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Title:

Development of new food-sharing relationships among nonkin vampire bats

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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 β , 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.

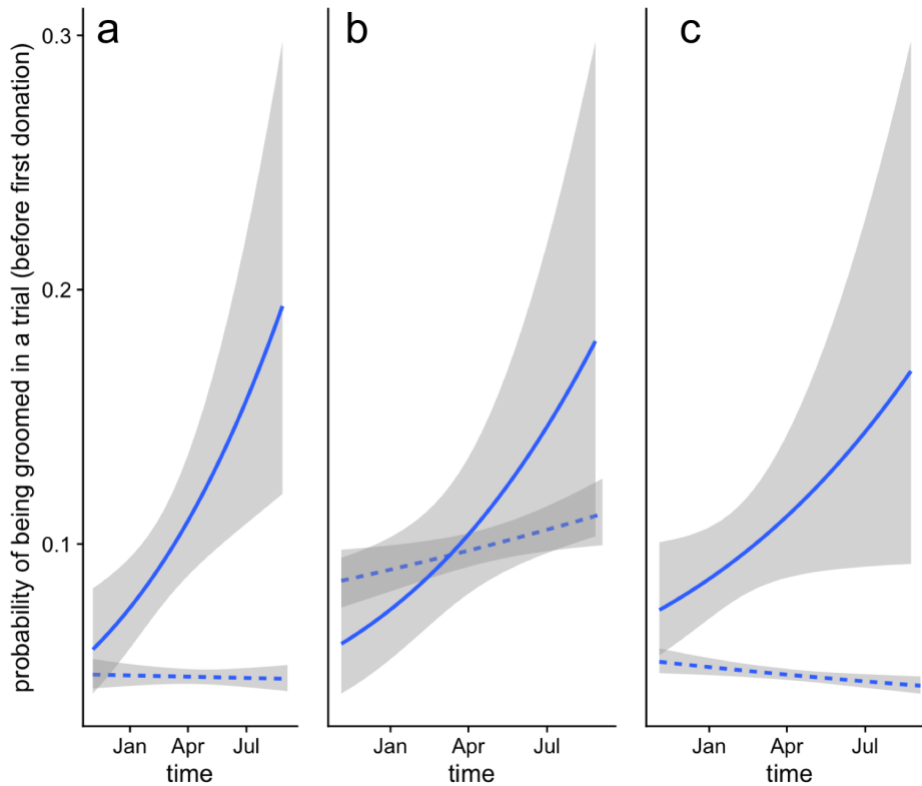
154 The empirical evidence for the ‘raise-the-stakes’ model (15) has been scarce in part
155 because it is a variation on the classic ‘tit-for-tat’ strategy in the iterated prisoner’s dilemma (1),
156 a model which is difficult to test unambiguously using natural forms of cooperation (4, 32).
157 Contingently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



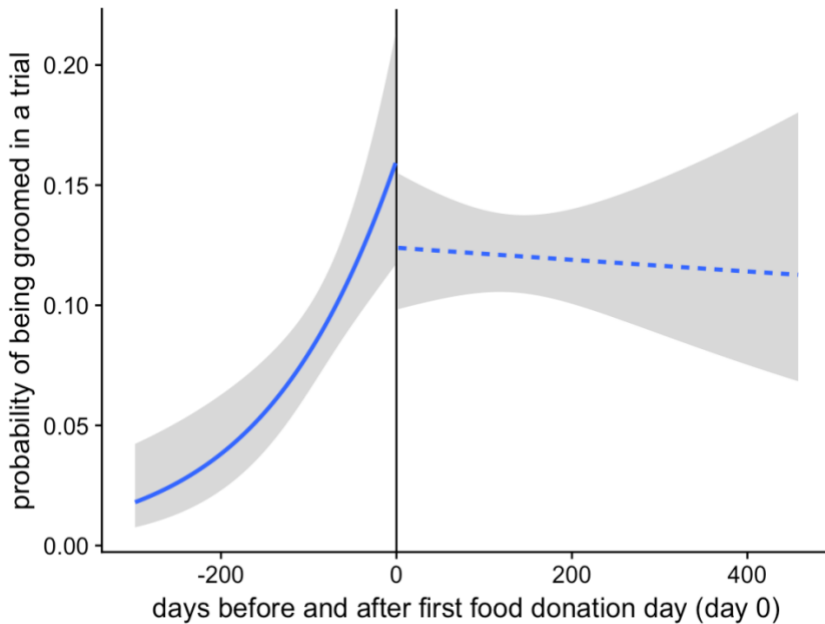
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184 **Fig. 1. Increasing A-to-B grooming led to new B-to-A food-sharing relationships.** In cases
185 where a new food-sharing relationship formed (solid line), the grooming rate towards the future
186 donor increased over time before the first donation 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

306 **References**

- 307 1 Axelrod R & Hamilton WD (1981) The evolution of cooperation. *Science* 211:1390-1396.
- 308 2 Noë R & Hammerstein P (1994) Biological markets: supply and demand determine the effect of
309 partner choice in cooperation, mutualism and mating. *Behav. Ecol. Sociobiol.* 35:1-11.
- 310 3 Trivers RL (1971) The evolution of reciprocal altruism. *Q. Rev. Biol.* 46:35-57.
- 311 4 Carter GG (2014) The reciprocity controversy. *Anim. Behav. Cogn.* 1(3):368-386.
- 312 5 Schweinfurth MK & Taborsky M (2018) Reciprocal trading of different commodities in Norway rats.
313 *Curr. Biol.* 28:1-6.
- 314 6 Rutte C & Taborsky M (2008) The influence of social experience on cooperative behaviour of rats
315 (*Rattus norvegicus*): direct vs generalised reciprocity. *Behav. Ecol. Sociobiol.* 62(4):499-505.
- 316 7 Fruteau C, Voelkl B, van Damme E, & Noë R (2009) Supply and demand determine the market value
317 of food providers in wild vervet monkeys. *Proc. Natl. Acad. Sci. USA* 106:12007-12012.
- 318 8 Samuni L, et al. (2018) Social bonds facilitate cooperative resource sharing in wild chimpanzees.
319 *Proc. R. Soc. B* 285(1888).
- 320 9 Seyfarth RM & Cheney DL (2012) The evolutionary origins of friendship. *Ann. Rev. Psych.* 63:153-
321 177.

- 322 10 Silk JB, et al. (2010) Strong and consistent social bonds enhance the longevity of female baboons.
323 Curr. Biol. 20(15):1359-1361.
- 324 11 Silk JB, et al. (2009) The benefits of social capital: close social bonds among female baboons
325 enhance offspring survival. Proc. R. Soc. B 276(1670):3099-3104.
- 326 12 Silk JB, Alberts SC, & Altmann J (2003) Social bonds of female baboons enhance infant survival.
327 Science 302(5648):1231-1234.
- 328 13 Connor RC (2010) Cooperation beyond the dyad: on simple models and a complex society. Philos.
329 Trans. R. Soc. Lond., Ser. B: Biol. Sci. 365(1553):2687-2697.
- 330 14 Carter GG, Farine DR, & Wilkinson GS (2017) Social bet-hedging in vampire bats. Biol. Lett.
331 13(5):20170112.
- 332 15 Roberts G & Sherratt TN (1998) Development of cooperative relationships through increasing
333 investment. Nature 394(6689):175-179.
- 334 16 Borgeaud C & Bshary R (2015) Wild vervet monkeys trade tolerance and specific coalitionary
335 support for grooming in experimentally induced conflicts. Curr. Biol. 25(22):3011-3016.
- 336 17 Bshary R (2002) Building up relationships in asymmetric co-operation games between the cleaner
337 wrasse *Labroides dimidiatus* and client reef fish. Behav. Ecol. Sociobiol. 52(5):365-371.
- 338 18 Kaburu SS & Newton-Fisher NE (2013) Social instability raises the stakes during social grooming
339 among wild male chimpanzees. Anim. Behav. 86(3):519-527.
- 340 19 Barrett L, Henzi SP, Weingrill T, Lycett JE, & Hill RA (2000) Female baboons do not raise the stakes
341 but they give as good as they get. Anim. Behav. 59(4):763-770.
- 342 20 Manson JH, Navarrete CD, Silk JB, & Perry S (2004) Time-matched grooming in female primates?
343 New analyses from two species. Anim. Behav. 67(3):493-500.
- 344 21 Fruteau C, Lemoine S, Hellard E, van Damme E, & Noë R (2011) When females trade grooming for
345 grooming: Testing partner control and partner choice models of cooperation in two species of
346 primates. Anim. Behav. 81:1223-1230.

- 347 22 Roberts G & Renwick JS (2003) The development of cooperative relationships: an experiment. Proc.
348 R. Soc. B 270(1530):2279-2283.
- 349 23 Van den Bergh B & Dewitte S (2006) The robustness of the " Raise-The-Stakes" strategy: Coping
350 with exploitation in noisy Prisoner's Dilemma Games. *Evol. Hum. Behav.* 27(1):19-28.
- 351 24 Wilkinson GS (1986) Social grooming in the common vampire bat, *Desmodus rotundus*. *Anim.*
352 *Behav.* 34:1880-1889.
- 353 25 Wilkinson GS (1984) Reciprocal food sharing in the vampire bat. *Nature* 308:181-184.
- 354 26 Delpietro H, Russo R, Carter G, Lord R, & Delpietro G (2017) Reproductive seasonality, sex ratio
355 and philopatry in Argentina's common vampire bats. *R. Soc. Open Sci.* 4(4):160959.
- 356 27 Wilkinson GS (1985) The social organization of the common vampire bat: II. Mating system, genetic
357 structure, and relatedness. *Behav. Ecol. Sociobiol.* 17:123-134.
- 358 28 Carter GG, Wilkinson GS, & Page RA (2017) Food-sharing vampire bats are more nepotistic under
359 conditions of perceived risk. *Behav. Ecol.* 28(2):565-569.
- 360 29 Carter GG & Wilkinson GS (2015) Social benefits of non-kin food sharing by female vampire bats.
361 *Proc. R. Soc. B* 282(1819):20152524-20152524.
- 362 30 Carter GG & Wilkinson G (2013) Does food sharing in vampire bats demonstrate reciprocity?
363 *Commun. Integr. Biol.* 6(6):e25783.
- 364 31 Clutton-Brock T (2009) Cooperation between non-kin in animal societies. *Nature* 462:51-57.
- 365 32 Noë R (2006) Cooperation experiments: coordination through communication versus acting apart
366 together. *Anim. Behav.* 71:1-18.
- 367 33 Fournier S & Yao JL (1997) Reviving brand loyalty: A reconceptualization within the framework of
368 consumer-brand relationships. *International Journal of Research in Marketing* 14(5):451-472.
- 369 34 Spunt RP & Adolphs R (2017) A new look at domain specificity: insights from social neuroscience.
370 *Nature Reviews Neuroscience* 18(9):559-567.
- 371 35 Behrens TEJ, Hunt LT, Woolrich MW, & Rushworth MFS (2008) Associative learning of social
372 value. *Nature* 456(7219):245-249.

- 373 36 King-Casas B, et al. (2005) Getting to Know You: Reputation and Trust in a Two-Person Economic
374 Exchange. *Science* 308(5718):78-83.
- 375 37 Ihle M, Kempnaers B, & Forstmeier W (2015) Fitness benefits of mate choice for compatibility in a
376 socially monogamous species. *PLoS Biol.* 13(9):e1002248.
- 377 38 Kern JM & Radford AN (2018) Experimental evidence for delayed contingent cooperation among
378 wild dwarf mongooses. *Proc Natl Acad Sci U S A* 115(24):6255-6260.
- 379 39 Kenny E, Birkhead TR, & Green JP (2017) Allopreening in birds is associated with parental
380 cooperation over offspring care and stable pair bonds across years. *Behav. Ecol.* 28(4):1142-1148.
- 381 40 Grutter AS (2004) Cleaner fish use tactile dancing behavior as a preconflict management strategy.
382 *Curr. Biol.* 14(12):1080-1083.
- 383 41 Soares MC, Oliveira RF, Ros AF, Grutter AS, & Bshary R (2011) Tactile stimulation lowers stress in
384 fish. *Nat. Comm.* 2:534.
- 385 42 Schirmer A, Jesuthasan SJ, & Mathuru A (2013) Tactile stimulation reduces fear in fish. *Front.*
386 *Behav. Neuro.* 7:167.
- 387 43 Radford AN (2012) Post-allogrooming reductions in self-directed behaviour are affected by role and
388 status in the green woodhoopoe. *Biol. Lett.* 8(1):24-27.
- 389 44 Radford AN (2011) Preparing for battle? Potential intergroup conflict promotes current intragroup
390 affiliation. *Biol. Lett.* 7(1):26-29.
- 391 45 Alarcón-Nieto G, et al. (2018) An automated barcode tracking system for behavioural studies in birds.
392 *Meth. Ecol. Evol.* 9(6):1536-1547.

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: β
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*

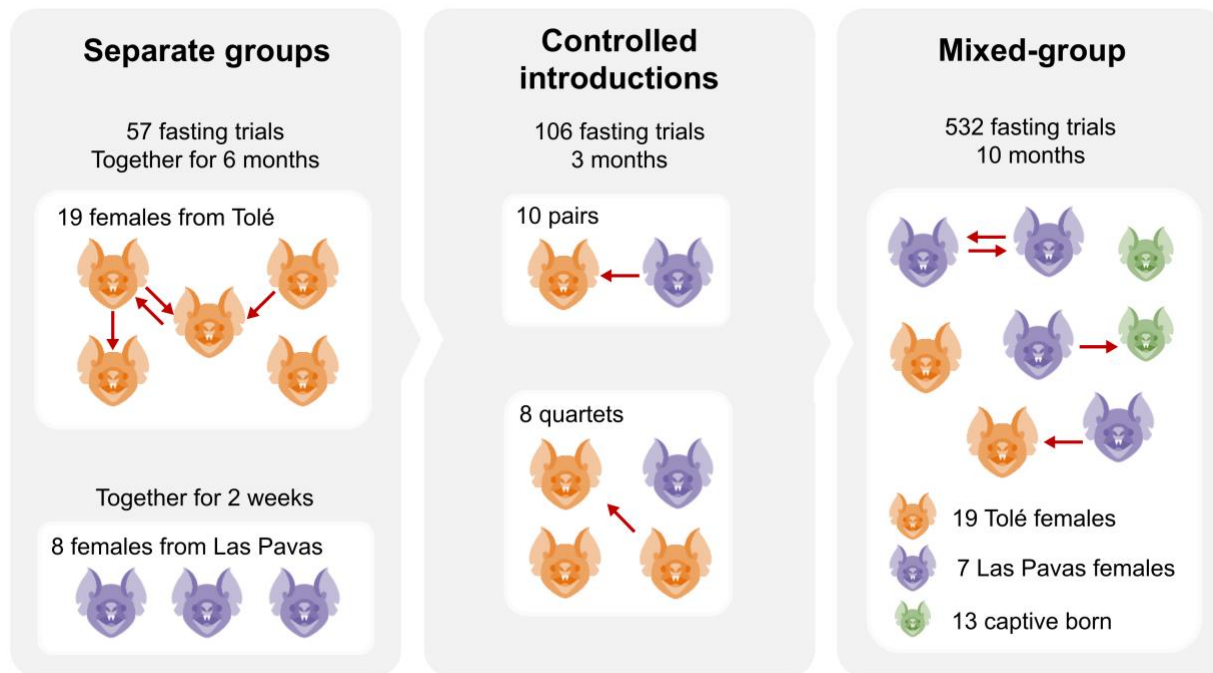
448 Grooming increased before but not after first donations in new relationships. The same
449 pattern (Fig. 2) was found in new relationships between adults (interaction: OR = 1.60, $p =$
450 0.033; before: OR = 1.49, $p = 0.024$; after: OR = 1.01, $p = 0.88$) and in new relationships with
451 captive-born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

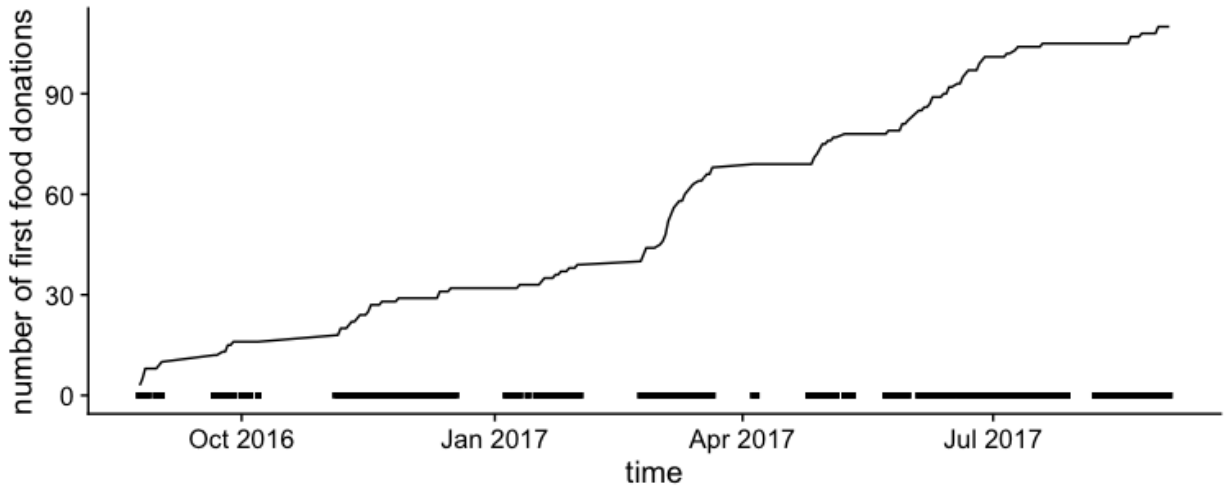


461

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



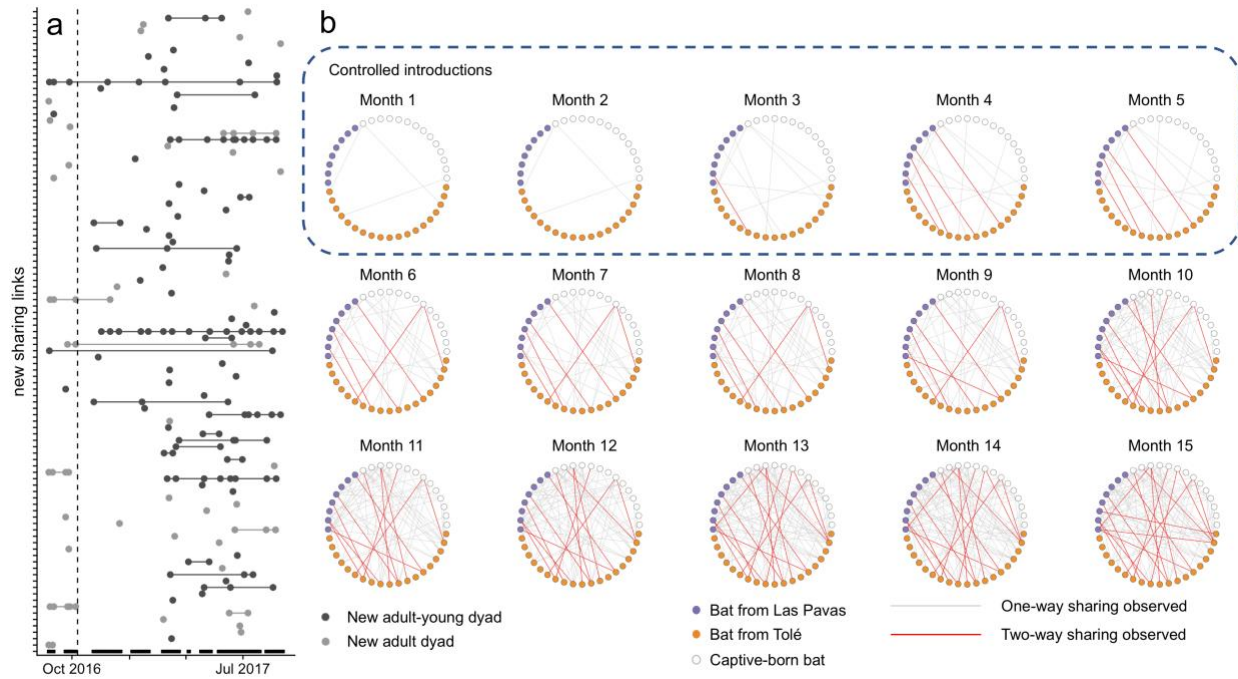
467

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.

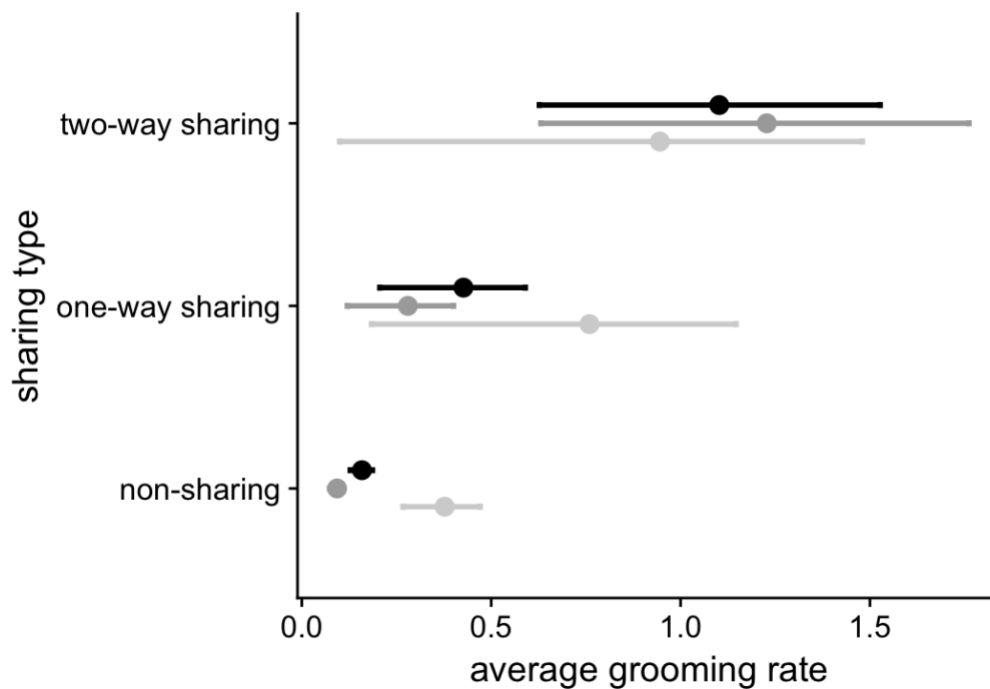
479 Panel B shows the monthly formation of the food-sharing network between Las Pavas bats

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

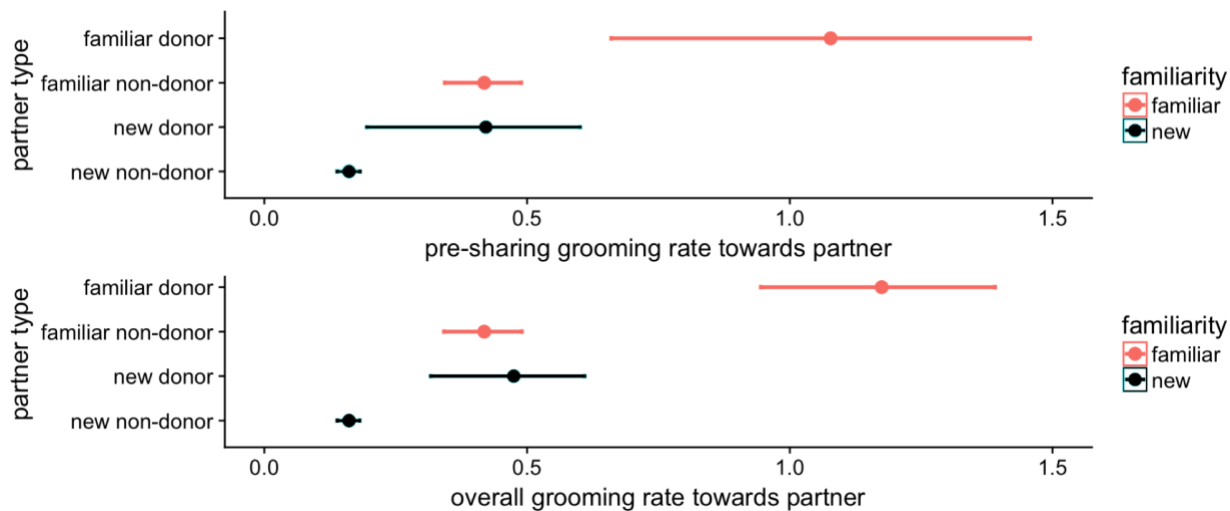


484

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.

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.

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/5z1tb1zv5v7fqt/AAA4D7fkeVIT7V_SArvy8D51a?dl=0

521