American Genetic Association

Journal of Heredity, 2018, 393–404
doi:10.1093/jhered/esx111
Original Article
Advance Access publication 8 December 2017

OXFORD

Original Article

How Social Structure Drives the Population Dynamics of the Common Vampire Bat (*Desmodus rotundus*, Phyllostomidae)

Maïlis Huguin, Nidia Arechiga-Ceballos, Marguerite Delaval, Amandine Guidez, Isaï Jorge de Castro, Vincent Lacoste, Arielle Salmier, Alvaro Aguilar Setién, Claudia Regina Silva, Anne Lavergne, and Benoit de Thoisy

From the Kwata NGO, 16 avenue Pasteur, 97300 Cayenne, French Guiana (Huguin and de Thoisy); the Laboratoire des Interactions Virus Hôtes, Institut Pasteur de la Guyane, Cayenne, French Guiana (Huguin, Guidez, Lacoste, Salmier, Lavergne, and deThoisy); the Laboratorio de Rabia, Instituto de Diagnóstico y Referencia Epidemiológicos. Mexico DF, Mexico (Arechiga-Ceballos); the Office National des Forêts, Cayenne, French Guiana (Delaval); the Laboratório de Mamíferos, Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá, Macapá, Amapá, Brazil (de Castro and Silva); and the Unidad de Investigación Médica en Inmunología, Coordinación de Investigación, Instituto Mexicano del Seguro Social, México DF, Mexico (Setien).

Address correspondence to Benoit de Thoisy, Kwata NGO, 16 avenue Pasteur, 97300 Cayenne, French Guiana, and Institut Pasteur de la Guyane, 23 avenue Pasteur, 97300 Cayenne, French Guiana. e-mail: benoit@kwata.net.

Received January 31, 2017; First decision April 13, 2017; Accepted December 1, 2017.

Corresponding Editor: Tomas Hrbek

Abstract

Social systems are major drivers of population structure and gene flow, with important effects on dynamics and dispersal of associated populations of parasites. Among bats, the common vampire bat (*Desmodus rotundus*) has likely one of the most complex social structures. Using autosomal and mitochondrial markers on vampires from Mexico, French Guiana, and North Brazil, from both roosting and foraging areas, we observed an isolation by distance at the wider scale and lower but significant differentiation between closer populations (<50 km). All populations had a low level of relatedness and showed deviations from Hardy–Weinberg equilibrium and a low but significant inbreeding coefficient. The associated heterozygote deficiency was likely related to a Wahlund effect and to cryptic structures, reflecting social groups living in syntopy, both in roosting and foraging areas, with only limited admixture. Discrepancy between mitochondrial and nuclear markers suggests female philopatry and higher dispersal rates in males, associated with peripheral positions in the groups. Vampires are also the main neotropical reservoir for rabies virus, one of the main lethal pathogens for humans. Female social behaviors and trophallaxis may favor a rapid spread of virus to related and unrelated offspring and females. The high dispersal capacity of males may explain the wider circulation of viruses and the inefficacy of bat population controls. In such opportunistic species, gene connectivity should be considered for management decision making. Strategies such as culling could induce immigration of bats from neighboring colonies to fill vacant roosts and feeding areas, associated with the dispersal of viral strains.

Subject area: Population structure and phylogeography, Reproductive strategies and kinship analysis
Key words: *Desmodus rotundus*, parasite spreading, population dynamics, sociality, structure, vampire bat

Bats (*Chiroptera*) are widely distributed across the world and are very diverse in their morphology, biology, and ecology (Fenton 1997). They are ranked the second largest species-rich mammalian order, accounting for 20% of all mammal species in the world. With their roles of seed dispersers, pollinators, and regulators of arthropod populations, they have major ecological functions and contribute to ecosystem services (Kunz et al. 2011). Because of difficult direct observation related to their nocturnality, small size, and cryptic behavior, many aspects of bat biology have been investigated with molecular approaches, namely population genetics, which has been widely used and has shown interspecific structuring variations in relation to bat behavior, mating, social systems, and movements (Rossiter et al. 2012; Moussy et al. 2013; Miller-Butterworth et al. 2014).

Bats are also a frequent source of pathogen spillover to humans and livestock, and a reservoir for emerging infectious diseases (Escobar et al. 2015). The ability of bats to effectively ensure virus amplification and dispersion is related to their resistance to and/or control of many infections (O'Shea et al. 2014). Neotropical bats are, notably, the main reservoir of rabies virus. Serological and molecular surveys of this virus have shown a high diversity of positive bat species, suggesting a wide distribution and circulation of the virus in Amazonia (Torres et al. 2005; Salmón-Mulanovich et al. 2009; Sodré et al. 2010; Almeida et al. 2011; de Thoisy et al. 2016) with a major role played by the common vampire bat *Desmodus rotundus* (Phyllostomidae).

Distributed from northern Mexico to northern Chile and the Amazon basin (Greenhall et al. 1983), the common vampire bat transmits the rabies virus throughout Latin America, causing lethal human cases and thousands of livestock deaths every year (Blackwood et al. 2013). Despite their major contribution to virus maintenance and dispersal, most studies carried out on *D. rotundus* relate to their physiological characteristics (Reddrop et al. 2005), their reproductive cycle (Wimsatt and Trapido 1952), the choice of host (Voigt and Kelm 2006) as well as their behavior and social structure (Wilkinson 1985a, 1985b; Trajano 1996). Adult females show a strong philopatry to their natal group, although adult males disperse and defend a roost where females roost (Wilkinson 1985a, 1985b). Besides being hematophagous, vampire bats have the particularity of practicing blood regurgitations and other social behaviors that strengthen social ties. According to Wilkinson (1984), exchanges are based on reciprocal altruism (Axelrod and Hamilton 1981; Trivers 1971), which affects the social structure of the population. Molecular approaches can never the less be reliable tools to decipher uncovered aspects of population dynamics. Several studies have been conducted on the genetic structure of vampire bat populations (Martins et al. 2009; Romero-Nava et al. 2014; Streicker et al. 2016).

Host social systems also have major effects on parasite population dynamics, structure, and dispersal (Streicker et al. 2016; van Schaik and Kerth 2014). Therefore, information on the vampire bat social system, spatial organization, and population connectivity is required for a better understanding of rabies virus dynamics (Streicker et al. 2016). Trophallaxis and social grooming contribute greatly to virus transmission due to direct saliva contact with broken skin and mucosa between individuals (Kobayashi et al. 2005; Velasco-Villa et al. 2006). The wide and high prevalence of rabies-neutralizing antibodies found in *D. rotundus* in French Guiana populations (de Thoisy et al. 2016) can be related to male dispersal (Wilkinson 1985b), favoring virus dispersal. Alternatively, the philopatry of females and population's social organization (Wilkinson 1984) might be the cause of population structuring, limiting interpopulation interactions and therefore the role of females in virus dispersal (Streicker et al. 2016).

In this study, 7 microsatellites and the mitochondrial DNA control region were used to investigate the genetic structure of *D. rotundus* at 2 spatial scales. Microsatellites were chosen because these are reference markers for many population genetics studies and molecular ecology (Huth-Schwarz et al. 2011; McCulloch 2012 ; Zeng et al. 2012). They are especially used for the analysis of demographic history, population structure at a fine geographic scale, and social structure (Selkoe 2006; Trinca et al. 2012; Lecompte et al. 2017). The aim of this study was to improve our knowledge on vampire bat populations, seeking to investigate how the genetic structure is impacted by social behavior at the intra- and interpopulation scales. Those results are discussed about 3 main issues, phylogeographic structuring, drivers of social organization, and potential to influence virus transmission.

Materials and Methods

Ethical and Legal Statements

Animals were captured, handled, sampled, and, whenever necessary, euthanized in accordance with ASM guidelines (Sikes et al. 2011), and in French Guiana under the supervision of researchers in possession of the national "animal experimentation level 1" degree. Bats are not protected by law in French Guiana, but the project was presented to the Conseil Scientifique Régional pour le Patrimoine Naturel de la Guyane, and approved by this council. Captures that occurred within protected areas (nature reserves) received approval of the Conseil Scientifique Régional du Patrimoine Naturel on 26 January 2010 and ad hoc authorizations (no. 2011–35 from the 30 May 2011, no. 35 and 59 obtained 21 March 2013 and 17 April 2013, respectively, and delivered by the Préfecture de la Guyane). All samples are kept in Cayenne, French Guiana, in the JAGUARS tissue collection (CITES FR973A).

Bat collection in Amapá (North Brazil) followed the Brazilian legislation and was authorized for IBAMA / Sisbio19140/2008, SEMA 1/2008 licenses. Samples of vampire bats were deposited in the tissue collection in Collection Scientific Amapá Fauna (CCFA) located at the Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá.

In Mexico, traditional rabies control is based on reduction of species populations. The samples used in this study were collected as part of the activities of Paralytic Rabies Control Campaigns of the States. Vampire bats were captured with mist nets complying with Mexican regulations (Norma Oficial Mexicana NOM-067-ZOO-2007 "Campaña Nacional para la prevención y control de la rabia en bovinos y especies ganaderas") in corrals and roosts.

Sample Collection and Study Areas

A total of 218 individuals were captured at 5 localities in French Guiana between 2009 and 2013 (Figure 1). The first 2 localities were breeding caves located in pristine primary lowland forests. The third and fourth localities were feeding areas located in pristine primary lowland forests, and the fifth was a feeding area located in edge habitat in a disturbed environment. The bats sampled in the first locality formed population 1 (below, pop1), the bats sampled in the second locality formed population 2 (pop2). The bats sampled in the third and fourth localities were pooled and formed population 3 (pop3) because exploratory analysis showed no genetic differentiation

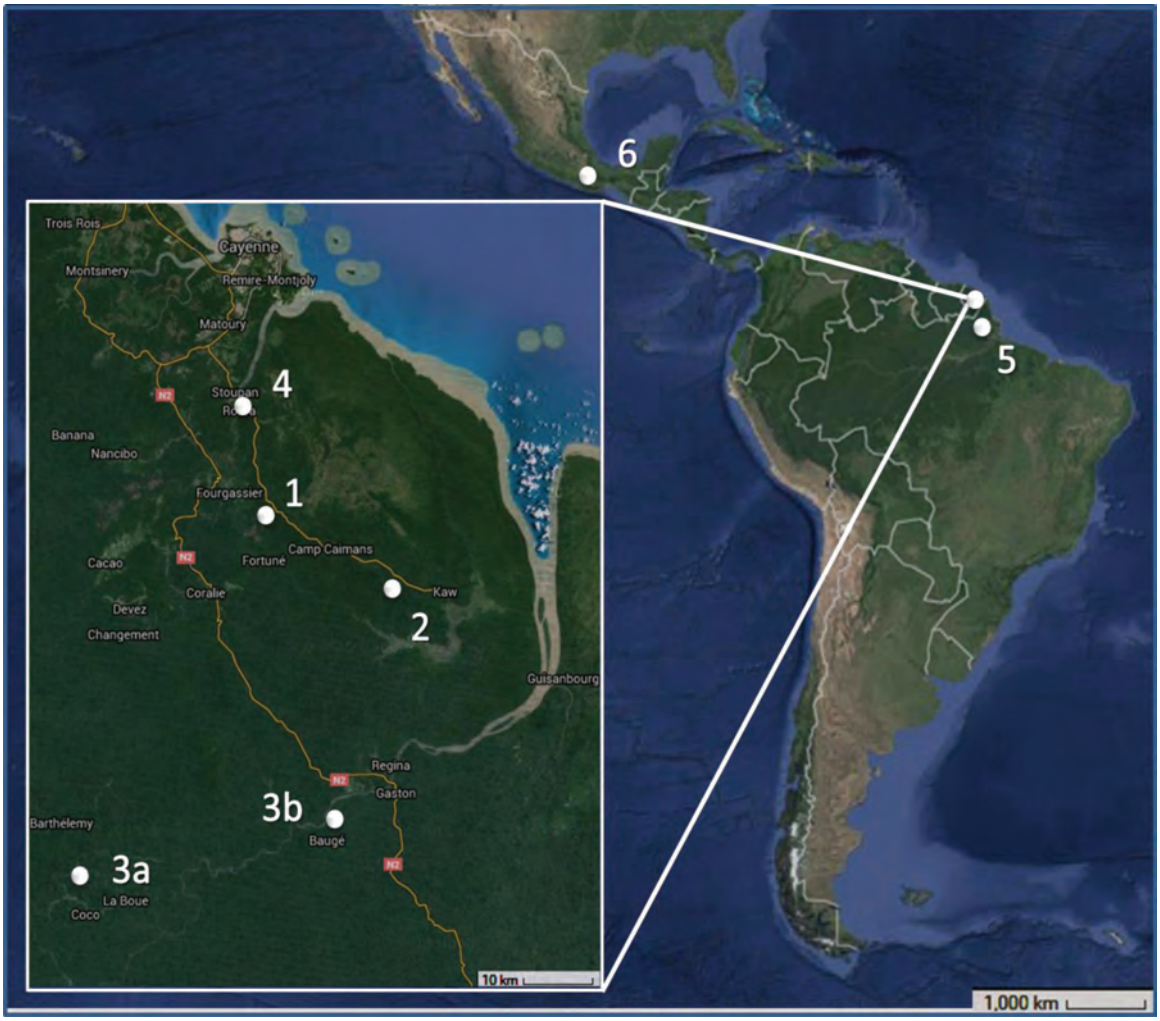

Figure 1. Geographical locations of *Desmodus rotundus* populations. Pop1 to 4 were sampled in French Guiana, pop5 was sampled in Brazil and pop6 was sampled in Mexico. Map data © Google 2017. See online version for full colors.

between them (F_{ST} = 0.003; P > 0.3). The bats sampled in the fifth locality formed population 4 (pop4).

The DNA samples of 39 individuals from Amapá State, northern Brazil, formed population 5 (pop5) and were sampled in 3 localities in the northeast of Amapá: Horto São Bento, Rebio Lago Piratuba, and Rebio Parazinho. The DNA samples of 82 individuals from Mexico formed population 6 (pop6) and were sampled in 4 localities: Morelos, Puebla, Veracruz, and Hidalgo.

In French Guiana, captures were made at night using Japanese mist nets (Ecotone®, Gdynia, Poland) as implemented in the region (Borisenko et al. 2008). Captures in breeding caves were performed inside with nets erected during the day time to localize the colony at rest and target captures on vampire bats only, to avoid disturbing other species. To prevent repeated disturbance of the colonies, captures were implemented by 3 persons only and lasted no more than 30 min. Whenever possible, females with their offspring were not captured and were directly released if they fell into the nets, so no young were sampled. All animals captured were kept in individual bags, blood-sampled (for detection of rabies virus neutralizing antibodies, e.g., de Thoisy et al. 2016) and measured at the mouth of the cave to limit disturbance inside. A wing tissue sample was taken using a 3-mm biopsy punch and stored in 95% ethanol at –20°C until DNA extraction. Bats were released directly after the procedure. In the caves, several capture/recapture sampling sessions were implemented, so that the animals could be marked (Passive Integer Transponder BackHome, Virbac, France, injected subcutaneously between the shoulders).

DNA Extraction, Microsatellite and D-Loop Control Region Genotyping

Biopsy punches were prelysed within the EasyMAG lysis buffer (BioMérieux, Marcy l'Etoile, France) at 4°C overnight, then in Tris–SDS buffer and proteinase K at 56°C before grinding. DNA was isolated using NucliSENS EasyMAG® bio-robot (BioMérieux, Marcy l'Etoile, France) following standard protocols for tissue. The DNA pellet extracted was resuspended in H_2O PPI and stored at –20°C until use.

Samples were genotyped using a panel of 7 microsatellite markers (Bardeleben et al. 2007; Ortega et al. 2002; Piaggio et al. 2008) as described in Supplementary Table s1). PCR was carried out in 12 µL with 1.5 µL of each primer (5 mM), 1.5 µL of buffer, 1.2 µL of dNTP (5 mM), 0.1 µL of BIOTAQ™, 0.4–0.6 µL of MgCL2 (50 mM) (Supplementary Table s2), and 2 µL of diluted DNA (1/10). PCR reactions consisted of initial denaturation at 94°C for 5 min,

followed by 40–45 cycles depending on the locus at 94°C for 30 s, 30 s at the annealing temperature (Supplementary Table s2), 72°C for 45 s, and final elongation at 72°C for 10 min. PCR products were mixed with Genome Lab SLS and GenomeLab DNA 400 size standard and run on a GenomeLab GeXP genetic analysis (Beckman Coulter®). Microsatellite alleles were sized using GenomeLab System software (Beckman Coulter®).

The primers F(mt) and P(mt) (Wilkinson and Chapman 1991) were used to amplify the hypervariable domain HVI of the mitochondrial DNA (mtDNA) control region (D-loop). PCRs were performed using a standard procedure with the BIOTAQ™ DNA Polymerase PCR kit (BioLine Reagents Limited, London, UK). PCR was carried out in a 50-µL PCR containing 10 ng of genomic DNA, 5 µL 10× buffer, 1.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.6 mM of each primer and 0.5 U BIOTAQ™ DNA Polymerase (BioLine, London, UK). The cycling conditions included an initial denaturation step at 95°C for 5 min followed by 35 denaturation cycles at 95°C for 45 s, annealing for 45 s at 55°C, and elongation at 72°C for 1 min. A final 7-min extension step at 72°C followed the last cycle. Beckman Coulter Genomics carried out sequencing (Beckman Coulter Genomics, Takeley, UK).

Microsatellite analysis

The presence of null alleles, large allele dropout, and stuttering were tested using Microchecker 2.2.3 (van Oosterhout et al. 2004). Tests for departure from HWE were performed for each population in Genepop3.4 (Raymond and Rousset 1995) under the hypothesis of heterozygote deficit. Linkage disequilibrium was tested using Genepop; genetic diversity indices and F_{ST} averaged over loci (Weir and Cockerham 1984) were obtained for and between populations and between females and males from Arlequin 3.5 (Excoffier et al. 1992) with 20 000 permutations and Genetix 4.0.5.2 (Belkhir et al. 2004) with the fixation index *F* of Wright (1969), according to Weir and Cockerham (1984). For all pairwise comparisons, all significance levels were adjusted using sequential Bonferroni correction (Rice 1989).

For each population sample and for each locus, the Bottleneck 1.2.02 program (Cornuet and Luikart 1996) computes the distribution of the heterozygosity expected from the observed number allele *k*, given the sample size *n* under the assumption of mutation-drift equilibrium. The distribution was obtained under the two-phase mutation model (TPM) recommended for microsatellite loci because it is a better fit to observe allele frequency data than the infinite alleles model (IAM) and the single-step model (SSM) (Di Rienzo et al. 1994). If a population has a normal L-shaped distribution, it is likely that this population has not experienced a recent reduction of their effective population size.

Relatedness

Relatedness (*r*) between individuals of unknown ancestry was estimated by Ml-relate 1.0 (Kalinowski et al. 2006), based on maximum likelihood estimates of relatedness, expected to be the most accurate method for such purposes (Milligan 2003). Ml-relate can adjust relatedness calculations to accommodate null alleles using a HWE test to detect a deficiency of heterozygosity (U-statistic of Rousset and Raymond 1995). Ml-relate uses the downhill simplex routine to find the maximum likelihood estimate of (*r*). Experience has shown that likelihood surfaces for (*r*) can have multiple peaks (Kalinowski et al. 2006). Therefore, the downhill simplex routine is started from the default values of 11 sets of points, one of which is {Unrelated, Full Sibs, Parent/Offspring}. The other 10 are random values. The relatedness coefficient (*r*) was estimated for each pair of individuals in each population by the software and the average (*r*) for each population was then calculated.

Gene Flow

The number of migrants per generation by pairwise population where $N_m = (1 - F_{ST})/4$. F_{ST} estimates of gene flow according to Wright (1969) was calculated with the Genetix4.0.5.2 program (Belkhir et al. 2004).

Population Structuring

Overall population structuring was inferred by the *dapc* function without any a priori information on the origin of individuals (discriminant analysis of principal components) of the Adegenet package (Jombart 2011) in the R 3.2.3 program (R Development Core Team 2008). Adegenet uses a multivariate analysis that does not rely on the assumption of HWE, the absence of linkage disequilibrium or specific models of molecular evolution to identify clusters within genetic data (Jombart 2008). The *dapc* function infers the number of clusters of genetically related individuals: data are first transformed using a principal component analysis (PCA) and subsequently clusters are identified using discriminant analysis (DA). *A*-score optimization and cross-validation were used to determine the optimal number of principal components (PCs) to maximize power of discrimination while also minimizing the risk of overfitting (9 and 14 were used, respectively). The Bayesian information criterion (BIC) was used to determine the most appropriate value of *K* and individuals were assigned to clusters using *DAPC*. The *assign.per.pop* slot was generated and indicated the proportions of successful reassignment (based on the discriminant functions) of individuals to their original clusters. High values indicate clear-cut clusters, while low values suggest admixed groups.

Hidden Structure

Cryptic subpopulations within each population were investigated using Baps 5.4 (Corander et al. 2004), identifying a hidden population structure through a Bayesian analysis and detecting a possible Wahlund effect. The program was run 50 times for each population to obtain the right number of *k* subpopulations on the basis of the estimated "log probability of data" The *k* with the highest posterior probability ("marginal likelihood") was considered the most likely number of subpopulations identified by baps. In accordance with De Meeûs guidelines (2012), F_{IS} was recalculated in the best distribution identified by baps for each population with Genetix 4.0.5.2 and noted *FisClus*. Then the *FisClus* was compared with the initial F_{IS} using a unilateral Wilcoxon signed-rank test for paired data (with the R 3.2.3 program) in order to identify geographical microstructure or social structure. For pop1 and pop2, F_{ST} averaged over loci were obtained with Arlequin 3.5 for each subpopulation whose number of individuals was greater than 3 (6 subpopulations for pop1 and 4 for pop2) and the relatedness coefficients were estimated with ml-relate 1.0 (Kalinowski et al. 2006) for each subpopulation whose number of individuals was greater than 1 (12 subpopulations for pop1 and 10 subpopulations for pop2, see below). As Baps may overestimate K due to the inference of a few small spurious subpopulations (Latch et al. 2006), the results of the Baps analysis were verified with Structure 2.3.4 (Pritchard et al. 2000) using admixture model (burnin: 100 000, chains after burnin: 50 000) in accordance with guidelines described in Pritchard et al. (2010). The

most likely K number of populations was defined both on the basis of the estimated "log probability of data," and with the Evanno's ΔK statistic based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005), implemented with Structure Harvester (Earl 2012).

For both algorithms (Structure and Baps), all inferred subpopulations were assumed to be panmictic and cryptic substructures were identified by minimizing Hardy–Weinberg and linkage disequilibrium within each of k clusters (Pritchard et al. 2000; Corander et al. 2004; De Meeûs 2012). Although Structure and Baps use different methods to search for the most likely number of subpopulations, both performed well, even at low levels of population differentiation (Latch et al. 2006). Nevertheless, Baps is more specifically used to identify the Wahlund effect and estimate the population substructure (Ravel et al. 2007; Dharmarajan et al. 2011).

MtDNA D-Loop Sequence Analysis

DNA sequences were edited and aligned with Mega 6.06 (Tamura et al. 2013). Gene diversity (H_s), nucleotide diversity (π) and genetic differentiation (F_{ST} averaged over loci) (Weir and Cockerham 1984) were obtained for each population from Arlequin 3.5 (Excoffier et al. 1992) with 99 999 permutations. Haplotype distribution was established using DNASP 5.10.1 (Rozas and Rozas 1995; Rozas 2009) and haplotype networks were constructed using the median joining network algorithm implemented in Network 5.0 (Bandelt et al. 1999).

Results

Microsatellites

Genetic Diversity and Basic Statistics

A total of 158 alleles were detected for the 7 microsatellite loci. All loci were polymorphic (range, 13–30 alleles per locus) for all populations. In some populations, markers showed low levels of estimated null allele frequency (Supplementary Table s3), which nevertheless assumes that populations are at HWE. However, all vampire populations deviated from HWE (see below); therefore, estimation was biased and significant null allele rates are likely the cause of those deviations toward a heterozygote deficit (Dąbrowski et al. 2014). Two markers showed likelihood of stuttering, but these markers were checked with at least 2 readings and were regarded as having no scoring errors; consequently, they were retained in the analysis.

Observed and expected heterozygosity, allelic richness, and gene diversity were moderate to high and consistent across all populations (Table 1). Pop4 had the greatest genetic diversity (0.730 ± 0.406) followed by pop5 and pop1, while Pop3 had the lowest genetic diversity (0.595 ± 0.330) followed by pop6 and pop2.

All populations presented deviations from HWE and the low significant inbreeding coefficient was explained by heterozygote deficiency (Table 1), but no linkage disequilibrium was detected. A recent bottleneck was detected in pop6 with a significant Wilcoxon signed-rank test ($P < 0.05$) and a normal-shift distribution. In pop4 a recent bottleneck was also detected with a shift in distribution of allele frequencies, but no significant test. Other populations had both a nonsignificant Wilcoxon signed-rank test ($P > 0.05$) and a normal-shift distribution.

The relatedness coefficient (r) was very low for overall populations ($r_{mean} = 0.07$). pop1 showed the strongest (r) value with 8% of related followed by pop6, while pop4 showed the lowest with 5% of related (Table 1).

Gene Flow

Between populations, estimated F_{ST} averaged over loci were low to high ($F_{ST_{mean}} = 0.084$; $P < 0.01$) and showed significant genetic structure between populations (Table 2). The genetic structure of the French Guiana populations (pop1 to pop4) appeared to be the lowest ($F_{ST_{mean}} = 0.031$; $P < 0.01$), while genetic differentiation between the Mexican population (pop6) and the Guianese populations seemed to be the strongest ($F_{ST_{mean}} = 0.183$; $P < 0.01$). The Brazilian population (pop5) showed moderate F_{ST} with the Guianese populations and a strong genetic structure with the Mexican population, although lower than between the Mexican and Guianese populations. N_m per generation varied greatly between population pairs and followed a similar pattern to F_{ST} values. pop2 and pop3 shared the highest N_m ($N_m = 20.26$) per generation and the lowest F_{ST}-values (0.009; $P < 0.01$), followed by pop1 and pop4 ($N_m = 11.94$; $F_{ST} = 0.023$; $P < 0.01$). Structuration discrepancies were evidenced in females and males. Considering females only, a significant but low genetic structure was observed between the 4 Guianese populations ($F_{ST_{mean}} = 0.046$; $P < 0.05$), pop3 showing moderate and the strongest F_{ST} with pop4 ($F_{ST} = 0.081$; $P < 0.05$) and pop2 showing the lowest with pop3 ($F_{ST} = 0.026$; $P < 0.05$). When focusing on males only, a lower and nonsignificant genetic structure was observed ($F_{ST_{mean}} = 0.019$; $P > 0.1$) except between pop1 and pop2 ($F_{ST} = 0.045$, $P < 0.05$) and between pop1 and pop3 ($F_{ST} = 0.034$, $P < 0.05$).

Population Structuring

The *dapc* analysis segregated the individuals into 6 genetic clusters (clus-a, b, c, d, e, and f) (Figure 2). The "*find.clusters*" function of Adegenet showed a clear BIC decrease until $k = 6$ clusters after which BIC increased. Those 6 clusters were highly differentiated (slot *asign.per.pop*; $P < 0.05$). The subdivision observed is associated with the geographic origin of the populations. clus-b and clus-d were mostly represented by the Mexican population (100% and 97%, respectively). clus-e was present in the Brazilian population and the

Table 1. Descriptive statistics for each population including observed heterozygosity (H_o), expected heterozygosity (H_e), average genetic diversity (H_s), P-value of test of Hardy–Weinberg equilibrium (HW), inbreeding obtained by ARLEQUIN, coefficient (F_{IS}) obtained by GENETIX, average relatedness coefficient (r) obtained by ML-RELATE and sample size (N)

Populations	H_o	H_e	H_s (±σ)	HW	F_{IS}	r	N
1	0.673	0.771	0.704 (0.382)	0.000	0.128*	0.080	60
2	0.647	0.735	0.696 (0.377)	0.000	0.120*	0.075	89
3	0.662	0.716	0.595 (0.330)	0.002	0.076*	0.074	55
4	0.648	0.747	0.730 (0.406)	0.004	0.136*	0.052	14
5	0.720	0.826	0.708 (0.386)	0.000	0.130*	0.059	39
6	0.621	0.727	0.694 (0.376)	0.000	0.147*	0.077	82

Table 2. Values below diagonal are F_{ST}-values, values marked with an asterisk (*) are significant ($P < 0.05$) obtained by ARLEQUIN with the method of pairwise genetic distances

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
Pop1		6.90	7.86	11.94	8.47	1.15
Pop2	0.036*		20.26	6.46	6.37	1.02
Pop3	0.036*	0.009*		6.41	6.37	1.07
Pop4	0.023*	0.039*	0.042*		6.56	1.07
Pop5	0.031*	0.039*	0.044*	0.045*		1.47
Pop6	0.181*	0.199*	0.193*	0.194*	0.146*	

Values above the diagonal are N_m values obtained by GENETIX.

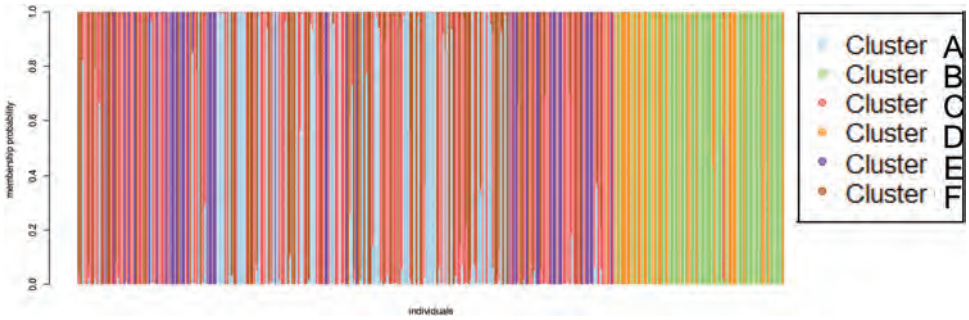

Figure 2. Distribution of clusters (*K*) by individuals estimated by *find.cluster* in ADEGENET R package represented by population. See online version for full colors.

Guianese populations sampled in the north (pop1, pop2, and pop4; Figure 1). clus-a, clus-c, and clus-f were shared by all the Guianese and Brazilian populations These 3 last clusters overlapped despite a significant differentiation between them (Figure 3).

pop1 and pop5 showed the same trend, mostly represented by clus-c (41% and 49%, respectively) and clus-e (27% and 32%, respectively) and poorly represented by clus-a (7% and 2%). Pop4 showed a close pattern and was mostly represented by clus-e and clus-f (28% and 38%, respectively). Pop2 and Pop3 showed a similar pattern and were mostly represented by clus-a (32% and 33%, respectively) and clus-c (30% and 34%) and poorly or not represented by clus-e (11% and 0%, respectively) (Figure 3).

Hidden Structure

The analysis of pop4 was not relevant with both programs probably because of its small sample size ($n = 14$). With Baps, 5 individuals of 14 were single in their subpopulation and the largest cluster contained 4 individuals. Structure failed to detect a stable number of subpopulations.

For the other 5 populations, the clustering algorithm used by baps identified a variable number of subpopulations (mean: 11.6; range: 8–14) in every populations examined; in overall populations, more than 75% of individuals in average were grouped into 3–6 main subpopulations.

FisClus of the combined populations was significantly lower than F_{IS} (Wilcoxon signed-rank; $P < 0.05$) (Table 3); it is likely that each population was composed of several genetically distinct entities. The genetic differentiation between the subpopulations of pop1 was high ($F_{ST_{mean}} = 0.131 \pm 0.043$; $P < 0.01$) while the genetic structure between subpopulations of pop2 was lower but significant ($F_{ST_{mean}} = 0.058 \pm 0.018$; $P < 0.01$) (Table 4). The average related coefficients (r) of pop1 subpopulations (r mean = 0.031 ± 0.042) and pop2 subpopulations (r mean = 0.025 ± 0.032) were weak and lower than the average r of all populations (r mean = 0.075 ± 0.010), just like pop1 r ($r = 0.080 \pm 0.129$) and pop2 r ($r = 0.075 \pm 0.124$).

In contrast to the results of baps, analysis with Structure revealed no evidence of cryptic genetic structure in 3–5 populations. Exceptions to this pattern were pop1 and pop2, wherein Structure identified the presence of 2 subpopulations.

MtDNA D-Loop

Sequence Characterization and Allelic Diversity

The first hypervariable segment (HVI) of the mitochondrial DNA (mtDNA) control region (D-loop) was amplified for 61 bats. Sequence alignment spanning 359 bp revealed 12 mtDNA D-loop haplotypes. Pop6 showed the highest gene diversity ($H_s = 0.8636 \pm 0.0786$) followed by pop5 and pop2. Nucleotide diversity was low for the overall population, pop2 showed the highest ($\pi = 0.007428 \pm 0.004881$) followed by pop6 (Table 5).

Structuring

Strong and significant genetic differentiation was found within the overall population ($F_{ST_{mean}} = 0.859$, $P < 0.05$) except between pop1 and pop4, and pop2 and pop3 (Table 6). The haplotype network revealed 2 main groups of haplotypes (Supplementary Figure s1). The first includes 7 haplotypes composed of all individuals from the Mexican population (pop6; H4–H10) with a maximum of 2 mutations between haplotypes. The second comprises 2 smaller groups, 1 with 3 haplotypes composed of all individuals from the Guianese populations (H1: pop2 and pop3; H2: pop1 and pop4; H3: pop2 and pop3), and the other with 2 haplotypes composed of all individuals from the Brazilian populations (pop5; H11 and H12) (Table 5). The highest number of mutations was found between the Mexican haplotype group and the Guianese/Brazilian groups with 48 mutations. Low sequence divergence was found between haplotypes from the same country ($\phi_{stmean\ FG} = 0.011 \pm 0.0073$;

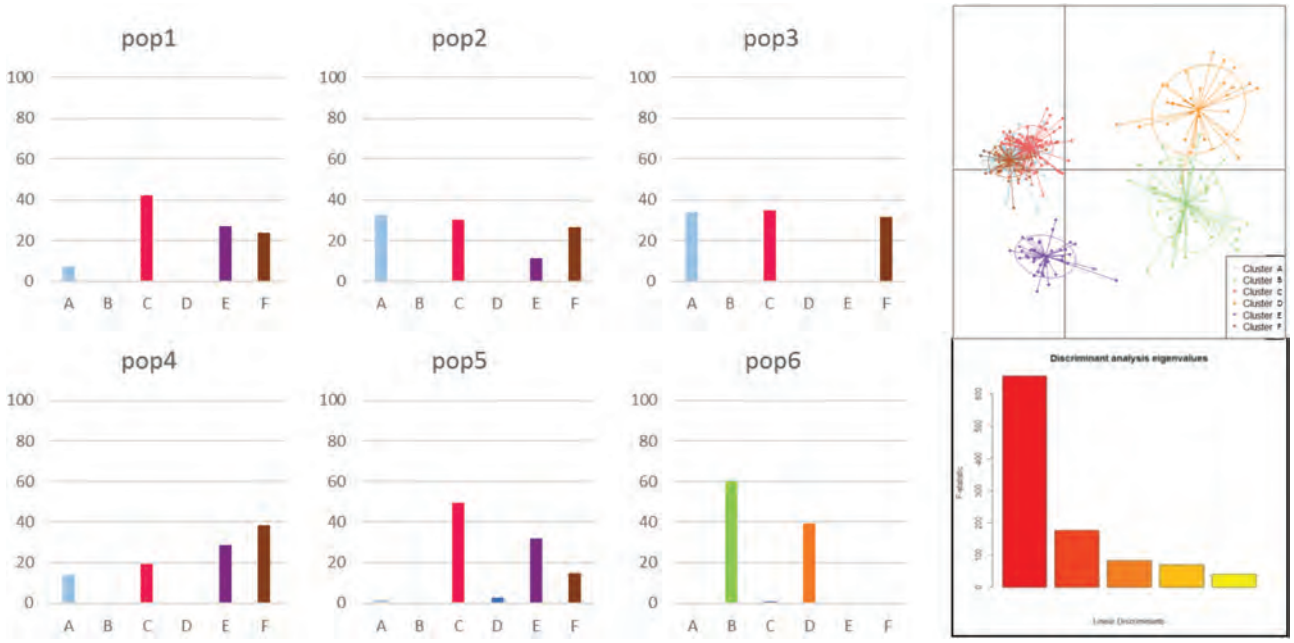

Figure 3. Discriminant analysis of principal component on overall populations. Six clusters have been found with BIC-value (top-right) and discriminant analysis eigenvalue (bottom-right) estimated by *Adegenet* package (Jombart 2011). The left part shows the contribution of the 6 clusters to the 6 populations. See online version for full colors.

Table 3. For hidden structure (BAPS analyses) reported number of clusters identified (*K*) with the higher for each population, mean (±standard deviation) of social group sizes, F_{IS} and FisClus for each population (recalculated after BAPS estimation) obtained by GENETIX 4.0.5.2

Populations	K	Mean (±σ)	F_{IS}	F_{IS}Clus
1	14*	4.286 (3.534)	0.128*	−0.040*
2	13*	6.923 (7.550)	0.120*	0.018*
3	10*	5.500 (5.162)	0.076*	−0.027*
4	8*	1.750 (1.090)	0.136*	−0.027*
5	13*	3.000 (2.746)	0.130*	−0.013*
6	8*	10.250 (12.111)	0.147*	0.079*

K-values are marked with an asterisk (*) when F_{IS}CLus is significantly lower than F_{IS} (Wilcoxon signed-rank; $P < 0.05$).

Table 4. F_{ST}-values between subpopulations of POP1 and POP2 whose numbers of individuals were greater than 3 obtained by ARLEQUIN 3.5 with the method of pairwise genetic distances

	Pop 1-a	Pop 1-b	Pop 1-c	Pop 1-d	Pop 1-e	Pop 1-f
Pop 1-a						
Pop 1-b	0.170*					
Pop 1-c	0.103*	0.122*				
Pop 1-d	0.083*	0.126*	0.115*			
Pop 1-e	0.087*	0.105*	0.074*	0.109*		
Pop 1-f	0.165*	0.185*	0.196*	0.113*	0.208*	
	Pop 2-a	Pop 2-b	Pop 2-c	Pop 2-d		
Pop 2-a						
Pop 2-b	0.031*					
Pop 2-c	0.070*	0.041*				
Pop 2-d	0.072*	0.057*	0.076*			

Values marked with an asterisk (*) are significant ($P < 0.01$).

Table 5. MtDNA D-loop descriptive statistics for each population including sample size (*N*), average genetic diversity (H_s), average nucleotide diversity (π) obtained by ARLEQUIN and haplotype distribution (H) obtained by DNASP

Populations	*N*	H_s (±σ)	π(±σ)	H
1	6	0.000 (0.000)	0.000 (0.000)	H2
2	10	0.533 (0.095)	0.007 (0.005)	H1/H3
3	12	0.409 (0.133)	0.006 (0.004)	H1/H3
4	12	0.000 (0.000)	0.000 (0.000)	H2
5	10	0.556 (0.074)	0.005 (0.003)	H11/H12
6	12	0.864 (0.079)	0.006 (0.004)	H4–H10

Table 6. MtDNA D-loop F_{ST}-values between populations obtained by ARLEQUIN 3.5 with the method of pairwise genetic distances

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
Pop1						
Pop2	0.600*					
Pop3	0.721*	−0.045				
Pop4	0.000	0.691*	0.785*			
Pop5	0.927*	0.827*	0.841*	0.948*		
Pop6	0.971*	0.953*	0.958*	0.979*	0.963*	

Values marked with an asterisk (*) are significant ($P < 0.01$).

$\Phi_{STmean\ BRZ} = 0.008 \pm 0.004$; $\Phi_{STmean\ MEX} = 0.007 \pm 0.003$) and was much higher between countries ($\Phi_{STmean\ FG\text{-}BRZ} = 0.0372 \pm 0.008$; $\Phi_{STmean\ FG\text{-}MEX} = 0.095 \pm 0.004$; $\Phi_{STmean\ BRZ\text{-}MEX} = 0.095 \pm 0.005$).

Discussion

In this study, the population structure and dynamics of *D. rotundus* was estimated at 2 spatial scales in Latin America from bi-parentally

inherited nuclear microsatellites and maternally inherited mtDNA haplotypes. These data provide information on population history, dynamics, and social structure, and are relevant to better assessing the role played by vampire bats as virus dispersers.

Phylogeographic Structure

Nuclear and mitochondrial markers showed a significant population genetic structure at the larger scale (French Guiana vs. Mexico; North Brazil vs. Mexico) and a lower structure at the regional scale (between French Guianese populations; French Guiana vs. North Brazil). This trend was confirmed by structure analysis that showed an ancient isolation where Mexican populations were represented in only 2 clusters (clus-b and d), which were not shared by the Guianese or Brazilian populations. In contrast, the distance between the Brazilian and Guianese populations was substantial but 5 times shorter and allowed gene sharing. Only low sequence divergence ($\phi_{STmean} = 0.009 \pm 0.002$) between haplotypes at the regional scale was also noted, both suggesting that the vampire bat seems to be a highly mobile species able to maintain gene flows over quite large distances. Divergence at the larger scale was higher ($\phi_{STmean} = 0.076 \pm 0.033$), but it is not unusual for haplotypes to be geographically isolated (McCracken et al. 1994). Moreover, nuclear markers indicated a low differentiation at the regional scale, showing the maintenance of recent gene flow. Additionally, the 4 ancestral clusters (clus-a, -c, -e, and -f) shared by populations from French Guiana and North Brazil suggest long-term past exchanges between those populations, likely supporting the connectivity of favorable habitat. On the other hand, the genetic structure identified could be driven by both: 1) social behaviors as shown by structuring discrepancy of nuDNA between females, for which the 4 Guianese populations were significantly structured ($F_{ST_{mean}} = 0.046$; $P < 0.05$), and males, for which the F_{ST} coefficients were very low and nonsignificant ($F_{ST_{mean}} = 0.019$; $P > 0.1$), except between pop1 and pop2 ($F_{ST} = 0.045$, $P < 0.05$) like pop1 and pop3 ($F_{ST} = 0.034$, $P < 0.05$) and 2) landscape history, which induced demography and gene flow changes. For instance, the refuge theory (Haffer 1969) suggests that contraction and subsequent expansion of forested areas during the last glaciation cycles would have created allopatry between populations of the same forest-dwelling species, leading to intra specific differentiation, as has been shown in birds, reptiles, amphibians, and mammals (Wüster et al. 2005; Weir 2006; Martins et al. 2009; Fouquet et al. 2012). Expansion of savannas in the Guiana Shield (Pennington et al. 2000) during the Pleistocene could explain why Guianese populations, although near each other, may be composed of distinct ancestral stocks. Impacts of these past forest cover changes may have been either direct, limiting dispersal since the species is susceptible to forest fragmentation (Martins et al. 2009), or indirect, since vegetation changes also influenced distribution and abundance of prey.

Social Genetic Structure

Many factors related to history (e.g., dispersal ability and colonization processes, isolation in geographical refugia) and the biology of species (e.g., mating system, social structure) may act on genetic structure and differently affect the variability of biparental and maternal inherited genes (Chesser and Baker 1996). The very low structure between pop1 (breeding) and pop4 (feeding) just as pop2 (breeding) and pop3 (feeding), and even not significant for mtDNA, suggests that the population structure identified in caves (i.e., in roosts) is maintained in foraging areas, although those areas are close to each other and ecologically connected. This structuring between the 2 roosts is supported by individual marking: 68 females and 52 males were identified in breeding cave 1, and 133 females and 33 males in breeding cave 2. After 7 years of monitoring and 9 capture sessions, no exchange was observed between the caves. This raises the question of a sort of territoriality of vampire bat with the use of exclusive foraging areas (Wilkinson 1985a, 1986). In other cases, the high differentiation detected in mtDNA differed strongly from the nuclear structure detected. This contrasted pattern has been found in many mammals, including bats (Kerth 2000; Castella et al. 2001; Arnold and Wilkinson 2015; Naidoo et al. 2016), suggesting female philopatry and male-mediated gene flow. Individual marking and recapture also support this result: in cave 1, 87% of recaptures ($n = 26$) were females, 71% of recaptures ($n = 21$) were females in cave 2.

All the populations examined in this study showed deviations from HWE and a low but significant inbreeding coefficient due to a heterozygote deficiency. Null allele, gene flow but also genetic drift may affect allele frequencies, as in some mammals, reptiles, and birds (Durrant et al. 2009; Plot et al. 2012; Spurgin et al. 2014) Considering these last factors vampire bats' social behaviors (Wilkinson 1984, 1985a, 1985b), and the fact that cryptic social structure may lead to deviations from HWE (Sugg et al. 1996), it seems that present deviations were likely caused by a Wahlund effect. The Wahlund effect (Wahlund 1928) is produced by a lack of consideration of substructure due to sampling or kin structure as shown in mammals (Svoboda et al. 1985) and arthropods (Ravel et al. 2007; Kempf et al. 2010; Dharmarajan et al. 2011).

In this study, the Wahlund effect was probably due to a hidden social structure for pop1 and pop2 given that sampled individuals came from single breeding caves, contrary to 4 other populations where individuals were sampled in multiple foraging areas. Some mammal species living in social groups may have genetic and demographic consequences (Kerth and van Schaik 2012), often leading to low genetic diversity, which decreases long-term viability (Hildner et al. 2003). Nevertheless, genetic diversity may be maintained despite sociality (Stiebens 2013; O'Donnell et al. 2016) as shown by present genetic diversity results ($Hs_{mean} = 0.688 \pm 0.047$), which are congruent with the analyses of Romero-Nava et al. (2014).

Considering this hidden structure characterized by several genetically distinct entities in each population (8–14 subpopulations), no inbreeding depression was detected in any population, consistent with Wilkinson (1985b), who observed that females avert some males to avoid inbreeding depression, showing random mating. Moreover, the genetic structure detected within populations (between pop1 subpopulations and between pop2 subpopulations) was significant, but this did not lead to high average levels of relatedness. The estimated mean relatedness in pop1 and pop2 subpopulations was very low and comparable to the mean relatedness calculated for the pop1 and pop2 populations. These results were also consistent with Wilkinson's estimates (1985b) whereby the low average degree of relatedness may be due to small litter size, high infant mortality, low adult mortality, and possible transfer of females from one subpopulation to another.

The presence of subpopulations with no inbreeding and low relatedness could be explained by vampire bat's social structure. The social structure is characterized by several groups of females, which may or may not be related, maintain a long-term association (Wilkinson 1985a), notably due to their long lifespan (Lord et al. 1976; Delpietro et al. 2017) and share a common set of diurnal roosts. Those associations may influence the mating system, as well as the genetic structure, and would appear sufficient to produce

some genetic heterogeneity within populations (Wilkinson 1985a) In this context, the question is what characterized these genetic entities detected if it was not only the kin structure.

A common theoretical condition for social living implies that the overall benefits of group living outweigh the costs in terms of evolutionary fitness (Hamilton 1964). In many mammals, relatedness was commonly used to estimate indirect fitness benefits, but despite a low level of relatedness, benefits may be still substantial (Kerth 2002) and association patterns such as social interactions may occur (Briga et al. 2012; Hirsch et al. 2013; Strickland et al. 2014). In *D. rotundus* social interactions such as allogrooming (Wilkinson 1986) and food sharing (Wilkinson 1984) occur between adult females, related and unrelated, as between females and young. Regurgitations of blood, likely based on reciprocal altruism (Carter and Wilkinson 2013, 2015; Wilkinson et al. 2016), are essential for the survival of the vampire bat, which has to consume blood daily (McNab 1973). To maximize inclusive fitness, reciprocal food sharing should occur among close kin; however, sharing with nonkin promotes reciprocal assistance by strengthening long-term social bonds (Carter and Wilkinson 2015; Carter et al. 2017). However, food sharing cannot be explained solely by kin selection or harassment (Carter and Wilkinson 2013). The theory of group selection claimed that the natural selection does not act only at the level of the individual but also at the level of the group and is based on the idea that the behavior of animals could affect their survival and reproduction and therefore their fitness (Darwin 1871; Wynne-Edwards 1962; Maynard-Smith 1964). This question has been debated (Wade et al. 2010; Marshall 2011; Van Veelen et al. 2012) although some evidence was provided, especially about effects of group phenotype on group fitness (Pruitt and Goodnight 2014; Pruitt et al. 2017). Considering the present results on the significant substructures in pop1 and pop2 and their low level of relatedness, like Wilkinson and Carter's observations and experiments mentioned above, the group selection hypothesis, added to kin selection, seems to be relevant to explain the distinct genetically entities (i.e., subpopulations) detected in this study.

Virus Spread

The population dynamics of parasites are influenced by both abiotic and biotic factors, and are highly dependent on the host's history and host spatiotemporal dynamics (Barrett et al. 2008; van Schaik and Kerk 2014). In mammals, males typically distribute themselves according to the presence of females, whereas female dispersion reflects the patchiness of resources and the importance of sociality (Wilkinson 1985a, 1985b, 1988; Clutton-Brock 1989; Arnaud et al. 2012). In polygynous taxa, males tend to disperse more to avoid inbreeding and/or competition, while females are more philopatric (Greenwood 1980; McCracken and Wilkinson 2000). Those drivers of host dynamics have important impacts for virus dispersal (Delpietro et al. 2017). *Desmodus rotundus* is the most important reservoir of rabies virus in Latin America (Schneider et al. 2009), and lessons from the genetic structure and population dynamics described above have potential implications for virus dispersal.

Our data suggested the gene flow would be sufficient to maintain virus circulation over a long distance at the regional scale. This high capability of males to disperse may explain the wide circulation of the lineage II of rabies virus detected in French Guiana (Lavergne et al. 2016) and circulating in northern Amazonia, without evidence of strong geographic structure signal (Condori-Condori et al. 2013). In foraging areas often visited by females, males could potentially spread rabies virus at a more local scale, in spite of gene flow restriction by natal philopatry, and female social behaviors may favor the rapid spread of the virus within social groups to related and unrelated offspring and adult females (Kobayashi et al. 2005; Velasco-Villa et al. 2006). In other social species, for example, the *Mandrillus sphinx* monkey, maternal kinship was also identified as a driver of simian immunodeficiency viruses (Fouchet et al. 2012). In the case of rabies dispersal, sociality, and trophallaxis should favor quick dispersal of the virus within subpopulations once introduced in the roosts. This behavior explains the equally distributed anti-rabies serologies observed within subpopulations as well as in adults and offspring (de Thoisy et al. 2016; BT & AL, unpublished data). This role of both genders in virus circulation is highlighted by comparable seropositivity in adult males and females, although social interactions in large roosts such as caves may favor virus transmission (37% and 55% of animals were seropositive in cave 1 and cave 2, respectively, vs. 23% at feeding sites).

Last, transmission of rabies virus is also favored by nonsocial factors. High vampire bat density and this bat's concentration around cattle may favor increased interactions between animals (de Thoisy et al. 2016). However, studies have shown an effect of livestock in favoring population expansion (Lima Ulbierta et al. 2017), reducing depredation on human beings (Gilbert et al. 2012; Streicker et al. 2016), contrary to extirpation of wildlife by hunting or deforestation, which could increase feeding on humans (Schneider et al. 2009; Stoner-Duncan et al. 2014).

Conclusions

This study investigated how fine-scale genetic signatures are influenced by the sociality of one of the most complex social systems reported in any bat species, which also acts as the main reservoir of one of the deadliest viruses. We infer that the social structure of the vampire bat affected the intra- and inter-population genetic structure and potentially virus transmission. At large scale, restricted gene flow was evidenced, but further work with better sample coverage, including the Guiana Shield and/or the southern region, is required to estimate the dispersal potential of *D. rotundus* and of viruses they host. At regional scale, a contrasted pattern between nuclear-inherited genes and mtDNA-inherited genes suggests a male-mediated gene flow and female philopatry. Physiologic demand such as blood feeding is probably the cause of social behavior and of the long-term association between females, which have contributed to spreading the rabies virus within the group and population. Subpopulations and potential social groups were revealed in roosting sites with no inbreeding depression, high genetic diversity and a low degree of relatedness, which corroborates observations of Wilkinson (1985a, 1985b) and suggest that both kin and group selection shape the social structure of vampires. The possibility of gene connectivity should be considered for decision-making on vampire-bat-transmitted rabies control because some strategies such as culling could induce the immigration of bats from neighboring colonies to fill vacant roost space (Streicker et al. 2012, 2016). These artificially induced dynamics may be associated with the dispersal of the virus, including possible (re)introduction of strains. Therefore, controls should probably be geographically coordinated (Blackwood et al. 2013), unless they fail to achieve the outcomes required by the principles for ethical wildlife control (Dubois et al. 2017).

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

Funding

This study was conducted within CAROLIA, RESERVOIRS, and INDIGEN programs supported by European (ERDF/FEDER) funds and assistance from Collectivité Territoriale de la Guyane and Direction Régionale pour la Recherche et la Technologie, "Investissement d'Avenir" grants managed by the Agence Nationale de la Recherche (CEBA, Ref. ANR-10-LABEX-25-01), and the project "Guyamazon II" funded by Ambassade de France in Brazil, IRD/AIRD (France), the French Guianan Region, CIRAD, FAPEAM, FAPEMA, and FAPEAP; and an European Commission "REGPOT-CT-2011-285837-STRonGer" grant within the FP7.

Acknowledgments

All field volunteers and owners and/or managers of capture sites are warmly acknowledged for their assistance in captures. In compliance with Advantages and Benefits Sharing policy in French Guiana, samples are kept in the tissue collection JAGUARS (http://kwata.net/la-collection-jaguars-pour-l-etude-de-la-biodiversite.html; CITES reference: FR973A) supported by Kwata NGO, Institut Pasteur de la Guyane, DEAL Guyane, and Collectivité Territoriale de la Guyane.

Data Availability

The primary data underlying these analyses are deposed as follows:
Sampling locations and microsatellite genotypes: provisional Dryad (doi: 10.5061/dryad.548gf).
DNA sequences: Genbank accessions MG563853–MG563911.

References

Almeida MF, Martorelli LF, Sodré MM, Kataoka AP, Rosa AR, Oliveira ML, Amatuzzi E. 2011. Rabies diagnosis and serology in bats from the State of São Paulo, Brazil. *Rev Soc Bras Med Trop*. 44:140–145.

Arnaud CM, Dobson FS, Murie JO. 2012. Philopatry and within-colony movements in Columbian ground squirrels. *Mol Ecol*. 21:493–504.

Arnold BD, Wilkinson GS. 2015. Female natal philopatry and gene flow between divergent clades of pallid bats (*Antrozous pallidus*). *J Mammal*. 96:531–540.

Axelrod R, Hamilton WD. 1981. The evolution of cooperation. *Science*. 211:1390–1396.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*. 16:37–48.

Bardeleben C, Campbell P, Lara M, Moore RL. 2007. Isolation of polymorphic tetranucleotide microsatellite markers for the silky short-tailed bat *Carollia brevicauda*. *Mol Ecol*. 7:63–65.

Barrett LG, Thrall PH, Burdon JJ, Linde CC. 2008. Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends Ecol Evol*. 23:678–685.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 2004. *GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations*. Montpellier (France): Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, université de Montpellier II.

Blackwood JC, Streicker DG, Altizer S, Rohani P. 2013. Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proc Natl Acad Sci U S A*. 110:20837–20842.

Borisenko AV, Lim BK, Ivanova NV, Hanner RH, Hebert PD. 2008. DNA barcoding in surveys of small mammal communities: a field study in Suriname. *Mol Ecol Resour*. 8:471–479.

Briga M, Pen I, Wright J. 2012. Care for kin: within-group relatedness and allomaternal care are positively correlated and conserved throughout the mammalian phylogeny. *Biol Lett*. 8:533–536.

Carter GG, Wilkinson GS. 2013. Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proc Biol Sci*. 280:20122573.

Carter GG, Wilkinson GS. 2015. Social benefits of non-kin food sharing by female vampire bats. *Proc R Soc Lond B Biol Sci*. 282:20152524.

Castella V, Ruedi M, Excoffier L. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *Evol Biol*. 14:708–720.

Chesser RK, Baker RJ. 1996. Effective sizes and dynamics of uniparentally and diparentally inherited genes. *Genetics*. 144:1225–1235.

Clutton-Brock TH. 1989. Mammalian mating systems. *Proc R Soc Lond B Biol Sci*. 236:339–372.

Condori-Condori RE, Streicker DG, Cabezas-Sanchez C, Velasco-Villa A. 2013. Enzootic and epizootic rabies associated with vampire bats, Peru. *Emerg Infect Dis*. 19:1463–1469.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ. 2004. BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*. 20:2363–2369.

Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*. 144:2001–2014.

Dąbrowski MJ, Pilot M, Kruczyk M, Żmihorski M, Umer HM, Gliwicz J. 2014. Reliability assessment of null allele detection: inconsistencies between and within different methods. *Mol Ecol Resour*. 14:361–373.

Darwin C. 1871. *The descent of man, and selection in relation to sex*. New York: Appleton and Company.

De Meeûs T. 2012. *Initiation à la génétique des populations naturelles: application aux parasites et à leurs vecteurs*. IRD éditions. 338.

de Thoisy B, Bourhy H, Delaval M, Pontier D, Dacheux L, Darcissac E, Donato D, Guidez A, Larrous F, Lavenir R, *et al*. 2016. Bioecological drivers of rabies virus circulation in a neotropical bat community. *PLoS Negl Trop Dis*. 10:e0004378.

Delpietro HA, Russo RG, Carter GG, Lord RD, Delpietro GL. 2017. Reproductive seasonality, sex ratio and philopatry in Argentina's common vampire bats. *R Soc Open Sci*. 4:160959.

Dharmarajan G, Beasley JC, Rhodes OE Jr. 2011. Heterozygote deficiencies in parasite populations: an evaluation of interrelated hypotheses in the raccoon tick, *Ixodes texanus*. *Heredity (Edinb)*. 106:253–260.

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB. 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proc Natl Acad Sci U S A*. 91:3166–3170.

Dubois S, Fenwick N, Ryan EA, Baker L, Baker SE, Beausoleil NJ, Carter S, Cartwright B, Costa F, Draper C, *et al*. 2017. International consensus principles for ethical wildlife control. *Conserv Biol*. 31:753–760.

Durrant CJ, Beebee T, Greenaway F, Hill DA. 2009. Evidence of recent population bottlenecks and inbreeding in British populations of Bechstein's bat, *Myotis bechsteinii*. *Conserv Genet*. 10:489–496.

Earl DA. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour*. 4:359–361.

Escobar LE, Peterson AT, Favi M, Yung V, Medina-Vogel G. 2015. Bat-borne rabies in Latin America. *Rev Inst Med Trop Sao Paulo*. 57:63–72.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*. 14:2611–2620.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*. 131:479–491.

Fenton MB. 1997. Science and the conservation of bats. *J Mammal*. 78:1–14.

Fouchet D, Verrier D, Ngoubangoye B, Souquière S, Makuwa M, Kazanji M, Gonzalez JP, Pontier D. 2012. Natural simian immunodeficiency virus transmission in mandrills: a family affair? *Proc Biol Sci*. 279:3426–3435.

Fouquet A, Noonan BP, Rodrigues MT, Pech N, Gilles A, Gemmell NJ. 2012. Multiple quaternary refugia in the eastern Guiana shield revealed by comparative phylogeography of 12 frog species. *Syst Biol*. 61:461–489.

Gilbert AT, Petersen BW, Recuenco S, Niezgoda M, Gómez J, Laguna-Torres VA, Rupprecht C. 2012. Evidence of rabies virus exposure among humans in the Peruvian Amazon. *Am J Trop Med Hyg*. 87:206–215.

Greenhall AM, Joermann G, Schmidt U. 1983. *Desmodus rotundus*. *Mamm Species* 202:1–6.

Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Anim Behav*. 28:1140–1162.

Haffer J. 1969. Speciation in amazonian forest birds. *Science*. 165:131–137.

Hamilton WD. 1964. The genetical evolution of social behaviour. I. *J Theor Biol*. 7:1–16.

Hildner KK, Soule ME, Min MS, Foran DR. 2003. The relationship between genetic variability and growth rate among populations ofthe pocket gopher, Thomomysbottae. *Conserv Genet*. 4:233–240.

Hirsch BT, Prange S, Hauver SA, Gehrt SD. 2013. Genetic relatedness does not predict racoon social network structure. *Anim Behav*. 85:463–470.

Huth-Schwarz A, Leon A, Ame RV, Moritz A, Kraus FB. 2011. Mating frequency & genetic colony structure of the neotropical bulble bee *Bombus wilmattae* (Hymenoptera: Apidae). *Apidologie*. 42:519–525.

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*. 24:1403–1405.

Jombart T, Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*. 27:3070–3071.

Kalinowski ST, Wagner AP, Taper ML. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol Ecol*. 6:576–579.

Kempf F, McCoy KD, De Meeûs T. 2010. Wahlund effects and sex-biased dispersal in *Ixodes ricinus*, the European vector of *Lyme borreliosis*: new tools for old data. *Infect Genet Evol*. 10:989–997.

Kerth G, Mayer F, König B. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. *Mol Ecol*. 9:793–800.

Kerth G, Mayer F, Petit E. 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). *Mol Ecol*. 11:1491–1498.

Kerth G, van Schaik J. 2012. Causes and consequences of living in closed societies: lessons from a long-term socio-genetic study on Bechstein's bats. *Mol Ecol*. 21:633–646.

Kobayashi Y, Sato G, Shoji Y, Sato T, Itou T, Cunha EMS, Samara SI, Carvalho AAB, Nociti DP, Ito FH, Sakai T. 2005. Molecular epidemiological analysis of bat rabies viruses in Brazil. *J Med Vet Sci*. 67:647–652.

Kunz TH, Braun de Torrez E, Bauer D, Lobova T, Fleming TH. 2011. Ecosystem services provided by bats. *Ann N Y Acad Sci*. 1223:1–38.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE Jr. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv Genet*. 7:295–302.

Lavergne A, Darcissac E, Bourhy H, Tirera S, de Thoisy B, Lacoste V. 2016. Complete Genome sequence of a Vampire Bat Rabies virus from French Guiana. *Genome Annoucements* 4:e00188–16.

Lecompte E, Bouanani MA, de Thoisy B, Crouau-Roy B. 2017. How do rivers, geographic distance, and dispersal behavior influence genetic structure in two sympatric New World monkeys? *Am J Primatol*. doi: 10.1002/ajp.22660.

Lima Ulbierta G, Sances de Siqueira TY, Graciolli G. 2017. White-winged vampire bat, *Diaemus youngi*, (Jentink, 1893) (Mammalia, Chiroptera): range extension in the Cerrado biome and new locality in Mato Grosso do Sul, southwestern Brazil. *Check List* 13:2128.

Lord RD, Muradali F, Lazaro L. 1976. Age composition of vampire (*Desmodus rotundus*) bats in the northern Argentina & southern Brazil. *J Mammal*. 57:573–575.

Marshall JA. 2011. Group selection and kin selection: formally equivalent approaches. *Trends Ecol Evol*. 26:325–332.

Martins FM, Templeton AR, Pavan AC, Kohlbach BC, Morgante JS. 2009. Phylogeography of the common vampire bat (*Desmodus rotundus*): marked population structure, Neotropical Pleistocene vicariance and incongruence between nuclear and mtDNA markers. *BMC Evol Biol*. 9:294.

Maynard-Smith JM. 1964. Group selection and kin selection. *Nature*. 201:1145–1147.

McCracken GF, McCracken MK, Vawter AT. 1994. Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. *J Mammal*. 75:500–510.

McCracken GF, Wilkinson GS. 2000. Bat mating systems. In: Crichton EG, Krutzsch PH, editors. *Reproductive biology of bats*. Cambridge: Elsevier, Academic Press. p. 321–362.

McCulloch ES. 2012. *Environmental & landscape determinants of population genetic structure & diversity of the great fruit-eating bat, Artibeus lituratus, in Atlantic forest remnants in South America* [Doctoral dissertation]. Lafayette: Faculty of the Louisiana State & University and Agricultural and Mechanical College.

McNab BK. 1973. Energetics and the distribution of vampires. *J Mammal*. 54:131–144.

Miller-Butterworth CM, Vonhof MJ, Rosenstern J, Turner GG, Russell AL. 2014. Genetic structure of little brown bats (*Myotis lucifugus*) corresponds with spread of white-nose syndrome among hibernacula. *J Hered*. 105:354–364.

Milligan BG. 2003. Maximum-likelihood estimation of relatedness. *Genetics*. 163:1153–1167.

Moussy C, Hosken DJ, Mathews F, Smith GC, Aegerter JN, Bearhop S. 2013. Migration and dispersal patterns of bats and their influence on genetic structure. *Mammal Rev*. 43:183–195.

Naidoo T, Schoeman MC, Goodman SM, Taylor PJ, Lamb JM. 2016. Discordance between mitochondrial and nuclear genetic structure in the bat *Chaerephon pumilus* (Chiroptera: Molossidae) from southern Africa. *Mamm Biol*. 81:115–122.

O'Donnell CF, Richter S, Dool S, Monks JM, Kerth G. 2016. Genetic diversity is maintained in the endangered New Zealand long-tailed bat (*Chalinolobus tuberculatus*) despite a closed social structure and regular population crashes. *Conserv Genet*. 17:91–102.

O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DT, Luis AD, Peel AJ, Plowright RK, Wood JL. 2014. Bat flight and zoonotic viruses. *Emerg Infect Dis*. 20:741–745.

Ortega J, Maldonado JE, Arita HT, Wilkinson GS, Fleischer RC. 2002. Characterization of microsatellite loci in the Jamaican fruit-eating bat Artibeus jamaicensis and cross-species amplification. *Mol Ecol Notes* 2:462–464.

Pennington RT, Prado DE, Pendry CA. 2000. Neotropical seasonaly dry forests and Quaternary vegetation changes. *J Biogeogr*. 27:261–273.

Piaggio AJ, Johnston JJ, Perkins SL. 2008. Development of polymorphic microsatellite loci for the common vampire bat, *Desmodus rotundus* (Chiroptera: Phylostomidae). *Mol Ecol Resour*. 8:440–442.

Plot V, de Thoisy B, Blanc S, Kelle L, Lavergne A, Roger-Bérubet H, Tremblay Y, Fossette S, Georges JY. 2012. Reproductive synchrony in a recovering bottlenecked sea turtle population. *J Anim Ecol*. 81:341–351.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155:945–959.

Pruitt JN, Goodnight CJ. 2014. Site-specific group selection drives locally adapted group compositions. *Nature*. 514:359–362.

Pruitt JN, Goodnight CJ, Riechert SE. 2017. Intense group selection selects for ideal group compositions, but selection within groups maintains them. *Anim Behav*. 124:15–24.

R Development Core Team. 2008. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. ISBN 3-900051-07-0. Available from: http://www.R-project.org.

Ravel S, T Meeûs De, Dujardin JP, Zézé DG, Gooding RH, Dusfour I, Sané B, Cuny G, Solano P. 2007. The tsetse fly *Glossina palpalis palpalis* is composed of several genetically differentiated small populations in the sleeping sickness focus of Bonon, Côte d'Ivoire. *Infect Genet Evol*. 16:116–125.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered*. 86:248–249.

Reddrop C, Moldrich RX, Beart PM, Farso M, Liberatore GT, Howells DW, Petersen KU, Schleuning WD, Medcalf RL. 2005. Vampire bat salivary plasminogen activator (desmoteplase) inhibits tissue-type plasminogen activator-induced potentiation of excitotoxic injury. *Stroke*. 36:1241–1246.

Rice WR. 1989. Analyzing tables of statistical test. *Evolution*. 43:223–225.

Romero-Nava C, Leon-Paniagua L, Ortega J. 2014. Microsatellites loci reveal heterozygosis and population structure in vampire bats (*Desmodus rotundus*) (Chiroptera: Phyllostomidae) of Mexico. *Biol Trop*. 62:659–669.

Rossiter SJ, Zubaid A, Mohd-Adnan A, Struebig MJ, Kunz TH, Gopal S, Petit EJ, Kingston T. 2012. Social organization and genetic structure: insights from codistributed bat populations. *Mol Ecol*. 21:647–661.

Rousset F, Raymond M. 1995. Testing heterozygote excess and deficiency. *Genetics*. 140:1413–1419.

Rozas J. 2009. DNA sequence polymorphism analysis using DnaSP. In Posada D, editor. *Bioinformatics for DNA sequence analysis*. Methods in Molecular Biology Series Vol. 537. (NJ): Humana Press. p. 337–350.

Rozas J, Rozas R. 1995. DnaSP, DNA sequence polymorphism: an interactive program for estimating population genetics parameters from DNA sequence data. *Comput Appl Biosci*. 11:621–625.

Salmón-Mulanovich G, Vásquez A, Albújar C, Guevara C, Laguna-Torres VA, Salazar M, Zamalloa H, Cáceres M, Gómez-Benavides J, Pacheco V, *et al*. 2009. Human rabies and rabies in vampire and nonvampire bat species, Southeastern Peru, 2007. *Emerg Infect Dis*. 15:1308–1310.

Schneider MC, Romijn PC, Uieda W, Tamayo H, da Silva DF, Belotto A, da Silva JB, Leanes LF. 2009. Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? *Rev Panam Salud Publica*. 25:260–269.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol Lett*. 9:615–629.

Sikes RS, Gannon WL. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal*. 92:235–253.

Spurgin LG, Illera JC, Jorgensen TH, Dawson DA, Richardson DS. 2014. Genetic and phenotypic divergence in an island bird: isolation by distance, by colonization or by adaptation? *Mol Ecol*. 23:1028–1039.

Stiebens VA, Merino SE, Roder C, Chain FJ, Lee PL, Eizaguirre C. 2013. Living on the edge: how philopatry maintains adaptive potential. *Proc Biol Sci*. 280:20130305.

Stoner-Duncan B, Streicker DG, Tedeschi CM. 2014. Vampire bats and rabies: toward an ecological solution to a public health problem. *PLoS Negl Trop Dis*. 8:e2867.

Streicker DG, Recuenco S, Valderrama W, Gomez Benavides J, Vargas I, Pacheco V, Condori-Condori RE, Montgomery J, Rupprecht CE, Rohani P, *et al*. 2012. Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proc Biol Sci*. 279:3384–3392.

Streicker DG, Winternitz JC, Satterfield DA, Condori-Condori RE, Broos A, Tello C, Recuenco S, Velasco-Villa A, Altizer S, Valderrama W. 2016. Host–pathogen evolutionary signatures reveal dynamics and future invasions of vampire bat rabies. *Proc Natl Acad Sci U S A*. 113:10926–10931.

Strickland K, Gardiner R, Schultz AJ, Frere CH. 2014. The social life of eastern water dragons: sex differences, spatial overlap and genetic relatedness. *Anim Behav*. 97:53–61.

Sodré MM, da Gama AR, de Almeida MF. 2010. Updated list of bat species positive for rabies in Brazil. *Rev Inst Med Trop Sao Paulo*. 52:75–81.

Sugg DW, Chesser RK, Stephen Dobson F, Hoogland JL. 1996. Population genetics meets behavioral ecology. *Trends Ecol Evol*. 11:338–342.

Svoboda PL, Choate JR, Chesser RK. 1985. Genetic relationships among southwestern populations of the Brazilian free-tailed bat. *J Mammal*. 66:444–450.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol*. 30:2725–2729.

Torres FD, Valença C, Filho R. 2005. First record of *Desmodus rotundus* in urban area from the city of Olinda, Pernambuco, Northeastern Brazil: a case report. *Rev Inst Med Trop Sao Paulo*. 47:107–108.

Trajano E. 1996. Movements of cave bats in soustheastern Brazil, with emphasis on the population ecology of the common vampire bat, *Desmodus rotundus* (Chiroptera). *Biotropica*. 28:121–129.

Trinca CS, de Thoisy B, Rosas FC, Waldemarin HF, Koepfli KP, Vianna JA, Eizirik E. 2012. Phylogeography and demographic history of the neotropical otter (*Lontra longicaudis*). *J Hered*. 103:479–492.

van Schaik J, Kerth G, Bruyndonckx N, Christe P. 2014. The effect of host social system on parasite population genetic structure: comparative population genetics of two ectoparasitic mites and their bat hosts. *BMC Evol Biol* 14:18.

Trivers RL. 1971. The evolution of reciprocal altruism. *Q Rev Blot*. 46:35–57

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICROCHECKER: Software for identifying & correcting genotyping errors in microsatellite data. *Mol Ecol*. 4:535–538.

van Veelen M, García J, Sabelis MW, Egas M. 2012. Group selection and inclusive fitness are not equivalent; the Price equation vs. models and statistics. *J Theor Biol*. 299:64–80.

Velasco-Villa A, Orciari LA, Juárez-Islas V, Gómez-Sierra M, Padilla-Medina I, Flisser A, Souza V, Castillo A, Franka R, Escalante-Mañe M, *et al*. 2006. Molecular diversity of rabies viruses associated with bats in Mexico and other countries of the Americas. *J Clin Microbiol*. 44:1697–1710.

Voigt CC, Kelm DH. 2006. Host preference of the common vampire bat (*Desmodus rotundus*; Chiroptera) assessed by stable isotopes. *J Mammal*. 87:1–6.

Wade MJ, Bijma P, Ellen ED, Muir W. 2010. Group selection and social evolution in domesticated animals. *Evol Appl*. 3:453–465.

Wahlund S. 1928. Zusammensetzung von Populationen und Korrelationserschinungen von Standpunkt der Vererbungslehreausbetrachtet. *Hereditas*. 11:65–108.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*. 38:1358–1370.

Weir JT. 2006. Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. *Evolution*. 60:842–855.

Wilkinson GS. 1984. Reciprocal food sharing in the vampire bats. *Nature*. 308:181–184.

Wilkinson GS. 1985a. The social organization of the common vampire bat. I. Pattern and cause of association. *Behav Ecol Sociobiol*. 17:111–121.

Wilkinson GS. 1985b. The social organization of the common vampire bat. II. Mating system, genetic structure, and relatedness. *Behav Ecol Sociobiol*. 17:123–134.

Wilkinson GS. 1986. Social grooming in the common vampire bat, *Desmodus rotundus*. *Anim Behav*. 34: 1880–1889.

Wilkinson GS. 1988. In: Greenhall AM, Schmidt U, editors. *Natural history of vampire bats*. Chicago: The university of Chicago Press. p. 86–96.

Wilkinson GS, Chapman AM. 1991. Length and sequence variation in evening bat D-loop mtDNA. *Genetics* 128:607–617.

Wilkinson GS, Carter GG, Bohn KM, Adams DM. 2016. Non-kin cooperation in bats. *Philos Trans R Soc Lond B Biol Sci*. 371:20150095.

Wimsatt WA, Trapido H. 1952. Reproduction and the female reproductive cycle in the tropical American vampire bat, *Desmodus rotundus murinus*. *Am J Anat*. 91:415–445.

Wright S. 1969. *Evolution and genetics of populations. Volume 2. The theory of gene frequencies*. Chicago: University of Chicago Press.

Wüster W, Ferguson JE, Quijada-Mascareñas JA, Pook CE, Salomão Mda G, Thorpe RS. 2005. Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Mol Ecol*. 14:1095–1108.

Wynne-Edwards VC. 1962. *Animal dispersion in relation to social behaviour*. Edinburgh: Oliver & Boyd.

Zeng L, Cheng Q, Chen X. 2012. Microsatellite analysis reveals the population structure & migration patterns of *Scomber japonicus* (Scombridae) with continuous distribution in the East & South China Seas. *Biochem Syst Ecol*. 42:83–93.