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Title:

Development of new food-sharing relationships among nonkin vampire bats

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Abstract

In an individualized animal society, social bonds can foster cooperation and enhance survival and reproduction. Cooperative bonds often exist among kin, but nonkin can also develop high-investment cooperative bonds that share similarities with human friendship. How do such bonds form? One theory suggests that strangers should 'test the waters' of a new relationship by making small initial cooperative investments and gradually escalating them with good partners. This 'raising-the-stakes' strategy is demonstrated by human strangers in short-term economic games, but it remains unclear whether it applies to helping in a natural long-term social bond. Here we show evidence that unfamiliar vampire bats (*Desmodus rotundus*) selectively escalate low-cost investments in allogrooming before developing higher-cost food-sharing relationships. We introduced females from geographically distant sites in pairs or groups and observed that bats 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% of female pairs, typically over 10-15 months, and developed faster when strangers lacked alternative familiar partners. A gradual grooming-to-sharing transition among past strangers suggests that 'raising the stakes' might be more evident when tracking multiple cooperative behaviours as new relationships form, rather than measuring a single behavior in an established relationship. 'Raising the stakes' could play a similar underappreciated role across a broader spectrum of social decisions with long-term consequences, such as joining a new social group or forming a long-term pair-bond.

Keywords: cooperation, social relationships, vampire bats

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Significance statement

Vampire bats form long-term cooperative social bonds that involve reciprocal food sharing. How do two unrelated bats go from being strangers to having a high-investment food-sharing relationship? We introduced unfamiliar bats and 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 did not have access to their original groupmates. The bats that formed new food-sharing relationships had a history of escalating reciprocal grooming up until the food sharing began. Our finding that unfamiliar nonkin vampire bats appear to gradually and selectively transition from low-cost to high-cost cooperative behaviors is the first evidence that nonhuman individuals 'raise the stakes' when forming new cooperative relationships.

Text

Animal societies are fundamentally shaped by repeated interactions among individuals over time, and repeated interactions allow for individuals to choose to cooperate based on their social history across different partners (1-3). Experiments have shown that organisms as diverse as animals, plants, and fungi can prevent exploitation by shifting cooperative investments towards partners that provide better reciprocal returns (4-7). However, a greater challenge has been testing if and how individuals prevent exploitation while forming stable social bonds. Although long-term bonds are known to enhance survival and reproduction (8-12), it remains unclear how they form. How do complete strangers form a long-term cooperative relationship?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

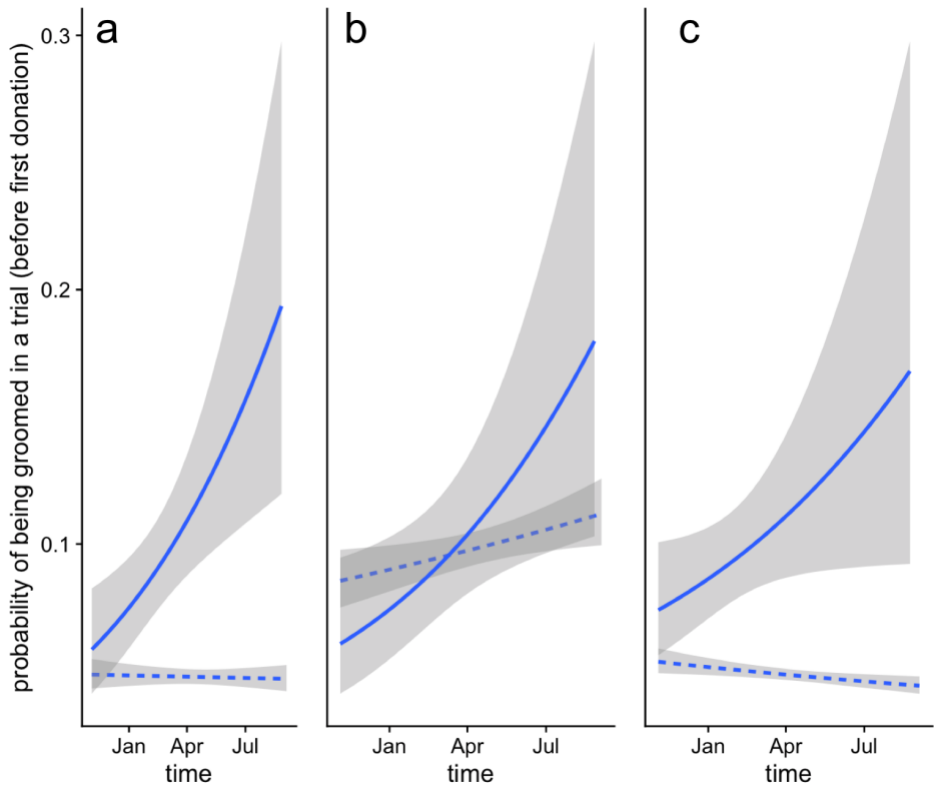

Fig. 1. Increasing A-to-B grooming led to new B-to-A food-sharing relationships. In cases where a new food-sharing relationship formed (solid line), the grooming rate towards the future donor increased over time before the first donation occurred (OR = 1.40, n = 33, p<0.0002), but the grooming rate towards 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

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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).

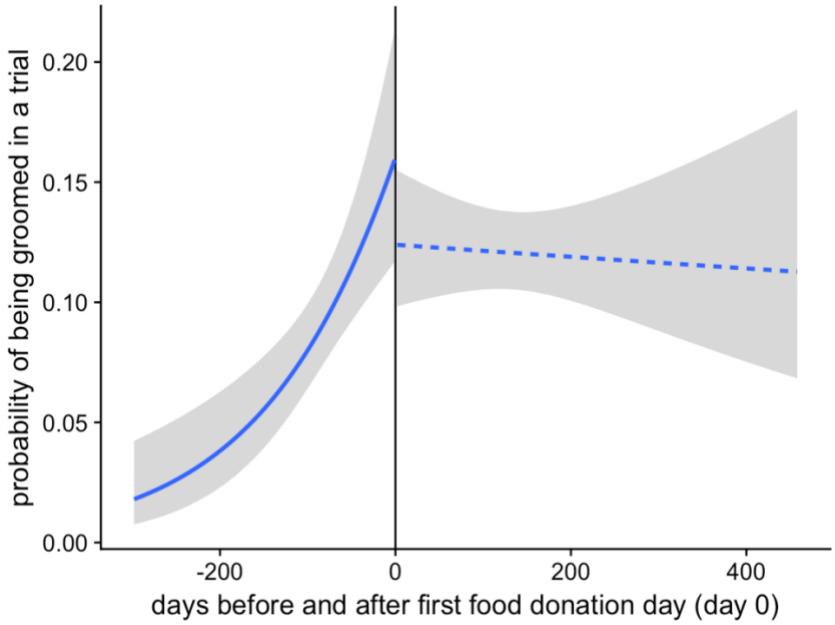

Fig. 2. Grooming rates increased before, but not after, new food-sharing occurred. The probability of a focal bat grooming a new future donor in a 1-h trial (y-axis) increased before the first day that the donor fed the focal bat (i.e. 'day zero'; OR = 1.4, p = 0.0004), but not after day zero (OR = 0.946, 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).

Methods

Animals

We used 41 common vampire bats (*Desmodus rotundus*) as subjects, including 19 female bats captured exiting a roost in Tolé, Panamá; 8 female bats captured foraging at a cattle pasture in Las Pavas, Panamá about ~215 km from Tolé; and 14 captive-born bats (8 females, 6 males).

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

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

Experimental design

We induced 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 sharing

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

We conducted fasting trials in each group during three experimental phases (SI 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 past strangers 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 sharing between previous strangers (SI 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 described above (19 Tolé, 7 Las Pavas, and 14 captive-born bats). These introductions in this combined group provided 11,823 more opportunities for new sharing.

Statistical analyses

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

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

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

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

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

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

Data 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;

References

1 Axelrod R & Hamilton WD (1981) The evolution of cooperation. Science 211:1390-1396.

2 Noë R & Hammerstein P (1994) Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behav. Ecol. Sociobiol. 35:1-11.

3 Trivers RL (1971) The evolution of reciprocal altruism. Q. Rev. Biol. 46:35-57.

4 Carter GG (2014) The reciprocity controversy. Anim. Behav. Cogn. 1(3):368-386.

5 Schweinfurth MK & Taborsky M (2018) Reciprocal trading of different commodities in Norway rats. Curr. Biol. 28:1-6.

6 Rutte C & Taborsky M (2008) The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): direct vs generalised reciprocity. Behav. Ecol. Sociobiol. 62(4):499-505.

7 Fruteau C, Voelkl B, van Damme E, & Noë R (2009) Supply and demand determine the market value of food providers in wild vervet monkeys. Proc. Natl. Acad. Sci. USA 106:12007-12012.

8 Samuni L, et al. (2018) Social bonds facilitate cooperative resource sharing in wild chimpanzees. Proc. R. Soc. B 285(1888).

9 Seyfarth RM & Cheney DL (2012) The evolutionary origins of friendship. Ann. Rev. Psych. 63:153-177.

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10 Silk JB, et al. (2010) Strong and consistent social bonds enhance the longevity of female baboons. Curr. Biol. 20(15):1359-1361.

11 Silk JB, et al. (2009) The benefits of social capital: close social bonds among female baboons enhance offspring survival. Proc. R. Soc. B 276(1670):3099-3104.

12 Silk JB, Alberts SC, & Altmann J (2003) Social bonds of female baboons enhance infant survival. Science 302(5648):1231-1234.

13 Connor RC (2010) Cooperation beyond the dyad: on simple models and a complex society. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 365(1553):2687-2697.

14 Carter GG, Farine DR, & Wilkinson GS (2017) Social bet-hedging in vampire bats. Biol. Lett. 13(5):20170112.

15 Roberts G & Sherratt TN (1998) Development of cooperative relationships through increasing investment. Nature 394(6689):175-179.

16 Borgeaud C & Bshary R (2015) Wild vervet monkeys trade tolerance and specific coalitionary support for grooming in experimentally induced conflicts. Curr. Biol. 25(22):3011-3016.

17 Bshary R (2002) Building up relationships in asymmetric co-operation games between the cleaner wrasse Labroides dimidiatus and client reef fish. Behav. Ecol. Sociobiol. 52(5):365-371.

18 Kaburu SS & Newton-Fisher NE (2013) Social instability raises the stakes during social grooming among wild male chimpanzees. Anim. Behav. 86(3):519-527.

19 Barrett L, Henzi SP, Weingrill T, Lycett JE, & Hill RA (2000) Female baboons do not raise the stakes but they give as good as they get. Anim. Behav. 59(4):763-770.

20 Manson JH, Navarrete CD, Silk JB, & Perry S (2004) Time-matched grooming in female primates? New analyses from two species. Anim. Behav. 67(3):493-500.

21 Fruteau C, Lemoine S, Hellard E, van Damme E, & Noë R (2011) When females trade grooming for grooming: Testing partner control and partner choice models of cooperation in two species of primates. Anim. Behav. 81:1223-1230.

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22 Roberts G & Renwick JS (2003) The development of cooperative relationships: an experiment. Proc. R. Soc. B 270(1530):2279-2283.

23 Van den Bergh B & Dewitte S (2006) The robustness of the" Raise-The-Stakes" strategy: Coping with exploitation in noisy Prisoner's Dilemma Games. Evol. Hum. Behav. 27(1):19-28.

24 Wilkinson GS (1986) Social grooming in the common vampire bat, Desmodus rotundus. Anim. Behav. 34:1880-1889.

25 Wilkinson GS (1984) Reciprocal food sharing in the vampire bat. Nature 308:181-184.

26 Delpietro H, Russo R, Carter G, Lord R, & Delpietro G (2017) Reproductive seasonality, sex ratio and philopatry in Argentina's common vampire bats. R. Soc. Open Sci. 4(4):160959.

27 Wilkinson GS (1985) The social organization of the common vampire bat: II. Mating system, genetic structure, and relatedness. Behav. Ecol. Sociobiol. 17:123-134.

28 Carter GG, Wilkinson GS, & Page RA (2017) Food-sharing vampire bats are more nepotistic under conditions of perceived risk. Behav. Ecol. 28(2):565-569.

29 Carter GG & Wilkinson GS (2015) Social benefits of non-kin food sharing by female vampire bats. Proc. R. Soc. B 282(1819):20152524-20152524.

30 Carter GG & Wilkinson G (2013) Does food sharing in vampire bats demonstrate reciprocity? Commun. Integr. Biol. 6(6):e25783.

31 Clutton-Brock T (2009) Cooperation between non-kin in animal societies. Nature 462:51-57.

32 Noë R (2006) Cooperation experiments: coordination through communication versus acting apart together. Anim. Behav. 71:1-18.

33 Fournier S & Yao JL (1997) Reviving brand loyalty: A reconceptualization within the framework of consumer-brand relationships. International Journal of Research in Marketing 14(5):451-472.

34 Spunt RP & Adolphs R (2017) A new look at domain specificity: insights from social neuroscience. Nature Reviews Neuroscience 18(9):559-567.

35 Behrens TEJ, Hunt LT, Woolrich MW, & Rushworth MFS (2008) Associative learning of social value. Nature 456(7219):245-249.

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36 King-Casas B, et al. (2005) Getting to Know You: Reputation and Trust in a Two-Person Economic Exchange. Science 308(5718):78-83.

37 Ihle M, Kempenaers B, & Forstmeier W (2015) Fitness benefits of mate choice for compatibility in a socially monogamous species. PLoS Biol. 13(9):e1002248.

38 Kern JM & Radford AN (2018) Experimental evidence for delayed contingent cooperation among wild dwarf mongooses. Proc Natl Acad Sci U S A 115(24):6255-6260.

39 Kenny E, Birkhead TR, & Green JP (2017) Allopreening in birds is associated with parental cooperation over offspring care and stable pair bonds across years. Behav. Ecol. 28(4):1142-1148.

40 Grutter AS (2004) Cleaner fish use tactile dancing behavior as a preconflict management strategy. Curr. Biol. 14(12):1080-1083.

41 Soares MC, Oliveira RF, Ros AF, Grutter AS, & Bshary R (2011) Tactile stimulation lowers stress in fish. Nat. Comm. 2:534.

42 Schirmer A, Jesuthasan SJ, & Mathuru A (2013) Tactile stimulation reduces fear in fish. Front. Behav. Neuro. 7:167.

43 Radford AN (2012) Post-allogrooming reductions in self-directed behaviour are affected by role and status in the green woodhoopoe. Biol. Lett. 8(1):24-27.

44 Radford AN (2011) Preparing for battle? Potential intergroup conflict promotes current intragroup affiliation. Biol. Lett. 7(1):26-29.

45 Alarcón-Nieto G, et al. (2018) An automated barcode tracking system for behavioural studies in birds. Meth. Ecol. Evol. 9(6):1536-1547.

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Supplementary Information

Development of new food-sharing relationships

Over 424 days, there was a 52% probability that two bats would form a new grooming relationship, and an 11% probability that two bats would form a new food-sharing relationship (all bats: 111 of 1036 potential relationships, 12154 donation opportunities; all females: 91 of 648 potential relationships, 7504 donation opportunities; wild-caught females: 38 of 243 potential relationships, 3202 donation opportunities). 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 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 fasting trials in the subsequent 10-month period when all the bats from both groups could freely interact (Fig. S3).

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

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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, 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 was true for Tolé bats (sharing: $\beta = 0.11$, $n = 342$, $p = 0.0008$; grooming: $\beta = 0.14$, $n = 460$, $p = 0.028$). During the mixed-group trials, new food-sharing relationships occurred gradually (mean days from introduction until first donation = 247, SD = 95, range = 66 – 556 days; Figure S2) and became more probable over time (OR = 1.56, $n = 3072$, $p = 0.0188$). We lacked the statistical power to test for increases in sharing amounts over time within new dyads; new donations that occurred later in time were not significantly larger ($\beta = 5.6$, $n = 37$, $p = 0.95$).

Isolated pairs started sharing faster

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 to 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, $p = 0.012$).

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,

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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

Thirteen of the previously unfamiliar pairs donated food in both directions during the study period, a greater number than expected if new donations were random (p<0.0002, 95% CI of expected relationships to be bidirectional = 1 to 9). The proportion of all 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).

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SI Figures and Tables

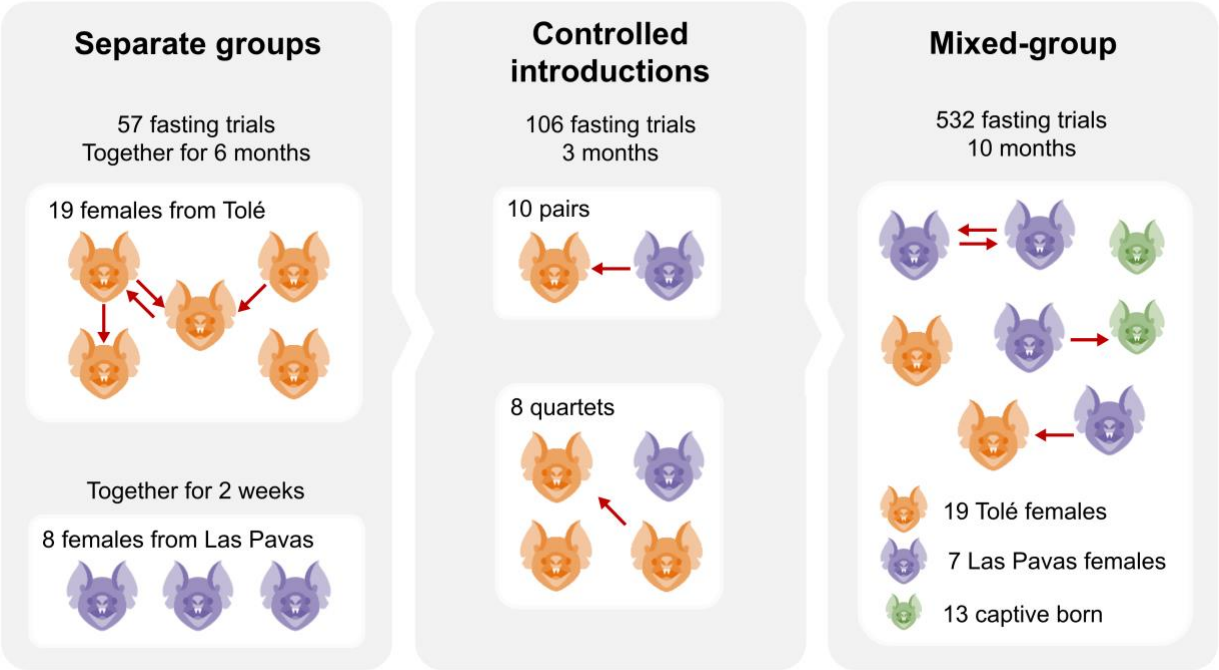

Figure S1 | Experiment overview

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

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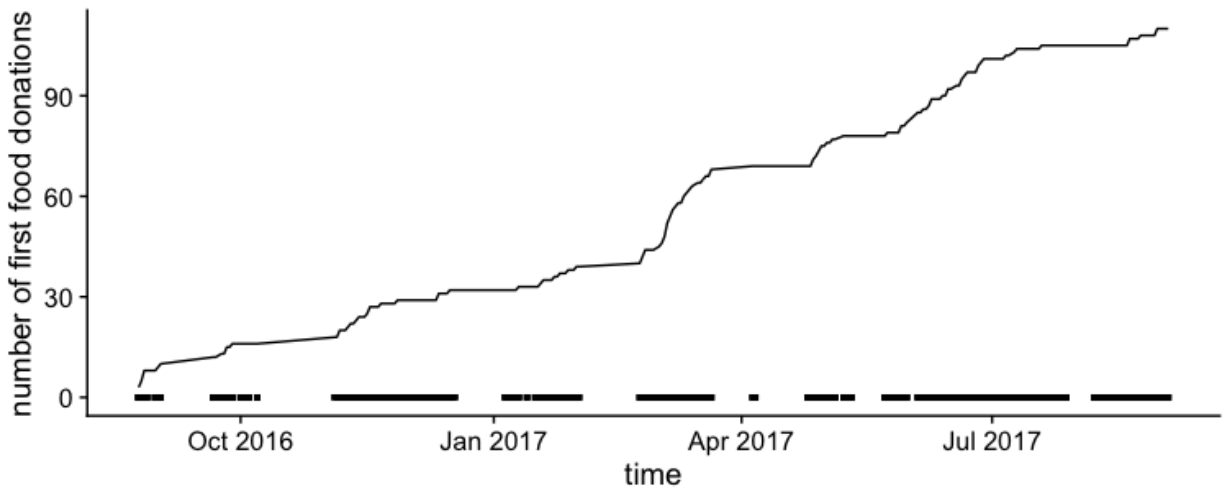

Figure S2 | First food donations over time

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

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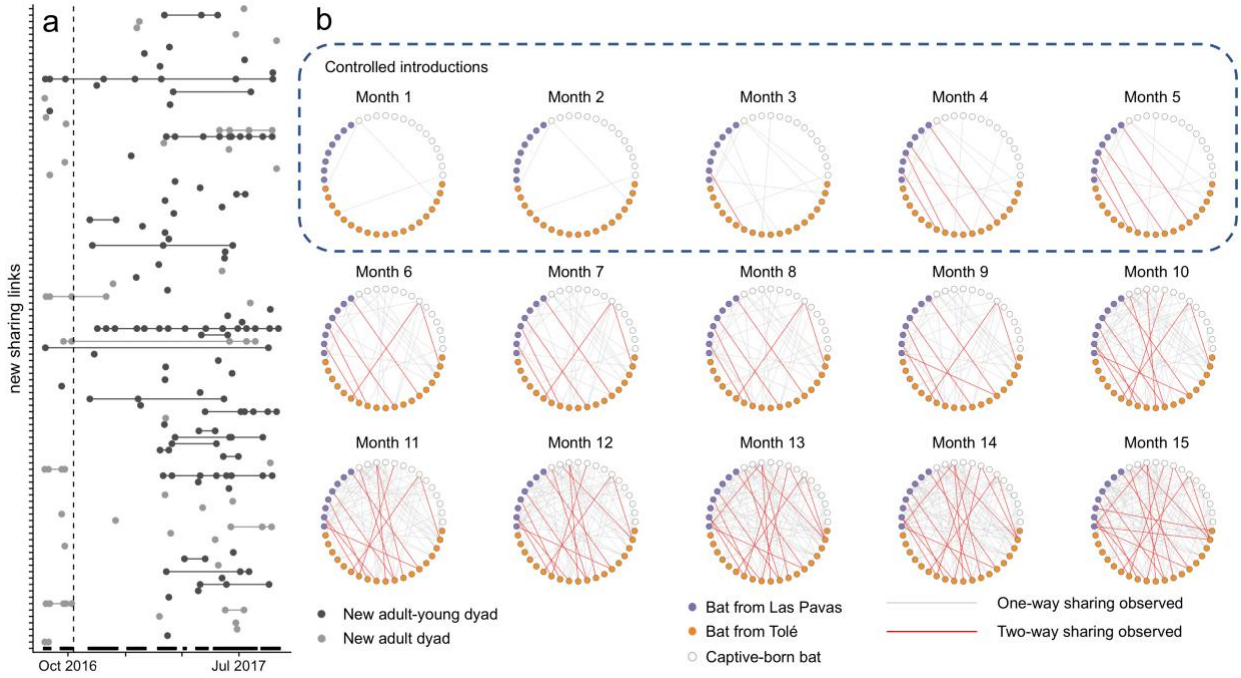

Figure S3 | Gradual development of new food-sharing relationships

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, 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).

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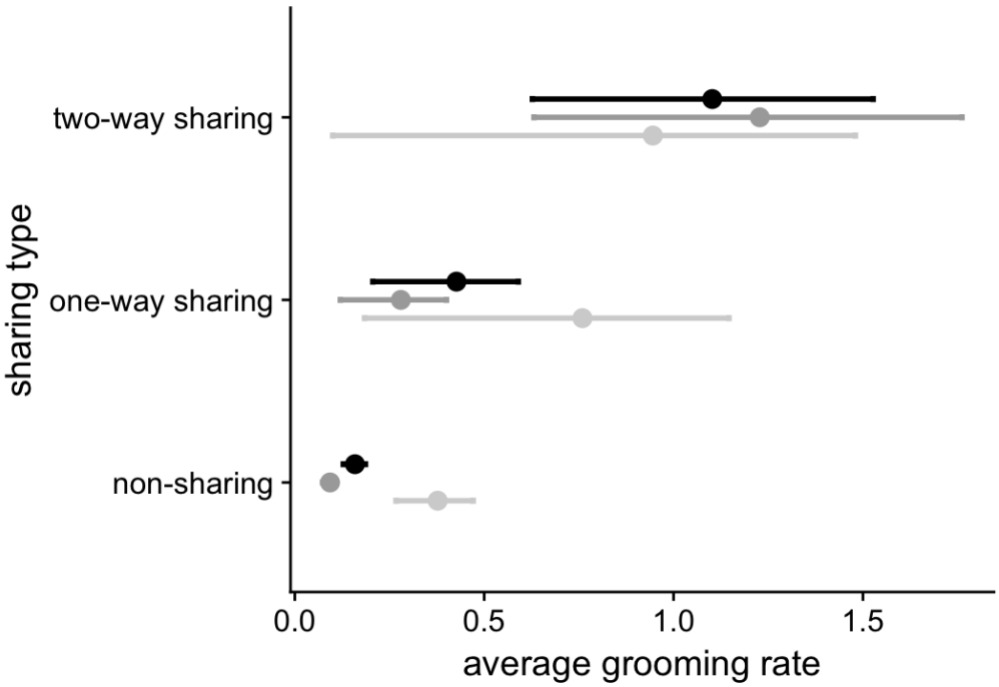

Figure S4 | Dyadic grooming rates predict new food-sharing relationships.

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).

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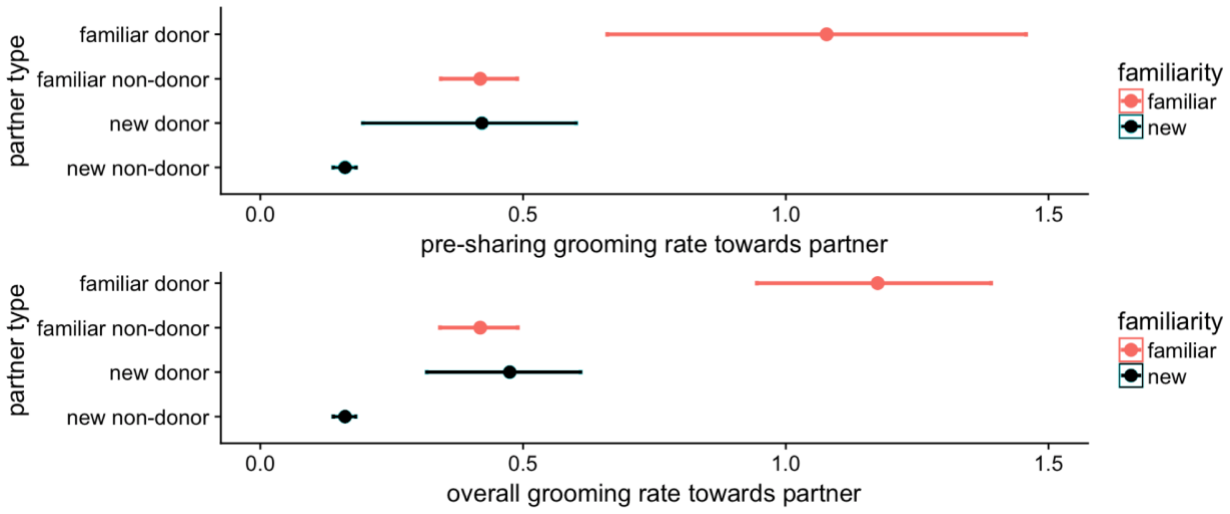

Figure S5 | Grooming rates are highest towards familiar donors. Mean grooming rates, with bootstrapped 95% confidence intervals, are shown towards each partner type (y-axis), for the days before first donation (top) and for all days (bottom).

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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.

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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. For preliminary observations, we also conducted seven one-day controlled introductions on 2016-07-02 without fasting trials that are not shown here.

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

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Summary of supplied data

Data S1. genotypes.csv

Microsatellite genotypes used to assess relatedness.

Data S2. vampire_maternal_kinship.csv

Maternal pedigree data

Data S3. new_bonds_data.Rdata

Food sharing and allogrooming data

Script S1. new_bonds_analysis15.R

R script for analyzing data

Temporary link for reviewers:

https://www.dropbox.com/sh/5z1tb1zvj5v7fqt/AAA4D7fkeVlT7V_SArvy8D51a?dl=0