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Can pipistrelles, *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825), foraging in a group, change parameters of their signals?

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Pipistrellus pipistrellus; *P. pygmaeus*; echolocation in the field and laboratory; monospecific and mixed-species groups.

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Abstract

Echolocation behaviour and the structure of calls of *Pipistrellus pygmaeus* and *Pipistrellus pipistrellus* were studied by using a time expansion bat detector. Echolocation signals were recorded in the field in south-eastern Moravia and northern Bohemia (Czech Republic) and in an *ad hoc* experimental laboratory. For each of the species, multivariate analysis of variance (MANOVA) indicated significant differences in calls produced inside the experimental room and in the open. Paired *t*-tests and MANOVA were also used to reveal influences of interindividual contacts in each of the cryptic species on the spectral patterns of call variables. Differences were found in the spectral variables of echolocation calls of an individual flying in the room alone and in a group of conspecifics. The possibility that bats use their flexibility to avoid mutual disturbances of echolocation calls was tested. We found that bats flying in a group modify the parameters of their echolocation signals according to the presence of other individuals of the same species. These differences can indicate jamming avoidance and recognition of own echoes. However, they did not change the parameters if individuals of another species were present. Social calls are more numerous when bats fly in a mixed-species group than in a monospecific group.

Introduction

Extensive screening of the distribution of *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*, performed within the last decade, demonstrated sympatric occurrence of the two cryptic species in most parts of Europe (Barratt *et al*., 1997; Mayer & von Helversen, 2001; Hulva *et al*., 2004). In the Czech Republic, the two species are sympatric in the lowlands of Moravia and central and southern Bohemia (Bartonička *et al*., 2002; Řehák *et al*., 2003). In the alluvia of big rivers, the two species usually use the same foraging sites. The syntopic occurrence of more species (e.g. *P. pipistrellus*, *P. pygmaeus*, *Pipistrellus nathusii*) can make species identification difficult (Jones *et al*., 1994). Individuals may change the parameters of their echolocation signals with respect to group foraging of their conspecifics (Obrist, 1995; Kazial, Burnett & Masters, 2001; Ratcliffe *et al*., 2004). Pipistrelles have a marked narrowband part of their echolocation calls and are able to avoid possible jamming, caused by the orientation pulses of their conspecifics, by shifting the frequency so that the echoes are not masked by calls or echoes from conspecifics (Miller & Degn, 1981). A more convincing and recent study was carried out by Ulanovsky *et al*. (2004). Bats also cease to identify targets when the interfering noise pressure approaches the peak echo sound pressure (Simmons *et al*., 1979).

Echolocation calls appear to be more conservative in their variability than social calls (Fenton, 1994). Nevertheless, they might contain some information on population membership or individual characteristics (Jones, 1995; Obrist, 1995). Variability of echolocation calls, found under natural or semi-natural conditions, can serve in interindividual communication within a foraging group or on occasional encounters with another bat (Barclay, 1982; Fenton, 1986). The great success in assigning echolocation calls (e.g. temporal parameters, asynchronous calling) to each bat (Habersetzer, 1981; Masters, Raver & Kazial, 1995) supports the idea that these calls are individually distinct, and suggests that the echolocation calls of a bat could communicate its individual identity and improve recognition of its own echoes. Body temperature (Huffman & Henson, 1993), geographical aspects (Law, Reinhold & Pennay, 2002), age (Jones & Ransome, 1993) and sex (Jones, Gordon & Nightingale, 1992) can all modify call frequency. These personal signatures must contain information on the sender's identity, and receivers must be able to detect those signatures as well as discriminate among them (Bohn *et al*., 2004).

However, shifts in echolocation calls can serve only to minimize jamming effects. These shifted conspecific signals present a good example of jamming avoidance behaviour (Schnitzler & Kalko, 2001). If modification of echolocation calls is connected only with jamming avoidance, it can

reduce the variability available to needful social information, for example recognition of members of the same colony whether they are related or not (Goodwin & Greenhall, 1961; Boughman, 1998; Wilkinson & Boughman, 1998). Social calls can potentially facilitate individual recognition. Interference with conspecifics was observed at high bat densities and often resulted in chases or other manifestations of aggression accompanied by social calls (Fenton, 1994). In pipistrelles foraging in a group, social calls were recorded more often than in other bat species (Lundberg & Gerell, 1986; Barlow & Jones, 1997). Theoretical studies suggest that individual signatures can be included in specific social calls rather than in echolocation calls (e.g. Ratcliffe *et al*., 2004). Several authors found that the number of social calls increases when conspecifics are present (e.g. agonistic interactions – Rydell, 1986; recruiting behaviour – Wilkinson & Boughman, 1998).

All available information supports the fact that recognition of own echoes is essential for bats. There is, however, no reason to presume that individual variation in echolocation calls is more important for 'a bat's identity' than integrated social calls. We hypothesize that parameters of echolocation calls and the number of social calls emitted are different among conspecifics and between the two cryptic species as well. Therefore, in our study, we focused on (1) comparing the echolocation calls of *P. pipistrellus* and *P. pygmaeus* flying in a room and in the field, (2) comparing the echolocation calls of an individual flying alone and in a group of conspecifics, (3) examining whether changes in the spectral parameters of echolocation calls of one species can be affected by the presence of its cryptic species and (4) examining whether the variability in spectral parameters is higher in a mixed-species flying group than in a monospecific group. We also tested the prediction that the increased number of social calls is due to the presence of conspecifics or of bats belonging to other species.

Materials and methods

Sound recordings

The changes in call structure of individual *P. pygmaeus* and *P. pipistrellus* foraging separately were studied in cluttered habitats in south-eastern Moravia and in northern Bohemia (Czech Republic) using a time expansion bat detector (D 980 Pettersson Elektronik, Uppsala, Sweden) and a Sony WM-D6C tape recorder (Sony, Tokyo, Japan). The study took place in 2002–2004, between April and mid-June, before the weaning of juvenile bats. Signals of *P. pygmaeus* and *P. pipistrellus* were also recorded under experimental laboratory conditions. The *ad hoc* laboratory was a room $6 \times 4 \times 2.7$ m, equipped with tables and chairs, in a brick building, in the roof of which a large colony of *P. pygmaeus* was situated. Female *P. pygmaeus* used in the experiment were netted from that colony. Adult female *P. pipistrellus*, netted in a nursery colony in northern Bohemia, were transported by car in linen bags to the experimental room and back within 2 days. Between experimental sessions the bats were kept in cages (30×30 cm wide, 50 cm high). All sessions were performed during night. The bats were fed with mealworm larvae *Tenebrio molitor* once a day, before the first session, and had access to water enriched by vitamins. During captivity, the light regime was natural and air conditions stable (25 °C, 50% humidity). The bats were captured and temporarily kept in captivity under licence no. 922/93-OOP/2884/93 of the Ministry of Environment of the Czech Republic.The authors have been authorized to manipulate with free-living bats according to the certificate of competency no. 104/2002-V4 (§ 17 of law no. 246/1992).

We recorded signals of the following bat assemblages: (1) mixed-species groups (two female *P. pipistrellus* and one female *P. pygmaeus* or vice versa), (2) monospecific groups (three or two females of eiher *P. pygmaeus* or *P. pipistrellus*) and (3) individual bats, either *P. pygmaeus* or *P. pipistrellus* (Fig. 1). Before releasing bats to fly, one bat of each group was marked by a standard aluminium band attached to its forearm and a piece of silver ribbon glued to its back. Silver ribbon was well visible on the flying bat during the whole session. No negative impact of marking on the bats' activity was recorded. Flying bats often landed on the walls of the laboratory, which enabled the researcher to identify the sequence of a flying individual when other bats were sitting or just starting to fly. It was possible to use long sequences (12-s storage time) in the bat detector. We were able to identify sequences of marked bats easier than by the discriminant function analysis method (e.g. King, 2005). Subsequently, identification of sequences to individual bats was made by determining similar interpulse intervals among successive calls in call sequences. The distance of the bats from the microphone was similar in each situation in the

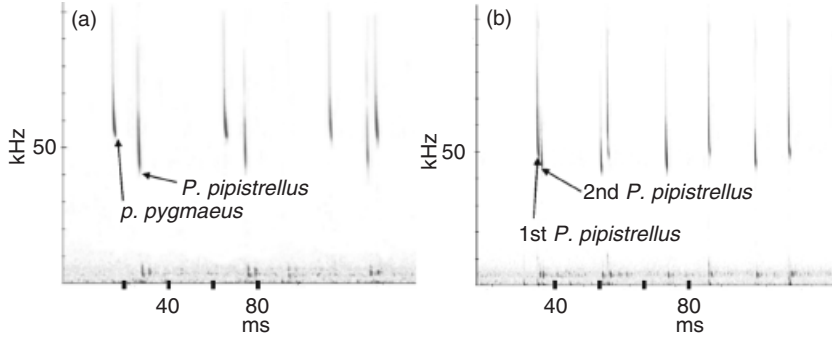

Figure 1 Representative sequences (in spectrograms) of the two *Pipistrellus* species studied: (a) –flying in a mixed-species group and (b) –flying in a monospecific group (only *Pipistrellus pipistrellus*) at roughly the same direction and distance from the microphone.

flight room and rather higher when bats were recorded in the field. Each session ended when the bats perched. They were then captured by a linen bag, the ribbon was removed from the backs of marked individuals and the bats were placed back in the cage. Female *P. pygmaeus* were released immediately to the roof of the same building, and female *P. pipistrellus* were transported back to their nursery colony in Bohemia the day when the experiments were finished. All experimental bats remained in good health and moved without any obvious problems after having been released into their original roosts. Echolocation calls were recorded using the same equipment as in the field. All recordings were finally analysed by Bat Sound 3.0.

Echo measurements

Only one sequence per individual or group was analysed. Only calls, not echoes, were measured. We used a 12-s storage time of the bat detector and analysed only sequences in the search phase (when times between two successive signals are not shortened; Murray, Britzke & Robbins, 2001) and with a good signal-to-noise ratio (*c.* 45 dB). We measured signal parameters using cursors on the PC screen to standardize the measurement procedure for individual echolocation signals (Fig. 2). The oscillograms and spectrograms were constructed from 512-point FFTs (time expansion, 10 times), using a sampling frequency of $f = 22.05$ kHz and Hamming window function with an 87% overlap between consecutive FFTs, giving a frequency resolution of 560 Hz and a time resolution of 0.30 ms. We manually measured temporal variables [interpulse interval, pulse duration, FM duration (time expansion, 10 times), QCF duration (time expansion, 10 times)], spectral variables (peak frequency, FO; frequency in −10 dB, Fmax10, Fmin10; frequency in −20 dB, Fmax20, Fmin20; frequency in −40 dB, Fmax40, Fmin40; FM bandwidth – difference between Fmax40 and Fmin40), duty cycle and repetition rate in pulses of echolocation calls recorded in both the room and the field directly from the FFT display (Fig. 2). Only spectral variables, however, were analysed to compare individual and group calls. Temporal variables reflect mainly 'clutter conditions' of the experimental room and were used to recognize which signal belongs to which individual (especially the interpulse interval; Ratcliffe *et al.*, 2004). Other parameters were used to analyse the differences in call structure between bats flying inside and outside. Social calls were analysed using a 12-s storage time of the bat detector. Records of 120 s from an individual flying alone, in a monospecific and mixed-species group, were examined to quantify the number of social calls.

Statistical analyses

The statistical software Statistica for Windows 6.0 was used for data analyses. All variables showed a normal distribution after arcsine transformation (Zar, 1984). The means of variables from three and eight signals (from 10 sequences inside and 10 outside for each species recorded at different sites) were tested and no significant differences were found [multivariate analysis of variance (MANOVA); *P. pipistrellus*, $F = 0.27$, NS; *P. pygmaeus*, $F = 1.24$, NS]. Therefore, we only used the first three signals per sequence (individual) and the computed mean values of variables were considered as one sample of one sequence (cf. Sendor *et al.*, 2002). MANOVA was used to check the differences between echolocation calls of *P. pipistrellus* and *P. pygmaeus* flying in a room and outside. Using MANOVA (and *post hoc* *t*-tests), we tested the differences in spectral parameters among members of a one-species group as well. Differences in spectral parameters when a bat flew alone and with conspecifics or in mixed groups were tested by paired *t*-tests. Bonferroni corrections were applied if multiple *t*-tests were used for the same dataset.

Materials

We recorded and analysed 70 sequences of *P. pygmaeus* and 41 sequences of *P. pipistrellus* foraging in the field, and

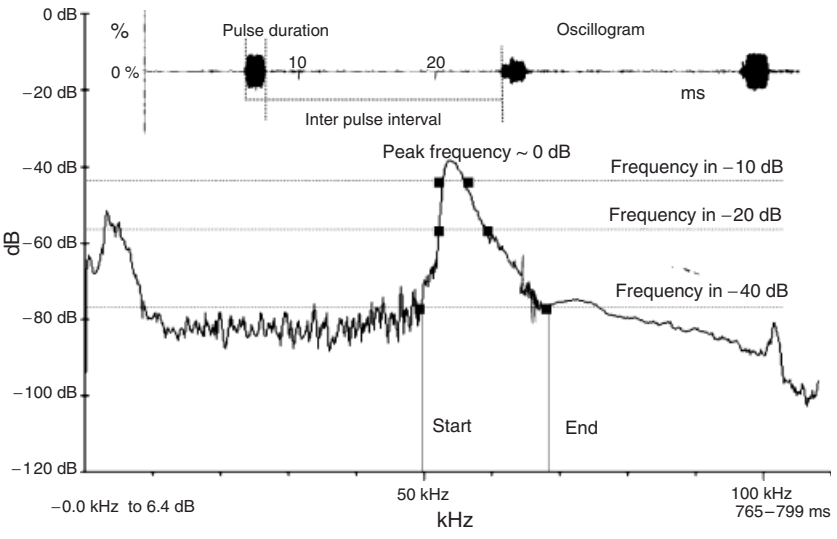

Figure 2 Studied parameters of a call of *Pipistrellus pipistrellus/pygmaeus* with defined measurement points in the oscillogram and power spectrum diagram.

30 single sequences from 30 individuals of each of the two species flying in the room. In the experimental room, we also analysed calls of 17 mixed-species groups, 14 monospecific groups of *P. pipistrellus* and 14 monospecific groups of *P. pygmaeus*. All individuals were recorded flying alone before including experimental groups (monospecific and mixed). To quantify the number of social calls, we examined 158 sequences of bats flying in all three situations.

Results

Differences between calls recorded in the room and in the field

Table 1 shows values of the call parameters studied using the calls of individuals of each species flying in the room and in the field. Significant differences were found in the spectral parameters of signals in both *P. pipistrellus* and *P. pygmaeus* (Table 2). Signals of both pipistrelles flying in the room show shorter temporal parameters and higher spectral parameters as well.

Differences between calls recorded in individually flying bats and in a monospecific group

We tested for differences in calls of 14 *P. pipistrellus* and 14 *P. pygmaeus* individual bats of the two species flying alone and in groups of conspecifics. Differences were found between spectral variables of the echolocation calls of an individual flying alone and when the same individual flew in a group of conspecifics in the room. Significant differences were found in peak frequency, Fmin10 and paired *t*-tests (Table 3). In most of the spectral parameters, the coefficient of variation (CV) was higher when an individual flew alone than when it flew in a group (e.g. *P. pipistrellus* peak frequency in group, CV = 1.9, alone, CV = 5.5; *P. pygmaeus* peak frequency in group, CV = 2.1, alone, CV = 9.3).

Differences between calls recorded of individually flying bats and of a mixed-species group

We tested changes in spectral variables if *P. pipistrellus* and *P. pygmaeus* were flying in mixed groups and alone in a room. No significant differences were found in all spectral parameters (*P. pygmaeus*, paired *t*-test, $n = 17$, NS; *P. pipistrellus*, $n = 17$, NS; Table 4). The CV was higher in most spectral parameters when a bat flew alone than when it flew in a mixed group (Table 4).

Differences between frequency range of calls recorded within monospecific and mixed-species groups

Significant changes were found in the frequency range of spectral shifts between members in one species and in mixed-species groups (*P. pygmaeus*, MANOVA, Wilks' $\lambda = 0.39$, *F*-value = 11.36, d.f. = 7, $P = 0.001$; *P. pipistrellus*, MANOVA, Wilks' $\lambda = 0.59$, *F*-value = 12.37, d.f. = 7, $P = 0.001$). Differences in frequency ranges were found in the peak frequency (*t*-test, Bonferroni correction in $\alpha = 0.0024$, *P. pipistrellus*, $t = 10.51$, $P < 0.001$; *P. pygmaeus*, $t = 8.85$, $P < 0.001$; Fig. 3) and in the start and end frequency in

Table 2 Results of multivariate analysis of variance

Analysis	Wilks' λ	*F*-value	Error d.f.	*P*-level
P.pyg inside vs *P.pip* inside	0.04	21.38	10.00	0.001
P.pip outside vs *P.pyg* outside	0.02	95.23	177.00	0.001
P.pip outside vs *P.pip* inside	0.17	23.00	42.00	0.001
P.pyg outside vs *P.pyg* inside	0.32	12.19	69.00	0.001

Statistics are based on spectral parameters shown in Table 1. Bonferroni correction was applied using $\alpha = 0.0125$, because multiple tests were used for the same dataset.
P.pip, Pipistrellus pipistrellus; P.pyg, Pipistrellus pygmaeus; vs, versus.

Table 1 Mean (± SD) values of 10 parameters of all studied sequences for *Pipistrelles pipistrellus* (*P. pip*) and *Pipistrellus pygmaeus* (*P. pyg*)

Species (number of individuals)	*P. pip* inside (30)	*P. pip* outside (?)	*P. pyg* inside (30)	*P. pyg* outside (?)
Number of sequences	30	41	30	70
Temporal parameters				
Pulse duration (ms)	3.6 ± 0.6	5.6 ± 1.2	3.1 ± 0.3	4.3 ± 0.9
Interpulse interval (ms)	80.3 ± 23.6	93.6 ± 23.4	62.3 ± 8.1	77.3 ± 6.2
QCF duration (ms)	2.6 ± 0.4	4.7 ± 1.4	2.2 ± 0.3	4.3 ± 0.8
FM duration (ms)	1.0 ± 0.3	1.0 ± 0.8	0.9 ± 0.2	0.9 ± 0.3
Spectral parameters				
Peak frequency (kHz)	48.4 ± 2.3	47.8 ± 1.7	58.3 ± 3.0	54.6 ± 2.2
Fmax40 (kHz)	81.5 ± 10.9	58.3 ± 6.3	87.1 ± 11.0	71.6 ± 8.0
Fmin40 (kHz)	41.3 ± 2.5	45.0 ± 2.1	48.8 ± 2.4	50.3 ± 2.3
FM bandwidth (kHz)	30.3 ± 8.1	9.3 ± 7.3	29.0 ± 11.2	15.8 ± 7.9
Other				
Duty cycle (%)	4.5 ± 1.0	5.8 ± 1.3	4.8 ± 0.6	5.7 ± 1.1
Repetition rate (pulses/s^{-1})	12.6 ± 2.6	10.4 ± 1.6	15.5 ± 1.9	12.3 ± 1.0

Fmax40 is the start frequency in −40 dB; Fmin40 is the end frequency in −40 dB (see Fig. 1). (?) we cannot exclude the multiple recording; this possibility was reduced by choosing different recording sites.

−10 dB (*P. pipistrellus*, $t_{Fmax} = 4.64$, $P < 0.001$, $t_{Fmin} = 7.39$, $P < 0.001$; *P. pygmaeus*, $t_{Fmax} = 4.94$, $P < 0.001$, $t_{Fmin} = 6.11$, $P < 0.001$).

Social calls

In addition to orientation signals, non-echolocation song-like calls were recorded. These social calls consisted of the rapid emission of very short three or four components (cf. Barlow & Jones, 1997) or only one component. We tested for differences in the number of social calls if *P. pipistrellus* and *P. pygmaeus* were flying alone, in monospecific and mixed groups in a room (ANOVA, $F = 52.39$, d.f. = 2, $P < 0.001$). No significant interspecific differences were found when the bats were flying alone (*t*-test, Bonferroni correction in $\alpha = 0.008$, $t = 0.96$, NS, $n_1 = 11$, $n_2 = 13$) and in monospecific groups ($t = 1.55$, NS, $n_1 = 55$, $n_2 = 49$). However, the bats emitted social calls significantly more often when flying in a group of conspecifics than alone ($t = -4.55$, $P < 0.001$, $n_1 = 24$, $n_2 = 104$) and more often in mixed than in monospecific groups ($t = -7.83$, $P < 0.001$, $n_1 = 104$, $n_2 = 30$).

Table 3 Results of paired *t*-tests when bats flew alone (14 individuals) and in one-species groups (14 groups with two or three members)

Species/variables	Shift in group (mean ± SD)	Paired *t*-test (*t*-values)
P. pipistrellus (*n* = 14)		
Peak frequency	2.1 ± 0.4	−1.76*
Fmax10	2.7 ± 3.1	−0.74
Fmin10	2.8 ± 0.8	−1.99*
Fmax20	7.5 ± 8.2	0.58
Fmin20	4.3 ± 1.1	−1.97*
Fmax40	10.0 ± 8.7	0.51
Fmin40	2.6 ± 2.7	0.61
P. pygmaeus (*n* = 14)		
Peak frequency	3.7 ± 1.1	−1.44*
Fmax10	4.4 ± 3.4	−0.57
Fmin10	1.7 ± 1.5	1.04
Fmax20	5.0 ± 9.3	0.33
Fmin20	2.7 ± 2.7	0.74
Fmax40	15.5 ± 10.3	1.16
Fmin40	3.3 ± 2.3	−0.16

*$P < 0.05$. Fmax10 and Fmin10, start and end frequency in −10 dB; Fmax20 and Fmin20, start and end frequency in −20 dB; Fmax40 and Fmin40,start and end frequency in −40 dB. Shift in group shows absolute values of differences between frequencies of bats flying alone and in one-species groups.

Discussion

Flying settings

In this study we examined frequency differences in echolocation calls between *P. pipistrellus* and *P. pygmaeus* flying in monospecific or mixed-species groups under laboratory conditions. Conditions in the experimental room were

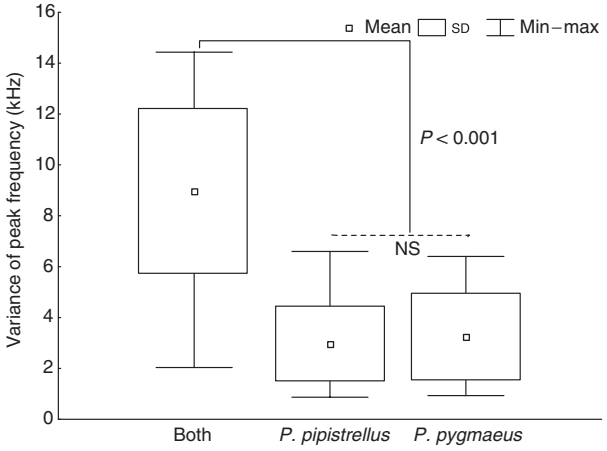

Figure 3 Significant changes (*t*-test, $P < 0.001$) in frequency scale of the peak frequency between members within a one-species group and a mixed-species group. Monospecific groups consisted of three or two females. No significant differences were found between one-species groups of *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*.

Table 4 Means (± SD) of call variables recorded when *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* were flying alone and in mixed groups in a room

Species/variables	*P. pipistrellus*		*P. pygmaeus*	
(*n* = 17 individuals or groups)	Alone	With *P. pygmaeus*	Alone	With *P. pipistrellus*
Peak frequency	48.4 ± 2.2 (5.5)	48.3 ± 1.5 (2.3)	58.3 ± 2.9 (9.4)	57.5 ± 1.8 (3.4)
Fmax10	56.0 ± 7.1 (55.1)	54.2 ± 3.4 (12.2)	67.2 ± 9.4 (88.6)	62.9 ± 2.7 (7.8)
Fmin10	46.2 ± 2.2 (5.1)	45.9 ± 2.3 (5.5)	54.3 ± 1.2 (1.7)	54.7 ± 2.1 (4.7)
Fmax20	64.9 ± 10.7 (125.4)	62.1 ± 5.5 (32.3)	74.3 ± 11.6 (145.4)	73.3 ± 9.2 (89.7)
Fmin20	44.3 ± 2.1 (4.9)	44.9 ± 2.3 (5.9)	52.8 ± 2.4 (6.1)	52.6 ± 1.9 (4.0)
Fmax40	81.5 ± 10.4 (118.2)	80.5 ± 10.3 (113.8)	87.1 ± 10.5 (120)	87.9 ± 13.6 (196.6)
Fmin40	41.4 ± 2.4 (6.3)	42.4 ± 2.9 (8.9)	48.9 ± 2.3 (5.5)	48.1 ± 3.4 (11.5)

Each mixed group consisted of two individuals which were also recorded when flying alone. No significant differences were found. Fmax10 and Fmin10, start and end frequency in −10 dB; Fmax20 and Fmin20, start and end frequency in −20 dB; Fmax40 and Fmin40, start and end frequency in −40 dB. Values of the coefficients of variance are shown in parentheses.

similar to a cluttered habitat; therefore, we chose signal sequences only from the cluttered forest to compare with the room signals. Significant differences were found between signals in the field and in the laboratory. Also, Mukhida, Orprecio & Fenton (2004) found significant differences in spectral parameters between *Myotis* individuals flying inside and in the field. The authors of that study observed that the difference between signals of one species flying inside and outside was higher than the difference of signals between two *Myotis* species flying anywhere. This made the identification of species unreliable. Although our recordings suggest high differences in peak frequency, these shifts were lower than differences among bats in different habitats (Kalko & Schnitzler, 1993; Bartonička & Řehák, 2005) and probably might not cause a confusion of the two species. Obviously, this kind of research does not offer reliable information on the absolute values of echolocation parameters. But we presumed that the shifts of spectral parameters found under experimental conditions would be similar to those in the field. Well-defined conditions of the experimental room are then more suitable to quantify the shifts in all parameters studied than are variable conditions in the field. The shorter durations recorded in the room suggest that the bats were flying closer to objects there (because calls become shorter when bats are flying closer to obstacles so that overlap between pulse and echo is avoided), and thus the room may have been a more cluttered situation than the field conditions.

Echolocation calls in foraging groups

Some studies of intraspecific variation in the search phase of echolocation calls demonstrated differences among bat species (e.g. Thomas, Bell & Fenton, 1987). Other authors reported group foraging activity in several bat species (e.g. McCracken & Bradbury, 1981; Hickey & Fenton, 1990). Collective foraging of *P. pipistrellus* and *P. pygmaeus* in the same hunting site was observed as well (Racey & Swift, 1985; T. Bartonička, Z. Řehák, & J. Gaisler, pers. comm.). When pipistrelles fly with their conspecifics they do not use a wider range of frequency parameters as found by Ratcliffe *et al*. (2004) in *Tadarida brasiliensis*, but they use higher frequencies than when flying alone. Laboratory studies indicate that jamming avoidance, if practised, would benefit bats foraging in groups in the field (e.g. Jones *et al*., 1994). In general, group foraging could provide benefits to members of a group (e.g. Barak & Yom-Tov, 1989; Wilkinson & Boughman, 1998). Its possible cost, however, is the shift of spectral parameters for correct identification of each individual's own echo. The absence of information about nearby conspecifics might be the cause for more air collisions. Our results show that bats may use shifts of spectral parameters for better identification of their own echoes during foraging with conspecifics. No significant differences in spectral parameters between individuals flying alone and in a mixed-species group support the assumption that differences in frequencies between *P. pipistrellus* and *P. pygmaeus* are sufficient to recognize own calls. Differences in bandwidth, that is the static frequency shift also found in other species (*Eptesicus fuscus*, Surlykke & Moss, 2000; *Tadarida teniotis*, Ulanovsky *et al*., 2004), seem to account partly for differences in jamming avoidance response (JAR). Experiments, when we recorded calls of two similar species of pipistrelles flying alone and together in the mixed group and found no changes in spectral parameters in one individual flying in these different situations, tend to show that the interference did not result from flying in a one-species group. If interference was causing frequency shifts, it would be found in the mixed-species group as well. We observed static and dynamic JAR described by Ulanovsky *et al*. (2004) in pipistrelles as well. Dynamic JAR was found only during first flights across the laboratory room. After habituation to the laboratory conditions bats used static JAR only, perhaps due to small distances between them. The fact that changes in call parameters can be used to identify the position of other individuals in a foraging group need not mean the presence of other social information (contrary to Fenton, 1984; Obrist, 1995). The higher CV of the spectral parameters of signals emitted by individuals flying alone, compared with those flying in a monospecific or mixed-species group, suggests that not echolocation but social calls contain the main information for use as individual signatures.

Social calls

Social calls emitted by a foraging bat could serve several functions: attracting mates, avoiding predators, defending foraging sites or advertising food patches. They can include information about sex and/or age (Boughman, 1998; Boughman & Wilkinson, 1998). Playback experiments demonstrated that bats *Phyllostomus hastatus* perceived acoustic differences and used the broadband signals to coordinate foraging movements of social group mates. In monospecific groups of our pipistrelles, social signals were mainly emitted at the beginning of flight and could have had an agonistic meaning. Infrequent recordings of these signals might arise from the fact that females originating from the same colonies were already familiar with each other before our experiments. In mixed-species groups, social calls were more numerous than in both individually flying bats and those flying with conspecifics and they were emitted continuously during the recording session. A foraging patch defence function of the calls, although frequent in the two species under study (Barlow & Jones, 1997; T. Bartonička, Z. Řehák, & J. Gaisler, pers. comm.), was not observed, but this might have been due to the absence of foraging under laboratory conditions. The main function of social calls emitted by pipistrelles foraging in groups remains to be tested in future experiments. However, the high number of social calls recorded and no aggressive behaviour observed in mixed-species groups, together with differences in diet between the two species (Barlow, 1997), support the assumption that social calls not only serve to defend foraging sites but also have other communicative functions.

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References

Barak, Y. & Yom-Tov, Y. (1989). The advantage of group hunting in Kuhl's bat *Pipistrellus kuhli* (Microchiroptera). *J. Zool. (Lond.)* **219**, 670–675.

Barclay, R.M.R. (1982). Interindividual use of echolocation calls: eavesdropping by bats. *Behav. Ecol. Sociobiol.* **10**, 271–275.

Barlow, K.E. (1997). The diet of two phonic types of the bat *Pipistrellus pipistrellus* in Britain. *J. Zool. (Lond.)* **243**, 597–609.

Barlow, K.E. & Jones, G. (1997). Function of pipistrelle social calls: field data and a playback experiment. *Anim. Behav.* **53**, 991–999.

Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P.A. & Wayne, R.K. (1997). DNA answers the call of pipistrelle bat species. *Nature* **387**, 138–139.

Bartonička, T. & Řehák, Z. (2005). Variability in echolocation calls of *Pipistrellus pygmaeus* (Leach 1825) in search flight in different habitats. *Acta Theriol.* **50**, 145–160.

Bartonička, T., Řehák, Z., Wolf, P. & Bryja, J. (2002). Small mammals of the Litovelské Pomoraví protected landscape area (Czech Rep.). Part 1. Bats (Chiroptera). *Lynx* **33**, 35–46.

Bohn, K.M., Boughman, J.W., Wilkinson, G.S. & Moss, C.F. (2004). Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication. *J. Comp. Physiol.* **190**, 185–192.

Boughman, J.W. (1998). Vocal learning by greater spear-nosed bats. *Proc. Roy. Soc. Lond. Ser. B* **265**, 227–233.

Boughman, J.W. & Wilkinson, G.S. (1998). Greater spear-nosed bats discriminate group mates by vocalizations. *Anim. Behav.* **55**, 1717–1732.

Fenton, M.B. (1984). Echolocation: implications for ecology and evolution of bats. *Q. Rev. Biol.* **59**, 33–53.

Fenton, M.B. (1986). Design of bat echolocation calls: implications for foraging ecology and communication. *Mammalia* **50**, 193–203.

Fenton, M.B. (1994). Assessing signal variability and reliability: "to thine ownself be true". *Anim. Behav.* **47**, 757–764.

Goodwin, G.G. & Greenhall, A.M. (1961). A review of the bats of Trinidad and Tobago. *Bull. Am. Mus. Nat. Hist.* **122**, 195–301.

Habersetzer, J. (1981). Adaptive echolocation sounds in the bat *Rhinopoma hardwickei*. *J. Comp. Physiol.* **144**, 559–566.

Hickey, M.B.C. & Fenton, M.B. (1990). Foraging by red bats (*Lasiurus borealis*): do chases mean territoriality? *Can. J. Zool.* **69**, 2477–2482.

Huffman, R.F. & Henson, O.W. Jr. (1993). Labile cochlear tuning in the mustached bat. I. Concomitant shifts in biosonar emission frequency. *J. Comp. Physiol.* **171**, 725–734.

Hulva, P., Horáček, I., Strelkov, P.P. & Benda, P. (2004). Molecular architecture of *Pipistrellus pipistrellus/pygmaeus* complex (Chiroptera: Vespertilionidae): further cryptic species and Mediterranean origin of the divergence. *Mol. Phyl. Evol.* **32**, 1023–1035.

Jones, G. (1995). Variation in bat echolocation: implications for resource partitioning and communication. *Le Rhinolophe* **11**, 53–59.

Jones, G., Gordon, T. & Nightingale, J. (1992). Sex and age differences in the echolocation calls of the lesser horseshoe bat, *Rhinolophus hipposideros*. *Mammalia* **56**, 191–195.

Jones, G. & Ransome, R.D. (1993). Echolocation calls of bats are influenced by maternal effects and change over a lifetime. *Proc. Roy. Soc. Lond. Ser. B* **252**, 125–128.

Jones, G., Sripathi, K., Waters, D.A. & Marimuthu, G. (1994). Individual variation in the echolocation calls of three sympatric Indian hipposiderid bats, and an experimental attempt to jam bat echolocation. *Folia Zool.* **43**, 347–362.

Kalko, E.K.V. & Schnitzler, H.U. (1993). Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behav. Ecol. Sociobiol.* **33**, 415–428.

Kazial, K.A., Burnett, S.C. & Masters, W.M. (2001). Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *J. Mammal.* **82**, 339–351.

King, B. (2005). *Changes in echolocation calls of* Eptesicus fuscus *when flying with conspecifics in a laboratory setting*. Honors thesis, The Ohio State University.

Law, B.S., Reinhold, L. & Pennay, M. (2002). Geographic variation in the echolocation calls of *Vespadelus* spp. (Vespertilionidae) from New South Wales and Queensland, Australia. *Acta Chiropterol.* **4**, 201–215.

Lundberg, K. & Gerell, R. (1986). Territorial advertisement and mate attraction in the bat *Pipistrellus pipistrellus*. *Ethology* **71**, 115–124.

Masters, W.M., Raver, K.A.S. & Kazial, K.A. (1995). Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. *Anim. Behav.* **50**, 1243–1260.

Mayer, F. & von Helversen, O. (2001). Sympatric distribution of two cryptic bat species across Europe. *Biol. J. Linn. Soc.* **74**, 365–374.

McCracken, G.F. & Bradbury, J.W. (1981). Social organization and kinship in the polygynus bat. *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* **8**, 11–34.

Miller, L.A. & Degn, H.J. (1981). The acoustic behaviour of four species of vespertilionid bats studied in the field. *J. Comp. Physiol.* **142**, 67–74.

Mukhida, M., Orprecio, J. & Fenton, M.B. (2004). Echolocation calls of *Myotis lucifugus* and *M. leibii* (Vespertilionidae) flying inside a room and outside. *Acta Chiropterol.* **6**, 91–97.

Murray, K.L., Britzke, E.C. & Robbins, L.W. (2001). Variation in search-phase calls of bats. *J. Mammal.* **82**, 728–737.

Obrist, M. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behav. Ecol. Sociobiol.* **36**, 207–219.

Racey, P.A. & Swift, S.M. (1985). Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. *J. Anim. Ecol.* **54**, 205–215.

Ratcliffe, J.M., ter Hofstede, H.M., Avila-Flores, R., Fenton, M.B., McCracken, G.F., Biscardi, S., Blasko, J., Gilliam, E., Orprecio, J. & Spanjer, G. (2004). Conspecifics influence call design in the Brasilian free-tailed bat, *Tadarida brasiliensis*. *Can. J. Zool.* **81**, 966–971.

Řehák, Z., Chytil, J., Bartonička, T. & Gaisler, J. (2003). Distribution of small mammals in the Biosphere Reserve Lower Morava (extended BR Pálava). Part II. Bats – Microchiroptera. *Lynx* **33**, 35–46.

Rydell, J. (1986). Feeding territoriality in female northern bats, *Eptesicus nilssonii*. *Ethology* **72**, 329–337.

Schnitzler, H.U. & Kalko, E.K.V. (2001). Echolocation by insect-eating bats. *Bioscience* **51**, 557–569.

Sendor, T., Roedenbeck, I., Hampl, S., Ferreri, M. & Simon, M. (2002). Revision of morphological identification of pipistrelle bat phonic types (*Pipistrellus pipistrellus* Schreber, 1774). *Myotis* **40**, 11–17.

Simmons, J.A., Lavender, W.A., Lavender, B.D., Childs, J.E., Hulebak, K., Rigden, M.R., Sherman, J. & Woolman, B. (1979). Echolocation by free-tailed bats (Tadarida). *J. Comp. Physiol.* **125**, 291–299.

Surlykke, A. & Moss, C.F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *J. Acoust. Soc. Am.* **108**, 2419–2429.

Thomas, D.W., Bell, G.P. & Fenton, M.B. (1987). Variation in echolocation calls frequencies recorded from North American vespertilionid bat: a cautionary note. *J. Mammal.* **68**, 842–884.

Ulanovsky, N., Fenton, M.B., Tsoar, A. & Korine, C. (2004). Dynamics of jamming avoidance in echolocating bats. *Proc. Roy. Soc. Lond. Ser. B* **271**, 1467–1475.

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

Zar, J.H. (1984). *Biostatistical analysis*. Englewood Cliffs, NJ: Simon and Schuster.