

## Assortative roosting in the two phonic types of *Pipistrellus pipistrellus* during the mating season

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## SUMMARY

The European pipistrelle, *Pipistrellus pipistrellus* consists of two phonic types that echolocate with frequencies of maximum energy (*FMAXE*) averaging 45 kHz and 55 kHz. These two phonic types occur in sympatry over much of Britain. Between 1993 and 1995 mating groups of *P. pipistrellus* were located in bat boxes between August and October, the main mating period for these bats. Mating groups comprised a single male and up to three females. Broad-band, time-expanded recordings of search-phase echolocation calls were made from bats immediately after release. The average *FMAXE* of each bat was calculated: bats with an *FMAXE* < 49 kHz were classed as 45 kHz phonic types and bats with an *FMAXE* > 52 kHz were classed as 55 kHz phonic types. Sixteen 45 kHz groups and ten 55 kHz groups were located. No mating group ever contained more than one phonic type, although both types were known to be present at several of the study sites. Males emitted calls of higher frequency than females for both phonic types. The study suggests that the two phonic types are reproductively isolated and supports the hypothesis that *P. pipistrellus* should be considered as two cryptic sibling species.

## INTRODUCTION

The pipistrelle bat *Pipistrellus pipistrellus* (Schreber 1774) is widespread throughout Europe (Stebbings 1988) and the most common bat in Britain (Harris *et al* 1995). *P. pipistrellus* has traditionally been considered to be one species, but Jones & Parijs (1993) found that their search-phase echolocation calls fall into two discrete categories,

with average maximum energy frequencies (*FMAXE*) at 45 kHz and 55 kHz. Maternity roosts were found to contain only one of the phonic types and differences in flight morphology, roost size and general appearance were noted (Jones & Parijs 1993). In continental Europe some areas appear to have only one of the phonic types, but in many areas both occur (Jones & Parijs 1993). The phonic types are sympatric over much of Britain and it has been suggested that *P. pipistrellus* is two cryptic species (Jones & Parijs 1993). Sequencing of the mitochondrial cytochrome *b* gene indicates two distinct clades with a sequence divergence of approximately 12% (Barratt *et al.* 1995). The two clades correspond unambiguously to the two phonic types (E.M. Barratt pers. com.).

*P. pipistrellus* employs a mating strategy of resource defence polygyny (Gerell & Lundberg 1985). Males set up territories at a roosting site (including artificial 'bat boxes') at the beginning of summer and are joined by up to ten females after they leave the nursery colonies (Gerell & Lundberg 1985; K.J.Park *et al.*, in prep.). Males defend territories against other adult males and are thought to advertise the presence of their day roost to females by performing a song-flight display at night. The male flies along a fixed route within his territory emitting low-frequency vocalisations (Gerell-Lundberg & Gerell 1994; Lundberg & Gerell 1986). Song-flight calls of the two phonic types differ in frequency, and in the number of components (Barlow & Jones, 1997).

If the two phonic types are separate species, reproductive isolation should occur. For the purpose of this study we have used the term mating group to describe roosting groups of reproductively active males and adult females in the same bat box during the mating season. We have looked at the composition of mating groups occupying artificial roost

boxes at four localities in the U.K. to see if the criterion of reproductive isolation is fulfilled.

## METHODS

The bat box sites are located mainly within the North Yorkshire Moors National Park (NYM), with additional sites in Dorset and Lancashire, on land managed by the Forestry Commission, and one site was in Surrey (see table 1). Between 1993 and 1995 mating groups were located by inspecting the bat boxes between August and October, the main mating period for these bats (Gerell & Lundberg 1985). In the NYM bats have been ringed since 1985, and the phonic types of bats in some mating groups, documented before 1993 were identified retrospectively. Only mating groups in which the phonic types of all the bats were known have been analysed.

All bats were ringed (excluding three bats in the Surrey mating group), identified, sexed, aged (immature or adult) and reproductive status was assessed. Visible cartilaginous epiphyseal plates in finger bones of bats under a few months old allowed young bats to be distinguished from adults (Racey 1988). Males of most small vespertilionid bats achieve sexual maturity in their first full season of food abundance following birth but in some species females may become receptive to males in their first autumn (Racey 1988). In this study it was assumed that all young of the year in the post-parturition mating season were sexually immature. Immature males were never found in the mating groups.

Bats were released from the hand several metres from the recording system. Recordings were made using a Pettersson D-980 ultrasound detector, in 10x time-expansion mode, and replayed to a Sony WM-D6C cassette recorder. One mating group (containing one male and two females) was recorded by K. E. Barlow using an Ultra Sound Advice S-25 detector to a Portable Ultrasound Signal Processor and replayed to a Sony WM-D6C cassette recorder. Jones & Parijs (1993) investigated the two systems used here, and found there was no systematic variation in the *FMAXE* of the calls in relation to the equipment used.

Recordings were analysed using a Kay DSP 5500 digital Sona-graph. Only search-phase echolocation calls were used for analysis (Jones & Parijs 1993). These calls have steep frequency-modulated (FM) sweeps with a constant-frequency (CF) tail. *FMAXE* was determined from power spectra with a resolution of 400 Hz. *FMAXE* was always located in the CF tail of the call. The *FMAXE* reported here for each bat represents a mean of between two and 31 calls analysed per bat. Some bats were recorded more than once but only the *FMAXE* from the first occasion was used, and variation between recordings was minimal.

A 2 x 2 contingency table of male and female phonic type within a mating group was compiled. Bats < 49 kHz were classified as 45 kHz phonic types and bats > 52 kHz were classified as 55 kHz phonic types (Jones & Parijs 1993). A single bat with an *FMAXE* averaging 50.11 kHz was classed as indeterminate type and left out of the table (see discussion). This bat probably flew close to clutter (background substrate such as vegetation, echoes from which might interfere with echoes of interest to the bat

(Neuweiler 1989)) and emitted broad-band calls with no evidence of a CF tail, resulting in considerable variability of *FMAXE*. To avoid pseudoreplication each male was entered into the contingency table only once with a randomly selected female he had been found with. The numbers of 45 kHz phonic type males found with 45 kHz phonic type females and the number of 45 kHz phonic type males found with 55 kHz phonic type females was entered. This was repeated for the 55 kHz phonic type males. The Fisher Exact test was used to test the probability of finding the resulting distribution by chance.

The number of females in the mating groups of each phonic type was compared. One mating group was picked at random for males that had been found in the presence of adult females more than once. To determine whether 55 kHz phonic type males roosted with 55 kHz phonic type females assortatively by frequency within phonic type, the *FMAXE* of males was correlated with the *FMAXE* of a randomly selected female he had been found with. Insufficient data were available for a similar analysis to be performed on 45 kHz bats. Analysis was carried out on MINITAB release 10 for Microsoft Windows (Ryan *et al.* 1985) and Sigmastat (Jandel Scientific).

## RESULTS

A total of 26 mating groups were found and 48 individual bats recorded (15 males and 33 females). Of these, four males and six females were captured in mating groups between two and six times, all other bats being captured just once. No bat recorded more than once was found to have changed its phonic type. Fig. 1 shows the distribution

of frequencies for the 46 bats for which exact *FMAXE* values were known (poor recordings of two bats prevented accurate determination of *FMAXE*). The distribution was clearly bimodal. The distribution of frequencies were approximately normal around 45.8 ( $\pm 1.78$ ) kHz and 54.6 ( $\pm 1.32$ ) kHz (means  $\pm$  standard deviations).

Fig 1 near here

None of the mating groups found contained bats of more than one phonic type. Of the 26 groups found, 16 were of the 45 kHz phonic type and 10 of the 55 kHz type. Using the contingency table described in methods, five 45 kHz males have been found in mating groups with females that were always of the 45 kHz phonic type and nine 55 kHz males have been found in mating groups with females that were always of the 55 kHz phonic type (Fisher Exact test,  $p < 0.001$ ).

Fig. 2 shows the *FMAXE* of each male against the *FMAXE* of each female he was found with. On some occasions the same male and female were found together more than once. The interval between finding the same male and female together varied from one week to two years. On all occasions where a male was found with the same female more than once within a season, the numbers of females, and the identity of some, in the mating group had changed. In both phonic types males had significantly higher frequency calls than females (mean *FMAXE* 45 kHz phonic type males =  $46.97 \pm 1.04$  kHz ( $n = 5$ ), females =  $45.10 \pm 1.39$  kHz ( $n = 16$ ),  $W = 82.5$ ,  $p = 0.026$ , Mann-Whitney; mean *FMAXE* 55 kHz phonic type males =  $56.16 \pm 0.93$  kHz ( $n = 7$ ), females =  $54.05 \pm 1.17$  kHz ( $n = 17$ ),  $W = 138.5$ ,  $p = 0.001$ ). Available data on forearm lengths of males

and females of both phonic types indicate that males are significantly smaller than females (K.E. Barlow, unpubl. data).

Fig. 2 near here

All mating groups found contained one male and between one and three females. There was an average of 1.20 ( $\pm 0.45$ ,  $n = 5$ ) females in 45 kHz phonic type mating groups and 1.78 ( $\pm 0.67$ ,  $n = 9$ ) in 55 kHz phonic type mating groups (means  $\pm$  standard deviations). There was no difference in the size of mating group between the phonic types ( $W = 26.5$ , n.s.).

Within the 55 kHz phonic type there was no correlation between the *FMAXE* of a male and a randomly selected female he had been found with (Pearson Product Moment Correlation,  $r = 0.278$ ,  $n = 7$ , n.s.).

Table 1 shows the location and group composition of each mating group found with the average *FMAXE* of each bat. The two phonic types were sympatric at many of these sites. Eighteen mating groups were found across seven sites in the NYM. Both phonic types of *P. pipistrellus* have been found in bat boxes and recorded at three of these sites though the 45 kHz phonic type predominates. On one occasion a male and female of the 45 kHz phonic type were found copulating in a bat box in the NYM. Activity transects carried out over the study period at several locations in the NYM found both phonic types present feeding (KJP & JDA, unpubl. data). Both phonic types have been found in the bat boxes in Lancashire though the three mating groups reported here are of the 55



kHz phonic type. Only bats of the 55 kHz phonic type were found in the boxes on the single visit to Dorset (four groups) and Surrey (one group) though maternity roosts of the 45 kHz phonic type have been found within 4 km of the site in Surrey (K.E. Barlow, pers. com.).

Table 1 near here
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## DISCUSSION

This study shows that assortative roosting between the two phonic types of *P. pipistrellus* occurs during the mating season. Although it was not possible to confirm that all these were mating groups (a male and female were found copulating in a bat box on one occasion) evidence from other studies (e.g. Gerell & Lundberg 1985) supports this assumption. This provides evidence of assortative mating between the two phonic types. The phonic types are sympatric across many of the sites where mating groups were located and this supports the proposal by Jones & van Parijs (1993) that *P. pipistrellus* is two cryptic species.

Only 5 % of bats recorded from maternity roosts by Jones & Parijs (1993) produced echolocation calls with a *FMAXE* of between 49 - 52 kHz and none of the bats whose social calls were recorded by Barlow & Jones (1997) fell within this range. Only one of the 48 bats recorded in this study was found to be intermediate in *FMAXE* (table 1). The echolocation calls of this bat were not representative of typical search-phase calls as the recordings were made before the bat flew in open space. When flying in clutter, *P.*

*pipistrellus* echolocation calls become broad-band, and lose their CF tail, which contains most of the signal's energy (Kalko & Schnitzler 1993). The bat with an *FMAXE* of 50.11 kHz is therefore not a bat of intermediate phonic type, but one that failed to emit search-phase calls of a suitable type for categorisation to phonic type. This bat was closer in frequency to a 45 kHz phonic type than a 55 kHz phonic type and genetic analysis has shown it to belong to the clade corresponding to the 45 kHz phonic type (E.M. Barratt pers. com.). The bat was a male found with two 45 kHz phonic type females.

*P. pipistrellus* mating groups contain one adult male and between one to ten females (Gerell & Lundberg 1985; Gerell-Lundberg & Gerell 1994; K.J.Park *et al.*, in prep.). The bats from Gerell & Lundberg's studies in Sweden are presumably of the 55 kHz phonic type (Ahlén 1981; Jones & Parijs 1993) and mating groups of comparable size are found in Britain. In this study there was no difference in the size of mating group between the two phonic types.

For both phonic types, males emitted echolocation calls of higher frequency than did females, and males are the smaller sex (K.E. Barlow, unpubl. data). *P. pipistrellus* therefore resembles *Hipposideros speoris* where males also emit calls of higher frequency than do females, though *H. speoris* does not show sexual size dimorphism (Jones *et al.* 1994). In other bat species, females emit higher frequency calls than males though there is no general rule that relates sexual dimorphism in call frequency to sexual size dimorphism (Jones 1995).

The lack of a positive correlation between echolocation call frequency of males and females within the same mating group for the 55 kHz phonic type suggests that assortative mating according to call frequency within phonic types does not occur, though the sample size here is too small to be able to draw any firm conclusions from this.

There is no experimental evidence as yet to support the assertion that the purpose of the males' song-flight call is to attract females, though circumstantial evidence suggests that such a function is likely (Gerell & Lundberg 1985; Gerell-Lundberg & Gerell 1994). If this is the case, females should be able to distinguish between phonic type on the basis of the major differences between their songflight calls found by Barlow & Jones (1997) and this would provide a mechanism for the assortative associations we have found.

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## FIGURE LEGENDS

Figure 1. Distribution of *FMAXE* of echolocation calls of 46 *P. pipistrellus* found in mating groups in the North York Moors National Park (25 bats), Lancashire (5 bats), Dorset (13 bats) and Surrey (3 bats). *FMAXE* is divided into 1 kHz bands and the number of bats with a mean *FMAXE* within each band is shown. For example, the 45 kHz band contains bats with a mean *FMAXE* of between 44.50 kHz and 45.49 kHz. Between 2 and 31 calls were recorded per bat and a mean value of *FMAXE* calculated. Poor recordings of two bats prevented accurate determination of *FMAXE* and these bats are not included.

Figure 2. The *FMAXE* of males plotted against the *FMAXE* of females they have been found with (open symbols = 45 kHz phonic type / closed symbols = 55 kHz phonic type). Each male is represented by a different symbol. Numbers in parentheses indicate the number of times the male was found with the same female. Note that males have calls of a higher frequency than females. A line of equality has been included to illustrate this.

## TABLES

Table 1. The location and group composition of the mating groups with the average *FMAXE* of each bat. <sup>1</sup> est. denotes those bats for whom an accurate *FMAXE* was not obtained but were assigned to a phonic type from heterodyned recordings, where the CF tail of the sound made a ‘slapping’ sound (Ahlén 1990). In the NYM, individuals found

in mating groups more than once have been assigned identities of M1 - M4 (male) and F1 - F6 (female).

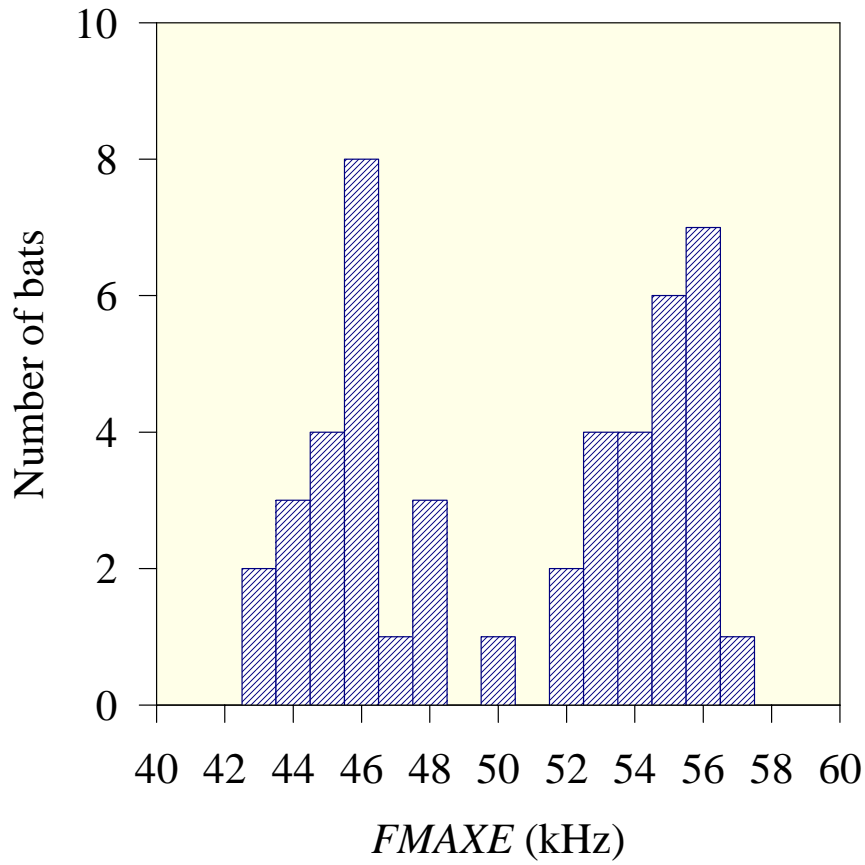
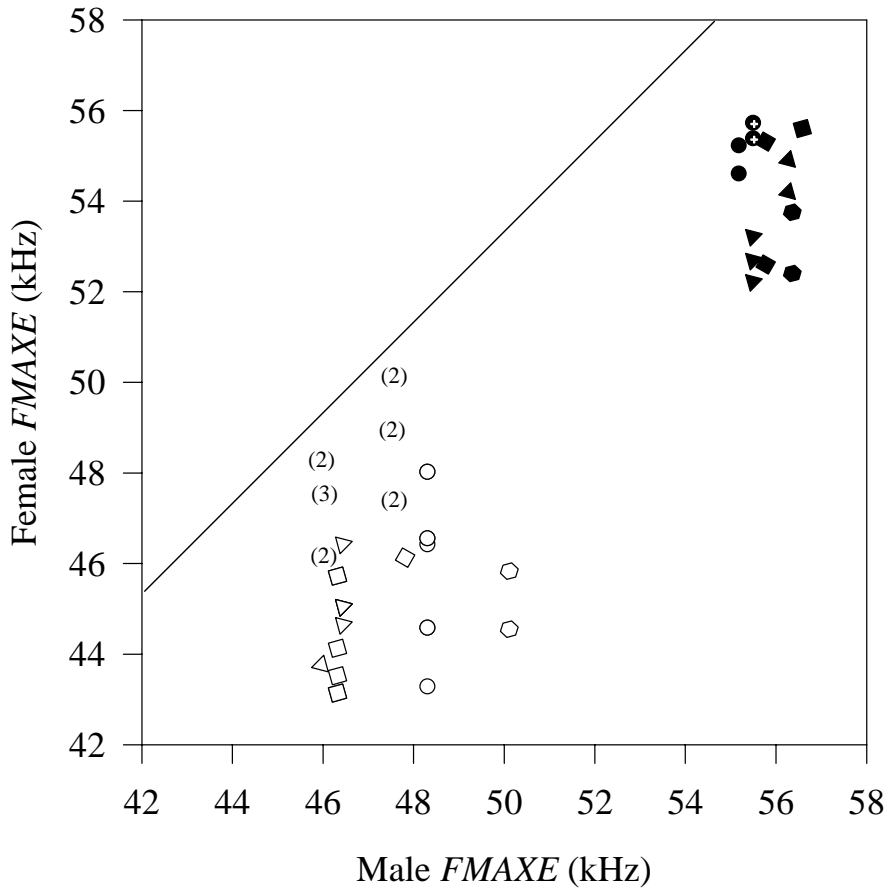


Figure 1





location (grid ref.)	date	sex	identity	<i>F</i> MAXE (kHz)	location (grid ref.)	date	sex	identity	<i>F</i> MAXE (kHz)
North York Moors: SE774007	14/09/88	m	M1	48.33 ± 1.22 (6)	SE896886	16/09/93	m		47.81 ± 1.33 (25)
		f		43.26 ± 0.71 (7)			f		46.13 ± 1.29 (15)
	19/10/89	m	M1	48.33 ± 1.22 (6)	SE930877	23/09/93	m		50.11 ± 1.07 (14)
		f	F1	44.56 ± 0.46 (5)			f		44.55 ± 0.74 (11)
	19/09/91	m	M1	48.33 ± 1.22 (6)	SE856849	23/09/93	m	M4	56.27 ± 0.72 (15)
		f	F1	44.56 ± 0.46 (5)			f		54.19 ± 2.04 (28)
		f	F2	48.00 ± 1.13 (2)			m	M4	56.27 ± 0.72 (15)
	09/09/93	m	M1	48.33 ± 1.22 (6)		23/08/95	m		54.90 ± 0.51 (8)
		f	F2	48.00 ± 1.13 (2)			f		
	30/09/93	m	M1	48.33 ± 1.22 (6)	Lancashire: SD510625	15/09/95	m		56.58 ± 1.33 (9)
f			46.40 ± 0.40 (5)			f		55.60 ± 1.39 (7)	
f		F3	46.53 ± 1.51 (6)		15/09/95	m		53 ± 1.00 est. <sup>1</sup>	
07/10/93	m	M1	48.33 ± 1.22 (6)			f		53.43 ± 0.89 (7)	
	f	F3	46.53 ± 1.51 (6)			f		53.87 ± 0.23 (3)	
SE857866	17/10/91	m	M2	46.32 ± 1.44 (31)		15/09/95	m		55 ± 1.00 est. <sup>1</sup>
		f		44.13 ± 0.27 (18)			f		53.88 ± 0.58 (24)
	f		43.52 ± 1.04 (5)	Dorset: SY894933	18/09/95	m		56.36 ± 0.51 (9)	
	16/09/93	m	M2	46.32 ± 1.44 (31)			f		53.75 ± 0.83 (11)
	f	F4	43.14 ± 0.59 (7)		18/09/95	m		52.40 ± 0.60 (9)	
30/09/93	f	F5	45.72 ± 1.31 (24)			f		55.48 ± 0.60 (13)	
	m	M2	46.32 ± 1.44 (31)			f		52.69 ± 0.49 (15)	
	f	F5	45.72 ± 1.31 (24)			f		53.22 ± 0.34 (25)	
SE914893	12/08/93	m	M3	46.43 ± 0.97 (13)			f		52.22 ± 0.76 (13)
		f	F6	45.05 ± 1.84 (8)	SY870932	18/09/95	m		55.20 ± 0.57 (2)
	23/09/93	m	M3	46.43 ± 0.97 (13)			f		54.58 ± 0.57 (9)
		f	F6	45.05 ± 1.84 (8)			f		55.20 ± 0.75 (5)
30/09/93	f		44.65 ± 1.06 (16)	SY924917	18/09/95	m		55.77 ± 0.63 (24)	
	m	M3	46.43 ± 0.97 (13)			f		52.60 ± 0.57 (2)	
	f	F6	45.05 ± 1.84 (8)			f		55.31 ± 0.48 (11)	
SE840953	13/09/94	m	M3	46.43 ± 0.97 (13)	Surrey: SU895440	3/10/93	m		55.52 ± 0.33 (5)
		f		46.43 ± 0.83 (24)			f		55.70 ± 0.95 (8)
	09/09/93	m		45.96 ± 0.83 (11)			f		55.36 ± 0.43 (10)
		f		43.76 ± 0.66 (10)					

Table 1