), but not after day

197 zero (OR = 0.946,  $p = 0.61$ ; interaction: OR = 1.69,  $p < 0.0002$ ). This effect was seen in new food-

198 sharing relationships with or without captive-born bats (SI).

199

## 200 **Methods**

### 201 *Animals*

202 We used 41 common vampire bats (*Desmodus rotundus*) as subjects, including 19 female

203 bats captured exiting a roost in Tolé, Panamá; 8 female bats captured foraging at a cattle pasture

204 in Las Pavas, Panamá about ~215 km from Tolé; and 14 captive-born bats (8 females, 6 males).

205 We studied adult females and their young, because these individuals form the basis of food-  
206 sharing networks in the wild, whereas adult males compete for access to territories and females  
207 and do not form stable bonds as often. To ensure familiarity within groups and unfamiliarity  
208 between groups, we housed the groups separately (Tolé bats for 6 months and Las Pavas bats for  
209 2 weeks) before the study began. Bats were marked with subcutaneous passive integrated  
210 transponders (Trovan Ltd. USA) and a visually unique combination of forearm bands (Porzana,  
211 National Tag, and birdbands.com). To feed bats, we provided refrigerated or thawed cattle or pig  
212 blood defibrinated with sodium citrate and citric acid.

213 We used a 3-4 mm biopsy punch to collect tissue samples in 80% or 95% ethanol, then used  
214 a salt–chloroform procedure for DNA isolation, and a LI–COR Biosciences® DNA Analyser  
215 4300 and the SAGA GT allele scoring software to genotype individuals at 17 polymorphic  
216 microsatellite loci. Allele frequencies were based on 100 bats from Tolé and 9 bats from Las  
217 Pavas, respectively. Genotypes were 99.9% complete. To estimate genetic relatedness, we used  
218 the Wang estimator in the R package ‘related’. To estimate kinship, we assigned a zero kinship  
219 to known unrelated individuals from different sites and to individuals with negative pairwise  
220 relatedness, and we assigned a kinship of 0.5 for known mother-offspring pairs or pairs with  
221 genetic relatedness estimates greater than 0.5. For all other pairs, we used genetic relatedness as  
222 the estimate for kinship.

223

#### 224 *Experimental design*

225 We induced allogrooming and regurgitated food sharing using a fasting trial, in which a  
226 focal subject was isolated from the group without food for a night and a day, then released back  
227 to the group of fed bats for 1 hour the following night. During the hour, all grooming or sharing

228 interactions with the subject were recorded using an infrared (IR) light and an IR-sensitive video  
229 camera. Each donation bout was estimated by the number of seconds that the unfed subject spent  
230 licking the mouth of a particular groupmate, starting at 5 seconds of uninterrupted licking. The  
231 dyadic donation size for that trial was estimated as the sum of these bouts. Bats were weighed  
232 before and after trials, and total observed mouth-licking durations predicted weight gain. Fasted  
233 subjects gained 51 mg of mass per minute of mouth-licking ( $R^2= 0.75$ , bootstrapped 95% CI: 45  
234 to 57 mg/min,  $n=619$ ,  $p<0.001$ ).

235 We conducted fasting trials in each group during three experimental phases (SI Fig. S1).  
236 First, we conducted 57 “baseline” trials to assess preliminary sharing rates between the 19 Tolé  
237 bats housed in a 1.7 x 2.1 x 2.3 m outdoor flight cage in Gamboa, Panama (1 group, 3420  
238 possible sharing interactions). Second, we conducted 106 “controlled introduction” trials to  
239 assess possible formation of new food-sharing bonds between past strangers introduced as either  
240 an isolated pair (one Las Pavas bat and one Tolé bat) or a quartet (one Las Pavas bat and three  
241 Tolé bats), housed in a 28 x 28 x 40 cm clear plastic observation cage (10 pairs and 8 quartets).  
242 These controlled introductions provided for 162 opportunities for new sharing between previous  
243 strangers (SI Table S2). Finally, we conducted 532 “mixed-group” trials to assess the formation  
244 of new sharing relationships when all bats were housed together in the flight cage described  
245 above (19 Tolé, 7 Las Pavas, and 14 captive-born bats). These introductions in this combined  
246 group provided 11,823 more opportunities for new sharing.

247

#### 248 *Statistical analyses*

249 During the baseline and mixed-group trials, we estimated food sharing as the number of  
250 seconds that a fasted subject spent mouth-licking a fed groupmate. During the controlled

251 introduction trials, however, when bats were forced in close proximity, we saw a greater  
252 frequency of begging, defined as mouth-licking that is clearly not food-sharing because the  
253 partner is turning away from the mouth-licking bat and the mouth-licking bat does not gain the  
254 weight that would be expected from food-sharing. To be conservative when measuring sharing,  
255 we therefore did not count mouth-licking as food sharing during the controlled introduction trials  
256 unless the subject weighed more than expected based on the average weight change for bats that  
257 did not perform any mouth-licking.

258 Durations of sharing and grooming were lognormal. To create a standard index of grooming  
259 rates, we therefore transformed the total duration of directed dyadic interactions in each trial  
260 using natural log ( $x+1$ ). We call these measures of the log duration per hour “rates”. When  
261 interaction bout length and probability had different meanings, we decomposed rates into two  
262 separate response variables: amounts (the magnitude of nonzero rates in a trial) and probabilities  
263 (the presence or absence of a nonzero rate in a trial). We used permutation tests with 5,000  
264 permutations for all hypothesis testing (p-values) and bootstrapping for all 95% confidence  
265 intervals. To approximate two-sided p-values, we doubled any one-sided permutation p-values  
266 that were below 0.05. Null distributions were not always centered on zero due to structure in the  
267 data, so caution must be taken when considering the observed coefficients.

268 To test for ingroup-outgroup biases in sharing for each site, we calculated observed  
269 coefficients for the effect of being from the same capture site on actor grooming rates, then  
270 calculated expected coefficients by permuting the grooming rates within each actor. To test the  
271 effects of kinship and reciprocal grooming on the formation of new food-sharing relationships in  
272 the mixed-group trials, we used multiple regression quadratic assignment procedure with double  
273 semi-partialing (MRQAP-DSP) via the netlogit function in the sna R package. This procedure

274 uses generalized linear models via the glm function in lme4 package to calculate observed  
275 coefficients and uses network-level permutations to get expected coefficients. Since MRQAP-  
276 DSP cannot test interaction effects, we compared observed and expected interaction coefficients  
277 using permutations in which we shuffled trial rates given by the actor among different possible  
278 receivers and then shuffled the trial rates received by the receiver among different possible  
279 actors. If the interaction coefficients were significant ( $p < 0.05$ ), we conducted separate MRQAP-  
280 DSP tests within each group.

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

290

#### 291 *Data availability*

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

293

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300

### 301 **Author contributions**

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

305

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394

## 395 **Supplementary Information**

### 396 *Development of new food-sharing relationships*

397 Over 424 days, there was a 52% probability that two bats would form a new grooming  
398 relationship, and an 11% probability that two bats would form a new food-sharing relationship  
399 (all bats: 111 of 1036 potential relationships, 12154 donation opportunities; all females: 91 of  
400 648 potential relationships, 7504 donation opportunities; wild-caught females: 38 of 243  
401 potential relationships, 3202 donation opportunities). We induced 12 of the 38 new food-sharing  
402 relationships between wild-caught adult females during the 106 “controlled introduction” fasting  
403 trials (Table S2), in which a single stranger Las Pavas bat was introduced to one unfamiliar Tolé  
404 bat (forming an isolated pair) or to three Tolé bats (forming a quartet). The 26 other new food-  
405 sharing relationships between wild-caught adult females developed gradually during the 532  
406 fasting trials in the subsequent 10-month period when all the bats from both groups could freely  
407 interact (Fig. S3).

408 During this mixed-group period, there was a 9% chance that a new food-sharing  
409 relationship would develop with one or two captive-born bats (7 females, 6 males, 3-19 months  
410 old; 73 of 787 potential relationships). The formation of new sharing relationships with captive-  
411 born bats showed a kinship bias (odds ratio (OR) = 13.85,  $n = 787$ ,  $p = 0.038$ ) due to a bias  
412 towards bats from the same source population (OR) = 1.73,  $p = 0.035$ ), but a kinship bias was  
413 not detected when controlling for the effect of source population (MRQAP-DSP,  $\beta = 0.21$ ,  $p =$   
414 0.13). In relationships between adults and younger captive-born bats, adult females performed on  
415 average 16 seconds more grooming (95% CI: 6 to 24 s) and 17 seconds more food sharing (95%  
416 CI: 5 to 28 s) per trial than their younger captive-born partners. Captive-born bats groomed each

417 other less than they groomed adult females, even when excluding the mother ( $\beta = -0.10$ ,  $n = 460$ ,  
418  $p = 0.012$ ).

419 During the mixed-group trials, Las Pavas bats were biased towards feeding and grooming  
420 other Las Pavas bats (sharing:  $\beta = 0.28$ ,  $n = 158$ ,  $p < 0.0002$ , grooming:  $\beta = 0.53$ ,  $n = 161$ ,  
421  $p < 0.0002$ ). The same was true for Tolé bats (sharing:  $\beta = 0.11$ ,  $n = 342$ ,  $p = 0.0008$ ; grooming:  $\beta$   
422  $= 0.14$ ,  $n = 460$ ,  $p = 0.028$ ). During the mixed-group trials, new food-sharing relationships  
423 occurred gradually (mean days from introduction until first donation = 247,  $SD = 95$ , range = 66  
424 – 556 days; Figure S2) and became more probable over time ( $OR = 1.56$ ,  $n = 3072$ ,  $p = 0.0188$ ).  
425 We lacked the statistical power to test for increases in sharing amounts over time within new  
426 dyads; new donations that occurred later in time were not significantly larger ( $\beta = 5.6$ ,  $n = 37$ ,  $p$   
427  $= 0.95$ ).

428

#### 429 *Isolated pairs started sharing faster*

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

436

#### 437 *Grooming trajectories over time predicted new sharing*

438 The age composition of new potential relationships affected the pre-donation grooming  
439 rate trajectories. For adult past strangers, the grooming probabilities increased for all recipients,

440 including those that never donated (OR = 1.12,  $p = 0.004$ ), and they increased significantly faster  
441 for grooming recipients that later donated (OR = 1.49,  $p < 0.0002$ ; interaction: OR = 1.45,  $p =$   
442 0.032, Figure 1b). For new potential relationships with captive-born bats, however, the grooming  
443 probabilities actually decreased for grooming recipients that never donated (OR = 0.9,  $p = 0.01$ ),  
444 but tended to increase for recipients that did later donate (OR = 1.32,  $p = 0.051$ ; interaction: OR  
445 = 1.70,  $p < 0.0002$ ), Figure 1c).

446

#### 447 *Grooming before versus after new sharing*

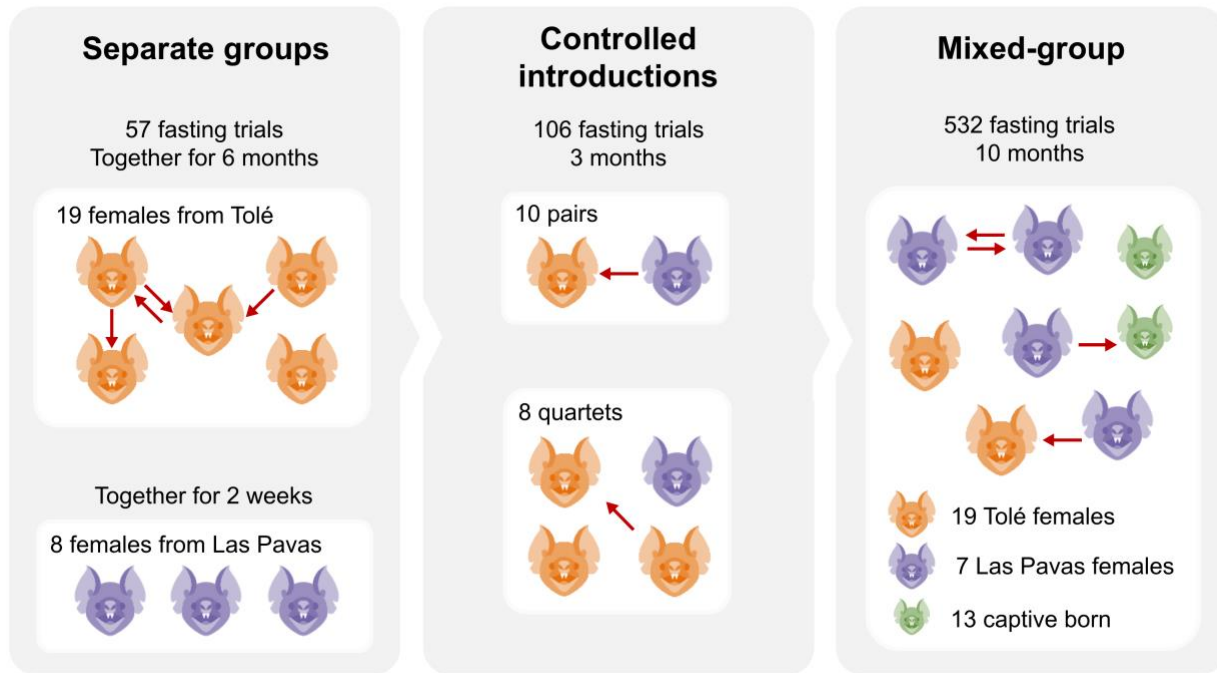
448 Grooming increased before but not after first donations in new relationships. The same  
449 pattern (Fig. 2) was found in new relationships between adults (interaction: OR = 1.60,  $p =$   
450 0.033; before: OR = 1.49,  $p = 0.024$ ; after: OR = 1.01,  $p = 0.88$ ) and in new relationships with  
451 captive-born bats (interaction: OR = 1.7,  $p = 0.0004$ ; before: OR = 1.32,  $p = 0.010$ ; after: OR =  
452 0.92,  $p = 0.6$ ).

453

#### 454 *Reciprocal development of food sharing*

455 Thirteen of the previously unfamiliar pairs donated food in both directions during the  
456 study period, a greater number than expected if new donations were random ( $p < 0.0002$ , 95% CI  
457 of expected relationships to be bidirectional = 1 to 9). The proportion of all previous trials in  
458 which bat A fed B predicted the occurrence of the first new reciprocal donation from bat B to A  
459 (OR = 6.00,  $n = 235$ ,  $p = 0.017$ ).

460 **SI Figures and Tables**

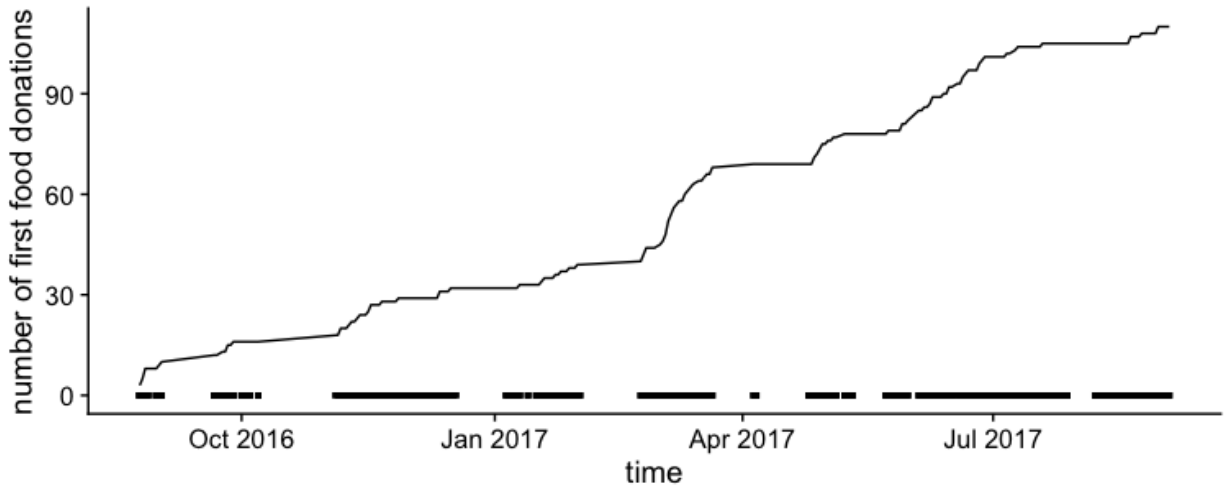


461

462 **Figure S1 | Experiment overview**

463 To see how vampire bats form new social bonds, we induced and sampled food sharing and  
464 grooming events between bats that are either previously familiar or unfamiliar. For details of  
465 controlled introductions, see SI Table 2. Icons from icons8.com used under a Linkware license.

466



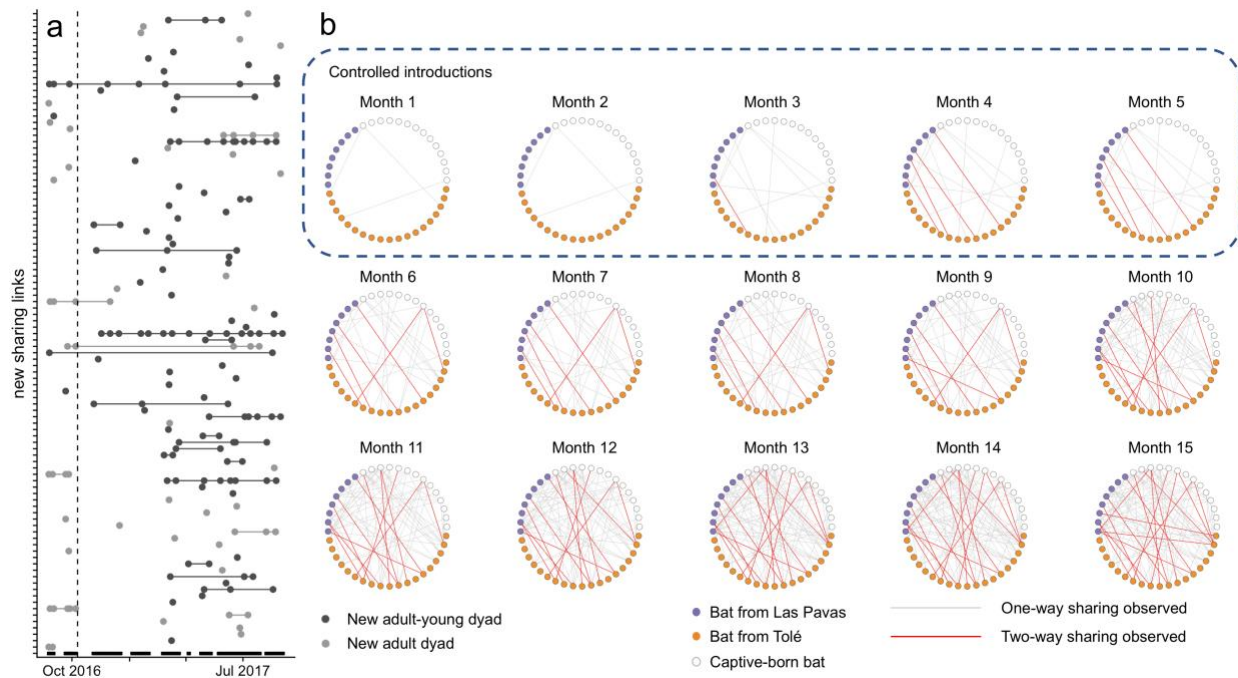
467

468 **Figure S2 | First food donations over time**

469 New food-sharing relationships accumulated gradually over time. Black rectangles above X-axis

470 show the occurrence of fasting trials.

471



472

### 473 **Figure S3 | Gradual development of new food-sharing relationships**

474 Panel A shows food donations (points) over time (x-axis) within new actor-receiver relationships  
475 (y-axis) between two adult females (grey points) or with a captive-born bat (black points).

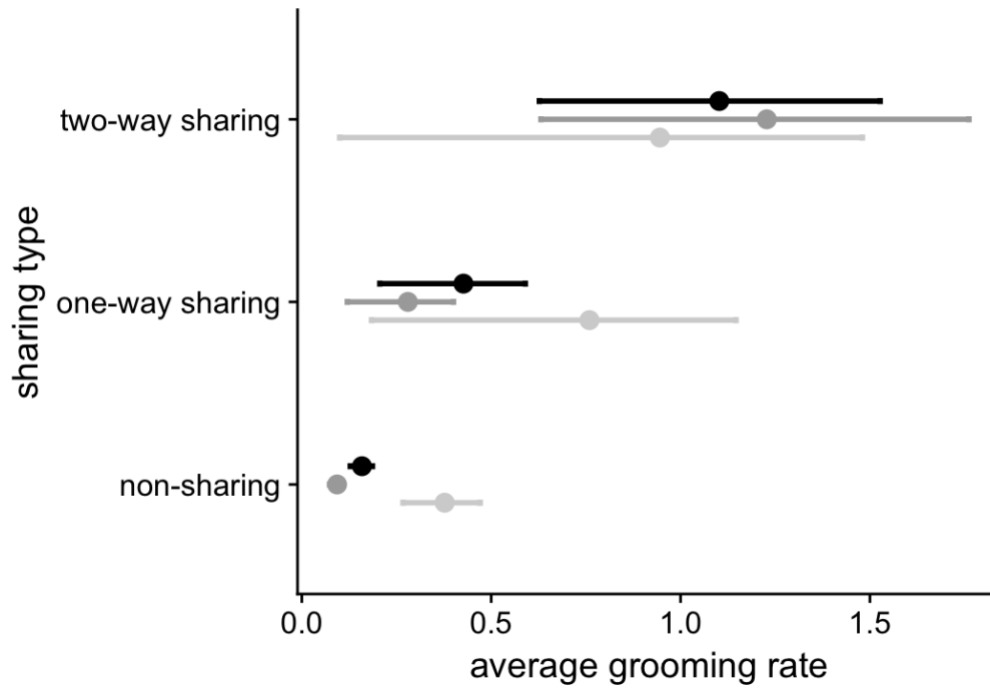
476 Repeated dyadic donations are connected by horizontal lines. The end of the controlled  
477 introduction period, after which all bats could interact freely, is shown by the vertical dotted line.

478 Black rectangles above the x-axis show the fasting trials, when new donations could be observed.

479 Panel B shows the monthly formation of the food-sharing network between Las Pavas bats  
480 (orange), Tolé bats (purple), and captive-born bats (white). Grey edges show one-way sharing  
481 and red edges show two-way sharing. Two-way sharing occurred more often than expected by  
482 chance (see results).

483



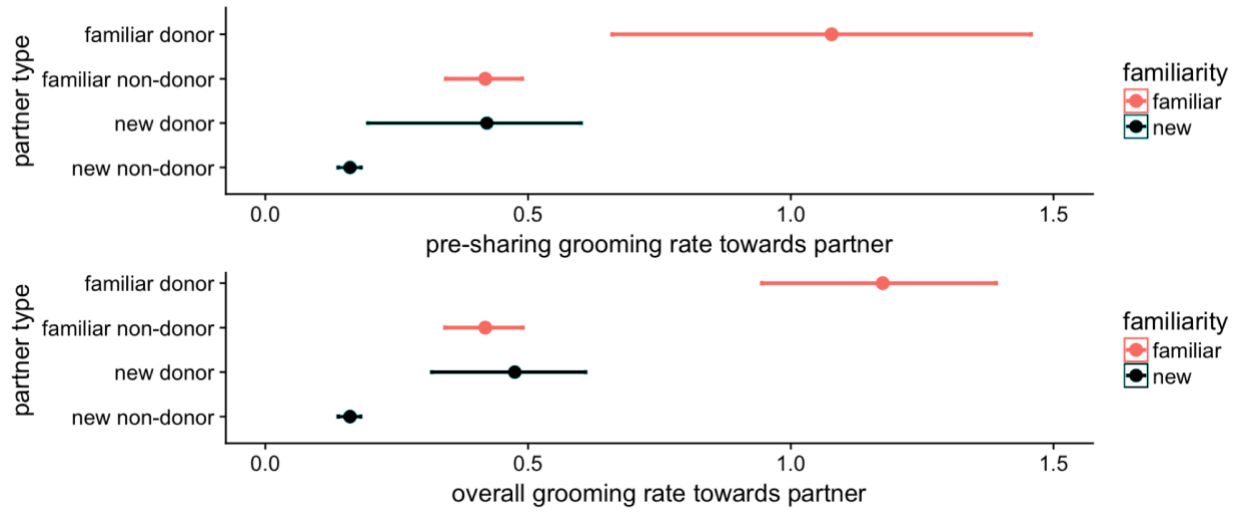


484

485 **Figure S4 | Dyadic grooming rates predict new food-sharing relationships.**

486 Mean within-dyad grooming rates, with bootstrapped 95% confidence intervals, are shown for  
487 three possible outcomes (y-axis) and for all potential relationships (black), potential relationships  
488 with captive-born bats (grey), and potential relationships between adult strangers (light grey).

489



490

491

492 **Figure S5 | Grooming rates are highest towards familiar donors.** Mean grooming rates, with

493 bootstrapped 95% confidence intervals, are shown towards each partner type (y-axis), for the

494 days before first donation (top) and for all days (bottom).

495

496 **Table S1 | Glossary.**

497 Definition of terms used in the text.

<b>Term</b>	<b>Definition</b>
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.

498

499

500 **Table S2 | Controlled introductions**

501 The same bats were used in multiple introductions. Bats were moved to and from groups to make  
 502 new combinations or because of health issues (pregnancy, weight loss). Bats not in a small cage  
 503 group during controlled introduction trials were kept with familiar individuals in a flight cage.  
 504 For preliminary observations, we also conducted seven one-day controlled introductions on  
 505 2016-07-02 without fasting trials that are not shown here.

506

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

507

508

509 **Summary of supplied data**

510 **Data S1. genotypes.csv**

511 Microsatellite genotypes used to assess relatedness.

512 **Data S2. vampire\_maternal\_kinship.csv**

513 Maternal pedigree data

514 **Data S3. new\_bonds\_data.Rdata**

515 Food sharing and allogrooming data

516 **Script S1. new\_bonds\_analysis15.R**

517 R script for analyzing data

518

519 **Temporary link for reviewers:**

520 [https://www.dropbox.com/sh/5z1tb1zv75v7fqt/AAA4D7fkeVIT7V\\_SArvy8D51a?dl=0](https://www.dropbox.com/sh/5z1tb1zv75v7fqt/AAA4D7fkeVIT7V_SArvy8D51a?dl=0)

521