Understanding social behaviour in mammals: Kin structure and roost fidelity in greater noctule bats

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Running Header: Kin structure of the giant noctule

ABSTRACT

Roost fidelity is an important aspect of mammalian biology. Studying the mechanisms underlying philopatry can help us understand a species' energetic requirements, ecological constraints and social organisation. Temperate bat species notably exhibit a comparatively high degree of female philopatry, resulting in maternity colonies segregated at the mitochondrial level. While cooperative behaviours between colony members are well documented across temperate genera, evidence for a genetic basis to these traits is limited. We focus on the greater noctule, *Nyctalus lasiopterus*, to study this behaviour in more depth. We make use of microsatellite data for 11 markers across 84 individuals residing in Maria Luisa Park in Seville, Spain. At the time of sampling, this site boasted the highest number of registered bats of this species, among which three social groups were observed to segregate spatially. We studied the distribution of pairs of individuals across relation categories and relatedness estimates relative to the social group of each individual. This analysis was complemented by information on roost-use frequency among a subset of genotyped bats. We found no significant relationship between roost use and genetic distance, but there was evidence of higher group-sharing with increased levels of relatedness. Mother-daughter pairs shared the same group more often than expected, as did pairs of individuals of relatedness above 0.36. We discuss the implications of these results in terms of the behavioural ecology of temperate bats and for conservation efforts aimed at preserving them.

Keywords: philopatry, genetic structure, microsatellites, relatedness, *Nyctalus lasiopterus*, Chiroptera

INTRODUCTION

Female philopatry, or fidelity to a birth site or region, is a common trait among mammals (Greenwood, 1980; Clutton-Brock & Lukas, 2012), including bats (Burland and Wilmer, 2001). It has been attributed to a number of factors such as familiarity with the habitat, mortality associated with finding new roosts, and kin cooperation (Handley & Perrin, 2007). Bats display an exceptionally high degree of female philopatry and colony fidelity (Kerth et al., 2002). While this can be partly explained by the temporal stability and distribution of the roosts used by cave-dwelling species (Lewis, 1995; Kerth, 2008), it is still common in tree- and foliage-roosting bats, for which roost availability may not constitute a limiting factor (Kerth, 2008). Roost switching is so common in tree-roosting species that it precludes roost fidelity as a plausible explanation for philopatry or group formation (Lewis, 1995; Kerth & König, 1999; Willis & Brigham, 2004; Chaverri et al., 2007). Instead, philopatry in female temperate tree-roosting bats has been attributed to familiarity with the natal area, roosts and foraging locations, or the maintenance of social relations (Kerth, 2008; Hyde et al., 2019).

Sociality theory posits that philopatry and traits linked to colony fidelity were selected for in order to take advantage of the benefits of group living. Strong female-biased philopatry together with high female longevity in bats (Barclay & Harder, 2003) should lead to the formation of matrilineal societies and open the door for the selection and fixation of cooperative behaviors (Emlen & Oring, 1977; Clutton-Brock, 2009). Indeed, reported interactions among females of bat colonies include communal nursing (Wilkinson, 1992), social grooming (Wilkinson, 1986; Koshurina, 1993; Kerth et al., 2008), food sharing (Carter & Wilkinson, 2013)

and information transfer about roost sites and foraging locations (Wilkinson & Boughman, 1998; Kerth & Reckardt, 2003; Chaverri et al., 2010). However, the extent to which these interactions are maintained by kin selection is unknown. Food sharing in *D. rotundus*, for example, was reported to be more dependent on reciprocity than relatedness (Carter & Wilkinson, 2013). Likewise, information transfer in both the Evening and Bechstein's bats showed no evidence of kin selection (Wilkinson, 1992; Kerth & Reckardt, 2003). For other behaviours, such as association and grooming, empirical support for different species is incongruent (Metheny et al., 2008; Patriquin et al., 2013).

Understanding the causes underlying philopatry and group formation is rendered particularly difficult by the fact that most colonies of tree-dwelling temperate bats are in fact fluid societies, where frequent roost changes result in day roost aggregations that are variable in size and composition (Kerth, 2008). However, studies monitoring roost associations have shown females will roost preferentially with some females rather than others, resulting in a non-random association pattern within the colony (Burland et al., 1999; Willis & Brigham, 2004; Metheny et al., 2008; Patriquin et al., 2013). Because tree-roosting bats occupy hollows not large enough for more than a small number of individuals, regular fission and fusion of subgroups could help maintain larger social groups spread over a given area (O'Donnell, 2000; Patriquin et al., 2016). A recent review of studies reporting pairwise association and genetic relatedness data across nine species of bats spanning four families found no relation between the strength of associations between kin and the occurrence of complex cooperative behaviours (Wilkinson et al., 2019). Rather, the authors observed that species that switched roosts often tended to present stronger associations between relatives.

The greater noctule bat, *Nyctalus lasiopterus* (Schreber, 1780), is a rare vespertilionid bat with a circum-Mediterranean distribution that roosts mostly in tree holes (Alcaldé et al., 2016). Western Andalusia, Spain, is unique for harboring several known maternity colonies of giant noctules in three main breeding areas (Santos et al., 2016). Females consolidate colonies in spring to breed, and remain until late August, when they disaggregate and disperse, presumably to mating and hibernation sites (Ibáñez et al., 2009), although some cases of permanent residency have been observed (C. Ibáñez and J. Juste, unpublished data). Few males have been found in these colonies, contrasting with other sites in northern Spain and Europe (Ibáñez et al., 2009).

In 2007, a pioneering study on the social structure of the greater noctule population of Maria Luisa Park (MLP) in Seville (Spain) revealed it follows a fission-fusion structure, with bats switching roosts on average every ~three days and segregated in three spatially distinct social groups (9-17% overlap), whereby the same tree holes were rarely used by bats of different groups (Popa-Lisseanu et al., 2008; Fortuna et al., 2009). Therefore, each social group can even be considered a differentiated colony. Moreover, the foraging areas of the three groups (extending over 40 km away from the park) overlapped almost completely. Popa-Lisseanu et al. (2009) further reported that females were loyal to their groups for at least five years, and that juveniles returned to their natal roosting areas along the study period. In a recent genetic analysis encompassing the region of Andalusia, Spain, and including four maternity colonies, we found that at least 31% of the females sampled shared their nursery colonies with mothers or daughters (Santos et al., 2016). However, the reasons for this high level of philopatry (despite the species' high dispersal ability, Burns & Broders, 2014) remain unclear. Here, we narrowed the geographic scale of our previous study to focus on the only population (in Seville) whose social

dynamics have been studied in detail (Popa-Lisseanu et al., 2008). Relying on bi-parentally inherited markers (microsatellites), we estimated average pairwise relatedness across as well as within the three social groups detected, and mapped out the distribution of closely related individuals (including mother-daughter pairs), to discriminate between the following hypotheses: 1) philopatry is the result of the low density of suitable roosts in the region, leaving females with little choice but to return to MLP. In this case, females should show no preference for any particular area of the park, and group composition should simply reflect proximity and a natural upper limit to the number of associations sustainable by individual bats (Kerth et al., 2011); 2) philopatry is driven by other factors, in which case females should choose to return to the same areas of the park (group or colony) they were born in (O'Donnell, 2000). In the first scenario, we would expect an even distribution of parent-offspring pairs in the park, while in the second these should be clustered within groups.

Materials and Methods

Focal Species and Study Population

The greater noctule, *N. lasiopterus*, is one of the largest and rarest vespertilionid bats (Ibáñez et al., 2004). Greater noctules typically roost in large, old trees. The sparse number of existing records suggests that the species' distribution is circum-Mediterranean and patchy from Morocco to Uzbekistan (Ibáñez et al., 2004). Very few breeding colonies are known, with a few maternity colonies having been described outside of the Iberian Peninsula: France, Hungary, Slovakia, Belarus and Russia (Ibáñez et al., 2004; Uhrin et al., 2006; Estók, 2011; Dubourg-Savage et al., 2013; Dombrovski et al., 2016).

The study population is located in MLP, Seville (lat 37°22′29″N, long 5°59′19″W). The 122 year old park extends over 38 ha and contains many large old trees that bear enough cavities to harbour the three distinct colonies. Most roost trees belong to *Platanus* spp., *Gleditsia triacanthos*, *Sophora japonica* and *Washingtonia filifera*. With a population previously estimated at ca. 500 individuals, but now in dramatic decline due to an invasive parakeet species (Hernández-Brito et al., 2018) that is taking over the roosting holes, it was once the largest known breeding site of giant noctules (Popa-Lisseanu et al., 2008).

Sampling

We analysed biopsy samples of 82 bats from MLP collected from 2004 to 2007. The sampling included 25 individuals that were radio-tracked to study the roost-switching behaviour of the population that was used to identify and define the social group dynamics in the previous study (Popa-Lisseanu et al., 2008). Therefore, group designations (I, II and III) were maintained from that earlier study.

Molecular Methods

Total DNA was extracted from 3 mm diameter wing punches using a modified version of the salt-based protocol developed by Aljanabi & Martinez (1997).

All individuals were genotyped at 11 nuclear microsatellite loci: Nle 2, 3 and 6-11 (developed for *Nyctalus leisleri*, see Boston et al. 2008); EF4 (developed for *Eptesicus fuscus*, Vohnof et al. 2002); P20 and P217 (Kaňuch et al., 2007). All were tested in muscle tissue prior to genotyping (see Supplementary Methods for a detailed description of DNA extraction,

amplification, sequencing, and microsatellite genotyping) and successfully employed in our previous study (Santos et al., 2016). Labelling followed Schuelke's procedure (2000).

Data Analysis

All nuclear markers were in linkage equilibrium as assessed with FSTAT v. 2.9.3.2 (Goudet et al., 1995). Using CERVUS, we estimated observed and expected heterozygosities, as well as deviations from Hardy-Weinberg equilibrium (HWE). Of the 11 microsatellites, three (Nle6, P20 and P217) deviated significantly from HWE (see Supplementary Table S1).

Pairwise and mean relatedness values (*r*) were estimated using ML-Relate (Kalinowski et al., 2006), which implements a corrected maximum-likelihood method for estimating relatedness that allows loci with null alleles to be incorporated into the analysis (Wagner et al., 2006). Three of the 11 loci used in this study showed the highest frequencies of null alleles (min=0.097, mean=0.187). Incidentally, this might have been the cause of the departure from HWE, as it would simulate a heterozygote deficiency. Because we did not want to abdicate the inferential power of three loci, we resorted to the method by Wagner et al. (2006), implemented in ML-Relate, and kept all 11 loci. Using this software, pairwise relations were classed as Mother-Daughter (MD), Full-Sibling (FS), Half-Sibling (HS), or Unrelated (U), and each attributed a value *r* of pairwise relatedness. Principal component analysis (PCA) was performed on the squared matrix of pairwise relatedness values.

With reference to the three groups within the park, pairwise relations were classed according to whether or not both individuals had been captured within the same group. We tested the null hypothesis that each group shared the same population mean as the whole population

using two-tailed t-tests as implemented in the function 'ttest_ind' of the python package 'scipy.stats' (Oliphant, 2007). The probability $P(O)$ of pairs of individuals falling across groups was estimated as $P(0) = P_{SR}(P_{SE} + P_{SH}) + P_{SE}(P_{SR} + P_{SH}) + P_{SH}(P_{SR} + P_{SE})$. The values P_{SE}, P_{SR} and P_{SH} were estimated as the proportions of individuals registered to roost in groups I, II and III at the time of sampling (81, 61 and 114 bats respectively, Popa-Lisseanu et al. 2008). The probability $P(I)$ of two individuals choosing the same group was estimated as 1 – $P(O)$. Analysis of molecular variance (AMOVA) of within-group relatedness estimates was conducted using the function 'f_oneway' of the python package 'scipy.stats' (Oliphant, 2007). We compared the expected and observed proportions across the data set and within the classes of relation MD, FS, HS and U, and then studied the deviation of observed within-group pairs as a function of relatedness values. We estimated the upper-tail *p*-value of observed proportions of same-group pairs within each class assuming a binomial probability $P(I)$. We further estimated upper-tail *p*-values of within-group proportions along windows of relatedness. Proportions were calculated among relationships presenting values within a 0.15 window of relatedness around a central value ranging between 0.075 and 0.5 in increments of 0.01. Window size was selected on the basis of the minimum, median and standard deviation of number of relations across windows of a given size (see Supplementary Figure S1).

We explored the relationship between genetic relatedness and roost use. For this, we resorted to the matrix of pairwise roost use similarity among 15 bats radio-tracked in 2004 used by Popa-Lisseanu et al. (2008) to infer the existence of social groups within the park (Supplementary Figure S2). Similarity was estimated using the Freeman-Tukey statistic (Krebs, 1989). All individuals considered in that study were genotyped. Pairwise relatedness and roost use similarity vectors were centred and scaled. The correlation (Pearson's r) between pairwise

genetic relatedness and roost-use similarity among the individuals for which this information was available was then estimated using the function 'pearsonr' of the python package 'scipy.stats.stats' (Jones et al., 2001). Given the bi-modal distribution of roost use after scaling (Fig. 4A), we further performed a Student's t-test between the sample of dyads presenting standardized roost use below 1 and that of dyads with standardized roost use above 1.

Results

No genetic structure was observed through PCA (Fig. 1a). One-way AMOVA of within-group relatedness estimates was non-significant ($p = 0.16$, d.f. = 1133). The result of the t-test of pairwise relatedness means between total and group III dyads was significant ($p < 0.01$, Table 1, Fig. 1B). Of the total number of possible associations within the park and groups (every female paired with every other female), the percentage of those exceeding a relatedness degree of 0.25 accounted for 6% of the total (Table 1). However, 81% of females shared their group with at least one close relative (Table 1), while 95% shared the park (considering close relatives found in both the same and different groups).

The distribution of parent-offspring pairs differed significantly ($p < 0.001$) from random expectations (Supplementary Figure S3). Proportions of FS, HS and U pairs of individuals within the same groups did not deviate significantly from the expected proportions (Fig. 2). However, a decreasing trend is observed when these groups are considered in order of decreasing relatedness.

When the same test is performed on pairs of individuals of increasing relatedness, the proportion of these pairs falling within the same group rapidly becomes significant ($r > 0.18$, Fig.

3). When only pairs within a moving window of relatedness are considered (size= 0.15, step= 0.03), the same proportion becomes significant above a relatedness level of 0.36 (Fig. 3).

Analysis of pairwise relatedness as a function of roost use revealed no significant association between the two variables (Pearson's r = 0.01, p = 0.91, Fig. 4B). Associated and non-associated individuals did not differ significantly in terms of relatedness (t = -0.3, p = 0.77, Fig. 4C).

Discussion

We found a significantly larger number of mother-daughter relations among individuals of the same social groups within the park than would be expected given a scenario of random roost choice. While this was not the case for the remaining classes of pairwise relations identified in our data set, a positive relationship with increasing relatedness is apparent. Analysis of within- versus among-group distribution of pairs of adult female *N. lasiopterus* along relatedness windows confirms this observation. The first result could be explained if we hypothesized that daughters follow their mothers to new social groups when they move or vice versa. However, the positive linear relationship observed with increasing relatedness would have us extend this argument to less related pairs. It seems thus more reasonable to explain these results as a tendency of females to return to their natal group roosts. In light of these results, and of previous studies on this site, we find it is safe to re-classify the social groups in MLP as separate colonies. Female fidelity to breeding colonies has been reported in a number of temperate bat species (Rossiter et al., 2002; Castella et al., 2008; Juste et al., 2009), leading, in the extreme, to the formation of closed societies as in *Myotis bechsteinii* (Kerth et al., 2000). The reasons for this

behaviour have been widely discussed (Moussy et al., 2013), and generally attributed to the females' acquaintance with their natal area and facilitated access to foraging and roosting sites, as well as benefits they might derive from social relationships and cooperative behaviors with conspecifics (Kerth, 2008; Kerth et al., 2011). Here, the observed lack of correlation between roost use and genetic relatedness suggests the return is not driven by family ties. The fact that despite this, returning greater noctule females not only show a considerable degree of philopatry but, in the case of MLP, seem to have a preference for the area of the park they were born in, allows us to refine our hypothesis regarding the basis of philopatry in this species. Given that the three groups have completely overlapping foraging areas (Popa-Lisseanu et al., 2009), distance to these cannot account for individual preferences of one group over another. We are also not aware of differences in roost characteristics between the three areas of the park that could account for the pattern observed.

Females thus seem driven by individual preferences for specific areas or roosts. One hypothesis that could account for this behaviour is associative learning by non-volant young when being carried by their mothers between day roosts (O'Donnell, 2000). Based on reports of observational learning, he reasoned that if young *Chalinolobus tuberculatus* could have their mother's social group pool of favourite roost trees imprinted through repetitive roost switching, group structure would be maintained. It had, however, been previously suggested by Wolz (1986) and Vaughan and O'Shea (1976) to explain observations of reduced roost fidelity in females just prior to or after offspring were weaned when compared to that of females in early lactation (in *M. bechsteinii* and *Antrozous pallidus* respectively). The same mechanism could be at play in *N. lasiopterus.* Philopatry is not only the safest bet regarding roost quality, but also a way of ensuring the proximity of close kin. Either of these could be the driver behind the

selection for such a behaviour. However, in light of our results regarding roost use, we can at least say that the second is no longer at play.

An alternative hypothesis is that roost fidelity functions to maintain social bonds, helping returning females reconnect with acquaintances made at specific roosts. Studying the social organization of a captive maternity colony of noctule bats Nyctalus noctula in captivity, Kozhurina (1993) reported the best predictors of social organization to be age and sex (92.6 % classification accuracy versus 29.6 % for family membership) and that the disparity between age classes remained over time. She concluded that the main cause of individual attachment among bats was the "attainment of a certain degree of comfort in the company of familiar individuals of predictable behavior" (Kozhurina, 1993). It is important to add that occasions of social grooming occurred strictly among members of the same social groups. A more recent study of the temporal and genetic components of associative dynamics of *Nyctalus leisleri* also found a stronger impact of cohort year on the composition of social groups inferred through network analysis (Nad'o et al., 2017). Although genetic relatedness was also observed to impact association between dyads significantly, this could be the result of different genetic profiles between cohorts due to male-biased gene flow. Cooperative behaviours and long-term associations among colony members, sometimes spanning several years, have been described in numerous bat species (Wilkinson & Boughman, 1998; Carter & Wilkinson, 2013). Kozhurina (1993) further observed that noctule bats start forming associations in their first year. If this is true for giant noctules, maintaining these bonds, either for the comfort derived from familiarity and predictability, or the increased likelihood of reciprocal cooperative behaviour, could explain why individuals tend to return to the area of the park they were born in. This theory has the advantage of providing an explanation

for the formation of social groups, which are a pre-requisite for the associative learning hypothesis.

In summary, we found that, if possible, female *N. lasiopterus* will seek to return to the specific areas or social groups they were born in, not just the region or breeding area. We further find that roost associations are not kin-based. While these findings confirm some of the predictions made by Popa-Lisseanu et al. (2008), it is likely that neither accounts for this behaviour entirely, but that instead both play a role. Both hypotheses explain the specificity of natal philopatry in giant noctules. Associative learning has the obvious adaptive advantage of allowing young adult females to benefit from their mothers' successful choice of colony. Strong social bonds leading to cooperative behaviours, such as allo-nursing or information sharing, bring fitness benefits to nursing mothers with already high energy expenditures, an explanation which seems particularly plausible for females of this species in light of their partially carnivorous habits (Ibáñez et al., 2001; Popa-Lisseanu et al., 2007). While both hypotheses seem plausible, each rests on certain assumptions regarding roosting behaviour or social interactions that lack confirmation in this species. There is one case of reported co-operative nursing in captivity (Kozhurina, 1993), but no study to date has captured roosting associations or behaviours with enough precision to infer the correlates of social organization in maternity colonies of *N. lasiopterus*. Likewise, not enough information is available on roost fidelity to correlate it with breeding status or to verify roost reuse. The lack of correlation between genetic and roost-use similarity among greater noctules, given the positive relation observed by Wilkinson et al. (2019) between this variable and frequency in roost-switching, supports the hypothesis that individual *N. lasiopterus* females do not change roost frequently over the

breeding season. If true, this would remove statistical power from an analysis of roost fidelity, but could serve to imprint specific roosts on new-born cubs.

Determining the relative importance of associative learning and social bonds in this species is crucial, as it could direct future conservation efforts regarding the few known maternity colonies. If females rely on the strength of social bonds in their choice of colony, moving whole groups without disrupting social structure might be possible. In order to discriminate between these two hypotheses, future studies should focus on roost fidelity and roosting patterns as well as on long-term associations. Evidence of roost reuse by individual females, as well as varying roost fidelity according to breeding status would point to associative learning. These will have to be compared with the frequency of long-term associations within the same colonies in order to determine the relative importance of social bonds. More generally, discovering the mechanism behind philopatry represents an important step in our understanding of the thought processes behind the individual decisions that structure mammalian societies, and has implications for conservation policies aimed at preserving healthy populations.

Acknowledgements

J. Nogueras and C. Ruiz helped collecting bat samples. We particularly thank J.L. García-Mudarra and J.M. Arroyos-Salas for valuable advice and their technical insight. We also acknowledge the staff of the Servicio de Parques y Jardines de Sevilla for their help and continuous support of our research. Logistical support was provided by the Laboratorio de Ecología Molecular, Estación Biológica de Doñana, CSIC (LEM-EBD). The regional government of Andalusia provided permits for collecting samples and handling of bats. This

study was partially funded by the Spanish MICINN CGL 2009- 12393, the PPNN 021/2002 and 1981/2010. JDS would like to acknowledge support from the Erasmus Student Mobility program.

Supplementary Data

Supplementary Methods – Detailed description of DNA extraction, purification, sequencing and genotyping.

Supplementary Table S1 – Summary statistics and PCR specifications for each locus over all colonies.

Supplementary Figure S1 - Exploratory analysis of the impact of window size on sampling across relatedness steps.

Supplementary Figure S2 - Intra-colony network of roost-sharing between giant noctules in Maria Luisa Park, Seville, Spain.

Supplementary Figure S3 - Fig. S3 Network of parent-offspring dyads among giant noctule bats roosting in Maria Luisa Park, Seville, Spain.

Data accessibility

Microsatellite genotypes, sample ID and location were deposited in the Dryad Digital Repository (doi: 10.5061/dryad.rc504). All data analysis scripts are accessible in the GitHub repository (doi: 10.5281/zenodo. 2653473).

Conflicts of interest

None declared

References

Aljanabi, S.M. & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* **25**, 4692–3.

Alcaldé, J., Juste, J. and Paunović, M. (2016). *Nyctalus lasiopterus*. The IUCN Red List of Threatened Species 2016: e.T14918A22015318. http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T14918A22015318.en. Downloaded on 24 November 2018.

Barclay, R.M.R. & Harder, L.D. (2003). Life histories of bats: life in the slow lane. In *Bat ecology*: 209–253. Kunz, T.H. & Fenton, M.B. (Eds.). University of Chicago Press.

Boston, E., Montgomery, I. & Prodöhl, P. a. (2008). Development and characterization of 11 polymorphic compound tetranucleotide microsatellite loci for the Leisler's bat, *Nyctalus leisleri* (Vespertilionidae, Chiroptera). *Conserv. Genet.* **10**, 1501–1504.

Burland, T.M., Barratt, E.M., Beaumont, M.A. & Racey, P.A. (1999). Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. *Proc. R. Soc. B Biol. Sci.* **266**, 975–980.

Burland, T.M. & Wilmer, J.W. (2001). Seeing in the dark: molecular approaches to the study of bat populations. *Biol. Rev. Camb. Philos. Soc.* **76**, 389–409.

Burns, L.E. & Broders, H.G. (2014). Correlates of dispersal extent predict the degree of population genetic structuring in bats. *Conserv. Genet.* **15**, 1371–1379.

Carter, G.G. & Wilkinson, G.S. (2013). Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proc. R. Soc. B Biol. Sci.* **280**.

Castella, V., Ruedi, M. & Excoffier, L. (2008). Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *J. Evol. Biol.* **14**, 708–720.

Chaverri, G., Quirós, O.E., Gamba-Rios, M. & Kunz, T.H. (2007). Ecological correlates of roost fidelity in the tent-making bat *Artibeus watsoni*. *Ethology* **113**, 598–605.

Chaverri, G., Gillam, E.H. & Vonhof, M.J. (2010). Social calls used by a leaf-roosting bat to signal location. *Biol. Lett.* **6**, 441–444.

Chaverri, G., Kunz, T.H. & Gillam, E.H. (2013). A call-and-response system facilitates group cohesion among disc-winged bats. *Behav. Ecol.* **24**, 481–487.

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature* **462**, 51–7.

Clutton-Brock, T. & Lukas, D. (2012). The evolution of social philopatry and dispersal in female mammals. *Mol. Ecol.* **21**, 472–92.

Dombrovski, V., Fenchuk, V., & Zhurauliou, D. (2016). New occurrence and the first breeding record of Nyctalus lasiopterus in Belarus. *Vespertilio*, **18**: 55-59.

Dondini, G. & Vergari, S. (2000). Carnivory in the greater noctule bat (*Nyctalus lasiopterus*) in Italy. *J. Zool.* **251**, 233–236.

Dubourg-Savage, M.-J. & Gaches, L. (2017). New data on *Nyctalus lasiopterus* in the Massif central (France). In *European Bat Research Symposium*. Donostia, Spain.

Emlen, S.T. & Oring, L.W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215–223.

Estók, P. (2007). Seasonal changes in the sex ratio of *Nyctalus* species in north-east Hungary. *Acta Zool. Acad. Sci. Hungaricae* **53**, 89–95.

Goudet, J. (1995). FSTAT (version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**, 485–486.

Greenwood, P.J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.* **28**, 1140–1162.

Handley, L. J. & Perrin N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* **16,** 1559-1578.

Hernández-Brito, D., Carrete, M., Ibáñez, C., Juste, J. & Tella, J.L. (2018). Nest-site competition and killing by invasive parakeets cause the decline of a threatened bat population. *R. Soc. Open Sci.* **5**.

Hyde, M.O., Silvis, A., Hallerman, E.M., Ford, M. & Britzke, E. (2019). Relatedness within and among Myotis septentrionalis colonies at a local scale. *Can. J. Zool*, ja.

Ibáñez, C., Juste, J., Garcia-Mudarra, J.L. & Agirre-Mendi, P.T. (2001). Bat predation on nocturnally migrating birds. *Proc. Natl. Acad. Sci.* **98**, 9700–9702.

Ibáñez, C., Guillén, A. & Bogdanowicz, W. (2004). Nyctalus lasiopterus — Riesenabendsegler. In *Handbuch der Säugetiere Europas*: 695–715. Krapp, F. (Ed.). Wiebelsheim: Aula-Verlag.

Ibáñez, C., Guillén, A., Agirre-Mendi, P.T., Juste, J., Schreur, G., Cordero, A.I., Popa-Lisseanu, A.G., Carretera, A., Haya, E., Lamparona, L. & Codosera, L. (2009). Sexual Segregation in Iberian Noctule Bats. *J. Mammal.* **90**, 235–243.

Juste, J., Bilgin, R., Muñoz, J., & Ibáñez, C. (2009). Mitochondrial DNA signatures at different spatial scales: from the effects of the Straits of Gibraltar to population structure in the meridional serotine bat (*Eptesicus isabellinus*). *Heredity*, **103**, 178.

Kalinowski, S.T., Wagner, A.P. & Taper, M.L. (2006). Ml-Relate: a Computer Program for Maximum Likelihood Estimation of Relatedness and Relationship. *Mol. Ecol. Notes* **6**, 576–579.

Kaňuch, P., Fornůsková, A., Bartonička, T., & Bryja, J. (2007). Multiplex panels of polymorphic microsatellite loci for two cryptic bat species of the genus Pipistrellus, developed by cross-species amplification within the family Vespertilionidae. *Mol. Ecol. Notes*, **7**, 871-873.

Kerth, G. (2008). Causes and Consequences of Sociality in Bats. *Bioscience* **58**, 737.

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., Perony, N. & Schweitzer, F. (2011). Bats are able to maintain long-term social relationships despite the high fission-fusion dynamics of their groups. *Proc. Biol. Sci.* **278**, 2761–7.

Kerth, G. & Reckardt, K. (2003). Information transfer about roosts in female Bechstein's bats: an experimental field study. *Proc. Biol. Sci.* **270**, 511–515.

Krebs, C.J. (1989). *Ecological methodology*. Harper & Row New York.

Kozhurina, E.I. (1993). Social Organization of a Maternity Group in the Noctule Bat, *Nyctalus noctula* (Chiroptera : Vespertilionidae). *Ethology* **93**, 89–104.

Lewis, S.E. (1995). Roost Fidelity of Bats: A Review. *J. Mammal.* **76**, 481–496.

Metheny, J.D., Kalcounis-Rueppell, M.C., Willis, C.K.R., Kolar, K. a. & Brigham, R.M. (2007). Genetic relationships between roost-mates in a fission–fusion society of tree-roosting big brown bats (*Eptesicus fuscus*). *Behav. Ecol. Sociobiol.* **62**, 1043–1051.

Moussy, C., Hosken, D.J., Mathews, F., Smith, G.C., Aegerter, J.N. & Bearhop, S. (2013). Migration and dispersal patterns of bats and their influence on genetic structure. *Mamm. Rev.* **43**, 183–195.

Naďo, L., Chromá, R. & Kaňuch, P. (2017). Structural, temporal and genetic properties of social groups in the short-lived migratory bat Nyctalus leisleri. *Behaviour* **154**, 785–807.

O'Donnell, C.F.J. (2000). Cryptic local populations in a temperate rainforest bat *Chalinolobus tuberculatus* in New Zealand. *Anim. Conserv.* **3**, 287–297.

Oliphant, T.E. (2007). Python for Scientific Computing. *Comput. Sci. Eng.* **9**, 10–20.

Patriquin, K.J., Palstra, F., Leonard, M.L. & Broders, H.G. (2013). Female northern myotis (*Myotis septentrionalis*) that roost together are related. *Behav. Ecol.* **24**, 949–954.

Patriquin, K.J., Leonard, M.L., Broders, H.G., Ford, W.M., Britzke, E.R. & Silvis, A. (2016). Weather as a proximate explanation for fission–fusion dynamics in female northern long-eared bats. *Anim. Behav.* **122**, 47–57.

Popa-Lisseanu, A.G., Delgado-Huertas, A., Forero, M.G., Rodríguez, A., Arlettaz, R. & Ibáñez, C. (2007). Bats' Conquest of a Formidable Foraging Niche: The Myriads of Nocturnally Migrating Songbirds. *PLoS One* **2**, e205.

Popa-Lisseanu, A.G., Bontadina, F., Mora, O. & Ibáñez, C. (2008). Highly structured fission–fusion societies in an aerial-hawking, carnivorous bat. *Anim. Behav.* **75**, 471–482.

Popa-Lisseanu, A. G., Bontadina, F., & Ibáñez, C. (2009). Giant noctule bats face conflicting constraints between roosting and foraging in a fragmented and heterogeneous landscape. *Journal of Zoology* **278**, 126-133.

Rossiter, S., Jones, G., Ransome, R. & Barratt, E. (2002). Relatedness structure and kin-biased foraging in the greater horseshoe bat (*Rhinolophus ferrumequinum*). *Behav. Ecol. Sociobiol.* **51**, 510–518.

Santos, J.D., Meyer, C.F.J., Ibáñez, C., Popa-Lisseanu, A.G. & Juste, J. (2016). Dispersal and group formation dynamics in a rare and endangered temperate forest bat (*Nyctalus lasiopterus*, Chiroptera: Vespertilionidae). *Ecol. Evol.* **6**, 8193–8204.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* **18**, 233.

Vaughan, T.A. & O'Shea, T.J. (1976). Roosting Ecology of the Pallid Bat, Antrozous pallidus. *J. Mammal.* **57**, 19–42.

Vonhof, M.J., Davis, C.S., Fenton, M.B. & Strobeck, C. (2002). Characterization of dinucleotide microsatellite loci in big brown bats (Eptesicus fuscus), and their use in other North American vespertilionid bats. *Mol. Ecol. Notes* **2**, 167–169.

Uhrin, M., Kaňuch, P., Benda, P., Hapl, E., Verbeek, H. J., Krištín, A., Krištofík, J., Mašán, P., Andreas, M. (2006). On the Greater noctule (Nyctalus lasiopterus) in central Slovakia. *Vespertilio*, **9**:183-192.

Wagner, A.P., Creel, S. & Kalinowski, S.T. (2006). Estimating relatedness and relationships using microsatellite loci with null alleles. *Heredity (Edinb).* **97**, 336–45.

Wilkinson, G.S. (1986). Social grooming in the common vampire bat, Desmodus rotundus. *Anim. Behav.* **34**, 1880–1889.

Wilkinson, G.S. (1992). Communal nursing in the evening bat, *Nycticeius humeralis*. *Behav. Ecol. Sociobiol.* **31**, 225–235.

Wilkinson, G.S. & Boughman, J.W. (1998). Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* **55**, 337–350.

Wilkinson, G.S., Carter, G., Bohn, K.M., Caspers, B., Chaverri, G., Farine, D., Günther, L., Kerth, G., Knörnschild, M., Mayer, F., Nagy, M., Ortega, J. & Patriquin, K. (2019). Kinship, association, and social complexity in bats. *Behav. Ecol. Sociobiol.* **73**, 7.

Willis, C.K.R. & Brigham, R.M. (2004). Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission–fusion model. *Anim. Behav.* **68**, 495–505.

Wolz, I. (1986). Wochenstuben-Quartierwechsel bei der Bechsteinfledermaus. *Zeitschrift für Säugetierkd.* **51**, 65–74.

Tables

Table 1: Mean relatedness estimates within groups and across the whole colony in Maria Luisa Park, Seville, percentage of associations with $r > 0.25$ among total possible associations, and percentage of females with close relatives within their groups as well as the whole colony.

			% associations	
Group	*Mean r*	*SD*	*r>0.25*	*% females*
I.	0.056	±0.106	6.3	89.2
II.	0.050	±0.099	5.6	75.0
III.	0.051	±0.099	5.6	78.6
Colony	**0.059**	**±0.098**	**5.7**	**95.2**

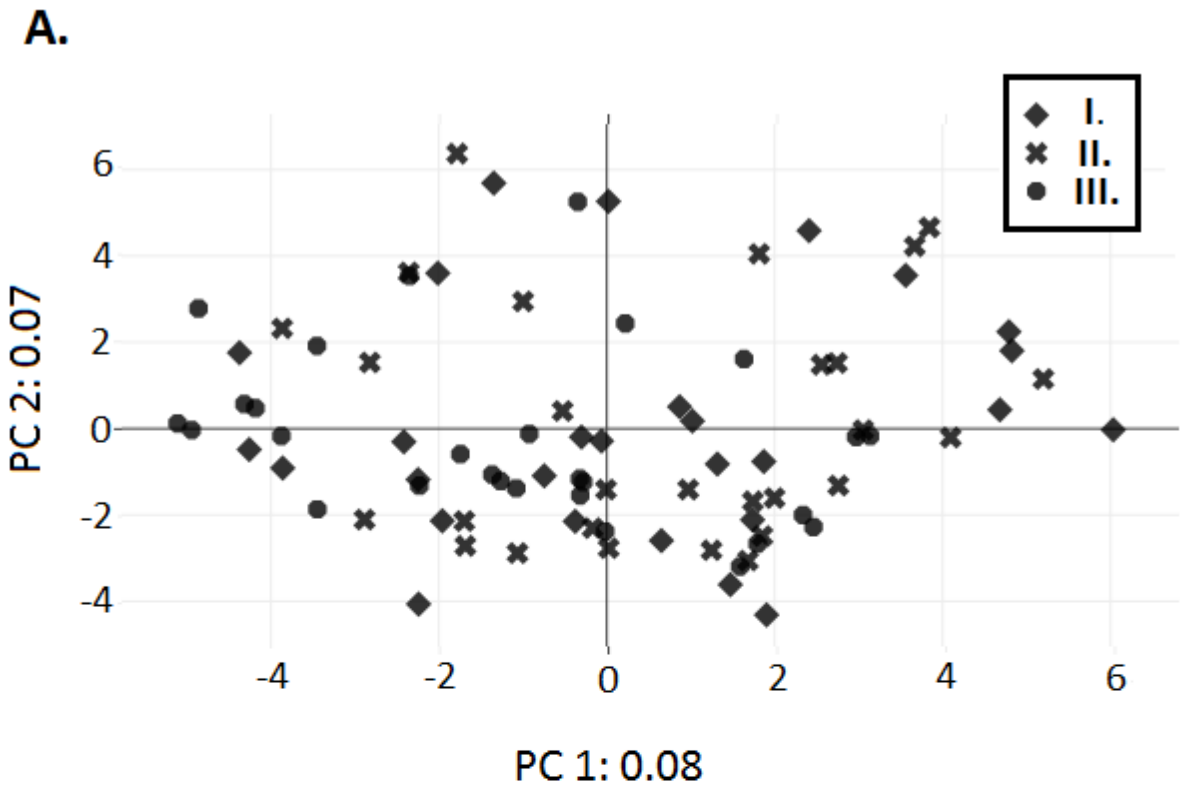
Figures

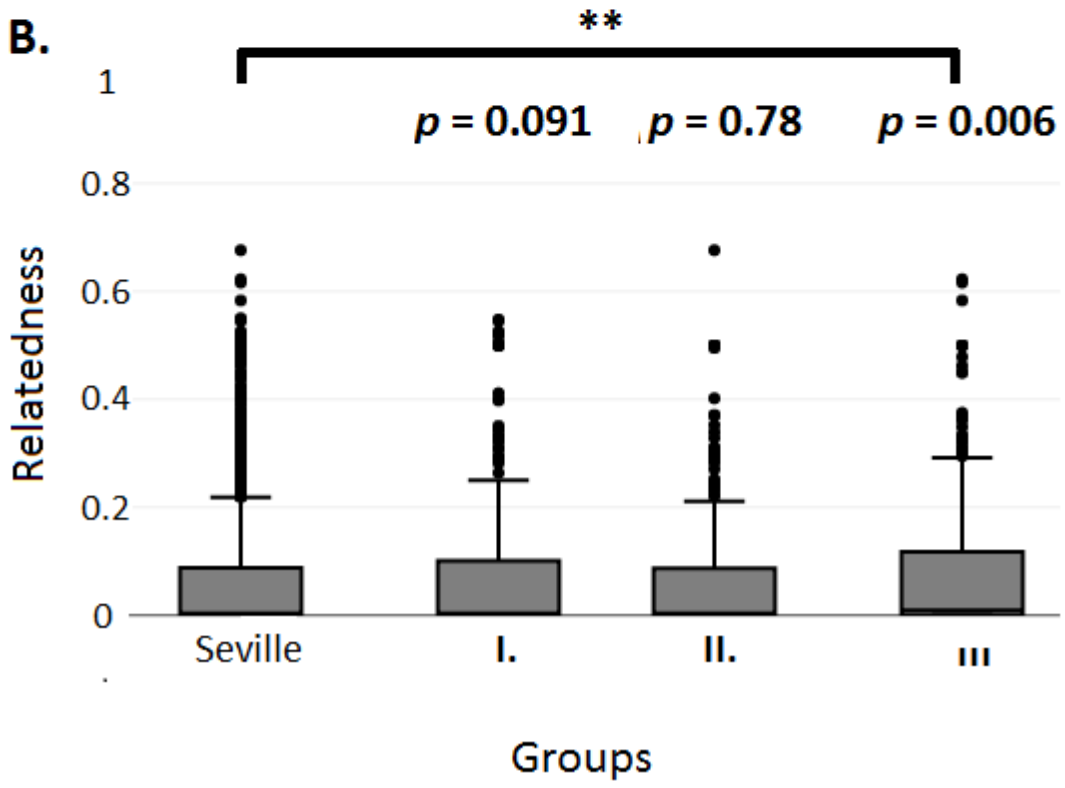
Fig. 1 Pairwise relatedness between adult female *Nyctalus lasiopterus* within and among social groups in Maria Luisa Park, Sevilla, Spain. **A**) Biplot of a principal component analysis based on a relatedness matrix. **B**) Boxplot of pairwise relatedness among individuals across the data set and within each social group. t-tests were calculated for group and colony sample means for each of the three groups (*p*-values shown above).

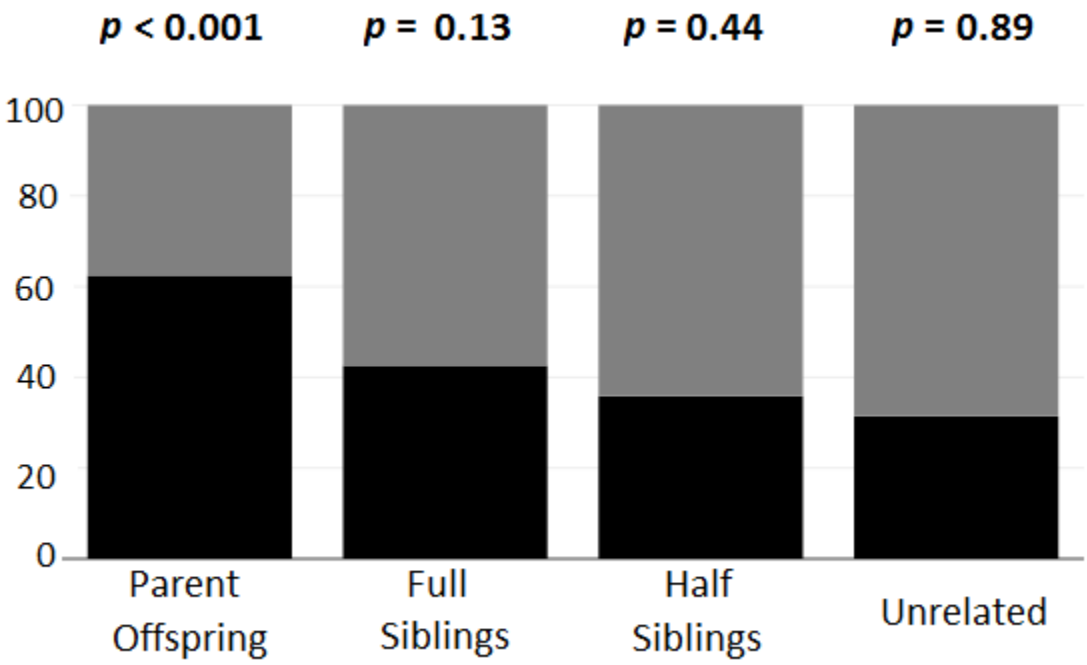
Fig. 2 Percentage of pairwise relations within and among social groups across relatedness categories. Grey: proportions of dyads between individuals from different groups; black: proportion of dyads between individuals from the same group. *p*-value of observed proportion of pairs from the same group for each class shown in bold.

Fig. 3 Group sharing proportion as a function of pairwise relatedness. Pairs of individuals were classified according to whether both were captured in the same area of Park Maria Luisa. This proportion was then plotted against the estimated relatedness of each pair. **A**) Proportion of dyads within the same group with relatedness values above abscissae. **B**) Proportion of dyads within relatedness windows that fall within the same group (window size= 0.15 *r*, step= 0.03 *r*). The upper-bound 95% confidence interval (lozenge-line) was estimated from a binomial distribution of $p = (I)$. The dashed line indicates the expected proportion $P(I)$ of within-group pairs.

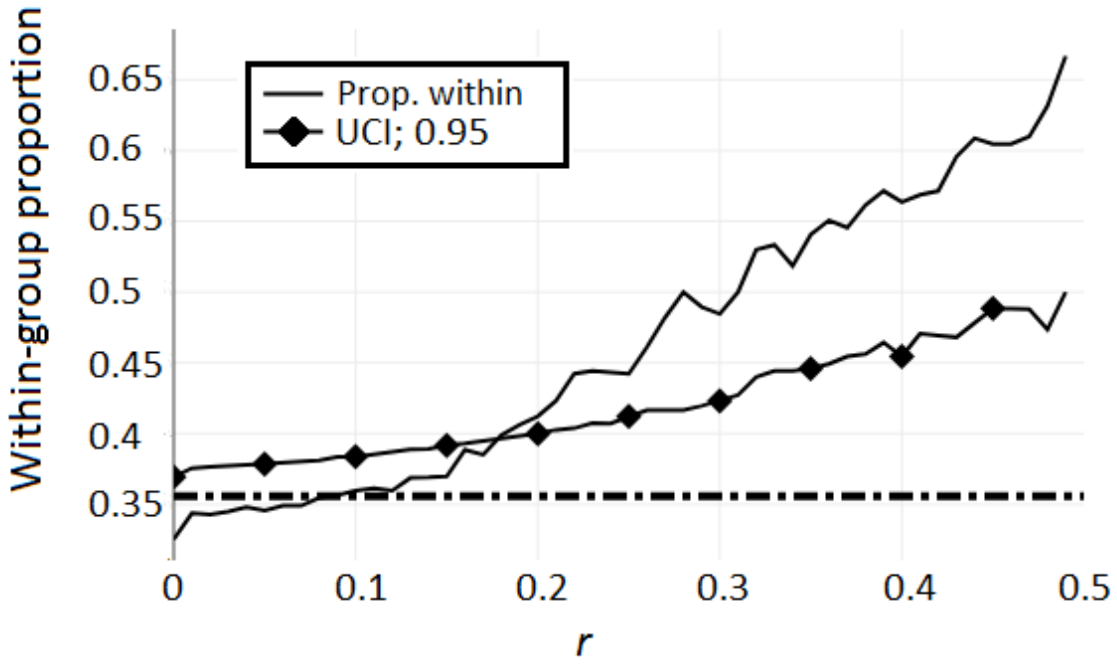
Fig. 4 Roost use and genetic proximity of giant female noctules in Maria Luisa Park, Seville, Spain. Genetic relatedness and roost use similarity were calculated for 25 radio-tracked bats roosting within the park. Estimates were centered and scaled across the colony. **A**) Density of standardized roost-use similarity (solid line) and genetic relatedness values (lozenge-line). Vertical dashed line indicates threshold used for association factor in C. **B**) Roost use similarity against genetic distances in standard deviations from the mean. **C**) Boxplot of genetic relatedness of pairs of individuals presenting a roost use Z score below versus above one.


A.
I.
II.
III.
PC 2: 0.07
PC 1: 0.08
6
4
2
0
-2
-4
-4
-2
0
2
4
6

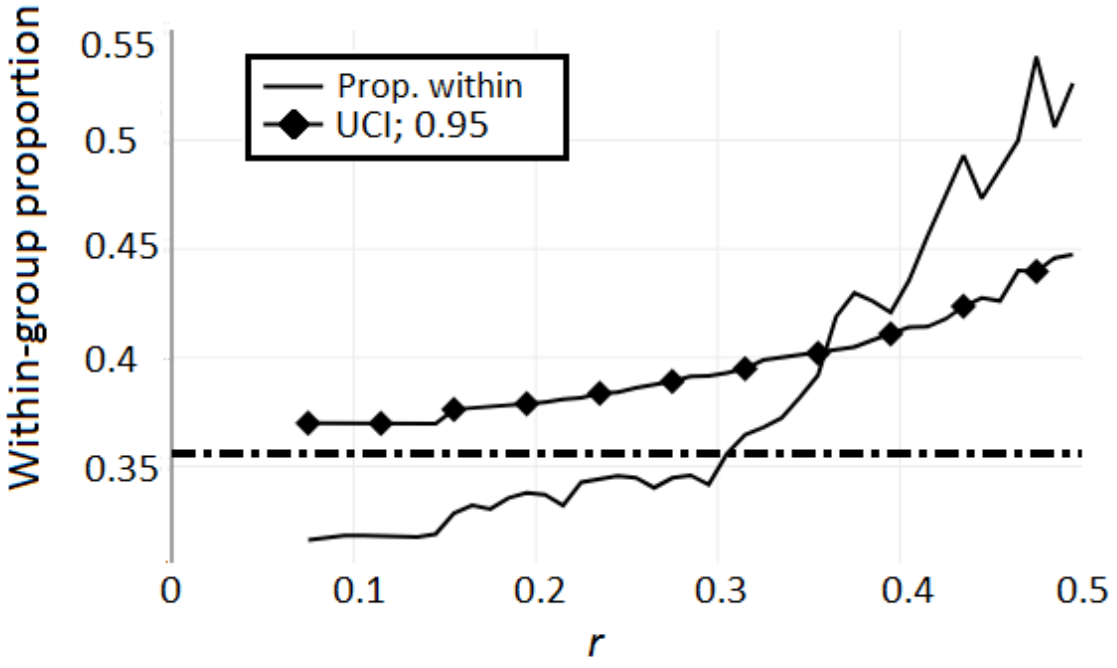

B.
**
p = 0.091
p = 0.78
p = 0.006
Relatedness
1
0.8
0.6
0.4
0.2
0
Seville
I.
II.
III
Groups

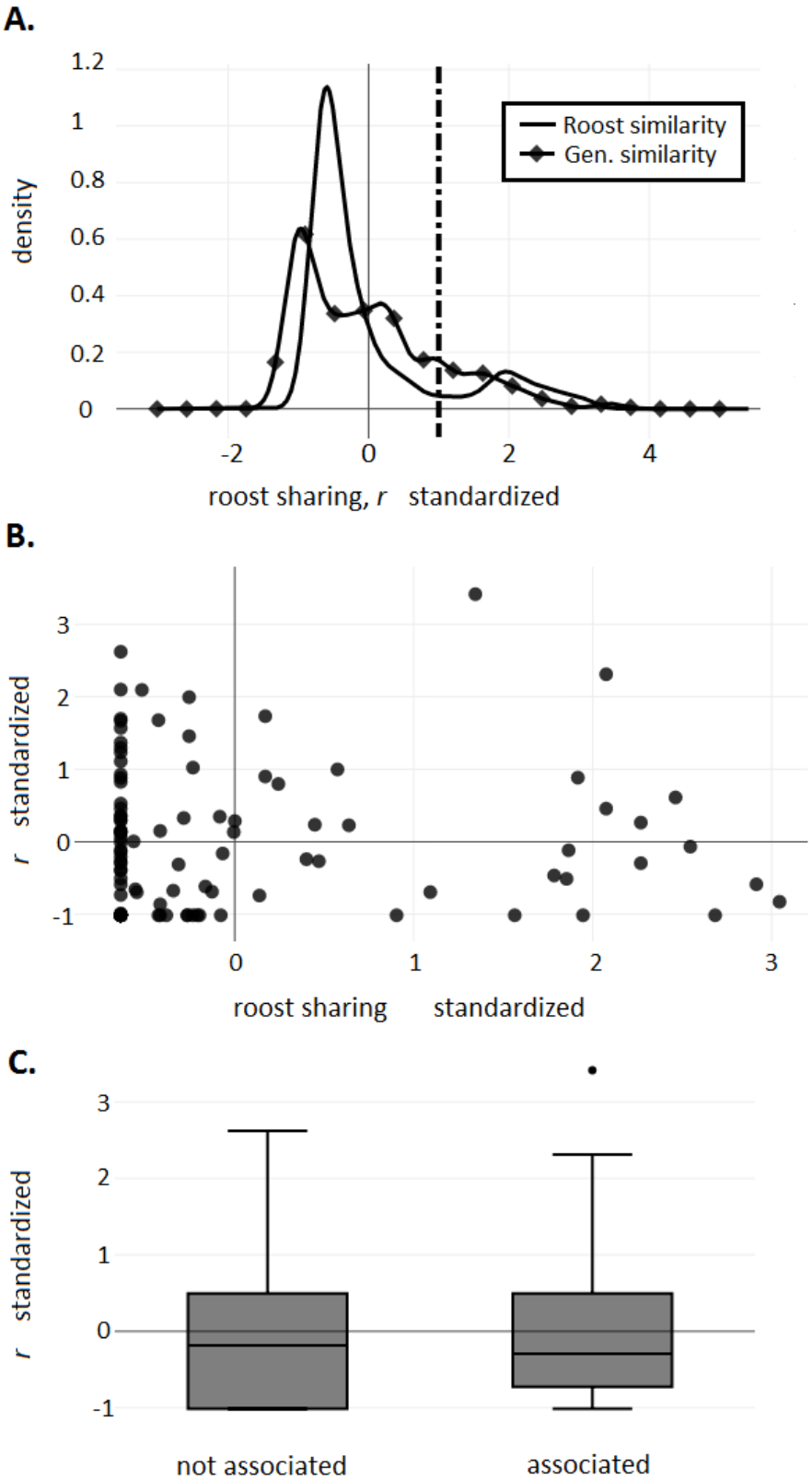

p < 0.001
p = 0.13
p = 0.44
p = 0.89
100
80
60
40
20
0
Parent Offspring
Full Siblings
Half Siblings
Unrelated

A.


Within-group proportion
0.65
0.6
0.55
0.5
0.45
0.4
0.35
Prop. within
UCI; 0.95
0
0.1
0.2
0.3
0.4
0.5
r

B.


Within-group proportion
0.55
0.5
0.45
0.4
0.35
Prop. within
UCI; 0.95
0
0.1
0.2
0.3
0.4
0.5
r


A.
1.2
1
0.8
0.6
0.4
0.2
0
density
Roost similarity
Gen. similarity
-2
0
2
4
roost sharing, r standardized
B.
3
2
1
0
-1
r standardized
0
1
2
3
roost sharing standardized
C.
3
2
1
0
-1
r standardized
not associated
associated