Report

Current Biology

Vampire Bats that Cooperate in the Lab Maintain Their Social Networks in the Wild

Highlights

- Captive grooming and food sharing in vampire bats predict associations in the wild
- New proximity sensors captured high-resolution social networks in a single tree
- Many social bonds persisted across different physical and social environments
- Both extrinsic constraints and intrinsic partner fidelity cause social structure

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In Brief

Ripperger et al. show that vampire bats maintain their social relationships after a dramatic change in the social and physical environment, from the lab back into the wild. However, the persistence of some, but not all, strong bonds suggests that both partner fidelity and partner switching play a role in stabilizing cooperation in social bonds.

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Report

Vampire Bats that Cooperate in the Lab Maintain Their Social Networks in the Wild

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SUMMARY

Social bonds, maintained by mutual investments of time and energy, have greatly influenced the evolution of social cognition and cooperation in many species [e.g., 1–8]. However, there are two pitfalls regarding "social bonds" as an explanation for social structure and cooperation [1, 9–11]. First, studies often incorrectly assume that frequent association implies partner fidelity based on mutual social preference, but even seemingly complex nonrandom interaction networks can emerge solely from habitat or spatial structure [12–16]. Second, the false appearance of partner fidelity can result from stable options in the "partner market" [1, 9–11, 17]. For instance, individuals might preferentially groom the same partner, even if the decision depends entirely on the immediate costs and benefits rather than relationship history. Given these issues, a key challenge has been testing the extent to which social structure is driven by the intrinsic relationship history versus the extrinsic physical and social environment. If stable bonds exist, they should persist even if the individuals are moved to a dramatically different physical and social environment. We tested this prediction by tracking social relationships among common vampire bats (*Desmodus rotundus*) moved from the lab to the wild. We show that allogrooming and food sharing among female vampire bats induced in captivity over 22 months predicted their assortativity and association rates when we subsequently tracked them in the wild with custom-made high-resolution proximity sensors. The persistence of many relationships across different physical and social environments suggests that social structure is caused by both extrinsic constraints and intrinsic partner fidelity.

RESULTS AND DISCUSSION

Bats in the Test Group Showed Symmetrical Cooperative Relationships in Captivity

To create the test group (n = 23), we captured female vampire bats from a large hollow tree, containing ~200 vampire bats, then housed them together in a captive colony of kin and nonkin females and their captive-born offspring. Mean kinship in this captive test group (r = 0.08) was equal to sampled wild roosts [18, 19]. To measure and strengthen their cooperative relationships, we repeatedly fasted individuals to induce social grooming and regurgitated food sharing, a costly form of cooperation that occurs predominantly among maternal kin but also among nonkin [20–22]. Consistent with past studies [6, 21, 22], food-sharing and social-grooming rates were symmetrical and correlated with each other, even when controlling for kinship. Food given was predicted by food received (β = 0.43, $p < 0.0002$) and kinship (β = 0.18, $p < 0.0002$) or by grooming received (β = 0.57, $p < 0.0002$) and kinship (β = 0.13, p = 0.0004). Grooming given was predicted by grooming received (β = 0.84, $p < 0.0002$) and kinship (β = 0.05, p = 0.029).

Overall, group-level social grooming among the wild-born adults in the test group increased with each month (R^2 = 0.51, p = 0.013), and the mean dyadic grooming rate increased more than expected by chance (by 6 s/trial, n = 272, p = 0.003, 95% CI of the mean change = +3 to +9 s/trial), but this effect was driven by increases in only some dyads. Although fasting trials increase the probability of the fasted subject receiving both food and grooming, we did not detect an increase over time in food donation size (mean = +2 s/trial, 95% CI = −2 to +5 s/trial).

The Previously Captive Test Bats Preferentially Associated with Each Other in the Wild

After measuring dyadic rates of social grooming and food sharing over a period of 22 months in captivity, we then released 23 of the previously captive vampire bats (test group) back into their hollow tree and tracked their social associations in the

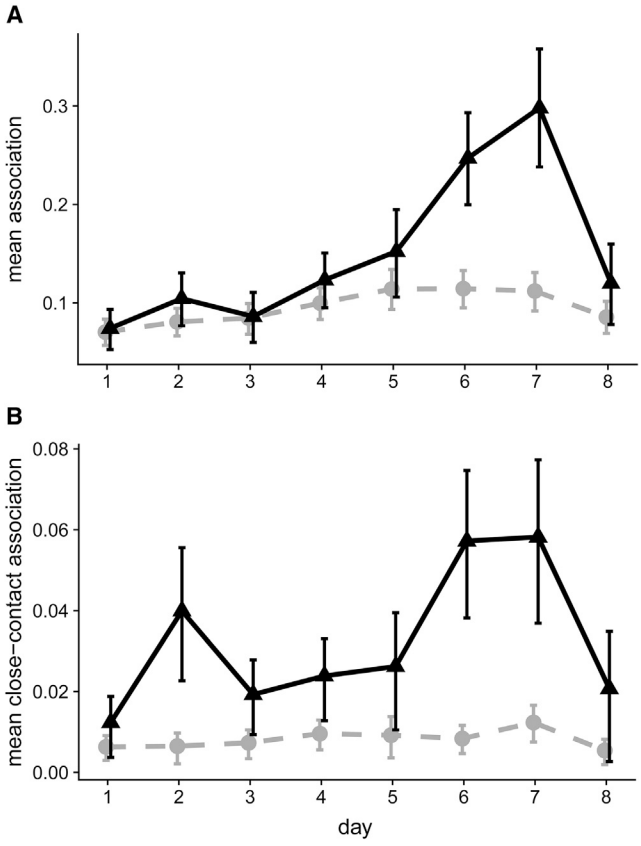

Figure 1. Assortativity of Test Bats over Time Based on Associations or Close-Contact Associations

(A) Associations.

(B) Close-contact associations.

Solid lines show the mean association rates for a test bat and another test bat (ingroup, black solid line) or a control bat (outgroup, gray dashed line). Error bars show bootstrapped 95% CI. See also Figure S1.

field using custom-built "next-generation" proximity sensors [23–25]. As a control group, we also placed the same sensors on 27 additional wild female vampire bats from the same tree. We analyzed roosting associations among all 50 bats for 8 days.

We expected to create assortativity. If the experience of constant association and repeated cooperative interactions in captivity led to stronger relationships in the wild, then bats in the previously captive test group should preferentially roost near each other. As predicted, the test bats had higher associations with each other than with the control bats ($t = 6.39$, $n = 23$ bats, $p < 0.001$; Figures 1 and S1). The association rates among the test bats were also higher than the association rates among the control bats even when excluding the captive-born test bats (mean association, test group = 26.0% [95% CI = 22.7%–29.2%], control group = 18.9% [95% CI = 16.7%–21.0%]; mean close-contact association, test group = 14.7% [95% CI = 12.2%–16.9%], control group = 9.1% [95% CI = 7.9%–10.2%]). We could see evidence for this assortativity in plots of the network and in photographs where small sub-groups of test bats closely associated (Figure S1).

Roosting Associations among Test Bats Were Stable but Less Stable Than among Control Bats

Despite the frequent fission-fusion dynamics of vampire bat colonies (where groups break apart and reconfigure each night when bats individually leave the roost to forage), we expected the bats to re-form similar proximity networks on each day. However, we expected the previously captive test group to have lower stability than the control group in the first few days after their release because their social environment changed far more than the control group. Bats in both the test group and control group roosted in dynamic spatial configurations that were more similar than expected by chance to the previous day, but certainly not identical (Figure 2).

The associations among the previously captive test bats were less stable than the control group in two ways. First, all six captive-born bats departed the study site by the sixth night (Table S1), suggesting a higher departure rate (100% [95% CI = 55%–100%]) compared to the wild-born test bats (24% [7%–50%]) and control bats (19% [6%–38%]). On days 1, 2, and 4, we observed four of the captive-born bats roosting in the tree hollow entrance or just outside of it, both at night and in daytime, and at least one bat had recent bite marks, suggesting a failure to integrate into the group. Second, even after controlling for arrivals and departures, the day-to-day similarities in roosting association were still lower for the test group (mean correlation = 0.46 [0.30–0.64]) than for the control group (mean correlation = 0.75 [0.72–0.77]; Figure 2A). This difference between the groups diminished, however, when testing the stability of close-contact networks (Figure 2B).

Captive Cooperation among Test Bats Predicted Their Roosting Proximity in the Wild

We expected that the dyadic social-grooming and food-sharing rates that we induced under controlled conditions in the lab should predict daily association networks in the wild. As expected, mean dyadic associations among the test bats were predicted by past rates of social grooming ($r = 0.11$, $p < 0.001$) and food sharing ($r = 0.09$, $p = 0.005$) in captivity, and kinship ($r = 0.04$, $p = 0.004$; Figures 3A and 3B). The same was true for close-contact association (social grooming, $r = 0.18$, $p < 0.001$; food sharing, $r = 0.15$, $p < 0.001$; kinship, $r = 0.03$, $p = 0.01$; Figures 3C and 3D). When defining associations using even higher thresholds of signal intensity, the correlations between networks of captive cooperation and wild association increased further (Figure S2).

In addition, we analyzed the overall association network among test bats as the total time together across the 8 days (rather than the average within days). We again found that association was predicted by both social grooming (MRQAP-DSP, associations, $\beta = 0.19$, $p = 0.003$; close-contact association, $\beta = 0.18$, $p = 0.008$) and food sharing (associations, $\beta = 0.10$, $p = 0.048$; close-contact association, $\beta = 0.17$, $p = 0.014$), even after controlling for kinship. Among the wild-born adult bats, individuals spent more time with their top five groomers than expected by chance (mean association = 15.7% of the study period, $n = 17$ bats, $p = 0.0064$). This result remained when using the highest three groomers or the highest groomer.

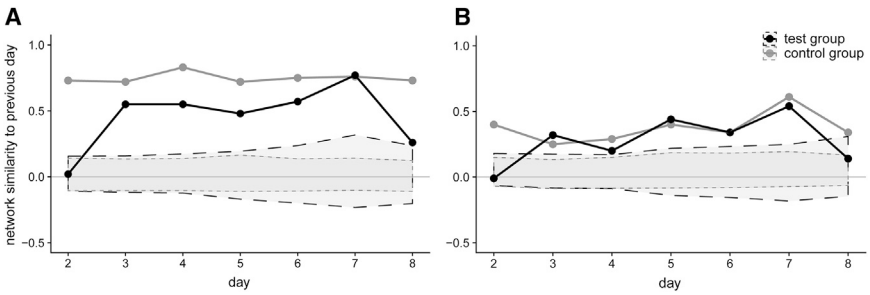

Figure 2. Day-to-Day Network Correlations in the Test and Control Bats over Time

Plots show the correlation of each day's association network with the previous day in the test group (black) and control group (gray).

(A) Day-to-day correlations for associations.

(B) Same for close-contact associations.

Solid lines and points show the observed effect size. Dashed lines and shaded areas show the corresponding 95% CI of the expected effect sizes under the null hypothesis. Only associations among bats present on both days were compared. See also Table S1.

Not All the Strongest Social Relationships Survived the Transition

Although stable social preferences do exist, the transition to a new physical and social environment was enough to break the associations between mothers and their captive-born daughters, which is one of the strongest social bonds in this species [6, 26]. Female vampire bats are typically philopatric [18], but all the captive-born bats departed from the wild colony before the study ended. The meaning of this outcome is difficult to interpret. The captive-born bats might have failed to develop certain natural behaviors. They might have used homing to attempt to fly back to their captive birth site [27]. The evidence of bites and the atypical behavior of roosting outside the tree even during the daytime suggested that at least some of the captive-born bats failed to socially integrate. Past field studies [18] also document three cases of new female vampire bats that failed to integrate into a social group. We do not yet understand what factors predict variation in social integration, but high-resolution association and interaction data will likely provide key insights.

Conclusions

The wild vampire bat association networks reflected a history of captive cooperation, and social preferences were evident even after a dramatic change in the physical and social environment. During their time together in captivity, social grooming rates increased among the test group. When released into the wild, they preferred to roost near each other compared to the control group. Despite frequent movements, their roosting associations were overall similar across days. The within-day association rates among the test bats in the wild were stronger than the same associations among the control bats. Since the bats in both groups were individually and haphazardly captured over the course of an entire night, greater assortativity in the test group is also consistent with the hypothesis that their social relationships strengthened during their time together in captivity. Within the test group, bats with stronger histories of cooperation in the lab roosted in closer proximity in the wild. Since we tracked the wild association networks for 8 days, the long-term stability of the test bat social networks is unclear. Future work should test whether relationships that form in captivity are less stable in the long-term than those that form in the wild. However, what is clear is that many social relationships persisted from captivity to the wild, and this observation is strengthened by the fact that we measured these relationships using both different behaviors and different methods. Taken together, these results strongly support the hypothesis that nonkin cooperative relationships observed in past studies with vampire bats [6, 20–22] are not an artifact of captivity, and that social bonds based on past events actually *cause* social structure.

This study also provides a necessary validation of this novel method for automated measures of proximity in the field, an emerging technology for generating the high-resolution data necessary to create dynamic networks. This method appears to have captured stable social relationships that lead to behaviors with fitness consequences in nature, e.g., regurgitated food sharing [20]. Grooming and food-sharing rates are good measures of relationships because they convey investments of time and energy, but they are typically estimated from relatively sparse data and require prolonged periods of observation (e.g., many months for food sharing [20, 22, 26]). In contrast, these automated proximity data are extremely high resolution (updated every 2 s) and directly capture association networks every minute, hour, or day.

The huge advantages of more frequent and reliable data for social network analysis have led to an increasing shift from laborious direct observations of interactions to automated measures of association from PIT tag readers [28], GPS tags [29], barcodes [30], and proximity loggers (including Encounternet [31] and BATS [23]), which can produce high-resolution dynamic networks that capture how social structure changes over time. Our "next-generation" automated proximity sensors are unique in their ability to track social networks of whole groups of small animals over time, even in hard-to-access sites such as small caves or hollow trees, while allowing us to examine how association network characteristics vary when associations are redefined at multiple temporal and spatial scales (e.g., in the same tree, in the same cluster, or close enough to groom and share food; Figures 3 and S2).

High-resolution proximity data can highlight how association networks depend largely on the spatial and temporal scale at which the researchers define associations. For example, Wilkinson [20, 32] defined association as vampire bats occupying the same tree at the same time, and he showed that adult female association predicted food sharing independent of kinship using 26 months of observations. His published data [33] show that about 200 vampire bats were divided into small groups spread among 14 trees, and there was a 91% chance each night that at least one adult female departed or joined a roosting group in a tree (n = 187 consecutive daytime observations of a roost). This frequent tree switching at his study site in Costa Rica was actually necessary to detect nonrandom social

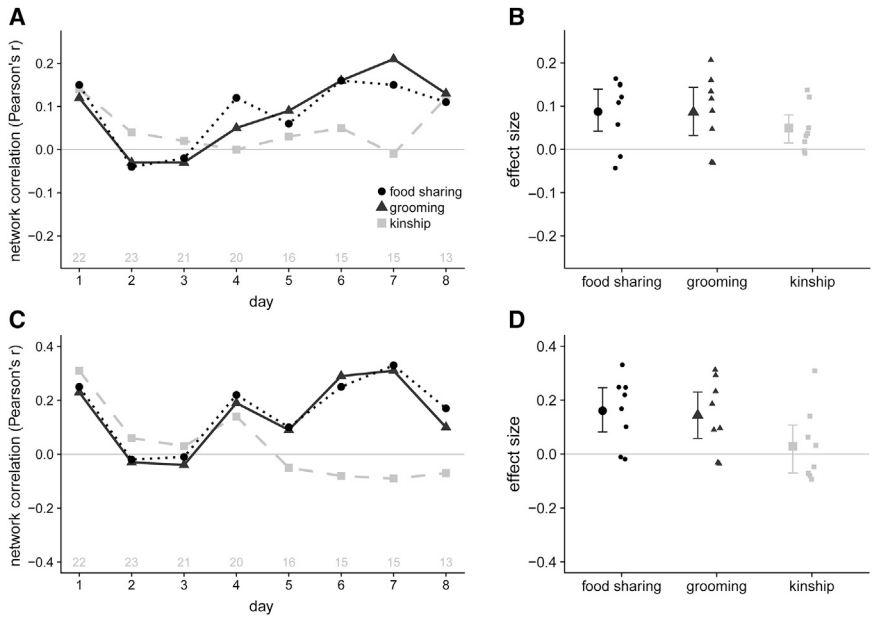

Figure 3. Cooperative Relationships Predict Roosting Proximity in the Wild

(A and C) Correlation between the daily wild association network and the captive rates of food sharing (black dotted line with circles), rates of social grooming (dark gray solid line with triangles), or kinship (light gray dashed line with squares) based on associations (A) or close-contact associations (C). Gray numbers above the x axis show the number of bats in the network.

(B and D) Mean effect size (observed – expected correlation) with bootstrapped 95% CI over all 8 days. The 95% CIs are based on only one observation per day and are more conservative than the p values in our permutation tests.

See also Figure S2.

network structure and fission-fusion dynamics [32]. By contrast, at our site in Panama, roughly the same number of bats inhabited just one tree and tree switching was less frequent. Yet we could observe similar fission-fusion dynamics occurring on shorter timescales and smaller spatial scales within a single tree.

This study adds to a growing body of evidence that vampire bats possess cooperative relationships that are analogous in form and function to "friendship-like" bonds seen in many primates [1, 2]. Social relationships vary in stability [34–36]. On one end of this spectrum are stable bonds with complete partner fidelity and no threat of partner switching, a scenario represented by two-player "partner control" models of reciprocity (e.g., reward, punishment, tit-for-tat) [35, 37] or interdependence (pseudo-reciprocity) [38–40], where relationship history or past partner actions matter, but not the social environment of alternative partners. On the other end of this spectrum are cooperative interactions without partner fidelity, where cooperation can be enforced by immediate partner switching [41], which depends largely on outside options in the social environment. Our results reject both endpoints of this spectrum because vampire bat social structure results from both intrinsic social preferences and the extrinsic physical and social environment. Social bonds were not an emergent byproduct of a stable captive environment, but the altered social environment also mattered and not all strong social bonds survived the transition to a new setting. Our results are consistent with the idea that both partner fidelity and partner switching play a role in stabilizing vampire bat cooperation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.10.024.

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AUTHOR CONTRIBUTIONS

S.P.R. and G.G.C. conceived the ideas and designed the study; N.D., A.K., B.C., and R.K. developed and tested the tracking technology; S.P.R.,

G.G.C., D.J., J.B.-M., N.D., and B.C. collected the data; S.P.R. and G.G.C. analyzed the data; and S.P.R. and G.G.C. led the writing of the manuscript. S.P.R., G.G.C., R.A.P., R.K., A.K., and F.M. contributed to acquiring funds. All authors contributed critically to the drafts, gave final approval for publication, and agreed to be held accountable for the work performed therein.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Data for reproducing all analyses	Figshare	https://doi.org/10.6084/m9.figshare.9037829.v3
Code for reproducing all analyses	Figshare	https://doi.org/10.6084/m9.figshare.9037814.v1
Experimental Models: Organisms/Strains		
Common vampire bat (*Desmodus rotundus*)	Wild	N/A
Oligonucleotides		
Microsatellite primers for estimating relatedness (see Table S2)	[26, 42, 43]	GenBank: EF591569– EF591580, PRJNA279293; AL2_27850
Software and Algorithms		
R 3.5	[44]	https://cran.r-project.org/

LEAD CONTACT AND MATERIALS AVAILABILITY

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

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study Species

The common vampire bat (*Desmodus rotundus*) is a 25-40 g bat that occurs from Northern Mexico throughout Central America to southern South America. It can often be found roosting in caves or hollow trees and feeds on blood of livestock in settled areas [45]. Females are mostly philopatric and form long-term associations within and between matrilines whereas males disperse after 12-18 months [18].

Study sites and sampling conditions

All study individuals were captured females or the offspring of a female captured from just outside a large hollow tree on a cattle pasture near Tolé, Panama (8°12'03"N 81°43'46"W). The main cavity inside the hollow trunk was about 1.5 m wide and 2.5 m high, and several smaller cavities branched off from the main one. The tree hollow was large enough to contain distinct social groups. We estimated the colony inside the tree to be about 200 vampire bats based on captures, visual estimation, and photographs.

On December 13, 2015, we captured 41 vampire bats outside the hollow tree using mist-nets until midnight and transferred them to a 1.7 × 2.1 × 2.3 m outdoor flight cage at the Smithsonian Tropical Research Institute in Gamboa, Panama. The *test group* included the 17 females caught in 2015 and their six female captive-born offspring (aged 10 to 19 months at time of their release back into the wild). Bats were marked for individual identification with subcutaneous passive integrated transponders (Trovan Ltd. USA) and a visually unique combination of forearm bands (Porzana and National Tag). Captive vampire bats were fed on blood from a slaughterhouse that was chemically defibrinated (with 44 g sodium citrate and 16 g citric acid per 19-L container) and stored by either refrigeration for up to six days or freezing. Before releasing captive-born bats in the wild, we confirmed that they could feed on a live animal [46].

After 22 months in captivity the test group was released at their point of origin, back into the hollow tree near Tolé. On the same day, we also created a *control group* to compare with the test group. On September 19, 2017, we used the same mist-netting procedure as we used to capture the test group until about 0200 h. We captured and fitted 27 adult females with proximity sensors, then we released these control group bats back into the roost between 0450 h to 0630 h on September 20. At 0812 h on September 20, we fitted the 23 test group bats with proximity sensors and released them back into the same tree. We observed the first two dropped proximity sensors beneath the colony on the morning of September 29. We therefore analyzed daily roosting associations from September 21 to 28.

Research permits

All experiments were approved by the Smithsonian Tropical Research Institute Animal Care and Use Committee (#2015-0915-2018-A9 and #2017-0102-2020) and by the Panamanian Ministry of the Environment (#SE/A-76-16 and #SE/AH-2-17).

METHOD DETAILS

Proximity sensor system

The proximity sensors used in this study were developed as a part of the BATS tracking system. The technological aspects of the system and its application are described in depth elsewhere [23–25]. The 1.8 g proximity sensor includes a 3D-printed plastic housing, a System-on-Chip for communication control and on-board data processing, a transceiver enabling communication with other proximity sensors or base stations in the sub-GHz band (903MHz), and a wake-up-receiver that activates full-system functionality from an energy-saving low-power mode. The proximity sensor broadcasts a signal every 2 s. The start of a 'meeting' is created when other proximity sensors are within line of sight and within the reception range of ca. 5 m. When no signal has been received by the partner sensor for 10 s, the meeting ends and is stored to on-board memory along with the ID of the meeting partner, a timestamp, the total meeting duration, and the maximum received signal strength indicator (RSSI). The raw meeting data is highly redundant since both nodes may store and download information from a single event. We therefore removed redundancy by fusing overlapping meetings. To facilitate binning associations by time, we split all meetings which crossed the h mark. The maximum meeting duration is therefore 60 min after post-processing.

A total of 60 proximity sensors can be operated simultaneously. Each sensor is powered by a 22mAh lithium-polymer battery enabling a runtime of about 10-14 days. Proximity sensors communicate with any and all base stations within range. Each base station contains a receiver for signals, which include transmitted meeting data. These transmissions also serve as 'presence' data for localizing individuals at a given time. Presence and meeting data are stored by a Raspberry Pi (Raspberry PI Foundation, Cambridge, UK) to a SD card along with the identity of the transmitting proximity sensor and the receiving base station and an absolute timestamp (UTC time) which is provided by a GPS unit. The Raspberry Pi hosts a WiFi hotspot allowing remote data access by users. We positioned a base station inside the roost to ensure regular download of data which has been stored by proximity sensors. We downloaded the data on a daily basis and stored them in a MySQL database. Due to a problem at the base station we lost all data past 1700 h on the last day of data logging, so the sampling period for day eight was 11 h rather than 12 h; however, we accounted for this different sampling period in our analyses.

Captive experiments

To assess cooperative relationships, we measured rates of social grooming and food sharing among the 23 captive individuals. To induce food sharing and social grooming among the test group bats, we conducted 533 fasting trials in the captive colony over a period of 22 months. During each fasting trial, a focal subject bat was isolated without food for 26 to 28 h, then reintroduced to the group and recorded for one h so that durations of food sharing and social grooming given and received between the fasted bat and all other bats could be scored from infrared-illuminated video footage. Each test bat was fasted and focal sampled in 12 to 23 trials (mean = 19.4 fasting trials per bat) in a mixed colony from two different wild populations [22].

Genetic relatedness

To measure relatedness, we extracted DNA from a 3-4 mm wing biopsy punch in 80% or 95% ethanol using a salt–chloroform procedure. We used a LI–COR Biosciences DNA Analyzer 4300 and the SAGA GT allele scoring software to genotype individuals at 17 polymorphic microsatellite loci (Table S2). Allele frequencies were based on 100 bats from Tolé and nine bats from another site, Las Pavas, Panama. Genotypes were 99.9% complete. We used the Wang estimator in the R package 'related' [47] to obtain an initial kinship estimate based on relatedness, then we assigned a zero kinship to known nonkin from different sites and to dyads with negative estimates. We also assigned a kinship of 0.5 for known mother-offspring dyads or dyads with relatedness estimates greater than 0.5.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were conducted in R using R Studio. We calculated all 95% confidence intervals (CIs) using bootstrapping with 5,000 iterations. For hypothesis-testing, we used permutation tests (see below) and α was 0.05.

Estimating cooperation rates in captivity

To construct food-sharing and social-grooming networks, we defined edges as the mean of natural log (x+1), where x is the total duration of social grooming or food sharing from bat A to B within the one-h trial where A could have been observed grooming or feeding B. We assumed that fasted subjects could be fed by all others present in a trial. To test whether group-level grooming rates increased over time in captivity, we fit a linear model with month as the predictor and mean duration of grooming per trial for that month as the response. To test whether dyadic cooperative relationships strengthened over time in captivity, we fit a linear mixed effects model, with the response being the duration of grooming received by the fasted subject from each partner during each trial. The fixed effect was trial rank order and the random effect was the groomer-receiver dyad. We extracted the fixed effect coefficient and compared it to the expected distribution of values when we fit the same model using datasets where the trial rank order was shuffled within each groomer-subject dyad. We compared the observed and expected increases in grooming rate per trial.

Interpreting meeting data from proximity sensing

To calibrate the proximity sensors, we tested the maximum RSSI values associated with meetings of varying distance of 2, 50, 100, 150, 300 and 500 cm (Figure S3) using pairwise combinations of six proximity sensors under the climatic conditions of the study site. We used the resulting calibration curve of RSSI by distance to interpret the biological context of meeting data. The RSSI values depend strongly on the distance between mobile nodes (Figure S3) and on antenna alignment with the highest signal intensity in parallel alignment [48], but antenna alignment for roosting tagged bats is typically close to parallel because the antenna orients vertically upward when bats hang upside down. To buffer the RSSI value against non-ideal conditions (e.g., when an antenna is temporarily bent against a surface or another bat), our system updates the RSSI every 2 s and keeps the maximum value. There are multiple opportunities during a single meeting to obtain a RSSI value in optimal orientation (close to parallel) as the roosting vampire bats frequently turn or slightly move their bodies, slightly changing the orientation of the antenna. Therefore, an association must be interpreted as a meeting where two bats have been associated for at least a few seconds rather than across the entire time of the meeting. To update the distance between bats we enforce a meeting interruption every 2,700 s to create a new meeting with a new maximum RSSI.

Thresholding at different maximum RSSI values (as a proxy for inter-individual distance) will affect network topology. For example, the mean network density was 47% (range across days = 38 to 61%) for associations and 12% (8 to 16%) for close-contact associations.

Daily association networks in the wild

To generate high-resolution association networks of roosting in proximity, we used the total duration of social meetings within the roost during each day. We defined 'days' as the period from 0600 to 1800 h, just before sunrise and sunset (Sept 21: 0607 h to 1814 h; Sept 28: 0606 h to 1810 h). Presence or absence of bats inside the tree were constant within each day because vampire bats never left the roost during the day. However, each day is separated by 12 h of active night time when bats are expected to individually leave the tree to forage and possibly to switch their roosting location [32]. We therefore used each daily association rate as an independent observation of roosting association.

To construct wild association networks, we used the proportion of time bats were in proximity based on two thresholds of proximity (called 'association' and 'close-contact association', Figure S3). Association rates (total time in meetings / sampled time) vary from 0 to 1. We defined 'association' as the sum of meeting durations with maximum signal strength in the top 10% of all meetings, corresponding to a maximum distance of about 50 cm, divided by the total sampled time. We defined 'close-contact association' as the sum of meeting durations with a signal strength in the top 1% of all meetings, corresponding to the expected signal intensity of two sensors within 2 cm, divided by the total sampled time. Overall, 73% of all possible dyads of tagged bats were associated at least once, and 29% were ever in close contact. A given dyad was likely to been associated on only four of the eight days (median days with an association = 4), and was likely to be in close contact on only two of the eight days. The within-day association rate predicted the number of days that two bats were associated at least once. The highest association rates in any dyad were 100% within a day and 73% over eight days. The highest close-contact association rates were 94% within a day and 26% over eight days.

Simulating random daily association networks

For testing assortativity and the effects of captive cooperation on wild roosting association across all days, we constructed realistic null models using network permutations of the bats within the roost that day to create 5,000 randomized networks for each day. To calculate p values, we compared the observed effect size (e.g., Pearson's correlation or t-statistic) to those effect sizes expected from the same procedure run using the randomized networks. This within-day network permutation test accounts for the fact that bats do not leave the tree at daytime and could not associate with others not in the tree that day.

Assortativity among the test and the control group

To test assortativity, we first fit a general linear mixed model with the dyadic mean association rate as the response, the dyad type (test/test, control/control, or control/test, i.e., mixed) as the fixed effect, and day as the random effect. We compared the observed t-statistics to those expected from applying the model to randomized network data.

Wild association network stability between days

Vampire bats could move within the tree or leave the tree for a different roost. To identify which individuals moved to a different roost, we extracted the nightly arrivals and departures of tagged bats from the tree on each day. We used a binomial test to compare the departure rates of the test and control bats. To assess network stability for the remaining bats in the test and control group, we constructed association networks for each day and used mantel tests to assess the correlation between each day's association network with the previous day's network using only the bats that were present on both days (see Figure 2). To compare the mean network correlations for the test and control group, we bootstrapped the 95% CI for mean correlations between the daily networks for days 2 through 8.

Effect of captive cooperation on wild association

Proximity and cooperation are almost certainly related because association predicts food sharing in the wild [20], forced association increases food sharing [22], and bats are attracted to calls of food donors [49]. To correlate captive cooperation and wild association,

we measured the correlation between daily dyadic association rates in the wild and three dyadic measures: kinship, captive grooming rates, or captive food-sharing rates (see Figure 3). As our effect size, we used the Pearson's correlation averaged across days, and compared this observed effect size to values expected from our randomized networks. To test whether this effect increased with a stricter definition of association based on closer proximity (i.e., a higher threshold of signal strength), we plotted the network correlations as a function of the signal strength value that was used to define associations (see Figure S2).

To test the effects of grooming or food sharing on association, while controlling for kinship, we used the multiple quadratic assignment procedure with double semi-partialling (MRQAP-DSP) in the 'asnipe' package [50]. However, this alternative permutation procedure does not constrain the permutations within each day. Rather than measuring the network correlations within each day for the bats that were present, this method tests whether social-grooming and food-sharing networks predicted the overall wild association network based on the total association times in the test group summed over all eight days. Bats that leave the site days earlier therefore have much lower associations with all other bats.

Finally, we directly tested if bats spent more time than expected by chance with their strongly bonded grooming partners. For this test, we excluded the captive-born bats which left the study site. Social grooming received by fasted bats in fasting trials is the best single measure for a social bond and predicts development of new bonds [22], so we tested whether each bat in the test group spent more time than expected over all eight days with its top five groomers in captivity. We calculated the mean dyadic association rate of each focal bat with its top five groomers and we then compared this value to those expected after randomly swapping the identity of the top five grooming partners across focal bats.

DATA AND CODE AVAILABILITY

All relevant data (https://doi.org/10.6084/m9.figshare.9037829.v3) and R code (https://doi.org/10.6084/m9.figshare.9037814.v1) for reproducing all analyses have been deposited on Figshare.