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

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HOW SOCIAL STRUCTURE DRIVES THE POPULATION DYNAMICS OF THE COMMONVAMPIREBAT(DESMODUS ROTUNDUS,PHYLLOSTOMIDAE) ?

Maïlis Huguin[1,2], Nidia Arechiga-Ceballos[3]*, Marguerite Delaval[4]*, Amandine Guidez[2]*, Isaï Jorge de Castro[5]*, Vincent Lacoste[2]*, Arielle Salmier[2]*, Alvaro Aguilar Setien[6]*, Claudia Regina Silva[5]*, Anne Lavergne[2], Benoit de Thoisy[1,2] §

* alphabetically ordered

§ corresponding author

1. Kwata NGO, 16 avenue Pasteur, 97300 Cayenne, French Guiana

2. Laboratoire des Interactions Virus Hôtes, Institut Pasteur de la Guyane, Cayenne, French Guiana

3. Laboratorio de Rabia, Instituto de Diagnóstico y Referencia Epidemiológicos. Mexico DF, Mexico.

4. Office National des Forêts, Cayenne, French Guiana.

5. Laboratório de Mamíferos, Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá, Macapá, Amapá, Brazil.

6. Unidad de Investigación Médica en Inmunología, Coordinación de Investigación, Instituto Mexicano del Seguro Social, México DF, Mexico

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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 structure. 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 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.

Key words

Sociality – structure – population dynamics – parasite spreading – vampire bat – Desmodus rotundus

INTRODUCTION

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 (Moussy et al., 2013; Miller-Butterworth et al., 2014; Rossiter et al., 2012).

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 (de Almeida et al., 2011; de Thoisy et al., 2016; Salmon-Mulanovich et al., 2007; Sodré et al., 2010; Torres et al., 2005) 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 & Trapido, 2005), the choice of host (Voigt & Kelm, 2006) as well as their behavior and social structure (Wilkinson, 1985a, b; 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 & 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 & Kerth, 2016). 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

inter-population interactions and therefore the role of females in virus dispersal (Streicker et al. 2016).

In this study, seven microsatellites and the mitochondrial DNA Control Region were used to investigate the genetic structure of D. rotundus at two 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; Pearse et al., 2006; Zeng et al., 2012). They are especially used for the analysis of demographic history, population structure at a fine geographic scale and social structure (Lecompte et al., 2017; Selkoe, 2006; Trinca et al., 2013). 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 inter-population scales. Those results are discussed about three 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 05/30/2011, no. 35 and 59 obtained 03/21/2013 and 04/17/2013, 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 five localities in French Guiana between 2009 and 2013 (Figure 1). The two first 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 between them (Fst =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 three 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 four 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 three 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, for example; de Thoisy et al., 2016) and measured at the mouth of the cave in order 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 pre-lysed 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 seven microsatellite markers (Bardeleben et al., 2007; Ortega et al., 2002; Piaggio et al., 2008) as described in supplemental data (supp data, Table s1). PCR was carried out in 12 µL with 1.5µL of each primer (5mM), 1.5µL of buffer, 1.2µL of dNTP (5mM), 0.1µL of BIOTAQ™, 0.4–0.6 µL of MgCL2 (50mM) (supp data, Table s2), and 2µL 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 (supp data, 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 10x 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 45s, annealing for 45s 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 & Rousset, 1995) under the hypothesis of heterozygote deficit. Linkage disequilibrium was tested using GENEPOP; genetic diversity indices and Fst averaged over loci (Weir & 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 & 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 & 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 & 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 ten 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 Nm= (1-Fst)/4. Fst (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 (nine and fourteen 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), Fis 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 Fis 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, Fst averaged over loci were obtained with ARLEQUIN 3.5 for each subpopulation whose number of individuals was greater than three (six subpopulations for POP1 and four 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 one (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 analysiss were verified with STRUCTURE 2.3.4 (Pritchard et al., 2000) using admixture model (burnin: 100,000, chains after burnin: 500,00)

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 sub-structures were identified by minimizing Hardy-Weinberg and linkage disequilibrium within each of k clusters (Corander et al., 2004; De Meeûs, 2012; Pritchard et al., 2000. 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 (Dharmarajan et al., 2011; Ravel et al., 2007).

MtDNA D-loop sequence analysis

DNA sequences were edited and aligned with MEGA 6.06 (Tamura et al., 2013). Gene diversity (Hs), nucleotide diversity (π) and genetic differentiation (Fst averaged over loci) (Weir & 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, 2009; Rozas & Rozas, 1995) 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 seven 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 (supp data, 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 towards a heterozygote deficit (Dabrowski et al., 2014). Two markers showed likelihood of stuttering, but these markers were checked with at least two 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 non-significant 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 Fst averaged over loci were low to high (Fst_{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 (Fst_{mean}=0.031; p<0.01), while genetic differentiation between the Mexican population (POP6) and the Guianese populations seemed to be the strongest (Fst_{mean}=0.183; p<0.01). The Brazilian population (POP5) showed moderate Fst with the Guianese populations and a strong genetic structure with the Mexican population, although lower than between the Mexican and Guianese populations. Nm per generation varied greatly between population pairs and followed a similar pattern to Fst values. POP2 and POP3 shared the highest Nm (Nm=20.26) per generation and the lowest Fst-values (0.009; p<0.01), followed by POP1 and POP4 (Nm=11.94; Fst=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 four Guianese populations (Fst_{mean}=0.046; p<0.05), POP3 showing moderate and the strongest Fst with POP4 (Fst=0.081; p<0.05) and POP2 showing the lowest with POP3 (Fst=0.026; p<0.05). When focusing on males only, a lower and nonsignificant genetic structure was observed (Fst_{mean}=0.019; p>0.1) except between POP1 and POP2 (Fst=0.045, p<0.05) and between POP1 and POP3 (Fst=0.034, p<0.05).

Population structuring

The dapc analysis segregated the individuals into six genetic clusters (CLUS-A, B, C, D, E & F) (Figure 2). The "find.clusters" function of ADEGENET showed a clear BIC decrease until k = six clusters after which BIC increased. Those six 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 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 three 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, five 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 five 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 to 6 main subpopulations.

FisClus of the combined populations was significantly lower than Fis (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 (Fst_{mean} =0.131±0.043; $p<0.01$) while the genetic structure between subpopulations of POP2 was lower but significant (Fst_{mean} =0.058 ±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±0.129) and POP2 r (r=0.075±0.124).

In contrast to the results of BAPS, analysis with STRUCTURE revealed no evidence of cryptic genetic structure in three to five populations. Exceptions to this pattern was POP1 and POP2, wherein STRUCTURE identified the presence of two 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 spanning359 bp revealed 12 mtDNA D-loop haplotypes. POP6 showed the highest gene diversity (Hs=0.8636 ±0.0786) followed by POP5 and POP2. Nucleotide diversity was low for the overall population, POP2 showed the highest (π=0.007428 ±0.004881) followed by POP6 (Table 5).

Structuring

Strong and significant genetic differentiation was found within the overall population (Fst_{mean}=0.859, $p<0.05$) except between POP1 and POP4, and POP2 and POP3 (Table 6). The haplotype network revealed two main groups of haplotypes (supp data, Figure s1). The first includes seven haplotypes composed of all individuals from the Mexican population (POP6; H4–H10) with a maximum of two mutations between haplotypes. The second comprises two smaller groups, one with three 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 two 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±0.0073; $\phi_{STmeanBRZ=}$ 0.008±0.004; $\phi_{STmean\ MEX=}$ 0.007±0.003) and was much higher between countries ($\phi_{STmean\ FG-BRZ=}$ 0.0372±0.008; $\phi_{STmean\ FG-MEX=}$ 0.095±0.004; $\phi_{STmean\ BRz-MEX=}$ 0.095±0.005).

DISCUSSION

In this study, the population structure and dynamics of D. rotundus was estimated at two 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 two 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 five times shorter and allowed gene sharing. Only low sequence divergence (ϕ_{STmean} 0.009±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 ove quite large distances. Divergence at the larger scale was higher (ϕ_{STmean} 0.076±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 four 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 four Guianese populations were significantly structured (Fst_{mean}=0.046; $p<0.05$), and males, for which the Fst coefficients were very low and nonsignificant (Fst_{mean}=0.019; $p>0.1$), except between POP1 and POP2 (Fst=0.045, $p<0.05$) like POP1 and POP3 (Fst=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 (Fouquet et al., 2012; Martins et al., 2009; Weir 2006; Wuster et al. 2005). 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 & 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 two 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 seven years of monitoring and nine 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 (Arnold & Wilkinson,

2015; Castella et al., 2001; Kerth, 2000; 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 ;Dharmarajan et al., 2011; Kempf et al., 2010).

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 four other populations where individuals were sampled in multiple foraging areas. Some mammal species living in social groups may have genetic and demographic consequences (Kerth & 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 (O'Donnell et al., 2016; Stiebens, 2013) as shown by present genetic diversity results (Hs_{mean}=0.688±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 life span (Delpietro et al., 2017; Lord et al., 1976) 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 (Hirsch et al. 2013; Strickland et al., 2014;

Briga et al., 2012). 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 & 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 non-kin promotes reciprocal assistance by strengthening long-term social bonds (Carter & Wilkinson, 2015; Carter et al.,2017). However, food sharing cannot be explained solely by kin selection or harassment (Carter & 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; Simon et al., 2012) although some evidence was provided, especially about effects of group phenotype on group fitness (Pruitt & 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 & 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 & Kerk, 2016). 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 (Arnaud et al, 2012; Clutton-Brock, 1989; Wilkinson 1985a, 1985b, 1988). In polygynous taxa, males tend to disperse more to avoid inbreeding and/or competition, while females are more philopatric (Greenwood, 1980; McCracken & Wilkinson, 2000). Those drivers of host dynamics have important impacts for virus dispersal (Delpietro et al., 2017). D. rotundus is the most important reservoir of rabies virus in Latin America (Schneider et al., 2009), and lessons from the genetic structure and population dynamicsdescribedabovehavepotentialimplicationsforvirusdispersal.

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; unpub. 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 (1985) 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).

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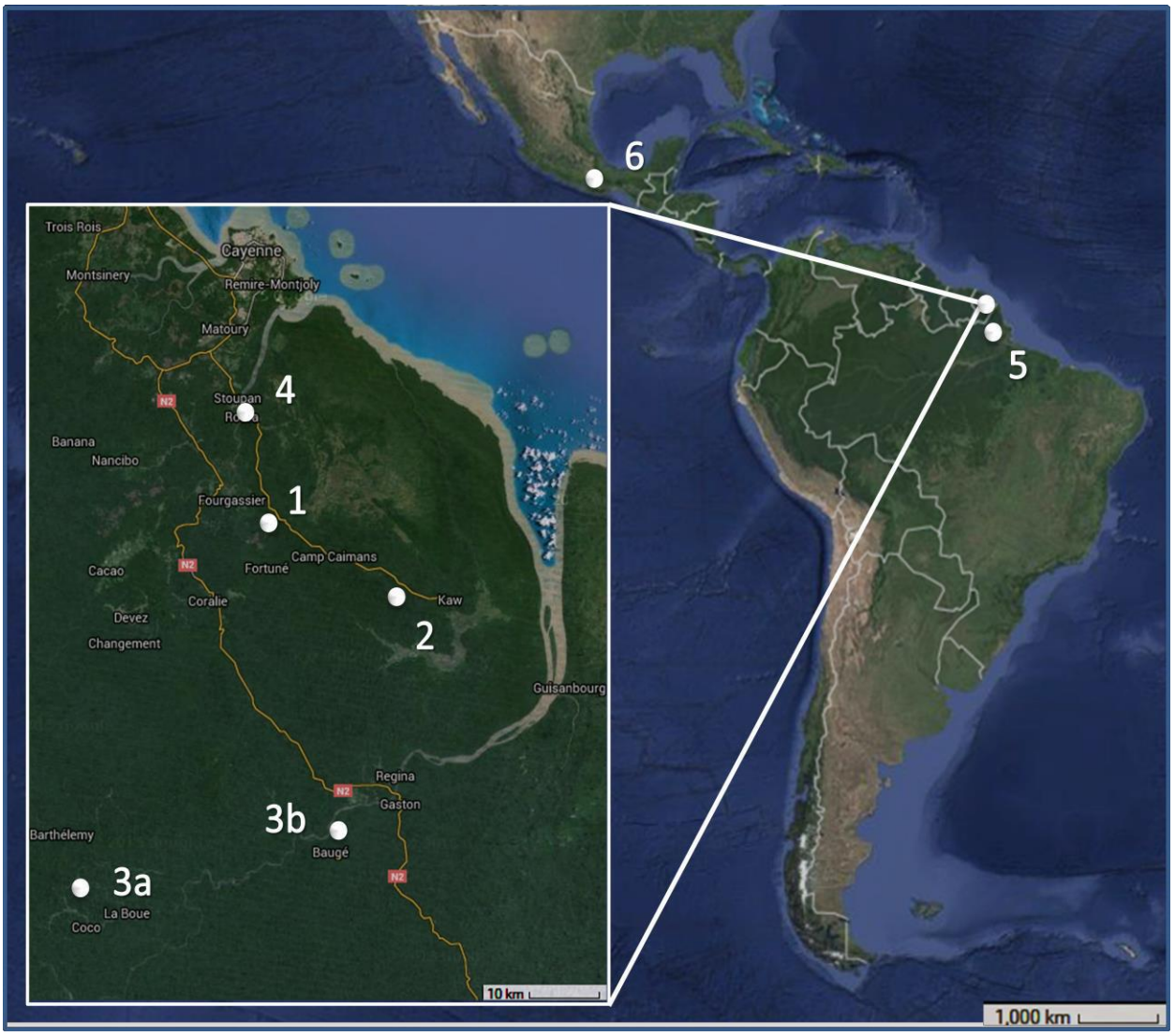

Figure 1. Geographical locations of *Desmodus rotundus* populations. POP1 to 4 were sampled in French Guiana, POP5 was sampled in Brazil and POP6 was sampled in Mexico. Map data © Google 2017

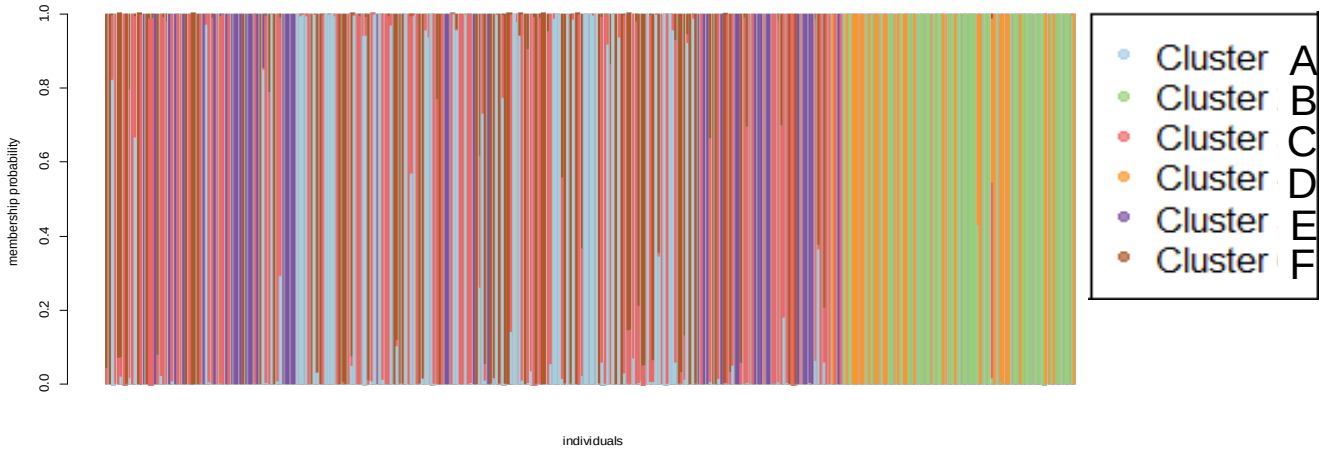

Figure 2. Distribution of clusters (K) by individuals estimated by *find.cluster* in ADEGENET R package represented by population

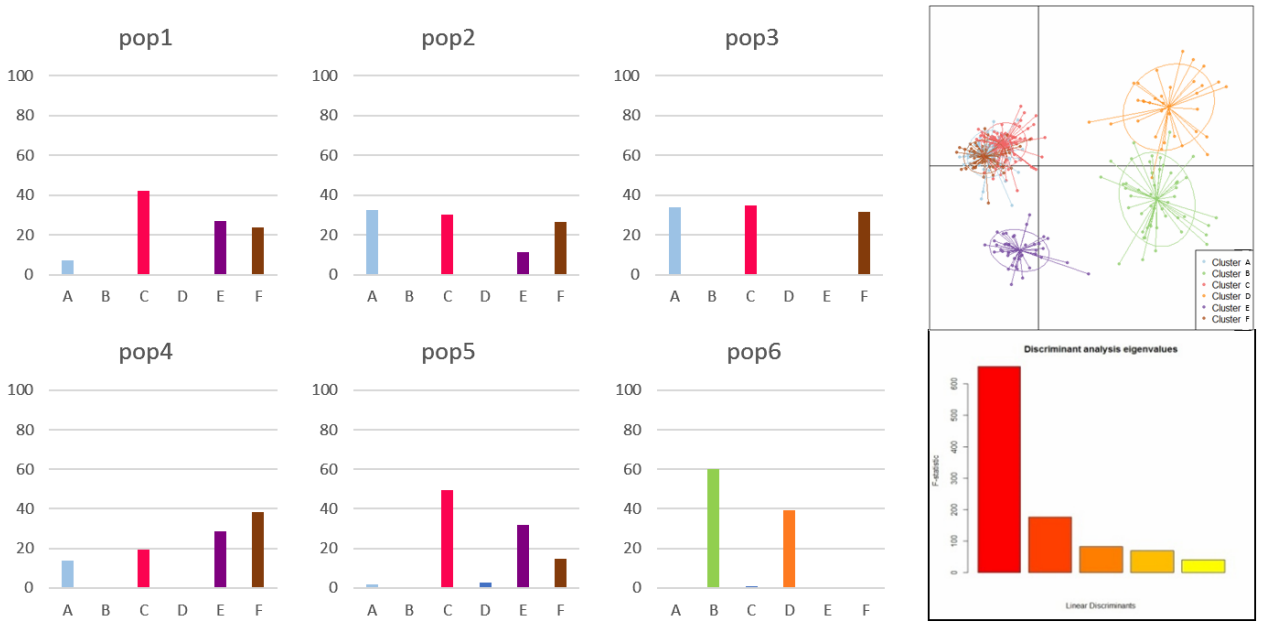

Figure 3. Discriminant Analysis of Principal Component on overall populations. Six clusters have been found with BIC-value (top-right) and discriminant analysis eigenvalue (bottom-right) estimated by *Adegenet* package (Jombart, 2011). The left part shows the contribution of the 6 clusters to the 6 populations.

Populations	Ho	He	Hs(±σ)	HW	Fis	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 1. Descriptive statistics for each population including observed heterozygosity (Ho), expected heterozygosity (He), average genetic diversity (Hs), p-value of test of Hardy-Weinberg equilibrium (HW), inbreeding obtained by ARLEQUIN, coefficient (Fis) obtained by GENETIX, average relatedness coefficient (r) obtained by ML-RELATE and sample size (N).

	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*	

Table 2. Values below diagonal are Fst-values, values marked with an asterix (*) are significant ($p<0.05$) obtained by ARLEQUIN with the method of pairwise genetic distances. Values above the diagonal are Nm values obtained by GENETIX .

Populations	K	Mean (±σ)	Fis	FisClus
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*

Table 3. For hidden structure (BAPS analyses) reported number of clusters identified (K) with the higher for each population, mean (±standard deviation) of kin group sizes, Fis and FisClus for each population (recalculated after BAPS estimation) obtained by GENETIX 4.0.5.2. K-values are marked with an asterix (*) when FisCLus is significantly lower than Fis (Wilcoxon signed-rank; $p<0.05$).

	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*	

Table 4. Fst-values between subpopulations of POP1 & POP2 whose numbers of individuals were greater than three obtained by ARLEQUIN 3.5 with the method of pairwise genetic distances. Values marked with an asterix (*) are significant ($p<0.01$)

Populations	N	Hs(±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 to H10

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

	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*	

Table 6. MtDNA Dloop Fst-values between populations obtained by ARLEQUIN 3.5 with the method of pairwise genetic distances. Values marked with an asterix (*) are significant ($p<0.01$).

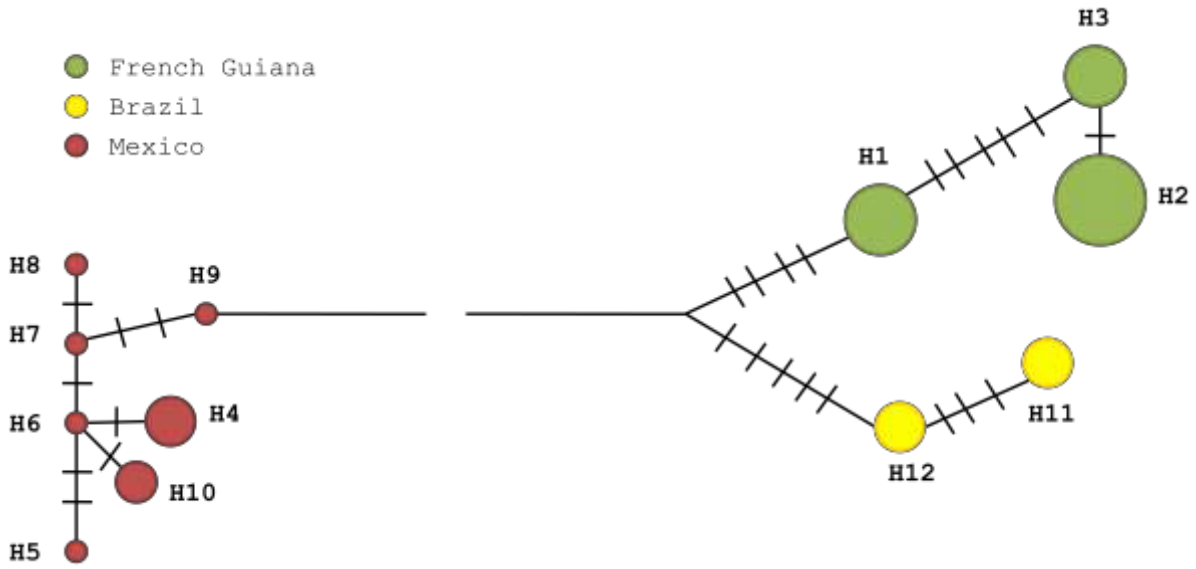

Figure s1. Haplotype networks of the mitochondrial DNA (mtDNA) control region (D-loop) at the nucleotide level for *D. rotundus.* Nodes are proportional to the frequency of bat carrying each haplotype, colored by the geographical location in which the bat was trapped. Hatch marks represent mutations. Interruptions in lines indicate the presence of more than ten mutations.

Loci		Primer sequence (5'-3')	Repeat motif	Size	Source
DERO3	C12F B02R	GCTGGGTCACCTAAGTATGG CAAATCAGATATACAAAGAAGCAAG	$(CA)_{17}$	100-200 pb	Piaggo et al., 2008
DERO4	B10F E01R	GAAGTTGGGGTGTCTATGG GGAGTTCTTTTAGCCTGTGC	$(GA)_{20}$	200-300 pb	Piaggo et al., 2008
DERO5	D12F D12R	ACATGCAAATCCATCTTGAT CCCAAATCCAAAACCTCAT	$(CA)_{11}(AC)_8$	100-200 pb	Piaggo et al., 2008
DERO9	C11F C11R	GTTAATAAGCCTTCAGGAAAAGC TCCTTCTGCACTCAAGAATTTTA	$(AG)_9$	100-200 pb	Piaggo et al., 2008
DERO11	C07F A02R	CCTAGGGCAAGAATGAGTATCC ACAGTATGGCACACAAACACG	$(TG)_9$	100-200 pb	Piaggo et al., 2008
CARO3	AAGG-117F AAGG-117R	GACTAAGATAGATAAATTGATGGATAA CAAAATGCTTTAGTTTCCTGAAT	$(AAGG)_{16}$	100-200 pb	Bardeleben et al., 2007
CARO6	AjA74F AjA74R	GGCAAAGGCTTTTACAAGTATG GCAGTGGAGGAGAAAGCTAGAC	$(GT)_7\ldots (GT)_4$	100-200 pb	Ortega et al., 2002

Table s1. Characteristics of the 7 microsatellite loci that were used in this study.

Loci	MgCl2 (µl)	PCR conditions	Cycles
DERO3	0.5	94°C/5min ; 94°C/30sec ; 52°C 30sec ; 72°C/45sec ; 72°C/10min	45
DERO4	0.5	94°C/5min ; 94°C/30sec ; 60°C 30sec ; 72°C/45sec ; 72°C/10min	45
DERO5	0.4	94°C/5min ; 94°C/30sec ; 53°C 30sec ; 72°C/45sec ; 72°C/10min	40
DERO9	0.4	94°C/5min ; 94°C/30sec ; 52°C 30sec ; 72°C/45sec ; 72°C/10min	40
DERO11	0.5	94°C/5min ; 94°C/30sec ; 58,5°C 30sec ; 72°C/45sec ; 72°C/10min	40
CARO3	0.6	94°C/5min ; 94°C/30sec ; 55°C 30sec ; 72°C/45sec ; 72°C/10min	45
CARO6	0.6	94°C/5min ; 94°C/30sec ; 60°C 30sec ; 72°C/45sec ; 72°C/10min	40

Table s2. PCR condition were used for the 7 microsatellite loci in this study

Loci	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6
Dero3	0,063*	-0,006	0,033	0,037	0,038	0,035
Dero4	0,024	0,110*	0,034	-0,072	0,052	0,089*
Dero5	0,042	0,039	0,045	-0,073	0,076*	0,019
Dero9	0,108*	0,030	0,028	0,067	0,038	0,120*
Dero11	0,065*	0,025	0,021	0,140*	0,010	0,082*
Caro3	-0,020	0,022	0,018	0,064	-0,000	-0,010
Caro6	0,086*	0,144*	-0,000	0,123*	0,168*	0,079*

Table s3. Null allele frequencis by locus and by population obtained MICROCHECKER 2.2.3 (Van Oosterhout and al., 2004) using method by Brookfield (1996). Null allele frequencis marked with an asterix (*) are significant.