Dispersal and group formation dynamics in a rare and endangered temperate forest bat (*Nyctalus lasiopterus*, Chiroptera: Vespertilionidae)

João D. Santos[1,2*], Christoph F.J. Meyer[2,3], Carlos Ibáñez[4], Ana G. Popa-Lisseanu[4] & Javier Juste[4]

[1] Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Avenue Agropolis, 34398 Montpellier, France

[2] Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal

[3] School of Environment and Life Sciences, University of Salford, Salford M5 4WT, United Kingdom

[4] Department of Evolutionary Ecology, Estación Biológica de Doñana (CSIC), Avenida Américo Vespucio s/n, 41092 Seville, Spain

*Corresponding author: João Santos, present address: Cirad, AGAP, TA A-108 / 03, Avenue Agropolis, 34398 Montpellier Cedex 5, France;

E-mail: joao.garcia_nolasco_dourado_santos@cirad.fr

Tel.: +330623720645

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Abstract

For elusive mammals like bats, colonization of new areas and colony formation are poorly understood, as is their relationship with the genetic structure of populations. Understanding dispersal and group formation behaviors is critical not only for a better comprehension of mammalian social dynamics, but also for guiding conservation efforts of rare and endangered species. Using nuclear and mitochondrial markers, we studied patterns of genetic diversity and differentiation among and within breeding colonies of giant noctule bats (*Nyctalus lasiopterus*), their relation to a new colony still in formation, and the impact of this ongoing process on the region-wide genetic makeup. Nuclear differentiation among colonies was relatively low and mostly non-significant. Mitochondrial variation followed this pattern, contrasting with findings for other temperate bat species. Our results suggest that this may indicate a recent population expansion. On average, female giant noctules were not more closely related to other colony members than to foreign individuals. This was also true for members of the newly forming colony and those of another, older group sampled shortly after its formation, suggesting that contrary to findings for other temperate bats, giant noctule colonies are not founded by relatives. However, mother-daughter pairs were found in the same populations more often than expected under random dispersal. Given this indication of philopatry, the lack of mitochondrial differentiation among most colonies in the region is probably due to the combination of a recent population expansion and group formation events.

Introduction

Studying natural populations in their habitat can prove difficult using traditional methods such as mark recapture and radio-telemetry (Clutton-Brock & Lukas 2012). This is particularly true when studying the dispersal habits of small, highly mobile and nocturnal animals such as bats. Furthermore, these methods provide estimates of individual mobility and dispersal, but not of their effective rate at the population level (Prugnolle & de Meeus 2002). In contrast, genetic methods that allow inferring the distribution of alleles across populations can provide estimates of gene flow, and thus information on the reproductive success of migrating individuals (Wright 1943; Slatkin 1987). The genetic structure of natural populations can result from a number of interacting factors, such as recent history, dispersal, mating system and group formation (Chesser 1991; Storz 1999; Parreira & Chikhi 2015). Dispersal ability in particular has been shown to be negatively correlated with genetic differentiation across a range of taxa (e.g. plants, Govindaraju 1988; mammals, Bohonak 1999), including temperate bats, where genetic population structure correlates negatively with the extent of migration (Burns & Broders 2014).

The formation of a new colony or social group is a rarely witnessed process that is particularly interesting for its effect on region-wide genetic variation and for providing information about the underlying social dynamics. Where groups consist of philopatric adults, the formation of a new group is usually the result of group fission (Alberts & Altmann 1995; Hoogland 1995; Thierry 2007; Kerth 2008; Armitage *et al.* 2011). However, the level of kinship among the members of the resulting groups varies across species. While in Savannah baboons (*Papio cynocephalus*) social bonds can supersede kin relations in the choice between emerging groups (van Horn *et al.* 2007), for a range of other primate species (Snyder-Mackler *et al.* 2014; van Horn *et al*. 2007), as well as African elephants (*Loxodonta africana,* Archie *et*

71*al*. 2006), hyenas (*Crocutta crocutta*, Holekamp *et al.* 1993) and yellow-bellied marmots
72(*Marmota flaviventris*, Armitage *et al.* 1987), females choose to remain or move together with
73close kin. The latter has also been documented for big brown bats, *Eptesicus fuscus*, in which
74average pairwise relatedness was higher than expected among individuals of three out of five
75matrilines following the formation of a new group (Metheney *et al.* 2008). Previous studies
76had found little or no correlation between the degree of association and relatedness levels
77among members of bat maternity colonies, including in this particular species (Kerth *et al.*
781999; Metheny *et al.* 2007). These estimates had, however, been obtained from established
79colonies. During colonization, higher levels of relatedness would likely facilitate cooperative
80behaviors, counterbalancing the increased risk incurred. Nevertheless, the structure and
81relationships within any group will be shaped by the composition of its founders, socially as
82well as genetically.

83 The giant noctule, *Nyctalus lasiopterus*, with a wingspan of up to 45 cm and weighing
84around 50 g, is the largest European bat species (Ibáñez *et al*. 2004). It is also one of the
85rarest, with only a few known breeding colonies in Spain, Hungary, and France (Ibáñez *et al*.
862004; Estók *et al*. 2007, Dubourg-Savage et al. 2013). A tree-roosting species, the giant
87noctule has a patchy circum-Mediterranean distribution throughout southern Europe (Iberia,
88France, Italy, the Balkans and Greece), North Africa, and Anatolia. The species' range also
89extends into the Caucasus, Iran, Kazakhstan, and the Urals (Ibáñez *et al.* 2004). The
90demographic dynamics observed in the Iberian Peninsula (Ibáñez *et al*. 2009) indicate that,
91similar to other temperate bats, giant noctule bats segregate sexually during spring and
92summer to form breeding colonies (Bradbury 1977; McCracken & Wilkinson 2000). These
93aggregations of giant noctule females form fission-fusion societies akin to those described for
94other temperate forest bats (Kerth & König 1999; Willis & Brigham 2004; Patriquin 2013) in

which frequent roost changes result in non-random associations between colony members (Popa-Lisseanu *et al*. 2008). The benefits of this social system and the factors underlying the individual decisions behind it are still under debate (Aureli *et al*. 2008; Sueur *et al.* 2011).

Colonization of new areas and the formation of new colonies are rare events that have seldom been described in bats, and on only one occasion has colonization been studied in detail from a genetic perspective (*Eptesicus fuscus*, Metheny *et al*. 2008). As part of a long-term study of giant noctule populations in southwestern Andalusia, Spain, we examined the influence of genetic relatedness on the formation of a new colony in Doñana National Park prior to 2007 and after 2010, following a temporary, unexplained 3-year abandonment. We sampled individuals regularly roosting in this new colony, in addition to three stable breeding colonies in the region. Using both nuclear and mitochondrial markers, we assessed genetic population structure and levels of genetic relatedness within colonies. To test the hypothesis that the colonizer group was kin-based, i.e. that the foundation of this new group was the result of a joint movement of related females, we first determined whether among-group genetic variance had increased after the establishment of this new colony. Subsequently, we estimated genetically-inferred relatedness and putative relations among individuals within colonies and within matrilines. We predicted higher levels of relatedness among colonizing females in Doñana National Park than expected by chance. Likewise, if related females moved together, we expected to find higher levels of average pairwise relatedness among females of the same matriline in the new group when compared to females carrying the same haplotypes in other colonies.

We discuss the implications of our findings with regard to the social habits of giant noctules and their demographic history in the region and, in a more general context, as to how

they advance our understanding of mammalian social structure and the role played by kinship in the formation of new colonies.

Materials and Methods

Study populations and Sampling

We sampled a total of 215 individuals present in four maternity colonies in southern Andalusia, Spain. The breeding colony in Doñana National Park (DNP) is located around a group of bat boxes in a small stand of mainly Eucalyptus trees near the marshes at the mouth of the Guadalquivir River (36.99º N, 6.44º W). Two breeding colonies of *N. lasiopterus* were recently reported from southwestern Andalusia (Ibáñez *et al*. 2009; Fig. 1): one in large, old plane trees (*Platanus sp.*) within 'Maria Luisa Park' (MLP) in the city of Seville (37.37º N, 5.59º W). This was the larger of the two colonies, with an estimated 500 bats roosting there in 2007 (Popa-Lisseanu & Ibáñez 2008). The other colony occupied a group of palm trees (*Washingtonia sp.*) located in the gardens of the zoo of Jerez de la Frontera (ZJF; 36.70° N, 6.15° W); this colony had an estimated population of 100-150 females. In contrast to these semi-natural colonies, the fourth population is found in a large natural Mediterranean mixed oak forest in 'Los Alcornocales Natural Park' (ANP) around 100-150 km southeast of Seville (36.31º N, 5.44º W) and has an estimated size of several thousand individuals that were sampled at different localities.

Samples consisted of wing punch biopsies (Wilmer & Barrat 1996) stored in 70% ethanol. We analyzed 84 samples from MLP, 52 from ANP, and 32 individuals from ZJF. A total of 47 individuals were sampled from the newly forming colony in DNP. This data set was split into: a) the Doñana 'original' colonizing group (D_O; N=23), consisting of samples collected between 2003 and 2005, and b), the Doñana 'recolonization' group (D_R; N=24),

sampled after the yet unexplained three-year breakdown (2007-2009), during the subsequent re-colonization process from 2010 to 2013. For both D_O and D_R we selected only females that were registered breeding in the colony during more than one year.

Molecular markers

Total genomic DNA was extracted from wing punches using a modified salt-based protocol (Aljanabi & Martinez 1997). The two hypervariable domains (HVI and HVII) of the mitochondrial control region were PCR-amplified using primers L15926 (Kocher *et al.* 1989) and CSBF-R (Wilkinson & Chapman 1991) for HVI, and L16517 (Fumagalli *et al.* 1996) and H607 (Wilmer *et al.* 1994) for HVII (forward and reverse primers, respectively). Sequences were aligned, visually inspected for ambiguities, and edited by hand using Sequencher v 4.9 (Gene Codes Corp., Ann Arbor, MI, USA). The final sequences were cropped to a length of 437 bp for HVI (including 103 bp of $tRNA_{thr}$ and the initial sequence and first repeat of the HVI region) and 397 bp for HVII.

All individuals were additionally genotyped at 11 nuclear microsatellite loci. As no specific microsatellites yet existed for *N. lasiopterus*, annealing temperatures and PCR mix concentrations were optimized for eight markers developed for *N. leisleri* (Nle 2,3 and 6-11; Boston *et al.* 2008), one developed for *Eptesicus fuscus* (EF4, Vohnof *et al.* 2002) and two developed for *Nyctalus noctula* (P20, P217; Mayer 1997). All were tested in muscle tissue prior to genotyping. Labelling followed Schuelke's procedure (2000).

See Appendix S1 in Supporting information for a detailed description of DNA extraction, amplification, sequencing, and microsatellite genotyping.

Data analysis

Mitochondrial DNA. The two mitochondrial fragments were concatenated and the number of haplotypes (*h*), haplotype diversity (*Hd*), nucleotide diversity (π) and the number of segregating sites (*S*) were calculated using DNASP v. 5.10.1 (Rozas *et al.* 2009). A median-joining network based on haplotypes was constructed using NETWORK (Bandelt *et al.* 1999). Through analyses of molecular variance (AMOVA, Excoffier *et al.* 1992) we assessed how genetic variation was partitioned among colonies, whereby we explored different grouping combinations to identify the one that maximized the among-group component of genetic variation. AMOVA was performed using the software ARLEQUIN v. 3.5.1.2 (Excoffier *et al.* 2005), which was also used to calculate ϕ_{st} values among colonies.

Microsatellites. All microsatellite loci were tested for genotyping errors using MICROCHECKER v. 2.2.3. Linkage disequilibrium among markers was assessed using FSTAT v. 2.8.3.2 (Goudet *et al.* 2001). Identification of loci under selection was performed using the software ARLEQUIN v. 3.5.1.2. Calculations of allele frequencies (including null alleles) across colonies, observed (H_o) and expected (H_e) heterozygosities, as well as deviations from Hardy-Weinberg equilibrium (HWE) were performed in CERVUS v. 3.0.6 (Kalinowski *et al.* 2007). Allelic richness was assessed using the R package 'hierfstat' (Goudet 2005).

Given the recent developments and ongoing debate about the various existing population differentiation measures and their appropriate use (Hedrick 1999; Jost 2008; Heller & Siegismund 2009; Meirmans & Hedrick 2011) we opted to estimate both D_{EST} and F_{ST}, the former for a more robust analysis and as a reference for future studies, the latter to facilitate comparison with results from previous studies. Both measures were calculated using the R package 'diveRsity' (Keenan *et al*. 2013). As for mtDNA, partitioning of genetic variation at the nuclear level was assessed with AMOVA in ARLEQUIN 3.5.1.2.

Genetic relatedness. Pairwise and mean relatedness values (*R*), both among colonies and for matrilines (between individuals with shared mitochondrial haplotypes), were estimated using ML-Relate (Kalinowski *et al.* 2006). This software implements a corrected maximum-likelihood approach that allows loci with null alleles to be incorporated into the analysis (Wagner *et al.* 2006). Mother-daughter pairs were identified, allowing not only to determine the number and proportion of close kin ($r > 0.25$) and of mother-daughter pairs within our data set, but also to examine the distribution of these dyads across colonies. Assignments inconsistent with mitochondrial haplotypes were excluded. For each colony, we estimated the proportion of close associations out of all possible pairs of individuals (% r > 0.25), as well as the proportion (%) of females with at least one close relative within the colony.

Results

Genetic diversity

A total of 15 haplotypes were found, which varied on average by only one substitution, comprising a total of 15 polymorphic sites. The two most common haplotypes were present in all colonies (Fig. 2) and together represented 86% of the individuals sampled. The remaining 13 haplotypes were found in two populations at most, six of them being present in only one. Colonies had between 4 and 8 haplotypes (mean 5.6 ± SD 1.52). Haplotype diversity ranged from 0.179 to 0.759 (total Hd = 0.578, Table 1), being lowest for ZJF and highest for D_O (first colonization attempt of Doñana), followed by ANP (the only two colonies located in a 'natural' habitat). The median-joining network showed a star-shaped structure around the two most frequent haplotypes (Fig. 2).

All microsatellite loci were polymorphic, with an average of 12 alleles, and all were in linkage equilibrium. H_o ranged from 0.347 to 0.850 (Table S1). Out of the 11 microsatellites, four (Nle9, Nle11, P20 and P217, see Table S1) deviated significantly from HWE, and were excluded from the genetic structure analysis. Selection acting on Nle11 could not be ruled out ($P< 0.05$), further supporting its exclusion. For estimating relatedness we resorted to Wagner & Kalinowski's (2006) method, implemented in ML-Relate, and kept all 11 loci.

Population differentiation

Mitochondrial differentiation according to ϕ_{st} averaged 0.11 ± 0.12 (range 0-0.36). This value was due mainly to ANP, which differed significantly from all other colonies (Table 2). For microsatellites, pairwise F_{ST} values between DNP's first colonization attempt (D_O) and the three other colonies were on average low (0.015 ± 0.01), ranging between 0 and 0.035 (Table 2). Significant pairwise differences among colonies, nonetheless, separated ANP from D_O, MLP, and D_R. Estimated values of population differentiation using D_{EST} (Table S2) differed slightly from those based on F_{ST}, yet both measures were significantly correlated (R^2=0.46, P=0.03). Nevertheless, no pairwise comparisons based on D_{EST} were significant.

The largest proportion of mitochondrial genetic variation was explained by the within-group component (mean=76.83 %, SD=3.02), whether DNP was included or not. Among-colony variation (among-group component) showed a slight decrease when either D_O or D_R were included in the analysis (Table 3). Exploring different grouping designs, we found that among-group variation was maximized when ANP was kept isolated, and MLP and ZJF united (I and III; Table 4). Again, this proved true, whether DNP was included or not. Adding either of the colonizer groups resulted in lower among-colony variation, whereas this component was maximized when the two colonizer groups were grouped together with MLP

and ZJF (II, III; Table 4). Nuclear variation was not affected by the different grouping strategies, with values of the among-group component always below 1 % (Table 4).

Relatedness estimates

Mean pairwise relatedness within colonies was very low (0.075 ± 0.10, Table 5). Average relatedness values within matrilines in the different colonies varied considerably but were altogether also low (0.055 ± 7e-2, Table 6), ranging from 0 (D_R, H2) to 0.345 (MLP, H5), although the latter consisted of only 2 females. Of the four haplotypes found in D_R, one was carried by only one female and two by unrelated females (H2 and H3, Table 6). Finally, average pairwise relatedness among females sharing H1 was low, with only three of its females being closely related ($r > 0.25$, Table 6). The number of females with at least one close relative in the same colony was high (62.5 - 93%, Table 5). Here, ZJF and ANP presented the lowest averages, 62.5 and 80.8% respectively. Relationship estimates based on microsatellite data revealed an elevated number of parental associations across all populations that involved approximately half the individuals sampled (57.1%, N=215, Table 5). As many as 72.7% of all paired females originated from the same colony. In MLP, this reulted in 43 of the 84 individuals (51%) roosting with their putative mothers/daughters. In ANP, 13 parental associations (involving 21 females, 40%) were found, while in DNP we only identified four (all within the post-2007 group). No such association was found among individuals from ZJF. As for inferred mother-daughter dyads pairing females from DNP together with females from other colonies, we found five involving females from D_O, and 12 involving females from D_R. Regarding the former, three out of five involved females from ANP (the two others assigned to MLP and ZJF), while in the latter, 9 out of 12 dyads involved females from MLP (two involved the same female from ZJF, the last one ANP). The number of mother-daughter pairs

was uncorrelated with variation in sampling year for each colony (R^2 = 0.0, P= 053), but increased significantly with the number of samples of each colony (R^2=0.90, P=0.009).

Discussion

Population structure and recent demographic expansion

We genotyped bats from three consolidated colonies and a recently colonized site (with two colonization events) and assayed variation both at nuclear and mitochondrial loci and levels of differentiation among the colonies. Haplotype diversity was highest in the D_O and ANP colonies, both situated in natural environments, whereas the two other stable colonies are located in urban parks. Mitochondrial and, to a lesser extent, nuclear differentiation of the ANP colony from the remainder further suggest a certain degree of genetic isolation and, since the former is mainly due to the presence of a private allele carried by 15.4% of its females, philopatry. The lack of any significant differentiation among the remaining sites indicates either a common, relatively recent origin, and/or high levels of gene flow mediated by dispersal in both sexes. Molecular variance analysis of different grouping designs, which returned higher values of among-colony variation when ANP was kept isolated and D_O and D_R were grouped together with MLP and ZJF, further supports this idea. Radio-tracking and a few ring-recovery data indicate movements between all the studied colonies, which could help to explain the lack of differentiation between them (Popa-Lisseanu *et al*. 2009). However, the lack of structure at the mitochondrial level should not be attributed to modern-day dispersal or group formation dynamics alone. The presence of the two most frequent haplotypes in every population and the star-shaped topology of the median-joining network both point to a recent population expansion (Fig. 2). Differences between putative original populations could account for the sharp differences in haplotype diversity found between the

first and second colonizer groups. Finally, different group formation processes (dispersal for D_O vs. budding for D_R) could also result in similar differences.

Regional kin structure

We estimated relationships among individuals based on shared nuclear alleles, and analyzed the distribution of close kin ($r > 0.25$) and mother-daughter pairs across the region. The number of females with at least one close relative in the same colony was unexpectedly high for some sites, particularly for the colony in the city park of Seville (MLP). However, it is the number of parental associations found within our complete data set and encompassing the whole area studied that stands out the most with 57.1% of parental associations found to be intra-colonial. The complementary 42.9% of these involved females from separate colonies, suggesting still, relatively frequent movements and thus significant gene flow between the colonies. A recent study revealed a negative correlation between wing loading, migration tendency and the magnitude of genetic differentiation among bat populations (Burns & Broders 2014). Our study sites are at most 150 km apart (MLP to ANP), and previous studies have not only indicated that *N. lasiopterus* can undergo long-distance migrations, but have also reported important movements in this particular region (Ibáñez *et al*. 2009; Popa-Lisseanu et al. 2009). We therefore expected a more even distribution of dyads, reflecting "regional philopatry" (*sensu* Vonhof *et al*. 2008). Instead, we found that 33.6% of females (a conservative estimate considering we could not sample all individuals in every colony) stayed in the same colony as their mothers or daughters. While this estimate falls predictably short of that found in colonies of non-migratory Bechstein's bats, characterized by strict female philopatry (72%; Kerth *et al*. 2002), it is higher than what was reported in big brown bats (9%; Vonhof *et al.* 2008), a species with an estimated migratory range of up to 288 km between maternity and winter roosts (Mills *et al*. 1975).

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310*Colonization of Doñana National Park*

311 We studied two consecutive colonization attempts of DNP by giant noctules in relation
312to the three closest known colonies of the species. We found considerable co-localization of
313female relatives, pointing to a high degree of philopatry and indicating that reported
314movements do not necessarily result in stable relocations. The lack of differentiation among
315all the colonies (except for ANP) could be due to the fact that these are too young for any
316differentiation to become apparent at the mitochondrial level. The formation of new groups or
317colonies involves the sampling of alleles from one or more parent groups. The degree to
318which founding individuals are related to one another will influence the genetic variation of
319the newly formed groups, and consequently the amount of among-group variation at the
320population level (Storz 1999). If the formation of the new colony in DNP was the result of
321random dispersal of females from different nearby colonies, following Slatkin's migrant-pool
322model (Slatkin 1977), we would expect the lack of genetic structure we observed. In that case,
323there may not have been sufficient time for philopatry to counteract this effect. On the other
324hand, if the new colony was the result of fissioning of closely related females from another
325colony (propagule-pool model, Slatkin 1977), the level of genetic relatedness among females
326of the new group would be higher and the genetic sampling less representative of the whole,
327increasing among-group variation. It is important to note that no ringed females (sampled or
328not) from the initial colonization were ever reported back in the new DNP recolonization
329group. While the re-colonizers of DNP harbor fewer haplotypes than its previous settlers (4
330and 8, respectively), an analysis of molecular variance failed to detect an increase of among-
331colony genetic variation after the creation of either group. The most parsimonious conclusion
332is that the Doñana, Seville and Jerez colonies are relatively recent and related. It is likely that

they are the result of an expansion of the natural population of *N. lasiopterus* living in the large area of *Quercus* spp. forest in Cadiz Province, encompassing most of Alcornocales Natural Park (ANP). This hypothesis is in agreement with the star-like distribution of the haplotype network. Nevertheless, the presence of private haplotypes in all new colonies points to the possibility of genetic additions from other colonies (or regions) apart from an ANP source. In summary, it seems likely that the lack of structure found is mostly due to recent demographic changes, not yet counteracted by the structuring effect of philopatry.

The only previous genetic analysis of the formation of a new group in temperate bats is a study of the tree-roosting big brown bat (*E. fuscus*) by Metheney *et al.* (2008). The studied colony fissioned, one group moving to a previously uninhabited area 7 km away from the original colony (Metheney *et al.* 2008). The authors found higher levels of relatedness in the seceding group than in the pre-fission one, suggesting that females from matrilines with higher relatedness levels had moved together, a pattern that was interpreted as ensuring the cooperative behaviors needed for group formation (Metheney *et al.* 2008). We found that average pairwise relatedness within the colonizer groups was nearly twice that of established colonies (Table 6) and four mother-daughter pairs were identified within D_R, indicating that colony formation in giant noctules does to some extent benefit from the coordinated move of related females. However, the presence of multiple haplotypes among the colonizers, leaving regional genetic structure unaffected, and the generally low pairwise relatedness values indicate a more complex scenario. The question remains open as to which individual-based considerations – such as proximity to foraging areas, temperature conditions, presence of kin or social partners – underlie the formation of a new group in this species. The presence of unrelated individual can either be explained by independent simultaneous movements of females, or cooperation and information sharing. Given their flight range (females can cover

357distances exceeding those between colonies during nightly foraging bouts – Ibáñez *et al*.
3582009; Popa-Lisseanu *et al*. 2009), it is reasonable to assume that independent discovery of
359roosts available at the new site by several females would have been quick. If the site's
360advantages were clear (i.e. unoccupied bat boxes, overcrowding of the remaining sites,
361proximity to Doñana's insect-rich foraging grounds), the arrival by unrelated females might
362have simply involved their individual choice to move, its speed giving the appearance of one
363coordinated movement. On the other hand, kinship-independent information transfer about
364novel roosts and their relative quality has been reported in Bechstein's bats (Kerth *et al*. 2002,
3652005) and could also, if confirmed in giant noctules, explain the simultaneous movement of
366several females to a newly available area. Our own analysis of parent-offspring dyads
367involving individuals from both the original and re-colonizing groups of Doñana identified an
368additional 6 dyads (42% more) in the latter group, the majority of these (9/11) related to
369females from Seville. Together with the small number of haplotypes in that group and the
370clustering with MLP in the AMOVA, our results seem to point to a common origin, in support
371of the latter hypothesis. However, because we are lacking exact information on the initial
372steps of the colonization, as well as on interactions among the colonizers prior to their
373movement, the dynamics of this process cannot yet be fully understood. It is possible that for
374a species of long-range fliers the decision to switch between colonies within this range is
375simply not under significant energetic restraints. On the contrary, at least three of the studied
376colonies (including the one in DNP) may be acting as a large social unit with frequent
377exchanges between them, despite their distance and the region's habitat heterogeneity (Popa-
378Lisseanu *et al*. 2009).

379 It is likely that the process of colonization is not a fixed species characteristic, but rather
380a plastic behavior molded by social and ecological factors. Group fission along matrilineal

lines documented for *E. fuscus* by Metheny *et al.* (2008) is probably not the norm, even within the same species, as suggested by the lack of genetic structure among the populations of big brown bats studied by Vonhof *et al*. (2008). Even though the existence of a fine-scale genetic structure has been reported in many mammalian societies (Altman *et al*. 1996; Ratnayeke *et al*. 2002; Nussey *et al*. 2005; Robinson *et al*. 2012), suggesting that kinship plays an important role in group choice during group fission, more research is needed to understand the relative roles played by kinship and social bonds. A predominance of the latter would explain the divergent results obtained across different bat species, in which average relatedness within social groups is remarkably low (Castella *et al*. 2001; Kerth *et al*. 2002; present study). We found evidence of philopatry, as well as of cooperation among kin during the formation of new breeding colonies in *N. lasiopterus.* However, the lack of suitable roosting grounds available in this heavily deforested region (Valbuena-Carabaña *et al*. 2010) is likely to play a strong role, and could impact the decision to remain with kin. Moreover, the crash of the D_O population in 2007 remains unexplained, but highlights the fragility of any colonization process.

In summary, further investigations into these unique populations will be essential to better understand bat social dynamics as well as help to efficiently design programs for the preservation of this rare and endangered species.

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Author Contributions

J.J., C.M., and J.S. conceived the study. A.P.-L. and C.I. collected the samples. J.S. performed the genetic laboratory work, analyzed the data, and wrote the first drafts of the manuscript. J.S., C.M. and J.J. critically revised and prepared the final version of the manuscript.

Data Accessibility

Mitochondrial DNA sequences have been uploaded to GenBank (Accession numbers: xxxx-xxxx). Microsatellite genotypes, sample ID and location, R scripts for F_{ST}, D_{EST} and allelic richness calculations were deposited in the Dryad Digital Repository (doi: xxxx).

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Detailed description of DNA extraction, purification, sequencing and genotyping.

Table S1 Summary statistics and PCR specifications for microsatellite loci.

Table S2 Pairwise D_{EST} values among populations based on microsatellite data.

645TABLES:

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649**Table 1**-- Genetic diversity in the mitochondrial and nuclear markers across all loci and by 650colony. The number of individuals sampled (***N***), as well as the variation in sampling time 651(***S**var*) are also given. [number of haplotypes (***h***), haplotype diversity (***Hd***), nucleotide diversity 652(***π***), number of polymorphic sites (***S***), observed (***Ho***) and expected (***He***) heterozygosity]

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			Mitochondrial				*Nuclear*	
Colony	***N***	***S**var*	***h***	***Hd***	***π***	***S***	***He***	***Ho***
D_O	23	-	8	0.759	0.00128	6	0.666	0.625
D_R	24	-	4	0.498	0.00079	3	0.681	0.680
MLP	84	1.24	6	0.354	0.00012	3	0.747	0.684
ANP	52	1.3	5	0.614	0.00135	8	0.761	0.647
ZJF	32	0.47	5	0.179	0.00022	2	0.787	0.675
Total	**215**	2.10	**15**	**0.578**	**0.00042**	**14**	**0.761**	**0.647**

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Table 2 -- Pairwise F_{ST} (above diagonal, microsatellite data) and ϕst (below diagonal, mtDNA) values among colonies of giant noctule bats in Andalusia, including Doñana's 'original' (D_O) and 'recolonization' (D_R) groups. Significant values ($P < 0.05$) are in bold, see text for population acronyms.

Colony	*D_O*	*D_R*	*MLP*	*ANP*	*ZJF*
D_O	-	0	0.0177	**0.0351**	0.0188
D_R	0.030	-	0.0111	**0.0267**	0.0098
MLP	**0.143**	0.003	-	**0.0093**	0.0017
ANP	**0.085**	**0.228**	**0.356**	-	0.0027
ZJF	0.029	0	0.011	**0.213**	-

660**Table 3** -- Partitioning of mitochondrial genetic variation among and within colonies of giant
661noctule bats in Andalusia, Spain. Genetic variation components were calculated without DNP,
662with D_O without D_R, and with D_R without D_O. All other colonies (ZJF, ANP, MLP) were kept
663separate. Contributions of 'among' and 'within' components given as percentage of the total
664variation.

Source of variation	DNP excluded	Following 1st colonization attempt (D_O)	Following 2nd colonization attempt (D_R)
Among colonies	26.5	20.6	22.4
Within colonies	73.5	76.4	77.6
P-value	<0.001	<0.001	<0.001

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Table 4 -- AMOVA-estimated variance components among colonies of giant noctule bats in Andalusia, Spain according to different grouping designs. Contributions of the different variance components are given as percentage of total variation. Significant fixation indices are also shown (* P<0.05, ** P<0.01, *** P<0.001).

	Group I		Group II		Group III		Group IV	
	mtDNA	*nDNA*	*mtDNA*	*nDNA*	*mtDNA*	*nDNA*	*mtDNA*	*nDNA*
Among groups	31.2	0.62	27.5	0.71	31.5	0.58	3.05	0.0
Among populations within groups	2.36	0.33	2.39	0.47	1.27	0.71	15.89	1.32
Within populations	66.45	99.05	70.1	98.82	67.27	98.72	81.05 99.14	99.14
F_{CT}	0.311	0.006	0.274	0.007	0.314	0.006	0.031	0.000
F_{ST}	0.335 ***	0.009 ***	0.299 ***	0.012 ***	0.327 ***	0.012 ***	0.189 ***	0.009 ***
F_{SC}	0.034	0.003	0.033 *	0.004	0.018	0.007 ***	0.163 ***	0.013 **

* Grouping structure: Group I: [MLP-ZFJ]-[ANP]; Group II: [MLP-ZFJ-Do]-[ANP]; Group III: [MLP-ZFJ-D_R]-[ANP]; Group IV: [ANP-ZFJ-Do-D_R]-[MLP]

673**Table 5** --Mean pairwise relatedness *R* within colonies, percentage of closely related dyads,
674percentage of females with close relatives within colonies, and number of parental
675associations per population (n_{par}).

Colony	R (mean ± SD)	% associations with *r*>0.25	% females with close relatives	n_{par}
D_O	0.046 (±0.090)	1.3	83.3	0
D_R	0.040 (±0.078)	1.0	91.3	4
DNP	0.085 (±0.109)	9.5	93.6.5	4
MLP	0.059 (±0.097)	6.6	97.6	39
ANP	0.052 (±0.091)	6.0	80.8	11
ZJF	0.048 (±0.076)	3.4	62.5	0
Total	0.059 (±0.090)	6.1	1	105

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678**Table 6** Average pairwise relatedness (± SD) among individuals with shared mitochondrial
679haplotypes roosting in the same colony, as well as the percentage of individuals found in any
680particular colony (columns) carrying a specific haplotype (rows). Only haplotypes carried by
681at least two individuals in the same colony are given. See text for the acronyms of the
682localities.

Haplotypes	*MLP*	*ANP*	*ZJF*	D_O	D_R
H1	0.062 (0.102) 52%	0.027 (0.0556) 10.4%	0.046 (0.071) 16.8%	0.030 (0.068) 8%	0.039 (0.073) 4.8%
H2	0.064 (0.141) 20.7%	0.052 (0.0955) 50%	0.009 (0.033) 12.1%	0.0183 (0.035) 10.3%	0.00 6.9%
H3	-	-	-	-	0.00 100%
H4	-	-	0 66.6%	-	-
H5	0.345 66.6%	-	-	-	-
H6	-	0.023 (0.110) 100%	-	-	-

683FIGURES

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686**Fig. 1** Location of the three maternity colonies and colonization site included in the study, as well as
687major towns and rivers. Grey areas indicate Natural and National parks of 'Los Alcornocales' and
688'Doñana', respectively.

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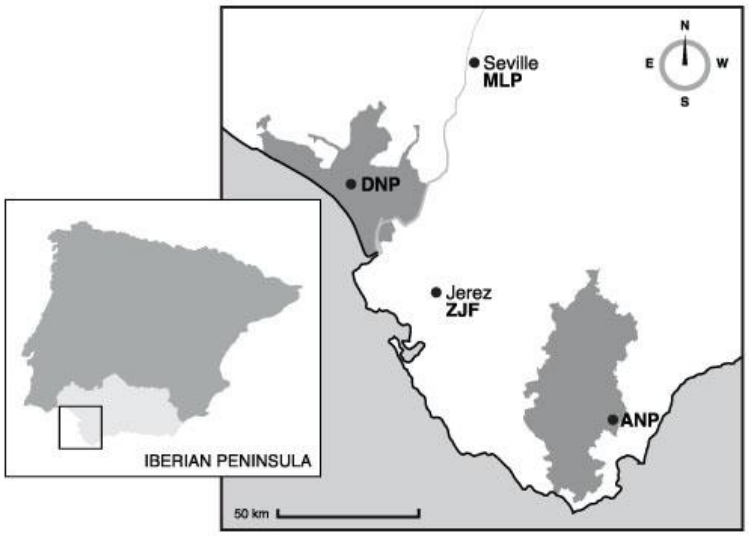

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Fig. 2 Parsimony-based network of mtDNA haplotypes using the median-joining algorithm. Circles correspond to haplotypes with size proportional to the number of individuals sharing this particular haplotype. Colors correspond to the four colonies/populations studied (see text for acronyms) and red numbers indicate the number of mutational steps needed to connect the haplotypes.