

Social organization and genetic structure: insights from codistributed bat populations

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Abstract

The impact of ecology and social organization on genetic structure at landscape spatial scales, where gene dynamics shape evolution as well as determine susceptibility to habitat fragmentation, is poorly understood. Attempts to assess these effects must take into account the potentially confounding effects of history. We used microsatellites to compare genetic structure in seven bat species with contrasting patterns of roosting ecology and social organization, all of which are codistributed in an ancient forest habitat that has been exceptionally buffered from radical habitat shifts. Over one thousand individuals were captured at foraging sites and genotyped at polymorphic microsatellite loci. Analyses of spatially explicit genotype data revealed interspecies differences in the extent of movement and gene flow and genetic structure across continuous intact forest. Highest positive genetic structure was observed in tree-roosting taxa that roost either alone or in small groups. By comparison, a complete absence of genetic autocorrelation was noted in the cave-roosting colonial species across the study area. Our results thus reveal measurable interspecies differences in the natural limits of gene flow in an unmodified habitat, which we attribute to contrasting roosting ecology and social organization. The consequences of ecology and behaviour for gene flow have important implications for conservation. In particular, tree-roosting species characterized by lower vagility and thus gene flow will be disproportionately impacted by landscape-scale forest clearance and habitat fragmentation, which are prevalent in the study region. Our method also highlights the usefulness of rapid sampling of foraging bats for assaying genetic structure, particularly where roosting sites are not always known.

Keywords: genetic autocorrelation, *Kerivoula*, microsatellites, monogamy, polygyny, *Rhinolophus*, roosting ecology

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Introduction

Numerous studies have considered the impacts of social structure and behaviour for genetic structure within social groups and vice versa (Sugg *et al.* 1996; Ross 2001). It is less clear how these factors routinely affect gene flow and population structure at broader land-

scape scales, where population and gene dynamics shape evolutionary processes as well as determine susceptibility to habitat fragmentation and stochastic events (Kraaijeveld-Smit *et al.* 2007; Saccheri *et al.* 1998). Attempts to assess the population consequences of social organization should take into account the potentially confounding contribution of historical processes. While behaviour largely determines microgeographical structure, especially within groups (Rossiter *et al.* 2005; Temple *et al.* 2006), broader patterns are

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often an artefact of past events, particularly in temperate regions where Pleistocene glaciations led to bottlenecks and/or rapid expansions (Hewitt 2004). At medium spatial scales, however, genetic structure may be a product of both past and current processes, with the former often obscuring the latter. Here populations affected by historical perturbations may have had insufficient time to reach drift-flow equilibrium, which depends on the generation time and population size (Nichols & Beaumont 1996).

To date, relatively few population genetic studies of vertebrates have been undertaken in a context appropriate for discriminating between contemporary gene flow and historical vicariance (Bossart & Prowell 1998). Even where these conditions might have been met (e.g. Sumner *et al.* 2001), it can be difficult to compare results from different populations, especially where they occupy separate geographical space and are under unique combinations of different environmental or biological factors. Indeed, most comparative approaches of population genetic structure have either synthesized multiple data sets, often from different areas (Duminil *et al.* 2010), or have examined codistributed taxa across broad scales (Castoe *et al.* 2007; Chen *et al.* 2010). Very few empirical studies have compared historically and spatially matched populations at smaller scales, to evaluate the population genetic consequences of ecology and life-history traits (Kraaijeveld-Smit *et al.* 2007; Rossetto *et al.* 2009).

To test for interspecies variation in genetic structure and gene flow at fine to medium spatial scales, we studied codistributed populations of seven species of bat: three woolly bats (genus *Kerivoula*, family Vespertilionidae) and four horseshoe bats (genus *Rhinolophus*, family Rhinolophidae). These taxa collectively exhibit a

range of roosting ecologies and group structures (see references in Table 1), and all can be readily captured in the same traps while foraging. We focused on populations that have almost certainly experienced the same climatic and historical processes, and which share an undisturbed ancient lowland rainforest. Palaeo-vegetation reconstructions show this forest has existed continually for at least 8000 years (Adams 1997) and probably predates the Last Glacial Maximum (Cannon *et al.* 2009). This would suggest the populations have remained undisturbed for thousands of generations and, given the spatial scale under consideration (<100 dispersal distances), are plausibly near to, or at, drift-flow equilibrium (see Barton 1992).

Studies of mammals indicate that males typically distribute themselves according to the presence of females, whereas female dispersion reflects the patchiness of resources, and the importance of sociality (Clutton-Brock 1989; Arnaud *et al.* 2012). Where resources are limited and/or sociality is adaptive, females form groups, and where these are defendable by males, polygynous mating will dominate (e.g. Le Boeuf 1974; McCracken & Wilkinson 2000). Conversely, where females do not form groups, then monogamous breeding systems can develop (Komers & Brotherton 1997; McCracken & Wilkinson 2000). Both such scenarios may be mediated by female choice, now recognized as common in mammals (Rossiter *et al.* 2005; Clutton-Brock & McAuliffe 2009). These breeding and social dynamics have repercussions for natal dispersal (Sinclair 1992; Clutton-Brock & Lukas 2012). In polygynous taxa, males tend to disperse more, to avoid inbreeding and/or competition, while females are more philopatric (Greenwood 1980; McCracken & Wilkinson 2000), although exceptions are not uncommon (e.g.

Table 1 Predictive framework for seven focal bat species sorted by roosting resource

Species	Body mass g (n)	Observations			Predictions		
		Roost _{1,3-10}	Social group _{1,3-10}	Local dispersion _{1,10,11}	Roost philopatry	Vagility	Genetic structure (fine to landscape)
<i>R. stheno</i>	9.0 (642) ₁	Cave	Large	Large stable hotspot	Strong	High	Weak structure
<i>R. lepidus</i>	6.5 (628) ₁						
<i>R. trifoliatus</i>	14.0 (436) ₁	Foliage	Singleton	Even/random	Weak	Low	Medium structure
<i>K. pellucida</i>	4.5 (564) ₁						
<i>K. lenis</i>	7.0 (53) ₂	Tree cavity	Small	Small mobile hotspot	Medium	Low	Strong structure
<i>K. papillosa</i>	9.0 (138) ₂						
<i>R. sedulus</i>	9.0 (119) ₁						

1, Kingston *et al.* (2006); 2, This study; 3, Csorba *et al.* (2002); 4, Hendrichsen *et al.* (2001); 5, Lekagul & McNeely (1988); 6, Medway (1983); 7, Payne & Francis (1998); 8, Fletcher (2006); 9, Heller *et al.* (1993); 10, Kingston (2000); 11, Struebig (2002).

Dechmann *et al.* 2007; Nagy *et al.* 2007). Increased variance in male reproductive success in polygyny reduces the effective population size, potentially increasing structure at local scales (Dobson *et al.* 2004). On the other hand, in monogamous systems, all-sex dispersal is probably more widespread because of equal competition for resources and mates (Dobson 1982; Dobson & Jones 1985), though males may disperse over shorter distances because mate competition between fathers and sons is less intense (Anderson 1989).

Bats exhibit an exceptionally wide range of roosting ecologies, breeding systems and social structures (McCracken & Wilkinson 2000; Kerth 2008). Our focal taxa can be broadly classified as either cave-roosting or tree-roosting, the latter of which can be divided into taxa that roost in the cavities of living and/or dead trees and those that roost in foliage. Each of the three woolly bat species form small mixed-sex colonies (~15 individuals), either in small tree cavities (*K. lenis*, *K. papillosa*) or in clusters of leaves (*K. pellucida*). Among the horseshoe bats, *R. trivoliatus* roosts singly under foliage and *R. sedulus* roosts singly or in pairs usually in hollows located in fallen trees, whereas both *R. lepidus* and *R. stheno* form large colonies (>1000 individuals) in caves (see Table 1). Variation in group size and associated social structure in the focal species is therefore probably related to roost availability (see Kunz & Lumsden 2003). All seven taxa are nonmigratory, though recapture and telemetry data suggest the cave-roosting colonial bats are more vagile than the tree-roosting species (see Table 1), possibly reflecting the uneven distribution of caves and need to avoid intra-specific competition (e.g. Struebig *et al.* 2009).

In this study, we compared patterns of spatial genetic autocorrelation among foraging bats across the seven focal species. We predicted that the two focal cave-roosting species would show low genetic structure at the spatial scales examined because of their greater ability to disperse and forage over larger home ranges. Conversely, we predicted that tree-cavity roosting species would show stronger spatial genetic structure because of short natal dispersal distances and small home ranges. Finally, owing to its lack of sociality and low roost philopatry, *R. trivoliatus* was expected to show weaker structure than other tree-roosting species yet more structure than its cave-roosting congeners because of its lower vagility (Table 1).

Methods

Study site and species

Fieldwork was undertaken in Krau Wildlife Reserve (KWR) in Pahang state, central Peninsular Malaysia

(3°40'N, 102°10'E) between 2000 and 2007. KWR covers approximately 62 000 ha of primary lowland Dipterocarp forest and supports the greatest diversity of insectivorous bats recorded for a single site anywhere in the Old World (Kingston *et al.* 2006). The seven focal species (*Kerivoula lenis*, *K. papillosa*, *K. pellucida*, *Rhinolophus lepidus*, *R. stheno*, *R. sedulus* and *R. trivoliatus*) all belong to an assemblage of forest-interior specialists (narrow-space foragers) that show ecomorphological adaptations for foraging on insects in clutter (see Arita & Fenton 1997; Kingston *et al.* 2003). Detailed dietary composition was not known.

Sampling design and construction of the Geographical Information System

All bats were captured using up to 10 four-bank harp traps (Francis 1989) set along trails within five permanent 1-km² study grids (S01–S05), two temporary sampling stations (S06 and S07), and along paths linking two grids (see Fig. 1). All sampling was conducted within a continuous and relatively homogenous tract of forest. Grids were intended as replicates and were thus free of major water bodies, edges or other landscape features that could impede dispersal and gene flow. Traps were set at least 50 m apart and were moved to a new position each night until all trails had been surveyed. As none of the bat species show seasonal movements, sampling was not set by season except to avoid the rainy period from late October to January. Grids were sampled based on a sequential rotation with time on each grid (from 4 weeks to 3 months) dependent on weather conditions.

For each permanent study grid, an unreferenced map was created with trap positions mapped using a tape measure and compass and correcting for slopes with a clinometer. To integrate the sites into a single Geographical Information System (GIS), raw distances and bearings were entered into the Coordinate Geography (COGO) package within ARC/INFO v 8.1, allowing the creation of arc and point coverages of the trap positions and distributing measurement errors over the data. Each unreferenced point-coverage was imported into ARCVIEW (3.2) and, for each trap, *x* and *y* coordinates generated. A single accurate position reading (<5 m) from each grid was then taken with a hand-held Global Positioning System (GPS) and used to geo-reference each point-coverage and integrate the five study grids into a UTM projection. Outputted *x y* coordinates were then used to generate intertrap distances within grids. Intertrap distances within grids ranged from 50 to ~1300 m providing fine-scale spatial resolution, while distances across the whole study area ranged up to 30 000 m providing landscape-level resolution. For the two temporary

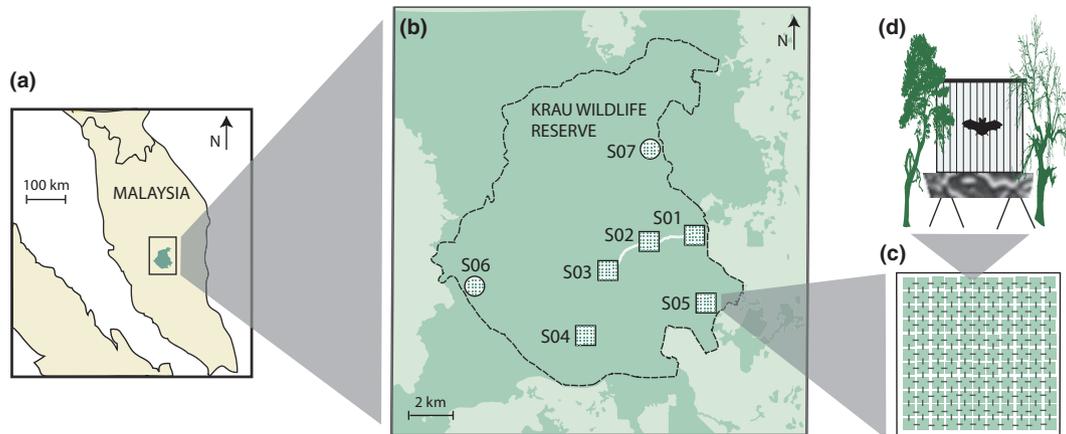


Fig. 1 Map showing (a) location of the Krau Wildlife Research within Malaysia and (b) the position of five permanent 1-km² study grids (S01–S05, squares), two temporary stations (S06 and S07, circles) and two trails used for sampling bats in this study. (c) Each study grid contains ten parallel trails running in each direction. (d) Harp traps (up to 14) were positioned along trails each day and moved daily to a new position until all trails were surveyed.

sampling stations and interconnecting trails, harp trap positions were measured directly with a hand-held GPS (accuracy of <5 m) and integrated into the GIS.

From this GIS, we also estimated the average recapture distance for each species from individual records obtained from long-term field data (including unsampled bats). For *K. pellucida*, recapture distances were based on an earlier study and so based on fewer data. Recapture distance for *K. papillosa* and *K. lenis* was based on subsets of individuals taken from our long-term database, which were confidently assigned to species retrospectively on the basis of body mass and length of forearm.

Bat capture and genetic analysis

Bats were captured at their feeding grounds during periods of peak foraging activity, either up to two hours after dusk or just before dawn. Unlike most studies of bats, no individuals were captured at their roosts, which were not known. All individuals were identified to species, sexed, aged (adult or juvenile), their body mass and length of forearm recorded, and fitted with a uniquely numbered wing band. Individuals of *Kerivoula pellucida* were not banded during this study because observations from an earlier study (Kingston 2000) indicated this taxon to be more prone to injuries from banding. For females, we also recorded reproductive condition (pregnant, lactating, postlactating or nonreproductive). All study species were easily identified with the exception of *K. papillosa* and *K. lenis*, which are morphologically similar. Although *K. papillosa* is on average larger than *K. lenis*, there is considerable overlap and therefore we used genetic methods to classify individuals into species retrospectively.

For all bats, prior to release, a 3-mm-diameter biopsy was also taken from each wing membrane with a dermatological punch (Stiefel Laboratories, Wooburn Green, UK) and transferred to 90% ethanol for storage at -20°C . Duplicate sets of samples were archived in Malaysia and the UK, and from the latter, DNA was isolated using the Promega Wizard[®] SV 96 Purification System (Promega, Southampton, UK) and used to amplify polymorphic microsatellite markers.

Rhinolophus species were amplified using a panel of 23 primers based on both *R. ferrumequinum* (Rossiter et al. 1999; Dawson et al. 2004) and *R. hipposideros* (Puechmaile et al. 2005; Struebig et al. 2011). *Kerivoula papillosa* were amplified with primers based on *Kerivoula papillosa* (Struebig et al. 2008a). Details of all markers used for each taxon are listed in Table S1 (Supporting information). Primers were labelled with 5'-fluorescein bases (HEX, FAM or TAMRA), and polymerase chain reaction (PCR) was carried out with 15 μL of the reaction mixture containing 10–50 ng DNA, primer concentrations of 0.667 μM , 0.33 mM of each dNTP, 2.0 mM MgCl_2 and 0.5 U AmpliTaq Gold[®] Taq polymerase in the manufacturer's buffer (PE Applied Biosystems, Warrington, UK). PCRs were performed on a DNA Engine Tetrad[®] (MJ Research, Waltham, MA, USA) using the following profile: 94 $^{\circ}\text{C}$ for 2 min; 34 cycles of 94 $^{\circ}\text{C}$ for 30 s, annealing temperature for 30 s, 72 $^{\circ}\text{C}$ for 30 s; 72 $^{\circ}\text{C}$ for 10 min. PCR products were run on an ABI Prism 3700 DNA Analyzer, and microsatellite alleles were sized using GENOTYPER 3.6 NT software (PE Applied Biosystems). To minimize genotyping error, all alleles were scored by one author (SJR). Genotyping was semi-automated: bin-sets were established to aid initial scoring, but all reads were individually inspected for each species-locus combination. All weak

reads were re-run to check for consistent scores, and particular effort was made to identify potential null alleles. Any individuals showing inconsistencies between runs were omitted from the analyses.

Genetic variation and taxonomic designation

For each species, we calculated the number and size range of allele per locus in the program SPaGeDi (Hardy & Vekemans 2002). For all seven species, estimates of observed and expected heterozygosity were calculated in GENALEX (Peakall & Smouse 2005), and tests for deviations from Hardy–Weinberg equilibrium were conducted in FSTAT, using a subset of individuals from one grid to avoid potential Wahlund effects (sample sizes are given in Table S1, Supporting information).

In the case of two morphologically similar species, *Kerivoula papillosa* and *K. lenis*, before obtaining descriptive statistics, it was first necessary to classify individuals into species. For this purpose, we used the Bayesian clustering method in STRUCTURE 2.3.3 (Pritchard *et al.* 2000) in which genotypes are sorted into a predefined number of separate clusters (K) to minimize deviation from Hardy–Weinberg equilibrium without reference to prior population information. We undertook ten replicate runs for values of $K = 2$ – 5 , in each case using a burn-in of 400 000 runs and 600 000 MCMC runs. We applied an ancestry model of no admixture and assumed uncorrelated allele frequencies between the two species. We used CLUMPP to ensure replicate runs yielded similar estimates of Q for individuals (cluster membership) (Jakobsson & Rosenberg 2007) and we displayed the results with DISTRUCT (Rosenberg 2004).

Genetic autocorrelation analyses

To characterize and compare species patterns of gene flow over the study area, we undertook multivariate spatial autocorrelation analyses of microsatellite genotypes following Smouse & Peakall (1999). First, we tested for autocorrelation at a microgeographical scale (henceforth termed 'fine-scale'), in which we calculated the global autocorrelation coefficient (r) among pairs of individuals at overlapping distance classes of increasing size from 0–100 to 0–1300 m. Fine-scale analyses focused on pairwise comparisons of bats sampled mainly within study grids. Upper and lower distances were considered to bracket the typical natal dispersal limits of the least vagile species, with 1300 m corresponding to the maximum intragrid distance (corner to corner). To reduce the potential effects of roost switching behaviour (see Discussion) on our estimates of fine-scale structure within grids, wherever possible we

selected animals that were captured within a single trapping session. For 'landscape-scale' analyses, we calculated r among bats sampled at five overlapping distance classes of increasing size, from 0–5000 to 0–25 000 m. These comparisons incorporate individuals captured in different grids and stations.

For each distance class, we assessed the significance of each value of r by comparing it with the 95% confidence limits of a null distribution of 999 permuted r -values (r_p), each one calculated by shuffling bats among the capture points within the given distance class. Positive autocorrelation was inferred if r exceeded the upper 95% limit of r_p (one-tailed test). As an alternative test of significance, we used bootstrapping to generate 95% error bars around observed r -values and examined whether the lower error bar was >0 .

Overlapping distance classes provide information on the decay in genetic autocorrelation with distance. We also repeated fine-scale and landscape-scale analyses using nonoverlapping distance classes. Hence, in the former case, r was calculated among individuals sampled 0–100 m apart, at increasing distances to 1200–1300 m apart, and for the latter case, r was calculated for classes ranging 0–5000 to 20 000–25 000 m. The use of nonoverlapping classes is more sensitive to oscillations in autocorrelation resulting from small sample sizes at some distances; however, this more common approach allows comparisons with some other studies.

To explore genetic structure at even finer spatial scales, we undertook two-dimensional analyses in which we calculated the local autocorrelation coefficient (rl) between each individual bat and its five nearest neighbours ($n = 5$ pairwise values; e.g. Double *et al.* 2005). This was repeated for each species for each permanent grid only, treating each grid as a replicate. Statistical significance was assessed by comparing rl with a null distribution of 999 permuted values (rl_p), each one generated by comparing the focal bat to its five nearest neighbours after random shuffling of bats among capture points within the given grid. Where positive autocorrelation was inferred, based on rl exceeding the upper 95% limit of rl_p (one-tailed test), we examined the age and sex of all six animals in the 'neighbourhood'. Finally, to explore patterns of genetic autocorrelation among individuals, we calculated rl for increasing numbers of nearest neighbours (5–17 bats). Step-like decreases in rl provided information on the number of related individuals. It is important to note that adjacent neighbourhoods will often contain some of the same animals and thus should not be considered independent from each other; however, these plots are useful for exploring substructure. All autocorrelation analyses were undertaken in GENALEX (Peakall & Smouse 2005).

Results

We captured bats at five permanent study grids, two temporary sampling areas and two interconnecting trails in the Krau Wildlife Reserve (Fig. 1). From seven focal species, we collected wing biopsies and genotyped seven species of bat (combined total = 1024 individuals at between 7 and 17 loci each; see Table 2). Bayesian clustering of multilocus genotypes at $K = 2$ was able to

Table 2 Samples sizes and number of loci scored for seven focal species

Species	Sample size	Loci used
<i>Kerivoula papillosa</i>	222 (125 ♂, 97 ♀)	13
<i>Kerivoula pellucida</i>	166 (88 ♂, 78 ♀)	11
<i>Kerivoula lenis</i>	76 (45 ♂, 31 ♀)	12
<i>Rhinolophus sedulus</i>	88 (51 ♂, 37 ♀)	11
<i>Rhinolophus stheno</i>	166 (68 ♂, 98 ♀)	15
<i>Rhinolophus trifoliatus</i>	152 (89 ♂, 63 ♀)	7
<i>Rhinolophus lepidus</i>	154 (57 ♂, 97 ♀)	11

unambiguously separate *Kerivoula papillosa* and *K. lenis*, in spite of their overlapping size range. Both taxa showed a degree of sexual size dimorphism, with females tending to be larger than males (Fig. S1, Supporting information). We repeated clustering for higher values of K and with models of admixture; however, no meaningful substructure was detected (data not shown). Following genotyping, analyses of F_{is} showed that between one and three loci deviated from Hardy–Weinberg per species, and therefore, these markers were excluded from all subsequent analyses. The final number of loci scored was seven to 15 per species (mean = 11.4). See Table 2 for a summary of the markers used and Table S1 (Supporting information) for full details including Hardy–Weinberg tests.

Estimated dispersal based on individual recapture records gathered as part of the longer-term study of bats at this site revealed clear interspecies differences. The shortest recapture distances were observed in the tree-roosting species *K. pellucida* and the largest in cave-roosting *R. lepidus*. The only focal species ever

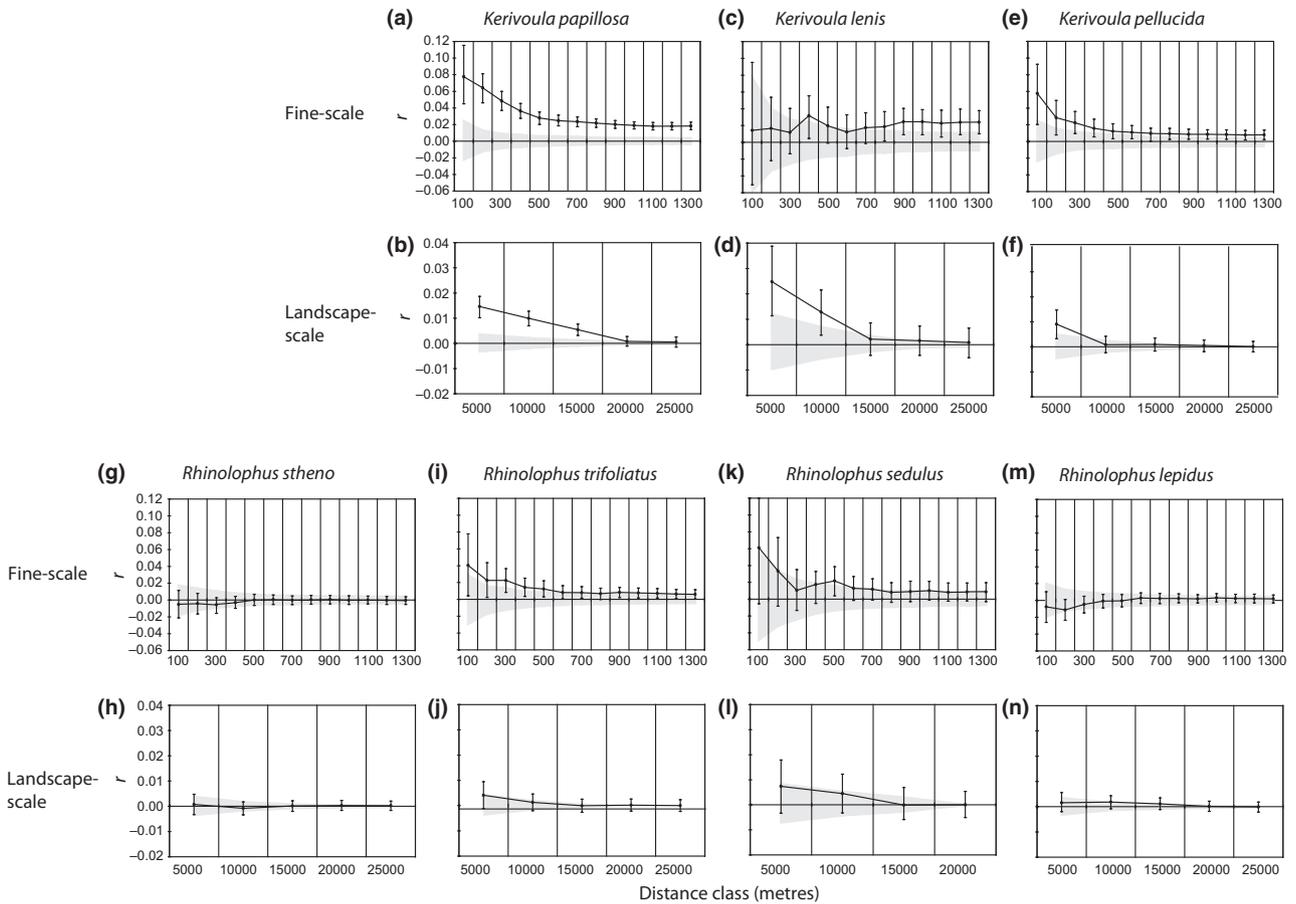


Fig. 2 Correlograms of r combined over sites for increasing distance classes, undertaken for seven species. For each species, the analysis is repeated for both fine-scale (0–1300 m) and landscape-scale (0–25 000 m) distances. The 95% confidence envelope around the null hypothesis is shaded grey, and the 95% intervals around r_c values are shown as bars.

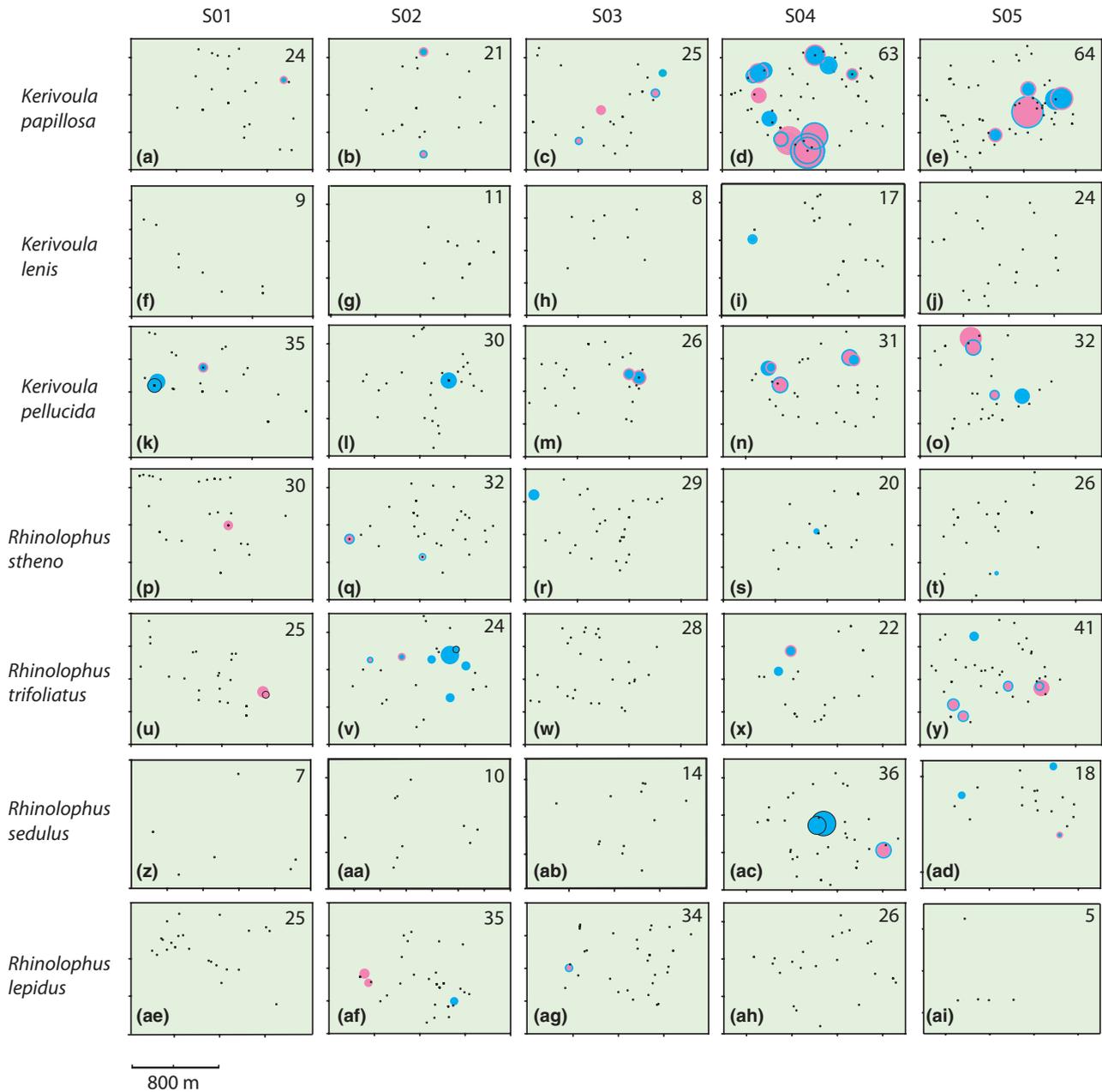


Fig. 3 Two-dimensional local spatial genetic autocorrelation analyses of each species across each study grid. Points represent individual capture positions, and bubbles depict significant local genetic autocorrelation based on a focal individual and its five nearest neighbours. Bubble radii are proportional to the magnitude of the autocorrelation coefficient (rI). The edge of each bubble is blue if the focal animal is male and pink if it is female, and the shading represents the sex of the majority (≥ 3) of the neighbours (blue = males, pink = females).

recaptured between grids was *R. stheno*, for which seven such cases were recorded (T. Kingston, unpublished data).

Multivariate spatial autocorrelation

Correlograms of genetic autocorrelation based on increasing distance classes revealed clear positive struc-

ture in four species that roost in trees: *Kerivoula papillosa*, *K. pellucida*, *Rhinolophus trifoliatus* and *R. sedulus*. Of the woolly bats, *K. papillosa* showed the highest positive structure, which was significant over all fine-scale distances, and up to around 20 000 m at the landscape scale (Fig. 2a, b). Results from *K. lenis* were erratic at very short distances because of low sample sizes, though positive structure was evident between around

700 and 15 000 m (Fig. 2c, d). In the smallest studied member of the genus, *K. pellucida*, positive structure persisted until 10 000 m (Fig. 2e, f). Among the horseshoe bats, positive structure was highest in *R. sedulus*, where it occurred over most distances and up to around 10 000 m (Fig. 2k, l). *R. trivoliatus* showed a similar pattern, albeit at fractionally lower coefficient values (Fig. 2i, j). In contrast, *R. lepidus* and *R. stheno*, both of which roost in large colonies, showed no evidence of positive structure at all distances (Fig. 2g, h, m, n).

Correlograms based on nonoverlapping distance classes also supported interspecies variation (Fig. S2, Supporting information). In *K. papillosa*, *K. pellucida*, *R. trivoliatus* and *R. sedulus*, positive genetic autocorrelation was significant (based on both tests—see Methods) for the first 100 m; however, while in *K. papillosa*, it remained significant until around 400 m, in the latter three taxa structure diminished after just 200 m (see Fig. S2a, e, i, k, Supporting information, respectively). This distance at which the genetic autocorrelation coefficient intersects the y -axis at $r = 0$ is sometimes referred to as the so-called genetic patch size (see Peakall *et al.* 2003). The remaining three species (*K. lenis*, *R. lepidus* and *R. stheno*) all showed no fine-scale positive structure (Fig. S2c, g, m, Supporting information, respectively), although in the former case, widely varying coefficients with large errors reflected the small sample sizes at many of the distance classes, thus casting doubt on the usefulness of this analysis for this comparatively rare species. At the landscape scale, species-specific trends remained and were more obvious. Clear positive structure was observed for the first 5000 m in all three *Kerivoula* species (Fig. S2b, d, f, Supporting information), as well as in *R. trivoliatus* and *R. sedulus* (Fig. S2j, l, Supporting information, respectively), though in the latter case, this was only based on observed coefficient falling outside the null envelope. No positive structure was seen at 10 000 m in any species except *K. papillosa*, in which it also disappeared at 15 000 m.

Genetic autocorrelation within neighbourhoods

Within permanent study grids, the position of hotspots of positive genetic autocorrelation within groups of neighbouring bats (each comprising six animals) provided additional insights into the spatial genetic consequences of roosting ecology and social structure. In *Kerivoula papillosa*, we detected strong positive structure in multiple neighbourhoods in the two grids S04 and S05 (Fig. 3d, e, respectively). The clustering and overlapping of these neighbourhoods suggest that the number of related animals exceeds six. Several cases of more moderate genetic structure were also observed in

K. pellucida (Fig. 3k–o), *Rhinolophus trivoliatus* (Fig. 3v, x, y) and *R. sedulus* (Fig. 3a–c), again with some evidence of neighbourhoods themselves being spatially correlated. Each of the remaining species showed only a handful of neighbourhoods with weak, albeit significant genetic autocorrelation.

In total, we recorded 71 significant ($P < 0.05$) 'six-bat-neighbourhoods', of which the vast majority (92%) comprised individuals of both sexes. Of significant neighbourhoods with high coefficients ($rl > 0.10$; $n = 33$), the majority ($n = 28$; 87.5%) contained at least some pregnant, lactating and/or postlactating females, indicating that these bats belong to maternity colonies. Six neighbourhoods were exclusively male, three in *K. pellucida*, two in *R. trivoliatus* and one in *R. lepidus*, and of these, four had rl values > 0.10 , suggesting that they were kin. Mean genetic autocorrelation (using a cut-off of $P < 0.1$) based on neighbourhood sizes of six bats showed a broadly negative relationship with recapture distance (Fig. 4), although this correlation was not testable because of small sample size.

Plots of genetic autocorrelation estimated for a wider range of neighbourhood sizes (Fig. 5) were broadly concordant with the findings of our two-dimensional analyses of neighbourhoods of six bats, and provided further information on genetic structure at very fine scales. *K. papillosa* exhibited the strongest genetic autocorrelation, especially in grids S04 and S05 (Fig. 5d, e), though it was evident in all grids and, on close inspection, appeared to undergo stepwise drop in strength at about 11 individuals (around the observed size of the roosting group). Data were too few to test in *K. lenis*; however, similar stepwise trends from high starting

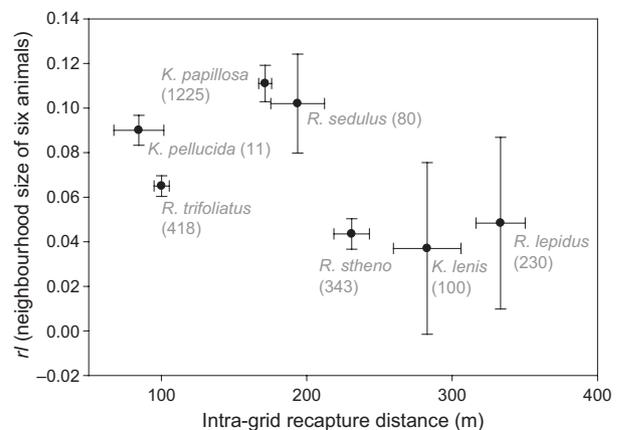


Fig. 4 Recapture distance (metres) versus local spatial genetic autocorrelation (rl) based on neighbourhood sizes of six bats, averaged across all five study grids. Data represent means with standard errors. Recapture distances are based on long-term banding data.

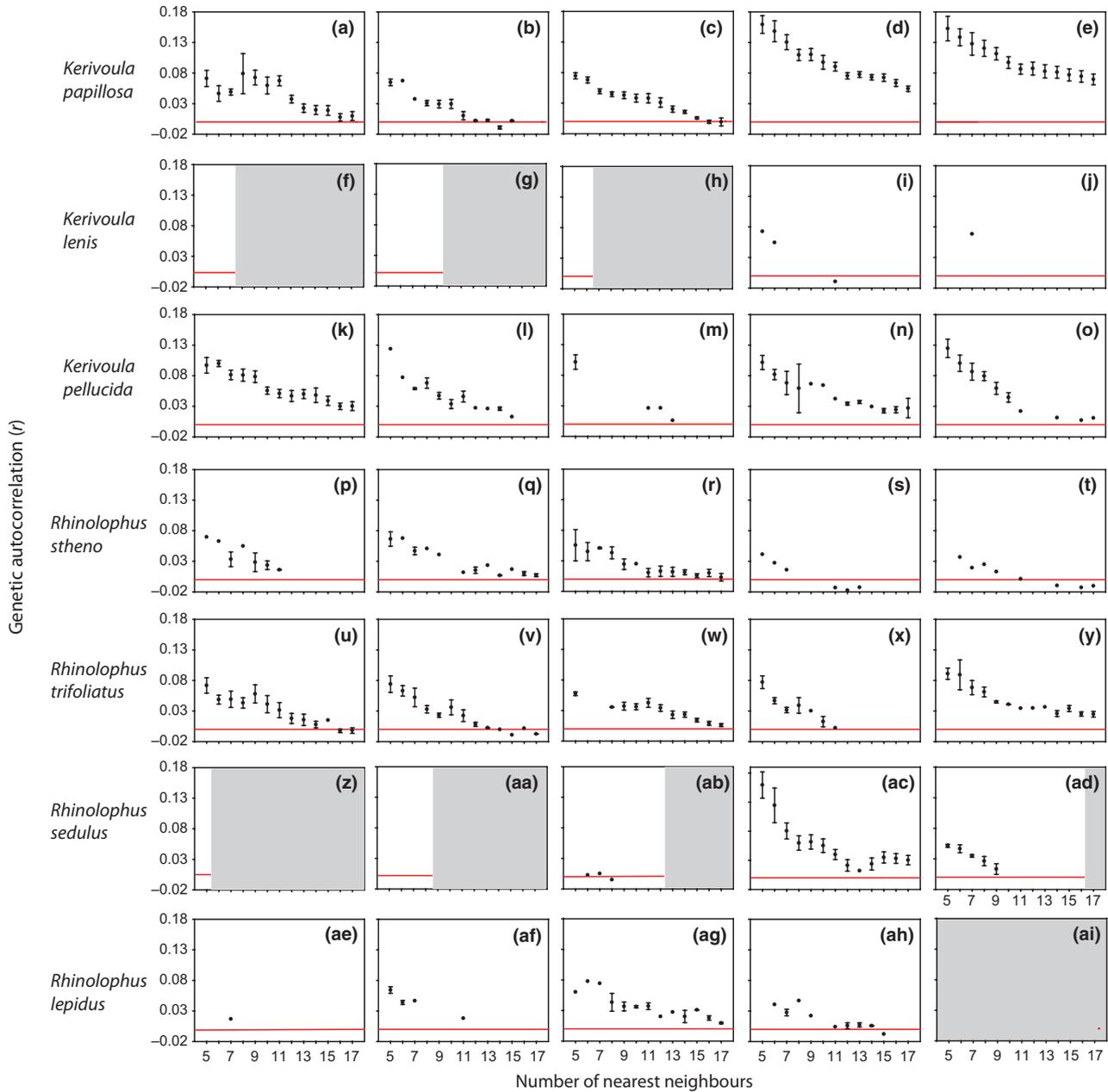


Fig. 5 Mean local genetic autocorrelation (rI) among neighbourhoods of increasing size (5–17 bats) shown for each species in each study grid. Means are based on significant coefficients only, and bars shown are standard errors. In each plot, the line at zero (depicting no autocorrelation) is shown. Shaded areas span neighbourhoods for which the significance of rI values was not testable (at $P < 0.05$) because of small sample sizes preventing the generation of meaningful null distributions by random shuffling of bats.

values were broadly observed in *K. pellucida* at about ten individuals. Among the horseshoe bats, *R. sedulus* was characterized by the strongest genetic autocorrelation, which appeared to decrease in a stepwise manner at around three and 12 bats in grid S04 (Fig. 5ac). *R. trifoliatus* also showed elevated though weaker genetic autocorrelation among small groups of bats (up to around 11 individuals; Fig. 5u–y). Consistent with our other results, estimates of genetic autocorrelation

were lowest in the cave-roosting species *R. lepidus* (Fig. 5p–t) and *R. stheno* (Fig. 5ae–ai), although some stepwise declines were also observed.

Discussion

Investigating the consequences of species traits for population genetic structure can shed valuable light on the interplay between behavioural, ecological and evolu-

tionary processes. However, these impacts can be easily obscured by the genetic legacies of older events. By comparing codistributed bat species in an ancient pristine habitat, so controlling for large-scale historical effects, we provide evidence that behavioural and ecological traits are strong determinants of genetic structure at landscape scales, which will have additional consequences for responses to habitat change.

Species variation in genetic autocorrelation

In general, the approach adopted of sampling individual foraging bats whose roosting sites were not known proved an effective means of assaying population genetic structure that otherwise would not have been possible. We found that the magnitude and pattern of global genetic autocorrelation across all sampling sites differed widely among the seven bat species studied.

As predicted, interspecific variation in genetic structure appeared closely linked to roosting ecology and associated social organization. Fine-scale analyses recovered similar signatures of high positive structure in the congeneric species *Kerivoula papillosa* and *K. pellucida*, both of which are known to roost in small groups of around 15 animals. For the former taxon, long-term mark-recapture data indicated restricted dispersal of up to 200 m (see Table 1), a value similar to foraging distances obtained from radio-tracking in Malaysia (Allen 2005; Fletcher 2006) and Indonesia (S. Rossiter & T. Kingston, unpublished data). For *K. pellucida*, banding data revealed even shorter dispersal distances, though these are based on fewer long-term recaptures and so should be treated with some caution. In the case of *K. lenis*, too few individuals were sampled for meaningful estimation of genetic autocorrelation at fine spatial scales; however, recapture records point to slightly greater dispersal distances than for the other *Kerivoula* species.

In species that favour temporary roost structures, including foliage or cavities in small trees, genetic structure might be reduced by regular roost switching behaviour; for example, if roosts are lost or become sub-optimal because of tree falls or parasite infestations, respectively (see Reckardt & Kerth 2007). Radio-tracking data for *K. papillosa* showed that males switched roost every 3–12 days and females every 2–10 days, though in both cases, this was nearly always to a roost on the study grid (Fletcher 2006). Given the fact that bats were not sampled at roosts, we aimed to minimize the potentially confounding effects of temporal heterogeneity by estimating spatial genetic structure from individuals captured within short time periods. However, this was not always possible and thus our snapshots of fine-scale

spatial genetic structure could be considered as conservative estimates, because they will incorporate animals sampled from pre- and postswitching events.

Fine-scale trends also held at the landscape scale, with all three *Kerivoula* species displaying broadly concordant trends characterized by strong positive structure at 5000 m that subsequently declined to negative structure within the bounds of the sampling area. Yet beyond these similarities, species differences were also evident, most notably in the rate at which genetic autocorrelation decreased. *K. pellucida* exhibited the most rapid decline, followed by *K. lenis* and then *K. papillosa* (Fig. 4b, d, f). While this order mirrors these species' respective body size (see Table 1), a parameter that is frequently seen to scale with home range due to resource requirements (e.g. Lindstedt *et al.* 1986; Haskell *et al.* 2002), it could be interpreted as contradicting expectations from the recapture data, which indicated *K. lenis* to be the most vagile of the three. This discrepancy could arise if distances to foraging sites exceed those over which gene flow routinely occurs or could reflect interspecific differences in population densities that are unknown but would influence neighbourhood size and so the scale at which genetic substructure occurs. Alternatively, vagility/natal dispersal inferred from mark-recapture data will be under-estimated if the distances travelled commonly exceed the dimensions of the study grid. Regardless of the reasons underlying uncertainty in the mark-recapture results, our genetic analyses suggest that despite close phylogenetic relatedness (see Khan *et al.* 2010), different species appear to vary subtly in the degree of gene flow across natural undisturbed habitat.

Wider variation in genetic autocorrelation was observed among the horseshoe bats, also following our predictions based on roosting habits and social organization. In *Rhinolophus sedulus*, autocorrelation trends across overlapping fine-scale and landscape-scale distance classes were similar to those of the woolly bats (*Kerivoula spp.*), to which they show ecological and social parallels such as small group sizes, the use of tree hollows for roosts and short foraging distances (<150 m, T. Kingston, unpublished data). In *R. tringifolius*, marked spatial genetic autocorrelation fits with expectations that gene flow and dispersal will be restricted in a solitary and putatively monogamous species for which roosts are not limited. Radio-tracking data show these animals forage over a small distance (<150 m; Kingston *et al.* 2006). Overall, patch sizes recorded for the tree-roosting bat species were similar to those previously reported for the terrestrial Australian bush rat *Rattus fuscipes* (Peakall *et al.* 2003) but smaller than those inferred for a medium-sized terrestrial carnivore, the Eurasian badger *Meles meles* (Pope *et al.* 2006).

Both horseshoe bat species that roost in large colonies in caves and rock/boulder outcrops off the grids showed remarkably similar genetic signatures, with almost no genetic autocorrelation among foraging individuals based on global tests and only limited evidence among nearest neighbours. In other words, on the whole, bats sampled near to each other tended to be no more related than if they were captured 25 000 m apart. Such a finding is perhaps to be expected for species with large home ranges and that forage some distance from the roost. It is noteworthy that seven individuals of *R. stheno* have been recaptured between grids over the duration of the longer-term trapping at Krau, which represent dispersal distances of 7.5–11.5 km (T. Kingston, unpublished data). For these taxa, therefore, average recapture distances from within grids will almost certainly hold little value as proxies for dispersal or vagility, as they represent repeated captures of the same individuals during commuting or foraging bouts, potentially far from their roost. Indeed, in the temperate cave-roosting horseshoe congeneric bat *R. ferrumequinum*, females are philopatric and can forage several kilometres from the roost, whereas gene flow among colonies is probably mainly mediated by mating events in which females visit solitary territorial males that frequently occupy smaller satellite caves (Rossiter *et al.* 2000).

Neighbourhood analyses

Results from local genetic autocorrelation among neighbouring bats further highlighted a link between social behaviour and population genetic structure. At the same time, however, intraspecific heterogeneity was also evident between replicates, probably partially a consequence of the greater noise associated with the smaller samples.

In tree-roosting species from both genera, we found evidence that multiple individuals captured in close proximity are likely to be related, a fact that is suggested but not implicit from the roosting ecology *per se*. As most bats—including the focal species—produce single offspring, elevated autocorrelation among groups of adult bats points to long-term natal area philopatry of one or both sexes coupled with polygynous breeding. The degree and sex-biased nature of natal philopatry in tropical bat species seems to vary considerably (e.g. Kerth 2008) and, as yet, is not known for *Kerivoula*. However, social group size and composition in this genus is consistent with some form of resource defence polygyny (such as harem defence) that has been described in several tropical bats (McCracken & Wilkinson 2000; Heckel & von Helversen 2002; Ortega *et al.* 2003). Genetic autocorrelation among neighbouring

R. sedulus was less obvious and was only really evident from one grid, where it declined rapidly. Again this broadly accords with field observations that have revealed *R. sedulus* occasionally roosts alone or in pairs (Heller *et al.* 1993), and also by a radio-tracking study that found five of seven individuals roosted in pairs, and two were solitary (Fletcher 2006).

Low yet measurable genetic autocorrelation among neighbours in the solitary foliage roosting *R. trilineatus* is best explained by limited dispersal from the natal area. Waser & Jones (1983) showed that natal area philopatry is more widespread than sociality in mammals and suggested that solitary taxa will therefore also be characterized by the spatial clustering of relatives, a condition more commonly associated with group living. To date, few studies have examined genetic structure and gene flow in solitary species; however, mounting results from a range of taxa, including primates (Kappeler *et al.* 2002), carnivores (Croteau *et al.* 2010) and rodents (Maher 2009), appear to suggest that philopatry does result in elevated relatedness among neighbours.

Lowest autocorrelation among neighbouring animals was observed in the two cave-roosting horseshoe bats consistently across grids, supporting the findings of a previous study that revealed low average relatedness within a horseshoe bat colony (Rossiter *et al.* 2002, 2005). Yet despite this overall trend, our analyses indicated the presence of a handful of putative relatives within grids in both species. Given the low chance of related animals occurring together by random, these cases may well be related bats commuting and/or foraging together. Other drivers of kin-biased foraging such as joint territorial defence (e.g. Brown & Brown 1995) are less likely in this system. Kin-biased foraging has been reported in a range of taxa, and an association between the relatedness of individuals and the extent to which they share foraging grounds has been documented in several bats, including the congeneric greater horseshoe bat (*R. ferrumequinum*; Rossiter *et al.* 2002).

Relating interspecies variation in genetic autocorrelation to ecological and behavioural differences relies on the assumption that all taxa are at a similar stage of drift-flow equilibrium and also that the different sets of loci examined do not vary markedly in their rates of mutation. The former condition cannot be proven, but is suggested by the inferred long history of forest in the Sundaland (Cannon *et al.* 2009) and the fact that much more recent anthropogenic perturbations have not occurred within the bounds of the forest under study (Struebig *et al.* 2008b). It is equally important to note that the greatest contrasts among taxa were more apparent over shorter distances, indicating that these probably reflect contemporary processes rather than variation in the extent of equilibrium. To mitigate the

potential effects of variation in the mutation rate of different loci, it would have been desirable to have applied a common set of markers across taxa. In reality, however, such an approach is impractical given that more 'universal' markers tend to have low polymorphism and so are unsuitable for the sorts of analyses conducted here. Although potential differences in mutation rates among loci precluded comparison of genetic diversity, these should have lower impact on signatures of autocorrelation for which error was estimated. Moreover, we detected contrasting structure among the four *Rhinolophus* spp. for which an overlapping set of markers was used. For these reasons, we can be reasonably confident that observed patterns will reflect, for the most part, species differences rather than the properties of the loci.

Implications for conservation

Genetic structure and gene flow are well known to have consequences for the viability of populations and species (see Traill *et al.* 2010). The observed associations between ecological and behavioural traits in forest-interior bats, and the spatial scale over which gene flow and drift occurs in undisturbed habitats, have important conservation implications. Our results, obtained from rapidly sampling foraging animals, can be viewed as a baseline or null level of genetic autocorrelation, against which the effects of historic processes, habitat change or even landscape features can be assessed. Anthropogenic modification of forests, including clearance, fragmentation and conversion to plantations, all operate at a landscape scale and are especially rapid in Southeast Asia (Sodhi *et al.* 2004), including the study region. Krau Wildlife Reserve was formerly part of a much larger contiguous forest block, although in recent decades, surrounding forest has been cleared for agriculture.

Numerous studies have predicted that species with large home ranges will be more sensitive to habitat fragmentation because of reductions in resource density (e.g. Haskell *et al.* 2002). However, such arguments tend not to account for ecological or behavioural specializations that might influence the ability of individuals to move between fragments. In a recent study of forest specialist birds, Callens *et al.* (2011) attributed the combination of low population structure and little dispersal to a relative loss of mobility caused by recent fragmentation. Similarly, many forest-interior bat species are considered to be highly vulnerable to habitat loss, and we predict that small species that roost in trees either alone or in groups are likely to be more susceptible to landscape-scale modification than those that form larger colonies

in caves (Struebig *et al.* 2008b). Indeed, low inferred vagility of some species within continuous forests could indicate that gene flow will be disproportionately disrupted by cleared areas in a fragmented landscape. This prediction is supported by our previous results which showed that tree-roosting taxa drop out of bat assemblages in smaller fragments (Struebig *et al.* 2008b) and also showed concordant reductions in allelic diversity (Struebig *et al.* 2011). In contrast, cave-roosting species rely on resources that are naturally rarer and less evenly distributed, such as karst outcrops, and thus may be better adapted for traversing greater distances to forage independent of body size. At the same time, however, cave-roosting species face other threats, from the loss of roosts to mining and tourism (Struebig *et al.* 2009; Kingston 2010). Ecological correlates of vulnerability have been identified in neotropical bats (Meyer *et al.* 2008) and the consequences of fragmentation on population genetic structure are clearly linked to dispersal ability (Meyer *et al.* 2009). We are currently in the process of comparing genetic structure across matching populations of these taxa in the surrounding denuded landscape to test these predictions from continuous populations in Krau Wildlife Reserve.

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S.J.R. and E.J.P. are interested in gene flow and genetic structure with a particular focus on bats; A.Z. and A.M. have backgrounds in the ecology and genetics of bats and other species; T.H.K. has wide interests in bat biology and S.G. applies spa-

tial analyses to address problems in biology. Research by T.K. and M.J.S. focuses on community ecology and conservation of forest bat species in Southeast Asia.

Data accessibility

Microsatellite data for the studied species: Dryad entry doi:10.5061/dryad.m61t20h4.

Supporting information

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

Table S1 Summary of markers used for genotyping for (a) four *Rhinolophus* species and (b) three *Kerivoula* species.

Fig. S1 Separation of *Kerivoula lenis* and *K. papillosa* based on Bayesian clustering of multi-locus genotypes for $K = 2$.

Fig. S2 Genetic autocorrelation combined over sites and loci for increasing distance classes undertaken for seven species.

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