

The advertisement song of *Pipistrellus nathusii* (Chiroptera, Vespertilionidae): a complex message containing acoustic signatures of individuals

HELENA JAHELKOVÁ^{1,3}, IVAN HORÁČEK¹, and TOMÁŠ BARTONIČKA²

^{1,3}Department of Zoology, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

²Institute of Botany and Zoology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

³Corresponding author: E-mail: hjahel@yahoo.com

Males of *Nathusius' pipistrelle* (*Pipistrellus nathusii*) are well known for their complex acoustic advertisement behaviour performed either as songflight (SF) or sedentary display (SD). We analysed phonologic, syntactic and semantic characteristics of these calls based on 2,924 acoustic records obtained from individual males repeatedly occupying 33 roosts in southern Bohemia from 1999 to 2006. Both SF and SD calls are composed of three main phonologically contrasting motifs (A, B, C) and under specific contexts often supplemented with two accessory motifs (D, E). Besides the major syntagmatic string ABC we recorded further 15 syntagms containing the main motif A and 10 syntagms without this motif. At the peak of the mating season and with the SD, the complexity of the vocalisation increases (increased variation in syntagmatic structure and syllabic composition of particular motifs, as well as in length of syntagmatic strings). The motif A corresponds to advertisement calls of congeneric species and is related to agonistic vocalisation, and B, C, D are specific for *P. nathusii*. B and C exhibit the largest between-individual but low within-individual variation, and their combination provides an acoustic signature of an individual and uniquely identified each particular male. Motif D corresponds to calls of mother-young communication and shows the largest syllabic variation. Motif E is a series of steep FM signals evocative of the act of landing. The message of a complete advertisement call (ABCED) could be thus: '(A): Pay attention, here is a *P. nathusii*, (B, C): I am male X, (E): land here, (D): we share a common social identity and common communication pool'. The individual characteristics of the performance of B, C, and D motifs were found to be invariant both within a season and over a series of successive years.

Key words: *Pipistrellus nathusii*, advertisement calls, individual recognition, vocalisation behaviour, phonology, semiotics

INTRODUCTION

Since the time of Griffin's (1958) "Listening in the dark" bats have been among the most popular subjects of bioacoustic studies. A vast majority of these studies have been engaged with various aspects of echolocation calls, while less attention has been paid to social calls and acoustic communication in general. Also echolocation calls have been proved to provide relevant social information (on individual identity, age and colony membership or information about food and roost resources), at least in specific contexts and in some species (Master *et al.*, 1995; Pearl and Fenton, 1996; Wilkinson and Boughman, 1998; Kazial and Masters, 2004). Nevertheless, the social aspects of bat calls have largely been ignored in echolocation studies. Social calls are more complex in their acoustic structure, they are highly variable and considerably influenced by contextual specificities, and for these reasons

the study of social calls has to include detailed information on behavioural context and semantic references, which are obviously hard to complete. Fenton (1985), who first reviewed this topic in general, stressed the essential role of acoustic communication in the social life of bats, which recent studies have largely supported (Jones, 1997; Altringham and Fenton, 2003; Davidson and Wilkinson, 2004). In every bat species where acoustic communication has been studied in detail, a rich repertoire of social calls has been found, of which at least some apparently serve in inter-individual recognition. Nevertheless, distinguishing the species-specific and individual-specific components of an acoustic repertoire is difficult, particularly under natural conditions when only incomplete information on behavioural context is available, and thus most experiments have been carried out in laboratories or under semi-natural conditions. Consequently, sufficiently comprehensive information is available for only those calls

that can be studied under such conditions, primarily mother-young communication in colonial bat species, such as the simple curved calls mediating the mother-pup recognition, e.g., in *Carollia perspicillata* (Porter, 1979), *Phyllostomus discolor* (Esser and Schmidt, 1989), *Molossus molossus* (Häussler *et al.*, 1981), *Tadarida brasiliensis* (Balcombe, 1990; Balcombe and McCracken, 1992), *Plecotus auritus* (de Fanis and Jones, 1995), *Pipistrellus pygmaeus* (de Fanis and Jones, 1996) and *Phyllostomus hastatus* (Bohn *et al.*, 2007).

Social calls can be classified into four major categories: (a) agonistic or aggressive calls (noisy broadband frequency calls), (b) distress calls (trill-like calls), (c) isolation and directive calls (tonal calls) and (d) advertisement or mating calls (song-like calls) (Pfalzer and Kusch, 2003). The calls in the last category are especially linked to complex social communication. Unfortunately, such calls are typically associated with very complicated and spatially divergent behaviour, such as display flights, chasing of intruders or mates, false landing, etc., and can not easily be investigated under laboratory conditions or in controlled experimental settings. Hence, a meaningful study of them must be performed under natural conditions, with a particular attention to any behavioural components accompanying the vocalisation, and avoiding disturbing effects, e.g. human intrusions. No wonder that detailed information on aspects of advertising vocal behaviour, its individual variation, and its interface with aspects of life history, have been until recently rather exceptional. Perhaps, the most complete information on subject is available for the Neotropic greater white-lined bat, *Saccopteryx bilineata*. In addition to a comprehensive survey of the bat's acoustic repertoire and its individual variation, the study provides an impressive illustration of how the social vocalisation is imbedded in other mechanisms of social integration (Behr and von Helversen, 2004; Davidson and Wilkinson, 2004), including the direct effects of vocalisation activity pattern and the acoustic structure of advertisement calls upon the reproductive success of the advertising male (Behr *et al.*, 2006).

In the temperate bats, whose social system is characterized by sexual segregation and spatial isolation of the sexes for most of the active season, a direct role of acoustic cues in social integration is apparently less pronounced and the advertisement acoustic display is limited to the mating period. Among the European bats, the acoustic advertisement performed by males during the mating period is particularly noticeable in *Vespertilio murinus* and

the members of genera *Nyctalus* and *Pipistrellus*. These bats are all fast aerial hawkers with conspicuous seasonal rearrangement in spatial organization of their population (often accompanied by long-distance migrations). They also tend to be r-strategists (regularly producing twins), with a polygynous mating system and active defense of territories by males during the mating season (compare Krapp, 2004 for details).

Increased attention has been devoted to acoustic behaviour in the genus *Pipistrellus*, particularly after separation of two cryptic species, *P. pipistrellus* and *P. pygmaeus*, based on the differences in terminal frequency of echolocation pulses (Jones and van Parijs, 1993). The differences in advertising calls provided one of the most robust arguments for their distinct species status (Jones, 1997). Despite differences, the advertisement calls in both cryptic species show a similar structure dominated by a series of loud, short FM pulses. These calls attract females and repel rival males, and outside mating season they appear in contexts of agonistic interactions at foraging sites (Barlow and Jones, 1997a, 1997b). The social calls of *Pipistrellus kuhlii* are of the same structure but at a lower frequency (Russo and Jones, 1999; Szkudlarek and Paszkiewicz, 2000; Jahelková, 2003). In contrast, the genus *Nyctalus*, the inner clade-group of the genus *Pipistrellus* (Mayer and von Helversen, 2001; Hoofer and Van Den Busche, 2003), exhibits broad variation in their advertising vocalisation: in *Nyctalus leisleri* only simple-curved low-frequency calls have been reported (von Helversen and von Helversen, 1994), whereas in *N. noctula* the social vocalisation is extremely varied and in male advertising vocalisations at least four distinct types of complex calls can be distinguished (Weid, 1994; Gebhard and Bogdanowicz, 2004), though some of them are produced also by females and/or are not restricted to the mating season (Kozhurina, 1996).

Seasonal male advertising vocalisation is a particularly conspicuous phenomenon in *P. nathusii*. Moreover, in that species the calls are of a quite regular structure and, at the same time, are more complex than those of the congeneric species. The advertisement calls are usually composed of three motifs: the first 'main motif' shows a structure similar to congeneric species, the second is formed by one steep FM note, and the third is a 'trill' of several 'V-shaped' syllables (Lundberg, 1989). A fourth motif formed by several simple-curved syllables can follow (Jahelková *et al.*, 2000; Jahelková, 2003; Russ and Racey, 2007).

The third component ('trill') is the most characteristic feature of an advertisement song of *P. nathusii*. The literature on the phonetic structure of this component suggest extreme variation: the lowest frequency values estimated from spectrograms by different authors were, for example, 22 kHz (Pfalzer and Kusch, 2003), 25 or 30 kHz (Lundberg, 1989), 30 kHz (Russ *et al.*, 1998), 35 kHz (Furmankiewicz, 2003), 35 kHz (Skiba, 2003), 40 kHz (Barlow and Jones, 1996), 47 kHz (Szkudlarek and Paszkiewicz, 2000) and 27–53 kHz (Russ and Racey, 2007). It is not clear whether these differences reflect differences between particular local populations, or whether broad variation is an inherent quality of each individual. Despite the recent analyses by Russ and Racey (2007), it is also not known to what degree the other components of a call promote individual specificity, what are the patterns of their within- and between-individual variation, and what factors influence syntactic the composition of these calls (e.g., repetitions or loss of particular components and other aspect of call structure).

The advertisement vocal activity can be recorded well before the beginning of mating season (Barlow and Jones, 1996; Russ *et al.*, 1998; Furmankiewicz, 2003) but whether the phonologic or syntactic structure of the calls change during the season is not known. Similarly, there are no data on whether individual characteristics (such as those reported by Russ and Racey, 2007) are retained over a period of more than one season.

The present paper, which is based on the results of a long-term field study on the reproductive biology of the species, tries to answer these questions. We report here data concerning (a) the phonologic design and syntactic structure of the advertisement calls of *P. nathusii*, (b) the seasonal variation in call characteristics, (c) the patterns of between- and within-individual variation in particular characteristics (both phonologic and syntactic) and the factors influencing them, (d) the phonologic parameters enabling individual recognition, and (e) semantic structure of the call.

MATERIALS AND METHODS

The study is based on field investigations conducted from 1999 to 2006 in the Třeboňsko lowland basin of southern Bohemia. The study area covers 5 km² (Fig. 1) and is situated in the vicinity of the town of Veselí nad Lužnicí (49°03'N, 14°50'E, altitude 420 m) in a variegated landscape densely patched with forests (oak, pine, birch, willow), fields, meadows, and fish ponds. Two maternity colonies of *P. nathusii* were found in this area, including 33 roosts occupied during the course of the study by at least 40 males. We tried to minimize

disturbance of animals, limiting our handling of males to capturing them once or twice per season, either mist netting them in vicinity of their roost or by capturing them within roosts (nest boxes, tree hollows, and fissures in man-made structures). In most instances visits to roosts were limited to visual checks without disturbing bats. In total 16 males were banded, while others were marked within a season by individual-specific staining of the pelage (which lasted through the season). Starting in 2002, all handled males were sampled for DNA by wing punctures (the results will be published elsewhere). Despite short-distance roost switching (3–30 m) each resident male occupied one territory per year. Therefore, for the purpose of this paper we distinguished the individual males by numbers (1 to 33) denoting their territories, which were centered on with the site of their regular sedentary display. The different males occupying the same roosts on different occasions or those roosting in the immediate vicinity of a territory (i.e., within a distance less than 15 m) are distinguished by letters supplementing the roost number (a, b, c).

Advertisement calls were recorded at the sites of sedentary displays during regular walks around a 6-km circular transect (Fig. 1) and during whole-night observations on three successive nights that were focused only on certain males (see Table 1 and below). These observations were performed (with few omissions) twice a month from May till September each year from 2000 to 2006. In total, 90 nights were spent observing male vocalisation behaviour.

In total, 2,924 advertisement calls (1,879 sedentary displays and 1,045 songflights) were analysed to reveal the seasonal course of vocalisation activity. This sample was contributed by 21 males in the study area and recordings obtained in 2001 from a single male in Lanžhot, Southern Moravia (48°43'N, 16°58'E, code lan — Table 1). All other analyses are based on 799 calls for which male individual identity was established with certainty by repeated recaptures and, for three bats (4, 12b, and 12c) radio-tracking. For five bats (4, 6a, 12a, 12b, and 17a) continual whole-night monitoring of vocalisation activity was repeatedly performed, which provided 557 calls allowing a detailed analysis of individual acoustic repertoires and their variation. During behavioural observations of maternity colonies, female and juvenile social calls were also recorded and analysed (1,321 calls).

The acoustic analysis was performed on the output of D240 and D240x bat detectors recorded by a digital DAT recorder (Sony TCD-D8, Sony MZ-RH10) and then transferred to a computer and examined with aid of Batsound 1.2 and Avisoft-SasLabPro 4.23 software. (The settings used in both programs were similar: sampling frequency 22050 Hz, Hamming window, FFT size 256, and FFT overlap 87.5%, what gives a frequency resolution 1,120 Hz and time resolution 0.15 ms). Incomplete records and those with poor resolution were excluded from the analysis, and the remaining ones were examined as to their composition, temporal structure, and the frequency characteristics of each particular component. The starting and ending frequencies were measured at 40 dB below the frequency with maximum energy (fmax). Additional frequency characteristics were measured at the centre of the spectrogram. Figure 2 shows abbreviations and terms denoting call motifs and illustrates acoustic parameters. The essential structural components of an advertisement call were called 'motifs' and are denoted with capital letters A, B, C, D, and E with submotifs denoted by numerals. Motifs are composed of a series of similar pulses called 'notes', which are here denoted with lower-case letters and numerals (a-1..a0..ax, c1..cx — Appendix).

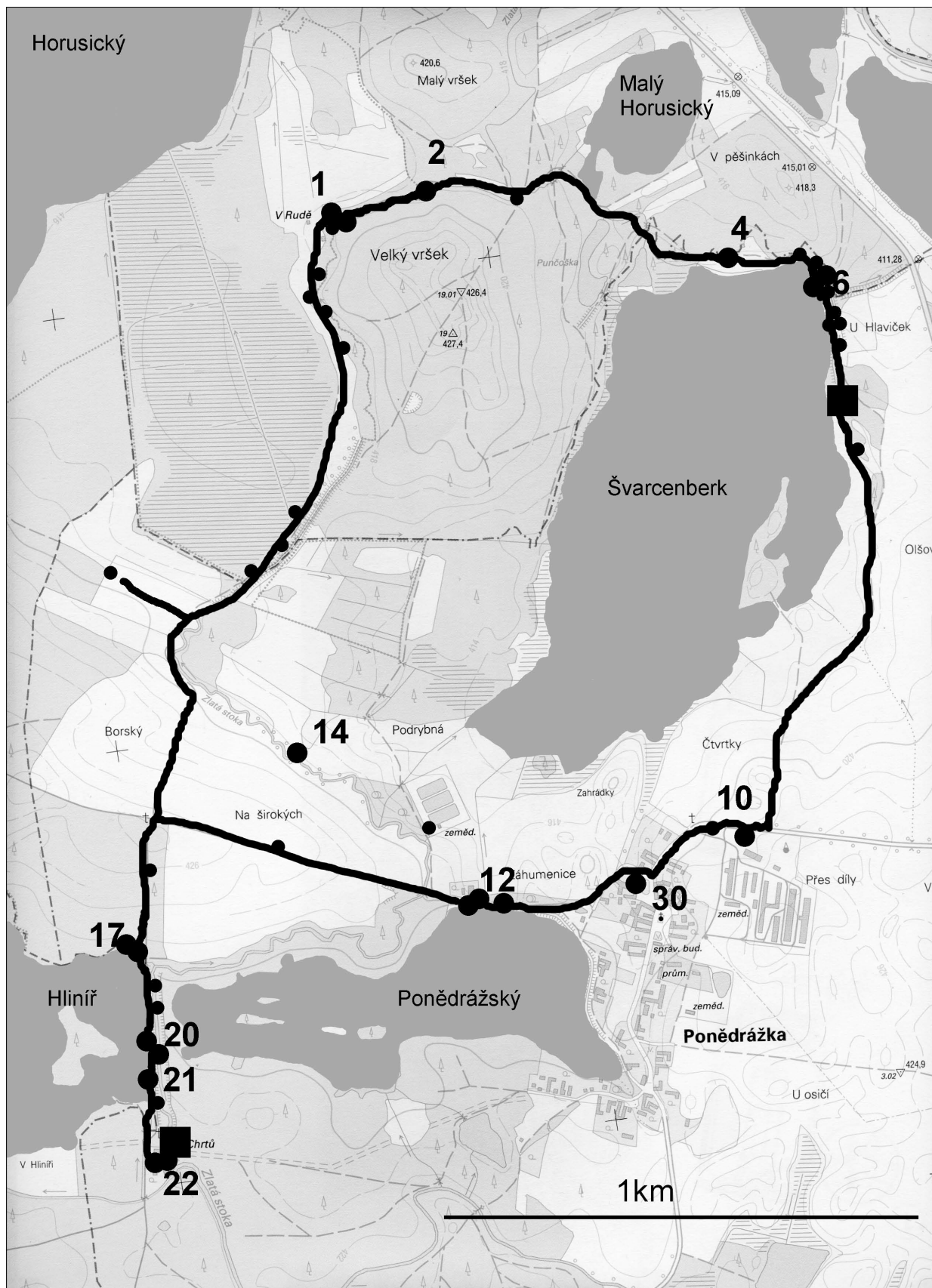


FIG. 1. Map of the study area showing the circular transect, roosts of maternity colonies (squares), and vocalisation sites of advertising males (dots with numbers). The numbers correspond to the codes of respective males as used in the present paper (see Appendix)

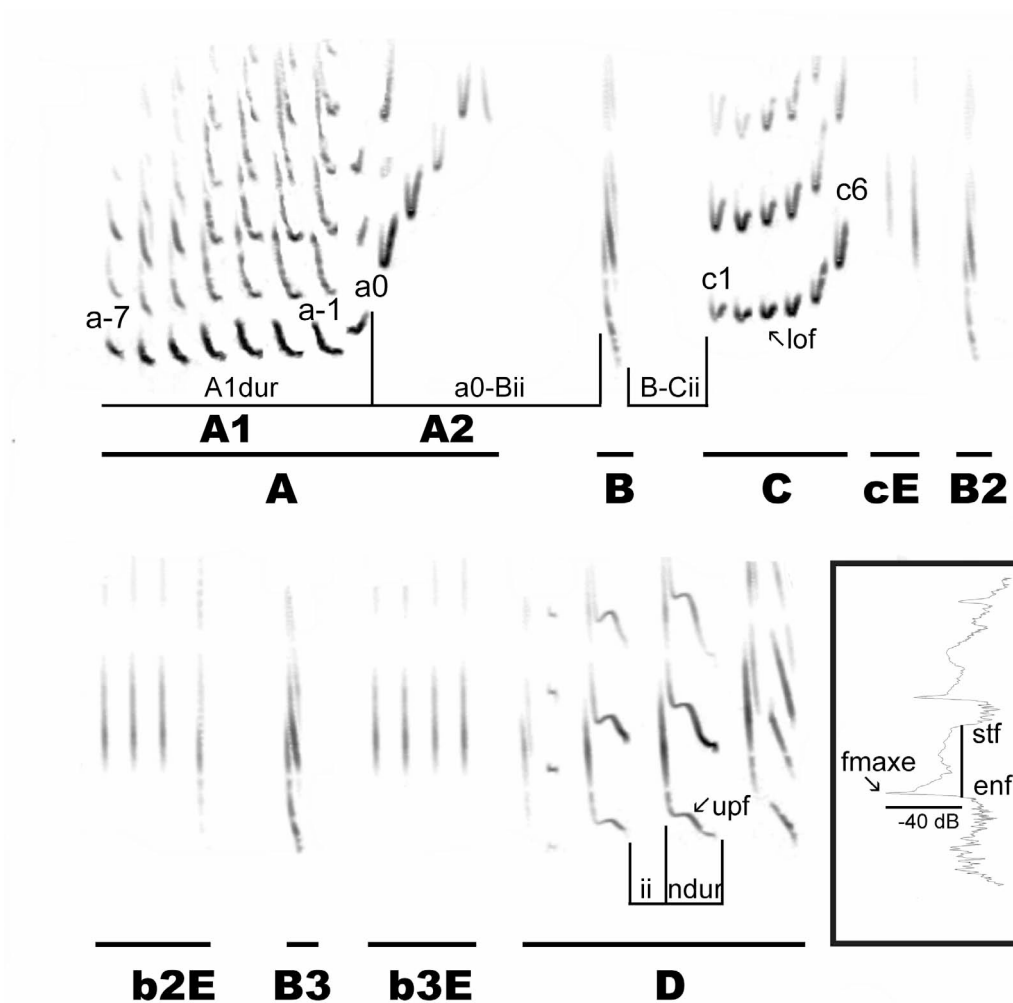


FIG. 2. Sound spectrogram of a typical complete advertisement call of *P. nathusii*, showing abbreviations and terms denoting major components of the call and illustrating the measurement of acoustic parameters (see Appendix for an explanation of terms and abbreviations). The box at the bottom right shows the power spectrum of a middle note of motif D, illustrating the measurement of frequency variables

The statistical analysis included computation of basic statistics for each individual male and examination of variation within and between individuals. Both in exploratory and confirmatory analyses, non-parametric statistics were applied (Spearman correlation coefficient r , Mann-Whitney U statistics, Kruskal-Wallis test). ANOVA with 'individual' as the factor was conducted to identify individually specific variation. Covariance and Pearson correlation ($1-r$) matrices were computed to analyse mutual relations among states of particular variables, the results being visualized by ordination techniques (UPGMA clustering and PCA). For each acoustic variable, the extent of within-individual variation relative between-individual variation was expressed using the W/B index, computed as a ratio of the mean span of within-individual variation (min-max) relative to the span of minimum and maximum mean values for all individuals under study. Significance of the differences in particular variables between SF and SD was tested using a Chi-square paired comparison test. The analyses were carried out using Microsoft Excel and Statistica 6.0 software.

Semantic analysis. Although there is perhaps no doubt about the general meaning of the advertising behaviour discussed in the present paper, the role and semantic meaning of individual

components of the display are apparently unknown. In this study we applied a technique used in the semantic analysis of languages — amplification of a component using data on its appearance in comprehended contexts (de Saussure, 1986).

RESULTS

1. Seasonal Dynamics in Advertising Calls

The number of males performing active acoustic advertising changed extensively during the season and in a similar way on every year of the study (Fig. 3). The peak in advertising activity was always found during August. Nevertheless, advertising calls were recorded throughout the season with the earliest incidence on April 18, 2004 and the latest record on September 20, 2005. The maximum number of advertising males recorded on a single transect was 30 on August 14, 2001.

TABLE 1. List of advertising males under study (1a–30), their incidence in the study area during 2001–2006 (supplemented with 1 male from South Moravia: lan), and total number of the analysed records. The detailed phonologic analyses were undertaken only with records in which both the male identity was confirmed for sure and which technical quality was acceptable in all respects ($n = 799$). Structural and syntactic analyses operated with all the complete set of call records ($n = 2,924$)

Male code	Year						Analysis (n calls)	
	2001	2002	2003	2004	2005	2006	Syntagmatic	Phonologic
1a	+	+	–	–	–	–	31	10
1	–	–	–	+	+	+	64	5
2	+	–	+	–	–	–	19	16
4	+	+	+	+	+	+	248	80
6a	+	+	+	+	+	+	151	49
6b	+	–	–	–	–	–	103	6
10	+	+	+	+	+	–	23	10
12a	+	+	+	+	+	+	225	225
12b	+	+	+	+	+	+	111	78
12c	–	–	+	+	+	+	91	21
12	–	–	–	–	+	+	39	10
14b	+	–	–	–	–	–	20	11
17a	+	+	+	–	–	–	268	125
17b	+	+	+	+	–	–	83	23
17	–	–	–	+	+	+	155	21
20a	+	+	–	–	–	–	43	28
20b	+	–	–	–	–	–	15	8
21	–	–	–	–	–	+	20	7
22a	–	–	–	–	+	–	26	13
22b	–	–	–	+	+	–	55	10
30	–	–	–	+	+	+	14	12
lan	+	–	–	–	–	–	31	31
Others							1,089	–
Total							2,924	799

2. Phonologic Structure of a Call

Below, we provide the data on (a) the general characteristics of acoustic design, (b) variation of particular call components (based on 799 calls of 22 males), and (c) within-individual variation. The latter aspect is here exemplified by the record obtained during 2001 from males 12a and 17a, which was not only particularly large (126 calls for 12a, and 111 for 17a) but also proportional in respect to parts of the season and situations, which was not the case for recordings of other males.

2.1. Motif A

(a) Typically, motif A consists of a two subseries: (A1) a cluster of multiharmonic FM notes followed by (A2) a sequence of V-shaped notes in which frequency steeply increases, all emitted at almost the same repetition rate (Fig. 2). The bordering note between the two syllabic series A1 and A2, denoted as a0, was characterized by fmax below 30 kHz (83% of syllables a0 had fmax under 25 kHz). Three major types of A1 syllables differing in shape were distinguished: L (with a quasi-constant part, ± 2 ms), I (enf-fmax, ± 2 kHz) and V (enf-fmax, ± 2 kHz).

(b) The number of notes in A1, i.e., those preceding a0, ranged from 2 to 15. A regular sedentary display usually contained 5 or 6 notes, but the number of notes can increase in the presence of another individual. A1 notes preceding a-4 were not analysed in detail due to their poor signal-to-noise ratio. A2 notes, i.e., those following a0, transformed into high frequency pulses with V-shaped structure. These high-frequency pulses had a lower intensity, probably due to atmospheric attenuation, and were therefore not included in detailed analysis. Syllables of A1 may contain a ‘hook’ at the beginning, and in several instances this ‘hook’ was prolonged and attached to a preceding note. In several instances we observed a gradual transition from an echolocation call to the A1 series: in these cases the first A notes were of exceptionally high frequency and the whole A1 series was thus ‘U-shaped’. Introductory notes of motif A dropped from high to low frequency (33.2–37.5 to 13.9–22.3 kHz) over the course of 5 to 10 notes.

(c) The most pronounced between-individual differences were in the number of A1 notes (A1nnote) and in the start (stf) and end frequency (enf) of a-1

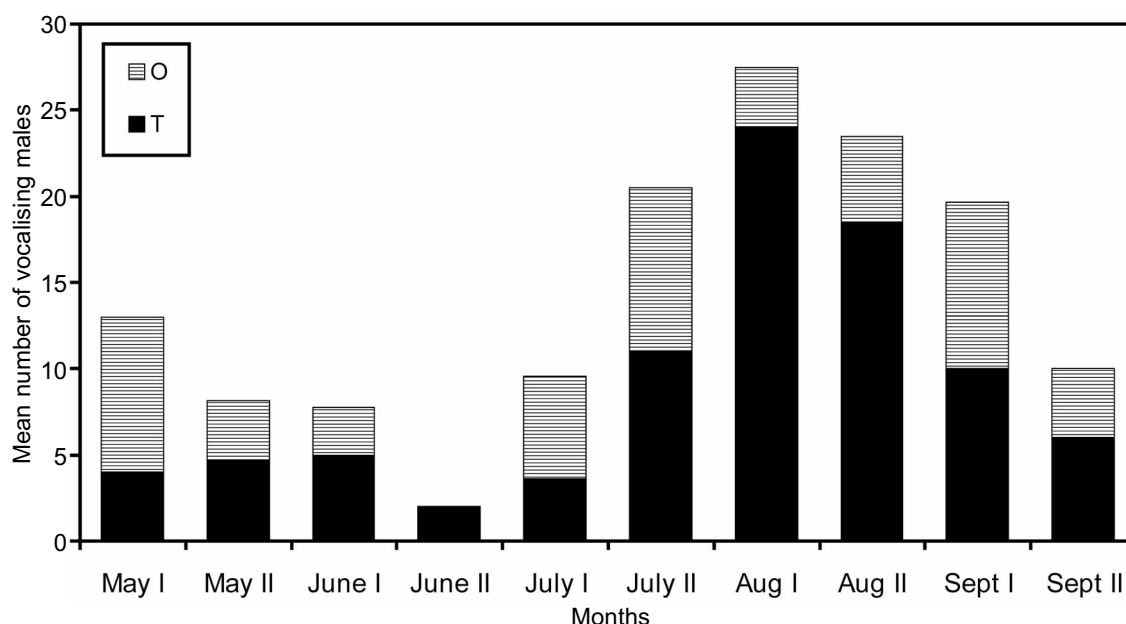


FIG. 3. Seasonal course of mean number of vocalizing males on a 6-km transect during the seasons 2000–2006. T — average number of advertising males on the standardised transect. O — average number of all vocalising males recorded during continuous whole-night observations at sites situated on the transect. I and II refer to the first and second half of particular months

(Mann-Whitney U test: $z_{A1note} = 7.63$, $z_{stf} = 13.50$, $z_{enf} = 11.07$, for all $P < 0.001$).

Within-individual analysis of variation of males 12a and 17a showed that: 1) In both males, syllable I prevailed throughout A1 with a gradual decrease from a-4 to a-1 (62–96% in 12a and 4–32% in 17a), and was replaced by the syllable L (4–37% in 12a, and 4–64% in 17a). 2) Syllable V first appears in a-2, prevails in the most variable syllable a-0 (51%), and is the typical form of notes in A2 (note a1: 100%). 3) Duration of particular notes within A series increases up to a-1 and then declines. The median duration of a-4 to a-1 differ by 4.1 ms in male 12a, and 3.2 ms in 17b, respectively. 4) The frequency of maximum energy (fmaxe) remains relatively constant within A1 (i.e. up to and including syllable a-1) while it increases again in A2. 5) The last note of A2 (with fmaxe around 104 kHz) often contains a ‘steep FM tail’ followed by a silent interval (Fig. 4). 6) Note a-1 showed low within-individual variation both in duration and fmaxe while, at the same time, both these parameters exhibited between-individual variation (Table 2). In contrast, a-0, the last A note with fmaxe ± 30 kHz, was the most variable, above all in duration, fmaxe and shape. 7) No essential differences in the structural design of motif A were found between SD and SF (χ^2 tested at $P < 0.05$). The population sample of these parameters ($n = 22$ males) show the same trends (compare ANOVA’s F in Table 3).

2.2. Motif B

(a) This motif was composed of a single multi-harmonic broadband FM note which was usually of a lower intensity compared to the notes of motifs A and C, and from which it differed markedly in its frequency characteristics and duration (Tables 3 and 4).

(b) Motif B typically separated motifs A and C. It was often inserted also after a complete ABC sequence, and during sedentary display it could be repeated in a short series with inserted C or E motifs (up to seven repetitions). During songflight it can appear alone among echolocation calls.

(c) Compared to other phonologic variables, the frequency characteristics of motif B exhibit extremely low values of W/B, i.e. their within-individual values vary within a narrow range while between-individual variation is broad (compare also ANOVA’s F in Table 3 and Fig. 5). The frequency variables benf and bfmaxe were positively correlated with fmaxe and enf of a1-3 of ($r = 0.46$ – 0.51 for benf, $r = 0.31$ – 0.67 for bfmaxe, for all $P < 0.001$). Correlation with a4 and a0 was weak and insignificant, as was correlation with the frequency variables of motif C ($r = -0.22$ to 0.01)

2.3. Motif C

(a) The motif C was typically composed of syllables of uniform ‘V-shape’, which sometimes changed into an ‘L-shape’ pattern at the last or penultimate

TABLE 2. Phonologic characteristics of motifs A, B and C in males 12a and 17a: number of notes in series A and C (Annote, Cnnote), median, minimum–maximum values of particular frequency (fmaxe, lof, stf, enf, in kHz) or temporal variables (ndur, ii, in ms) for index notes a1, B, and c3. Sample sizes (*n* total) are shown in *italics*

Motif A	A nnote		a-1 note									
			ndur		stf		fmaxe		enf		ii	
	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a
	5	6	6.7	6.5	23.75	31.1	17.7	18	13.7	15.9	2.8	3
	2–10	3–10	4.8–9.5	3.8–9.1	18.1–30	22–37.6	15.4–22.4	16.7–22.3	10.6–21.5	12.5–23.3	0–4.9	0–5.1
	<i>180</i>	<i>106</i>	<i>180</i>	<i>106</i>	<i>180</i>	<i>106</i>	<i>180</i>	<i>106</i>	<i>180</i>	<i>106</i>	<i>180</i>	<i>106</i>
Motif B	a-b ii		B note									
			ndur		stf		fmaxe		enf		b-c ii	
	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a
	58.2	50.1	4.3	4	52	39.1	24.9	20.5	17.3	11.2	20.6	18.2
	<i>158</i>	<i>103</i>	<i>77</i>	<i>95</i>	<i>77</i>	<i>95</i>	<i>77</i>	<i>95</i>	<i>77</i>	<i>95</i>	<i>158</i>	<i>95</i>
Motif C	C nnote		c3 note									
			ndur		stf		lof		enf		ii	
	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a	12a	17a
	5	6	4	4.1	32.6	48	26.6	37.15	26.75	48.5	1.9	1.9
	3–6	4–8	2.6–5.2	3.4–5.6	29.2–37.4	42.7–51.8	24.7–29.6	35.8–40.2	23.1–40	45–59.5	0.8–3.1	0–2.6
	<i>133</i>	<i>51</i>	<i>133</i>	<i>51</i>	<i>133</i>	<i>51</i>	<i>133</i>	<i>51</i>	<i>133</i>	<i>51</i>	<i>133</i>	<i>51</i>

notes. As a rule, motif C was performed after motif B and was separated from C by a silent interval.

(b) Number of notes within a C cluster ranged from 3 to 11. In a regular sedentary display the motif consisted mostly of 5 or 6 notes and followed a quite uniform design. The third note (c3) had the highest relative intensity. The duration of C notes continually increased to c3 and then decreased, except for the last syllables with ‘steep FM tail’. The increase of the lowest frequency of syllables (lof) was pronounced within series. In complex calls, motif C can terminate with very high frequency syllables of a poor resolution similar to A2 series, or with a ‘steep FM tail’ on the last syllable. Notes c2 and c3 exhibited the least within-individual variation among c notes and their lof and enf were found to vary within very narrow limits specific to the particular individual (compare extreme values of the W/B index and ‘individual’ factored ANOVA’s *F* — Table 3 and Fig. 5).

The frequency characteristics (particularly lof and enf) of individual notes c1 to c5 were all mutually closely correlated ($r = 0.77–0.99$ for lof, $r = 0.77–0.96$ for enf, $r = 0.57–0.96$ for stf, for all $P < 0.001$). There was also a good correlation between lof and enf within motif C ($r = 0.77–0.99$, $P < 0.001$) and highly significant correlations to benf ($r = -0.26$ to -0.35 , $P < 0.001$). Only faint and mostly insignificant correlations were found between frequency variables and the duration of particular notes ($r = -0.02–0.14$), but significant correlations were found between duration and the number of notes of the C series and also the A1 series

($r = 0.55–0.77$ for Cnnote, $r = 0.52–0.79$ for Cdur, $r = 0.30–0.40$ for A1dur, $r = 0.42–0.60$ for A1nnote, for all $P < 0.001$).

(c) Males 12a and 17a, for which the largest samples were available, differed markedly in all frequency parameters and number of C notes (Mann-Whitney *U* test, $z_{\text{Cnnote}} = 6.77$, $z_{\text{stf}} = 10.50$, $z_{\text{lof}} = 10.50$, $z_{\text{enf}} = 10.50$, for all $P < 0.001$), while no differences were found in temporal parameters of particular notes. Both bats showed a tendency to shorten the interval between notes toward the end of the series, starting from c4–c5 in male 12a, and from c3–c4 in male 17a. The two males differed also in the sequence of lowest frequencies within a single C string, though this depended on the number of notes: the number of notes and the difference in lof between successive notes within a C series were all negatively correlated at very high statistical significance level (12a: lofc2–c1, $r = -0.39$; lofc3–c2, $r = -0.43$; lofc4–c3, $r = -0.63$; lofc5–c4, $r = -0.64$, for all $P < 0.001$; 17a: lofc3–c2, $r = -0.56$; lofc4–c3, $r = -0.78$; lofc5–c4, $r = -0.81$; lofc6–c5, $r = -0.69$, for all $P < 0.001$ — Fig. 4).

2.4. Motif D

(a) Motif D was a series of long FM/qCF multi-harmonic notes, mutually separated by long silence intervals that varied greatly both in duration and frequency design. Motif D was an inconstant but regular component of advertisement calls. It appeared in 66% of records from late summer period, but in only 21% of those recorded in early the period (May to July). D motif was further characterized

TABLE 3. Phonologic characteristics of particular notes of three main motifs (A, B, C) in the total sample ($n = 799$, 22 males): a list of median and range for main frequency (fmaxe, lof, stf, enf, in kHz) and temporal variables (ndur, ii, in ms), supplemented with index of within/between individual variation (W/B), and F -values (in italics) of one-way ANOVA for respective variables. All F values for 'individual' as the factor, were significant at $P < 0.001$, except for that of a0stf with $P < 0.01$

Parameter	A			B		C					
	a4	a3	a2	a1	a0		c1	c2	c3	c4	c5
ndur	4.07	4.94	6.33	7.2	5.55		2.49	3.3	3.85	3.9	3.88
	1.4–9.0	2.2–7.8	3.8–9.3	3.8–9.5	1.6–9.0		1.2–3.9	2.0–6.2	2.4–6.5	2.3–6.8	1.0–10.6
	0.404	0.511	0.516	0.554	0.573		0.405	0.321	0.390	0.342	0.367
	15.50	16.86	14.77	10.39	6.79		18.60	22.89	22.61	21.48	13.40
fmaxe, lof	17.23	17.66	18.09	18.09	21.96		36.6	36.4	36.75	37.9	40.48
	12.0–32.3	11.0–25.3	15.0–25.0	15.3–24.1	16.4–30.0		24.1–61.2	24.5–54.3	24.7–55.6	25.2–55.1	28.5–64.9
	0.315	0.316	0.401	0.444	0.685		0.126	0.101	0.105	0.217	0.299
	21.85	18.51	14.05	13.63	3.382		651.20	1,437.50	1,096.30	300.06	46.25
stf	24.55	26.27	27.56	27.56	28.95		41.7	45.6	46.5	47.7	49.52
	12.5–37.5	14.2–35.7	18.0–35.7	17.0–37.6	19.0–38.3		28.2–66.3	28.4–67.2	28.4–68.5	31.1–67.6	32.3–70.5
	0.466	0.413	0.364	0.353	0.538		0.213	0.179	0.206	0.262	0.328
	18.09	38.51	5.09	33.11	2.15		218.87	394.55	343.64	239.80	52.85
enf	13.5	13.9	14.62	15.05	24.6		43.93	44.6	44.1	48.67	52.11
	9.4–21.5	9.4–20.9	11.2–25.8	10.0–32.3	12.5–45.7		24.7–66.8	23.8–65.9	23.1–66.3	27.3–68.1	33.1–75.0
	0.510	0.421	0.387	0.388	0.583		0.152	0.136	0.180	0.263	0.381
	11.25	25.61	24.11	13.41	4.32		474.63	614.35	319.08	113.31	37.86
ii	3.99	3.56	2.83	2.54	3.85		3.12	2.5	2.1	2.1	2.03
	1.2–9.7	0.8–7.5	0–6.0	0–6.4	0–9.9		1.2–4.6	0–4.1	0–4.0	0–4.8	0–4.2
	0.322	0.356	0.431	0.460	0.498		0.421	0.305	0.375	0.443	0.460

TABLE 4. Overall temporal (dur, ii, Aii, all in ms) and structural (nnote) characteristics of advertisements calls in total sample ($n = 799$, 22 males). A-A is an interval between first notes of two consecutive calls within a continuous display. Median, minimum–maximum, and individual F values are shown

ABC	A1		B	C		a0-B	B-C	A-A
dur	nnote	dur	dur	nnote	dur	ii	ii	Aii
163.7	6	50	3.92	6	34.54	55.22	19.95	625.3
55.4–220.2	2–12	16.8–88.6	1.96–5.1	3–10	17.5–93.3	38.02–67.8	15.2–31.9	264.4–2519.6
–	16.80	25.93	–	42.63	55.84	17.26	15.69	–

by particularly large syllabic variation. The most contrasting and common syllables were classified into the categories L, N, I, U, V, W, M, IC (see Fig. 6). In syllables that contained a qCF part (L, N, U, IC), we rather measured the middle frequency of the qCF part (mif) instead of fmaxe, which was also found in the ‘hook’ or ‘tail’ of these syllables.

(b) Particular syllables could occur separately or in clusters of 2 to 9 notes. Furthermore, motif D appeared as a single cluster (D1 in 54.76% of recordings) or as a series of distinct clusters separated by silent intervals longer than treble the average interval between notes of the previous cluster, i.e. two (26.04%), three (10.71%), four (4.91%), five (1.79%), six (0.74%), seven (0.60%), eight (0.30%) or even nine clusters (0.15%). When the *D* motif was performed as a series of a single note, the respective interval was at least three times longer than the note duration. Further details of the first three clusters, which represent 91.51% of the total sample of *D* notes, are shown in Table 5. We found no rule

for the distribution of particular syllables within a cluster, except that syllable I predominantly appeared as a terminal note, and syllable W almost never appeared at the beginning of a cluster.

(c) Motif D clearly represented the most variable component of calls, both between and within individuals. Variability appeared in the syllabic combinations, in structural rearrangements of particular syllables within a call, and in the syntactic composition of particular clusters. Although the major syllables appeared in the repertoire of almost all males under study, we found clear between-individual differences in preferences for particular syllables, as well as in the appearance of rare syllables or of major syllables of distinctive frequency design (Table 6).

2.5. Motif E

(a) Motif E was a series of weak steep FM pulses quite uniform in their repetition rate and syllabic structure. It appeared with moderate frequency in

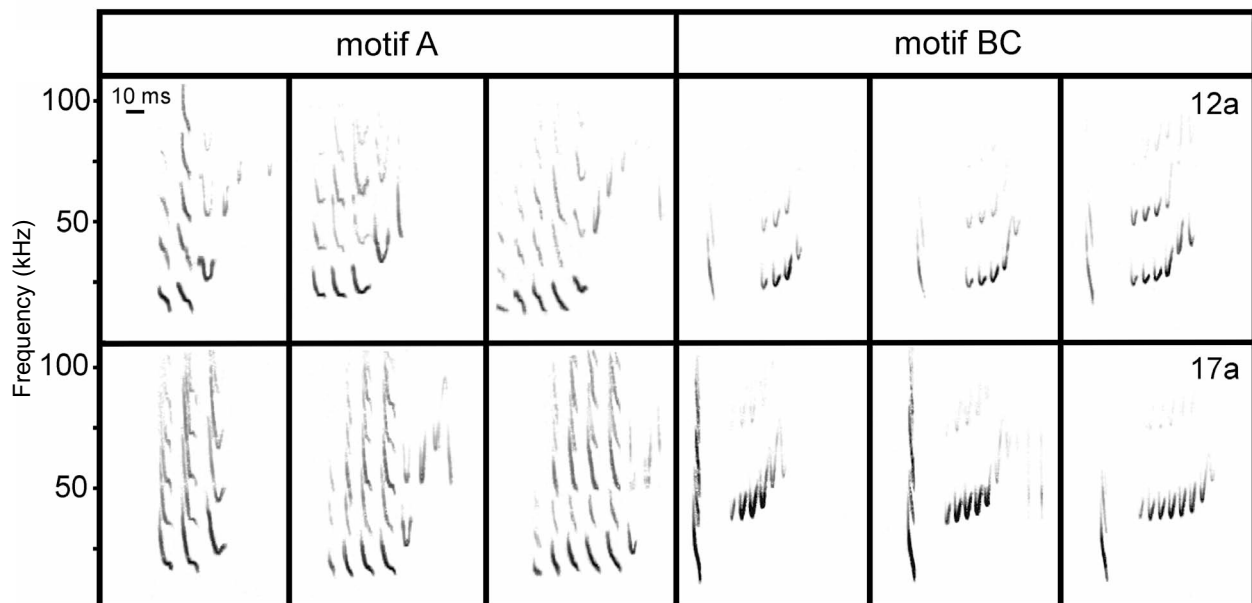


FIG. 4. Variation in motifs A and syntagma BC in males 12a (upper row) and 17a (lower row). Note differences in the end frequency of motif B, and the lowest frequency of motif C and within-individual variation in number of notes

complex calls within intervals between main motifs of ABC calls or as continuing motif after C (denoted as cE) or B (bE). In motif B pulses of motif E were often found as a series of distinct clusters separating syllables of motif B (b1E, b2E etc.). All notes composing the E motif were almost identical and represent a single specific syllable: an FM sweep of short duration (0.58–3.60 ms) starting at roughly 70 kHz and dropping down to 20 kHz (Fig. 2). The frequency of maximal energy ranged

from 26.6 to 62.45 kHz with median 44.69 ± 7.32 kHz.

(b) Motif E appeared almost exclusively in sedentary displays. Quite typically it was associated with multiple repetitions of motif B, where it separated particular B clusters, and it was also found between motifs C-B, B-D, C-D, and less often between D-D clusters. The maximum number of cluster repetitions of motif E was 6. A single E cluster appeared in 65.5% of all cases, the most common

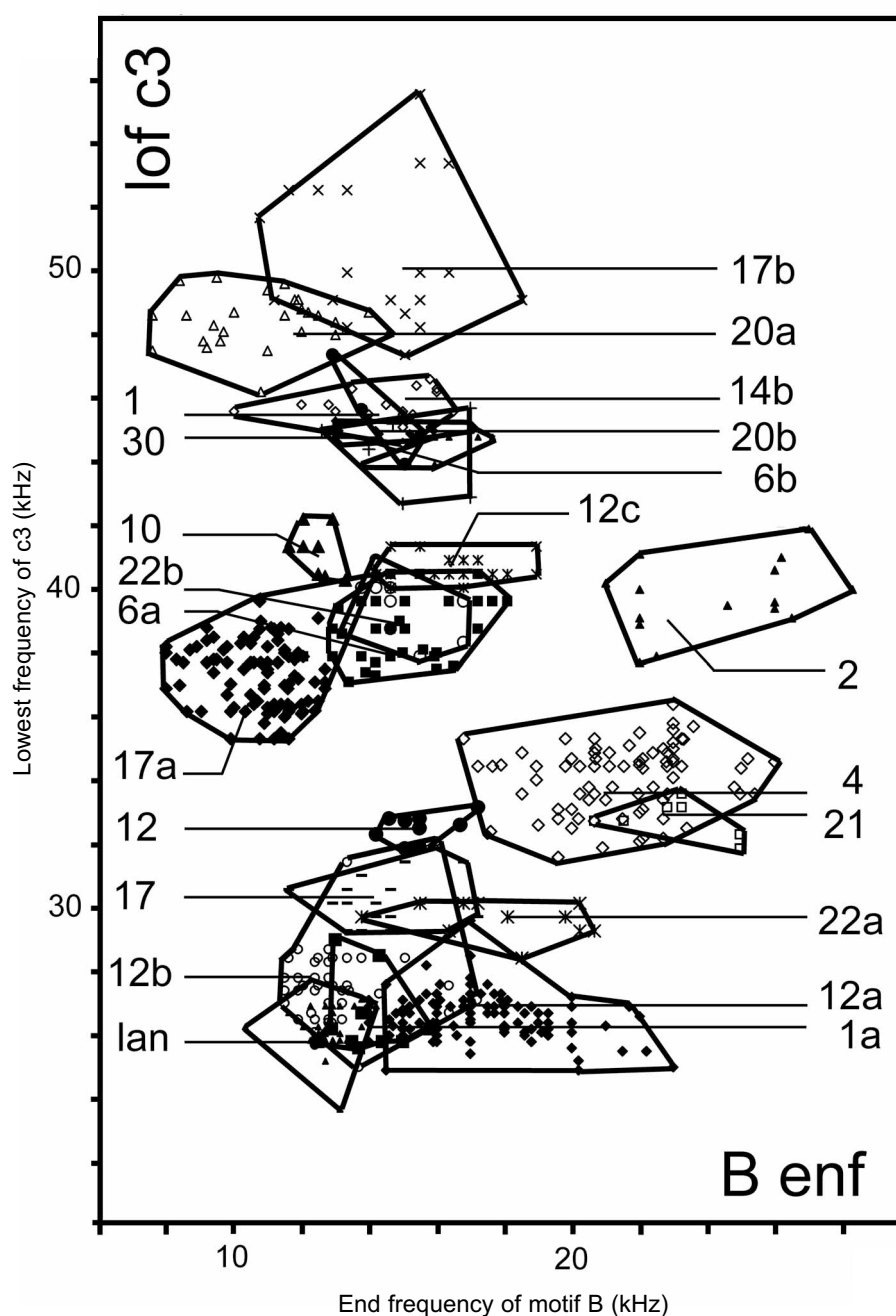


FIG. 5. Scatter plot of the lowest frequency of c3 (lof c3) versus end frequency of motif B (Benf). Note the limited extent of variation in individual males, and clear differences among them in the respective variables

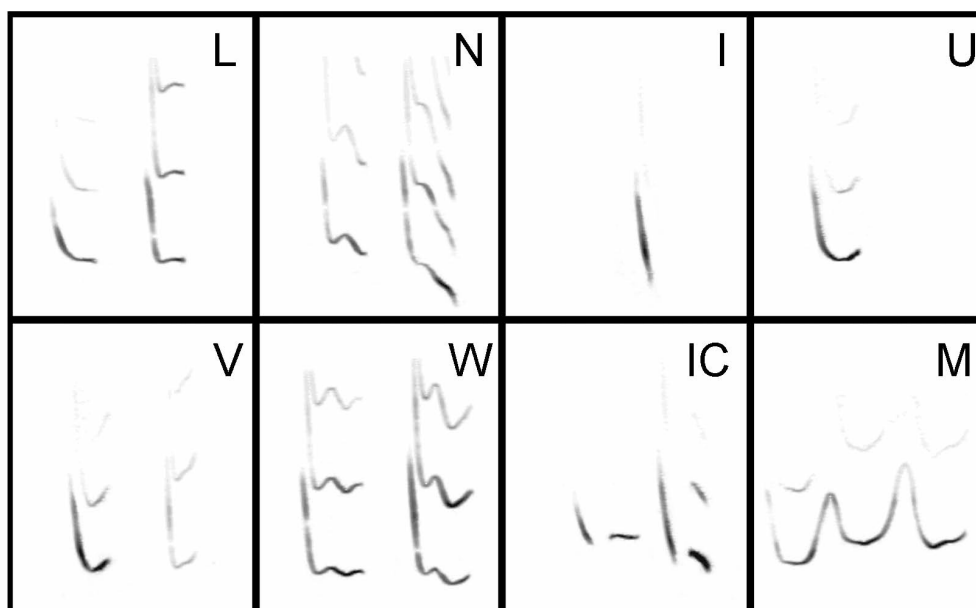


FIG. 6. Sonograms of the major syllables of the motif D. Besides differences in shape, the syllables are defined by the relation between middle frequency of the quasi-constant frequency region (mif) and end frequency (enf)

being b2E: 23.7%, b3E: 7.2%, b4E: 2.6, b5E: 0.6%, b6E: 0.3%.

(c) In contrast to its syllabic uniformity, the number of notes composing a single E cluster varied greatly, from 1 to 18, but most often consisted of 4 to 7 notes (Ennote: median = 5 ± 3.23 , variance = 10.43 — Table 7). In four males we recorded, in addition to typical E notes, a series of ‘single element distress calls’ after motifs B or C that had significantly longer duration and lower frequency than E notes ($n = 58$, median: ndur 2.68 ± 0.46 , ii 6.17 ± 0.87 , fmaxe 27.5 ± 2.47).

3. Syntax of Calls

As a rule, advertisement calls are emitted in a continuous series at nearly constant repetition rate

(1.2 per second on average). Nevertheless, more than a quarter of all calls differed considerably from this average, and within-individual variation was comparatively large, particularly due to multiple repetitions of certain motifs that often accompanied sedentary display vocalisations, or under a changed behavioural context (e.g., presence of other individuals).

Individual motifs did not appear randomly within a call but formed clearly marked syntagmatic strings. The central component of the string was the sequence ABC, which was retained in almost all the calls despite the temporal and contextual variation produced by the appearance of the satellite syntagmatic sequences (Fig. 7).

The ABC syntagma was a core structure of 94.8% of all calls (97.1% in sedentary display,

TABLE 5. A comparison of phonologic characteristics of the advertisement calls that included D motif in males 4, 6a, 12a, 12b and 17a: median frequencies of B note (Benf), c3 note (lof c3), and the first cluster of motif D (D1 fmaxe, mif) (all in kHz), and number of notes (median and range) in successive D clusters (D1–D3 nnotes)

Parameter	4	6a	12a	12b	17a
B enf	21.83	14.80	17.33	12.65	11.35
lof c3	33.73	37.79	26.17	27.22	36.69
D1: fmaxe, mif	32.80	25.40	21.85	31.30	28.50
D1 nnote	3	2	5	4	4
	1–6	1–6	1–9	2–7	1–7
D2 nnote	3	4	6	4	4
	2–4	1–6	1–9	3–6	1–5
D3 nnote	3	3	4	3	3
	1–4	3–5	2–7	1–5	1–5
n total (clusters of D)	80	49	86	78	77

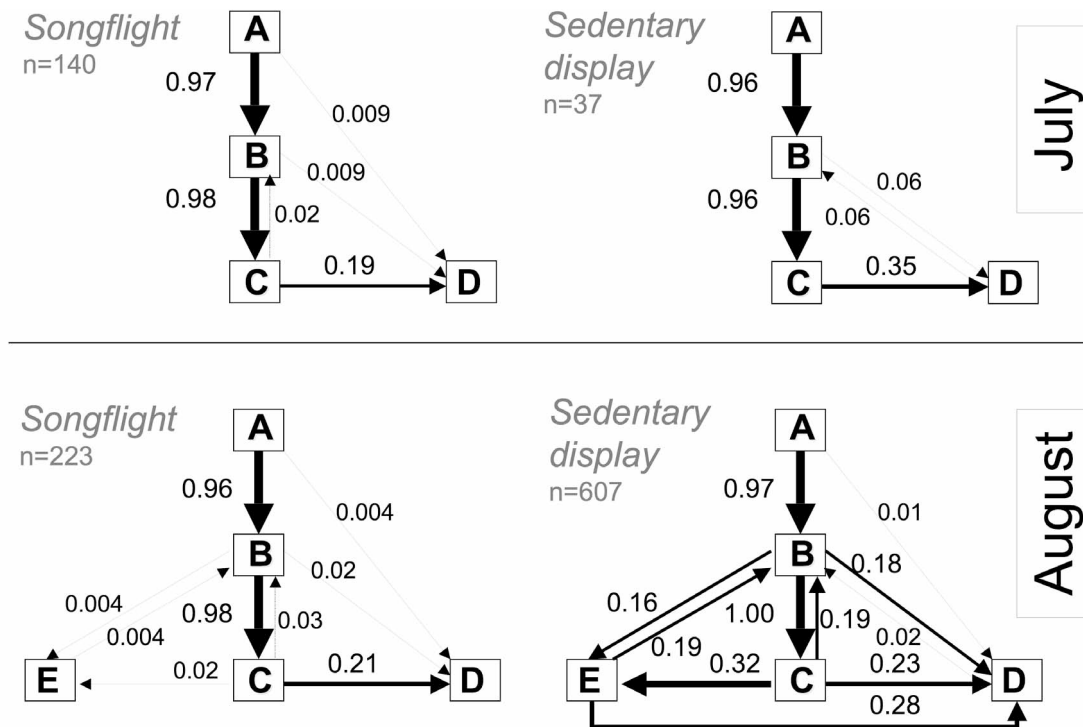


FIG. 7. Transition probabilities of the motifs of advertisement calls during songflight and sedentary display in July (upper) and August (lower). The numbers refer to frequencies of incidence of particular node in the total sample (n gives the number of samples analysed)

91.1% in songflight). It formed the complete call in 38.6% of the analysed records and represented a core element of ABCD calls (a further 47.5% of all calls — see Table 8). Calls composed exclusively of the syntagma ABC prevailed at the beginning of season (May–July) and were performed mostly during songflight, whereas ABCD-calls prevailed at the peak of the mating season (August–September) and also during regular sedentary display. Less frequently strings of single motifs A, B, C or D were emitted separately or in simple syntagmatic composites (AB or BC). Fragmentary calls composed of one or two motifs appeared predominantly in songflight display and at the beginning of the season, while the more complex calls (four motifs with repetitions) were almost exclusively produced during sedentary display and at the peak of the mating season (Tables 8, 9, and 10). The syntax of the more complex calls, i.e. those with multiple repetitions of motif B or BC, and/or with long D-sequences, was further complicated by the insertion of E motifs (steep FM pulses) in between main motifs: BEB, BED, CEB, CED. 19.2% of advertisement calls included E notes, though the number of notes in a cluster and/or rate of appearance of motif E in songs varied among particular males.

The duration of the song increased considerably with multiple repetitions of the basic motifs B and C

and with the insertion of motifs D and E and repetitive sequences of them. As demonstrated by results of both the correlation analysis and PCA (Fig. 8), particularly compact integration is apparent within motif C, while the relations among A notes are obviously weaker and A seems to be unrelated to other elements in motifs A, B or C. Worth mentioning is that in respect to their variation dynamics (visualized in the factor space of PCA), motifs C and B represent orthogonal, i.e. completely independent, structures. Except for relations between *benf* and *cstf* ($r = 0.29$ – 0.05 , $P < 0.05$), the correlations between *benf* and other frequency variables are markedly weak and not significant ($r = -0.22$ to 0.13 , $P > 0.1$). Also worth noting is that *bnfur*, a parameter otherwise unrelated to any other variable ($r = -0.14$ to 0.22 , $P > 0.1$) exhibits highly significant negative correlations with *clorf* ($r = -0.64$ to -0.77 , $P < 0.001$) and *cenf* ($r = -0.30$ to -0.55 , $P < 0.001$).

4. Within- versus Between-individual Variation, Non-overlapping Acoustic Characteristics, and Possibility of Individual Recognition

Almost all variables studied, both phonologic and syntactic, show considerable variation, with between-individual variation exceeded within-

TABLE 6. Phonologic characteristic of advertisements calls of males 4, 6a, 12a, 12b, 17b in successive seasons 2001 to 2005: median frequencies of B note (Benf), c3 note (lof c3), and the first cluster of motif D (D1fmaxe, mif; all in kHz), median number of notes in successive three clusters (D1–D3 nnote), and percentages of particular syllables in motif D. The results of two-way ANOVA for year and individual (ind) as factors are also presented; the variables with highly significant effects ($P < 0.01$) in bold

Parameter	4a				6a				12a			12b		
	2001	2003	2004	2005	2001	2002	2003	2005	2001	2003	2004	2001	2003	2004
<i>n</i> calls	42	20	13	5	34	10	2	3	41	30	15	43	20	15
B enf	20.67	22.55	22.10	22.00	14.64	15.30	14.35	14.90	17.40	17.50	17.10	13.14	12.40	12.40
lof c3	34.50	33.95	33.10	33.40	37.70	39.60	38.70	39.00	26.60	26.40	25.50	27.56	27.60	26.50
nnote D1	3	3	3	3	2	2	1	1	5	5	5	4	4	4
nnote D2	3	3	2	3	4	3	0	0	5	6	5	4	4	3.5
nnote D3	3	2	3	0	4	3	0	0	4	4.5	7	5	3	3
mif, fmaxe D1	32.20	33.59	32.80	34.00	24.80	27.50	25.43	30.70	21.50	22.50	21.00	30.40	31.70	33.00
Syllabic frequencies														
Motif D: L%	21.19	7.69	15.79	6.67	9.86	6.25	0	0	32.76	38.64	39.06	11.17	12.16	11.32
N%	45.76	69.23	55.26	73.33	5.63	18.75	0	0	4.02	6.82	0	59.78	55.41	39.62
I%	20.34	16.92	13.16	13.33	26.76	12.50	33.33	25	12.64	5.30	10.94	6.15	4.05	11.32
U%	0	3.08	5.26	6.67	11.27	0	0	0	21.84	35.61	34.38	6.15	16.22	13.21
V%	5.08	1.54	5.26	0	1.41	6.25	0	0	13.22	7.58	9.38	7.82	1.35	3.77
W%	7.63	1.54	5.26	0	1.41	0	0	0	1.15	1.52	0	7.26	10.81	20.75
IC%	0	0	0	0	2.82	0	0	0	14.37	4.55	6.25	1.68	0	0
M%	0	0	0	0	40.85	56.25	66.67	75	0	0	0	0	0	0
<i>n</i> Dnotes	118	65	38	14	71	16	3	4	174	132	64	179	74	53

individual variation. Frequency variables showed this pattern more prominently than temporal variables (e.g., ndur and ii vs. lof, enf, or stf). One-way ANOVA showed significant individual variation in nearly all frequency and temporal variables, except a0stf ($F = 2.15$, $P = 0.03$) and aldur ($F = 2.70$, $P = 0.02$). Individual differences were particular strong for the frequency components of c2, c3, and B, especially lof of c2 and c3 and B enf, lof, which also had the smallest W/B index (within-relative to between-individual variation). The variable fmaxe showed much higher variation, apparently because fmaxe often occurred in 'hook' or 'tail' part of a pulse in A and C notes. In contrast, the overall variation in all these characteristics in the total sample was considerably higher. High variation was also found in many temporal characteristics: duration (or number of notes) of both A and C motifs, intervals between individual calls, the occurrence of particular D syllables, and the number of notes within D and E motifs. In these variables, discrepancies between within- and between-individual variations were much less pronounced than in the above mentioned frequency variables, of course.

Based on individual differences in c2 and c3 frequency variables and their orthogonality to B variables, we developed a procedure for acoustic recognition of individual males and applied it for field identification of particular males. The identity of individuals provided by the procedure was tested by

recapture of marked males and by radiotracking of three males. In all instances reliable individual recognition was confirmed. The procedure included the following steps: (i) identification of c2 and c3lof for the male in question and comparison of these values with a list of individual-specific ranges recorded for particular males from previous investigations. Discrimination between the males with similar lof c3 was pursued with comparisons focused on (ii) end frequency of B (B enf), and (iii) shape and representation of particular D syllables. Individual identity was further confirmed by examination of the following characters: (iv) difference between start or end frequency of c3 and its lowest frequency (stf-lof, enf-lof); (v) end frequency of a-1(enf a-1); (vi) differences of lof c1 to cx ('shape of motif C') during regular sedentary display; (vii) mean number of syllables during regular sedentary display; (viii) relationship among relative intensity of parts A, B, and C; and (ix) tendency to join neighbouring notes in regular sedentary display. In most instances, the first two variables, c3lof and enf, were sufficient for a reliable discrimination (these variables are plotted for the 22 males in Fig. 5).

In each of the characteristics mentioned in the preceding paragraph the between-individual variation markedly exceeded within-individual variation, and individuality was retained over at least several years. There is a negligible effect of between-season variation contrasting with a pronounced effect of

TABLE 6. Extended

Parameter	17a		<i>F</i> (2+3)	<i>P</i> (2+3)	<i>F</i> (ind)	<i>P</i> (ind)	<i>F</i> (year)	<i>P</i> (year)
	2001	2003						
<i>n</i> calls	58	19						
B enf	11.50	11.20	60.32	0.000	92.29	0.000	0.49	0.748
lof c3	37.20	36.18	100.68	0.000	114.38	0.000	1.40	0.356
nnote D1	4	4	27.11	0.001	32.45	0.000	1.46	0.338
nnote D2	4	3.5	1.75	0.280	2.03	0.227	0.81	0.568
nnote D3	3	3.5	2.61	0.151	1.65	0.299	2.31	0.192
mif, fmaxe D1	28.50	28.60	17.59	0.003	25.59	0.001	2.51	0.170
Syllabic frequencies								
Motif D: L%	5.08	2.90	10.09	0.010	13.05	0.006	1.39	0.358
N%	27.92	18.84	11.77	0.007	19.17	0.003	1.00	0.486
I%	8.63	13.04	5.46	0.037	8.40	0.017	3.30	0.111
U%	18.78	15.94	4.27	0.061	5.48	0.041	0.19	0.936
V%	9.64	15.94	3.28	0.101	4.02	0.074	0.97	0.498
W%	26.90	31.88	19.98	0.002	29.13	0.001	0.40	0.801
IC%	3.05	1.45	2.88	0.127	3.67	0.088	1.20	0.415
M%	0	0	13.51	0.005	15.02	0.005	0.76	0.595
<i>n</i> Dnotes	197	69	8.06	0.016	3.84	0.081	7.13	0.027

individual specificity, at least for c3lof, benf, and the design of motif D (Dnote, fmaxe, percentages of L, N, W, M syllables).

5. Semantic Analysis

5.1. Contextual Influences upon Acoustic Variables

We found no essential difference in phonologic characteristics (both spectral and temporal) or in overall composition of the major motifs between sedentary and songflight display (as confirmed by χ^2 test, except for c3ii, a1stf and benf, which showed significant differences at $P < 0.05$ between SF and SD). The main syntactic string (ABC) composed the vast majority of calls both in songflight (91%) and sedentary display (96%). There were, however, differences in appearance of complex calls (i.e., those including motifs D and E and/or repetitions of main motifs), which were strictly associated with sedentary display. In contrast to the ABC string, which was apparently an obligatory component of advertisement calls, motifs D and E appeared as accessory components associated with sedentary display and thus, presumably, supplement the basic message of a call with information related to the contextual background of the sedentary display.

The sedentary display was mostly performed just at entrance of the male's roost, or in the nearest vicinity of it. At the peak of the mating season in August, about half an hour prior to dawn, males either sat at the entrance hole of their roosts and performed sedentary display, or joined the morning swarm and vocalised during flight, probably with members of their harem.

The most pronounced changes in temporal characteristics and syntax of advertisement calls were observed when a foreign individual of the same species was present. Resident males sharing neighbouring roosts apparently did not cause such responses — at several sites we regularly observed neighbouring males performing simultaneously, either in sedentary display or songflight, without any acoustic characteristics of their standard calls being affected. Nevertheless, the appearance of a foreign individual (as evidenced by echolocation calls appearing in the time-expansion records simultaneously with advertising call of the male) affected the display's acoustic characteristics in 70% of the cases. The typical responses of an advertising male to echolocation calls of another individual included (Table 11): (a) increased number of notes in A clusters (up to 15) and C clusters (up to 11); (b) increased repetition rate of ABC(D), which often resulted in (c) changes in syntax: occurrence of simple syntagms with high repetition rate in song-flight or more complex syntagms in sedentary display (such as the long sequences of motif D incorporating syntagms BE and BC), (d) a change in relative intensity of the advertisement call, probably caused by the bat's crawling out of his roost (direct observation).

An increase in repetition rate can be also accompanied by changes in a call and syllabic structure: incomplete fusions of particular notes in motifs A, C, and D; a shift of frequency in the first several syllables of motif A which resulted in appearance of 'U-shape' of motif A1 (originating from a gradual transition from echolocation calls in song-flight or from motif D in sedentary display). During

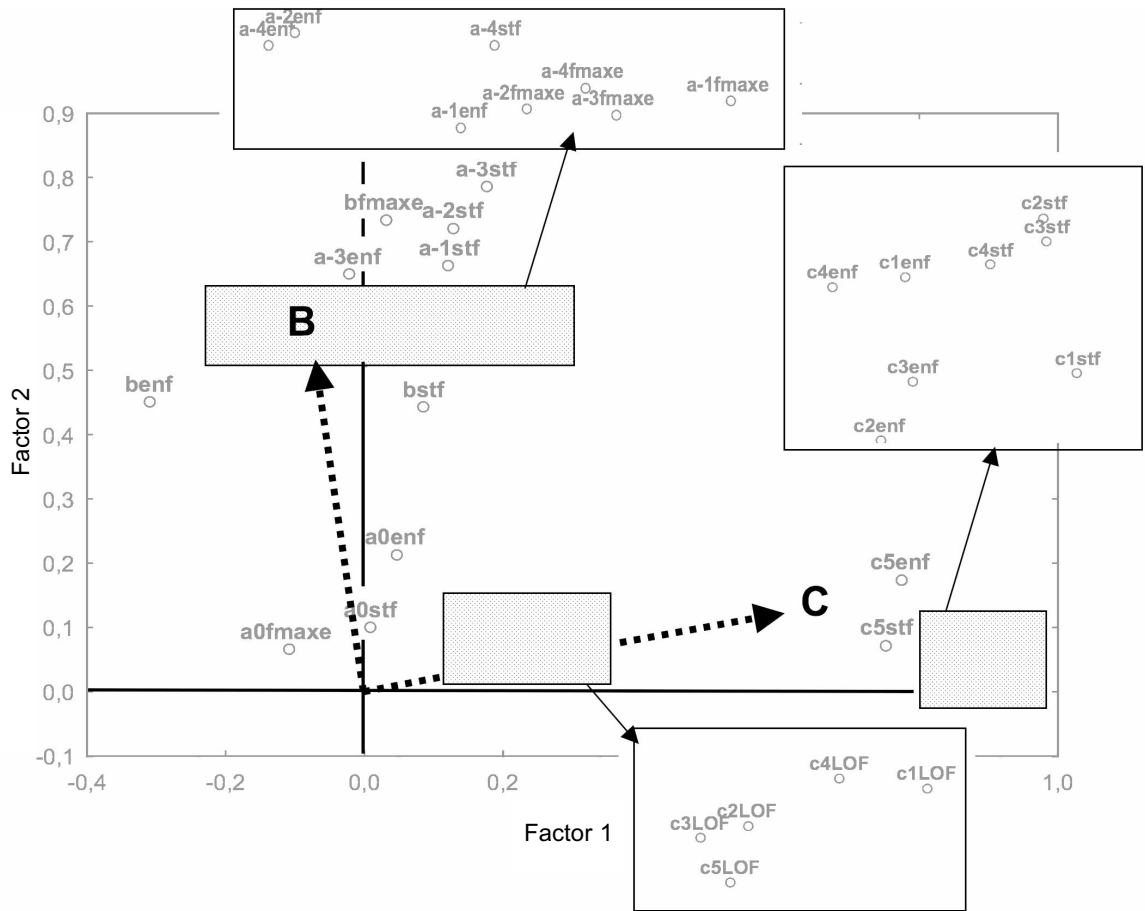


FIG. 8. Plot of the first two principal components for centroids of the frequency variables of motifs A, B, and C (1-*r*, varimax normalized, *n* = 799). The dotted arrows are vectors, nearly orthogonal to each other, to the centroids of all B and C variables

songflight, up to 10 steep FM notes similar to ‘single element distress calls’ (Russ *et al.*, 2004) can appear after motifs B or C (after syntagmatic strings

ABCB, ABC, or BCB). Unfortunately, the sex and status of alien individuals was not determined in most instances.

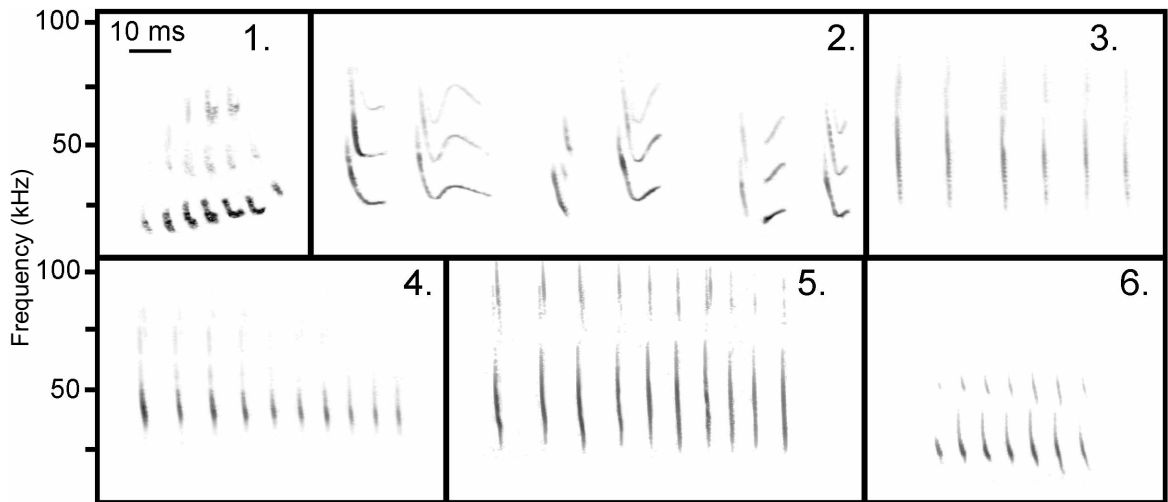


FIG. 9. Spectrograms of the calls similar to motifs A, D and E. 1. Multi-element distress call similar to motif A. 2. Calls from juveniles and females recorded in maternity colony. 3. Motif E. 4. Phase I of a feeding buzz. 5. Landing in the roost. 6. Series of single-element distress calls

TABLE 7. Motif E in advertisement calls of males 4, 6a, 12a, 12b, 17a: percentages of calls which included motif E, percentages of the syntagmatic strings including motif E, number of notes in cE (E series following motif C) and bE (E series following motif B) cluster, and temporal characteristics (ndur, ii in ms) of single E notes. Syntagms with multiple repetitions within a string are marked with (*)

Parameter	4	6a	12a	12b	17a
(1) % calls with motif E	14.52	19.2	14.09	39.64	19.78
(2) Syntagmatic context of motif E					
ABCEB	0	3.45	0	0	0
ABCED	61.11	44.83	81.25	72.73	30.19
ABCEBD	0	13.79	3.13	2.27	3.77
ABCEBED	5.56	31.03	15.63	11.36	43.4
ABCEBEBD	0	0	0	0	9.43
ABCEBEBED	5.56	6.9	0	6.82	9.43
ABCEBE(BE)*BD	0	0	0	2.27	1.89
ABCEBEBE(BE)*D	8.33	0	0	2.27	1.89
ABCEBEBE(BE)*D	0	0	0	2.27	0
others	19.44	0	0	0	0
<i>n</i> total (syntagms)	36	29	32	44	53
(3) cE nnote (median)	3	7	7	4	5
(min-max)	1-6	2-18	1-10	1-17	1-9
(4) bE nnote (median)	5	12	6	7	6
(min-max)	2-10	3-14	2-8	1-15	2-13
<i>n</i> total of subseries cE and bE	56	42	35	63	98
(5) E syllables, ndur (median)	1.31	1	1.5	1.31	1.53
(min-max)	0.73-3.30	0.58-2.60	0.8-3.60	0.8-2.46	0.87-3.48
(6) E syllables, ii (median)	6.31	6.1	8.7	6.45	7.7
(min-max)	4.86-9.58	4.28-7.50	4.8-16.2	4.1-14.9	5.4-13.93
<i>n</i> total of E notes	105	48	72	87	112

5.2. Origin of Particular Components of a Call

We compared the acoustic design of particular components of male advertising calls with the records of the acoustic repertoire of females and young (including behavioural context) obtained during observations in maternity colonies. Apparent similarities were found in syllabic design and syntactic pattern to motifs A, D, and E (Fig. 9):

1) motif A: repetitive low-frequency FM notes are typical characteristics of 'multiple element distress calls', which both males and females produce. Similar structures were also recorded in the maternity colony roost during emerging and landing of particular individuals as a gradual transition from irritation buzzes (Fig. 9). We also recorded low frequency multi-component calls ($n = 5$, nnote 7 to 14), probably produced by females, both when flying close to the maternity roost and in the territories of two males. Despite common syntactic design, the syllabic characteristics of these calls differed in having lower frequency (A1-note fmax: median, a-5 to a-0 12.2 kHz), narrower bandwidth, and shorter A-note duration (A1-note dur: median, a-5 to a-0 2.9 ms).

2) motif D: single notes or clusters composed of syllables typical of motif D were repeatedly recorded in the two maternity colonies ($n = 1,321$ calls). The D-like calls of mothers and pups ($n = 1,789$ notes) included seven syllabic types: L (41.3%), N (16.3%), I (27.5%), U (8.7%), V (1.1%), W (0.6%), and IC (4.6%). Most often they appeared separately or in loose sequences separated by longer intervals (68.8%), and in the form of double-note calls (24.9%). The maximum number in a cluster was five notes.

3) motif E: steep FM signals are typical of the terminal phase of echolocation during foraging, or are used in situations where there are higher demands on orientation (e.g., landing at the roost). Very short duration signals of broad bandwidth and high repetition rate are typical for these types of calls, which are similar to motif E. Compared to motif E, the notes of terminal phase I of the feeding buzz ($n = 91$, median: ndur 1.65 ± 0.26 , ii 7.9 ± 1.9 , fmax 42.00 ± 1.85) show lower variability, whereas landing at the roost was much more variable in frequency and temporal parameters, apparently due to differences in spatial situation and

TABLE 8. Syntagmatic structure of advertisement calls recorded at the early (May–June) and late season (August–September): percentages of respective syntagmatic strings in sedentary (SD) and songflight display (SF). *n* calls: total number of calls recorded. Others: the rare strings ABCDB, ABCDBCB*, ABCDBDB*, BCD, BCB, BCBCD, BD, B(B)*D, BD(B)*D, BCD(BC)*D, DBCBBB*, mostly recorded at single instances only. Motifs (or syntagms) with multiple repetitions within a string are marked with (*)

Syntagma	May–June			August–September			<i>n</i>
	SF	SD	<i>n</i>	SF	SD	<i>n</i>	
A	0.57	0.00	3	2.32	0.28	17	20
B	1.33	0.00	7	0.19	0.06	2	9
AB	1.71	0.00	9	2.70	0.11	16	25
BC	3.61	0.00	19	1.54	0.00	8	27
BC(B)*	0.57	0.00	3	0.77	0.00	4	7
ABC	71.16	53.85	410	62.16	21.83	718	1128
ABCB	0.00	0.00	0	0.97	0.77	19	19
ABCB(B)*	0.38	0.00	2	0.00	0.17	3	5
ABCBC	1.52	0.00	8	0.19	0.11	3	11
ABCBCB	0.38	0.00	2	0.19	0.06	2	4
ABCBC(BC)*	0.57	0.00	3	0.00	0.00	0	3
AD	0.38	0.00	2	0.00	0.61	11	13
ABD	0.38	1.54	3	0.77	0.72	17	20
ABCD	17.46	43.08	120	25.48	62.68	1269	1389
ABCB	0.00	0.00	0	1.54	7.55	145	145
ABCBB	0.00	0.00	0	0.19	1.87	35	35
ABCBB(B)*D	0.00	1.54	1	0.00	1.54	28	29
ABCBCD	0.00	0.00	0	0.00	0.17	3	3
Others	0.00	0.00	0	0.97	1.49	32	32
<i>n</i> total	100.00	100.00	592	100.00	100.00	2332	2924
ABC string present	91.08	98.46	546	90.73	96.75	2226	2771

context of particular landing efforts ($n = 104$, median: $\text{ndur } 1.74 \pm 0.58$, $\text{ii } 10.25 \pm 3.88$, $\text{fmaxe } 48.20 \pm 4.13$).

DISCUSSION

European pipistrelle species are polygynous bats with a mating system described as a resource-defence polygyny; where the resource is a territory with a roost (Gerell-Lundberg and Gerell, 1994). In contrast to other species of the genus in which the seasonal dispersal is limited mostly to the local scale (cf. Gaisler *et al.*, 2003; Taake and Vierhaus, 2004), *P. nathusii* is a migratory species well known for its long distance seasonal migrations (Masing, 1988; Lina, 1990; Ahlén, 1997; Strelkov, 2000; Petersons, 2004). In Western and Central Europe, where the species is quite rare throughout the year and the resident population is mostly composed by males, the mass appearance of advertising males of this species in the late summer is particularly conspicuous (Arnold *et al.*, 1996; Vierhaus, 2004). The peak abundance of advertising males was in August (here our data are in agreement with the summary of Furmankiewicz, 2003). Advertising males exhibit high roost fidelity and return to the same area

each year (Rachwald, 1992), as we also found in the present study by repeated recaptures of particular males.

Typical advertisement calls have also been recorded in spring, both in our study and by other

TABLE 9. Syntagmatic structure of advertisement calls recorded from males 4, 6a, 12a, 12b and 17a: percentages of respective syntagmatic strings. Motifs (or syntagms) with multiple repetitions within a string are marked with (*)

Syntagma	4	6a	12a	12b	17a
B	0.00	0.66	0.00	0.91	0.00
AB	0.00	1.32	0.00	0.00	0.37
BC	0.40	0.66	1.78	1.80	0.75
BC(B)*	0.81	0.00	0.00	0.90	0.37
ABC	53.23	39.07	52.00	23.42	35.08
ABCB	0.81	0.66	0.00	0.00	1.87
ABCB(B)*	0.00	0.00	0.00	0.00	1.49
ABCBCB	1.21	0.00	0.00	0.00	0.00
ABCBC(BC)*	0.00	0.00	0.89	0.00	0.00
AD	0.40	0.00	0.89	0.00	0.37
ABD	0.40	0.66	0.00	0.00	1.12
ABCD	35.08	31.13	37.33	62.17	14.92
ABCB	1.21	19.21	4.44	3.60	27.98
ABCBB	0.81	1.99	0.44	2.70	8.21
ABCBB(B)*D	3.23	3.97	0.00	0.90	6.72
ABCBCD	0.00	0.00	0.89	0.00	0.00
Others	2.42	0.67	1.33	3.60	0.75
<i>n</i> calls total	248	151	225	111	268

TABLE 10. Within-season variation (May–June versus August–September) in syntagmatic structure of advertisement calls in the total sample. Note the differences in ratio of songflight (SF) to sedentary display (SD), in total number of syntagmatic strings recorded and in percentage of ABCD and more complex strings (ABCB, ABCB(B)*, ABCBD, ABCBBD, ABCBB(B)*D, ABCBCD, ABCBC, ABCBC(BC)* and further 11 complex strings (listed in Table 8) versus ABC and the simple strings (A, B, AB, BC), respectively

Display	<i>n</i> calls	% calls	<i>n</i> strings	Percentages of syntagmatic strings			
				ABC	ABCD	simple	complex
May–June							
SF	527	89.02	13	63.34	15.53	6.42	2.54
SD	65	10.98	4	5.91	4.73	0	0.17
<i>n</i> total	592	100	14	69.25	20.26	6.42	2.71
August–September							
SF	518	22.21	14	13.81	5.66	1.49	0.9
SD	1814	77.79	16+	16.98	48.76	0.34	10.69
<i>n</i> total	2,332	100	18+	30.79	54.42	1.83	11.59

authors (Barlow and Jones, 1996; Russ *et al.*, 1998), but we found that the total amount of vocalisation activity was lower, and calls were noted mainly in the early part of the night (see also Furmankiewicz, 2003). We also found that the vast majority of advertising calls recorded prior to August were emitted during flight, and that their structure was mostly either ABC or simple, with more complex calls being quite exceptional. Provisionally, we agree with Barlow and Jones (1997b) that emitting advertising calls in spring is probably connected with territory defence by males, including their foraging grounds. Nevertheless, despite lack of direct evidence for a mate-attraction function of calls and for mating during spring migrations is available (Heise, 1982; Schmidt 1994a, 1994b), neither possibility should be completely rejected.

In these respects the general phenomenology of advertisement behaviour in *P. nathusii* is similar to that of other European representatives of the genus. The major difference is, of course, in the phonologic structure of the calls. *P. nathusii* shares with other pipistrelles the initial component of the song, motif A (or, more exactly, the A1 series, since the A2 series of *P. nathusii* differs from that of other species). This component appears to be a genus-specific type of social call that appears in various situations, both to repel and attract conspecifics (Barlow and Jones, 1997b). The syllables of motif A (multiple low frequency FM signals with harmonics) appear regularly in the ‘multiple element distress calls’ of pipistrelles, and presumably are derived just of them. In the experiments of Russ *et al.* (2004), all three species were attracted to loudspeaker emitting

TABLE 11. Structural changes in advertisement calls recorded from males 4, 6a, 12a, 12b and 17a during overnight observations under presence of another individual in vicinity. They included most often change in number of notes of both motifs (A1nnote, Cnnote) and shift from basic syntagms (ABC, ABCD) to either very complex or simple syntagmatic strings. OI = presence of other individuals

Parameter	4	6a	12a	12b	17a
A1 nnote in regular SD	5–6	6–7	4–5	6–7	5–6
C nnote in regular SD	6–7	6–7	4–5	6–7	6–7
A1 nnote at OI	12	15	10	15	11
C nnote at OI	11	10	7	10	9
SD + presence of other individuals					
Change in A1 nnote (<i>n</i>)	8	–	3	1	0
Change in C nnote (<i>n</i>)	18	–	7	1	1
Change in syntax (<i>n</i>)	20	–	2	0	3
No change (<i>n</i>)	5	–	6	0	1
<i>n</i> total (calls)	28	0	13	1	4
SF + presence of other individuals					
Change in A1 nnote (<i>n</i>)	3	12	4	10	5
Change in C nnote (<i>n</i>)	2	3	2	5	3
Change in syntax (<i>n</i>)	3	3	0	2	2
No change (<i>n</i>)	4	11	6	0	3
<i>n</i> total (calls)	12	27	11	10	8

distress calls of the other species. Nevertheless, in contrast to other pipistrelles, in *P. nathusii*, the A1 series is supplemented with the A2 series, and motif A represents merely the initial element of the advertisement calls, which is completed by further 2–4 motifs of different structure. The structural core of the call is the string of motifs A-B-C, which we recorded in nearly 95% of the 2,960 calls analysed. This core can be complemented with other motifs or modified by the repetition or deletion of elementary motifs.

In agreement with previous reports, we confirmed extremely large variation in the syntactic structure of calls within the population. Besides the normal combinations of motifs A, B, and C described in the literature, e.g., ABCBC, ABCC, AB, AC, ABBC, A, B, C (Szkudlarek and Paszkiewicz, 2000), and ABCBC with multiple repetitions of BC (Russ *et al.*, 1998) we found a total of 15 syntagms containing the main motif A and 10 syntagms without this motif. It is highly probable, of course, that the syntax of advertising calls is even diverse and that new syntagms will be discovered in other males. The isolated appearance of single motifs A, B, and D during songflight and sedentary display is of particular interest. Motif C was recorded separately only in two cases in one male, while motif E as an isolated call was never recorded. The splitting of syntagma ABC(D) to single motifs A, B, C, and D may be due to a tendency to stress of the special semantic information of each motif.

Factors found to affect the syntagmatic structure and temporal characteristics of males' calls included time of year, the mode of display (sedentary or songflight), and current situation at the site (i.e., the presence of other individuals). The vast majority of advertisement calls were produced as sedentary display (in this aspect *P. nathusii* differs from other pipistrelle species — Russ and Racey, 2007). It may be, given that vocalisation from the entrance of the roosts requires no demands on the spatial orientation, all the energy of vocalisation can be invested in the acoustic performance, which then becomes quite variable and is often enriched by insertions of additional motifs D and E in the intervals between the main syntagms A, B, and C. Alternatively, vocalisation in songflight might be used for long-distance communication and sedentary display for short-distance communication, which allows it to attain more complexity. We might then hypothesize that songflight is preferentially performed when establishing new territories, whereas sedentary display advertises the resident status of a male to its conspecifics.

The greatest within-individual variation in the syntax of complex calls was observed at the peak of the mating season, which corresponds well both to the highest proportion of sedentary displays at that time, and, presumably, also to the highest number of actually formed harem groups (H. Jahelková, unpublished data). It seems most probable that the amount of variation in the advertising calls produced by a male is positively related to his attractiveness to females, as has been demonstrated in *Saccopteryx bilineata*, where males with greater repertoire complexity tend to have more females in their territories (Davidson and Wilkinson, 2004). If so, it is not surprising that most of display activity in *P. nathusii* is performed from the sedentary position, which for respiratory and energetic reasons is probably more suitable for production of long and structurally varied songs than is songflight. If the expected effects of song quality upon efficacy in attracting females and in harem formation are to emerge, we should also expect that the calls must carry information distinguishing individual males. Such a role for social calls is reasonable, since social calls are complex enough to code individual-specific information, as we have shown (as is seldom done) in this study (Fenton *et al.*, 2004; Siemers, 2006).

Like Russ and Racey (2007), we found that the pattern of between-individual variation is, for almost all phonological variables, considerably affected by individual peculiarities. Thus, almost all these variables potentially bear individual-specific information. We have shown, however, that only certain variables exhibit the narrow range of within-individual variation expected for those that actually code the individual identity. For instance, the broad within-individual variation in most of the analysed parameters of motif A does not convincingly support the use of this motif for individual recognition, although this possibility has been suggested for *P. pipistrellus*, for example (Pfalzer and Kusch, 2003). In contrast, motifs B and C of *P. nathusii*, typically exhibited narrow within-individual variation while combined with large between-individual variation. We demonstrated that a combination of basic frequency parameters of motifs B and C allows reliable individual identification in most instances, and perhaps serves as a core element of the acoustic signature of a male. The combination of the lowest frequency of the third syllable of motif C (lof c3) and end frequency of B (B enf) can even be applied even as a quick tool for individual identification in the field. We have confirmed the discriminating power of these parameters at other sites with abundant

advertising males, such as in Central Bohemia and Romania (Danube Delta: Crisan, Sfintu Gheorghe — H. Jahelková, unpublished data). In addition, we have demonstrated that the individual-specific values of these (and several other) phonologic variables are invariant to contextual changes and season, even in males recaptured over a period of five years.

We have not found calls similar to motif B or C in any behavioural context other than the advertising display, but Russ *et al.* (2004) described signals with the syllabic structure of motif B as a 'single element type' of distress call. In some instances, typically when other individuals were present, we recorded a series of several these 'single element distress calls' as a supplementary series after motif B that apparently replaced motifs C or E. The trill call of motif C, in a form of 'V-shaped' syllables or a 'wavy line' can be found in other species as a part of calls emitted during 'excitement': e.g., the territorial and courtship song of *S. bilineata* (Behr and von Helversen, 2004), or similar calls of *N. noctula* (Weid, 1994) and *Myotis myotis* (Zahn and Dippel, 1997).

In addition to the specific motifs B and C, which distinguish the advertising call of *P. nathusii* from those of congeneric species, the most noticeable trait is the quite variable motif D. Its incidence dramatically increased at the peak of mating season when it represented a nearly obligatory component of a call. Motif D is characterized by extreme variation in within- as well as between-individuals, both as concerns its frequency and its syllabic composition. Presumably, it provides information on the social identity of the male and/or on his social and reproductive status. We assume that types of notes that are similar to mother-youth calls contain information about colony membership and facilitate females' choice of a suitable mate. The developmental origin of the simple curved advertising calls of males from isolation calls of pups has been suggested by Pfalzer and Kusch (2003).

Another accessory motif of the advertising call, motif E, was recorded only during sedentary displays. The high repetition rate and short duration of notes in motif E are also typical of the terminal phase of foraging signals and landing on objects (Kalko and Schnitzler, 1998). It seems that this motif may have evolved either from steep FM of terminal phase of approach, and approach and landing, or from feeding buzz. By emitting pseudo-landing calls, a male may perhaps increase his attractiveness to other individuals and thus, conceivably lure in more females. The results of Ciechanowski and

Jarzebowski (2004), who demonstrated that females preferred only a few successful males, are worth mentioning in this context. Nevertheless, actual evidence of females choice based on males vocalisation is lacking.

In conclusion, the advertising calls of *P. nathusii* include both species-specific information (motif A) and individual-specific information (motifs B and C). Since the syntagma BC (and calls of similar structural design) were recorded only in the context of male advertising or territorial behaviour, we assume that motifs B and C probably represent the essential core of the advertising call. This being the case, it is perhaps not by chance that these components, which contain the greatest individual specificity, are the most pronounced. Moreover, the syntactic reduction consequent on excitation by the appearance of foreign individual tend to result in simplified multiple repetitions of syntagma BC. In contrast to B and C, motif A is apparently derived from basic social calls of European pipistrelles, which is widely used in the context of agonistic interactions. It is the most intense part of the advertisement call (audible with bat detectors up to 100 m — Gerell-Lundberg and Gerell, 1994) and apparently is adapted for a long-distance communication. Motif D, when used as a part of the advertising call, is specific to *P. nathusii* (Jahelková, 2003), and its extremely broad syllabic pool may provide information on social identity. Motif D also commonly appears as part of mother-young communication among pipistrelle species (de Fanis and Jones, 1996). Similar frequency modulated signals, either single notes or note clusters, also represent a common component of the acoustic repertoire of many other bat species (Fenton, 1985).

The semantic mirror of an advertising call of *P. nathusii*, resulting from the present analysis, is thus: a general alerting signal (motif A), the most intense part of the call, which contains generic and species identity, is followed by two structurally divergent components (motifs B and C) that identify a particular male, which in turn may be supplemented with further information specific to the individual or to the local population (motif D), and, finally, a signal suggestive of landing (motif E), which is inserted optionally within semantic complex conveying individual identity (B, C, and D). Hence, translated into human words, the message ABCED could be approximately: (A) 'Pay attention: I am a *P. nathusii*, (B, C) specifically male 17b, (E) land here, (D) we share a common social identity and common communication pool'.

ACKNOWLEDGEMENTS

We are grateful to Vladimír Hanák and Radek Lučan for their excellent assistance in the field, to Jiří Gaisler and P. N. Clifford for reading of earlier version of manuscript, Zdeněk Řehák for providing us his own records for comparative purposes and Elisabeth K. V. Kalko for her suggestion on methods of acoustic analyses. Last but not least we thank to two anonymous reviewers for numerous critical comments and suggestions that substantially improved the paper. The work was supported by the grants GAČR 42/201/813, MSM 0021620828 and GAUK 43/203/359.

LITERATURE CITED

- AHLÉN, I. 1997. Migratory behaviour of bats at south Swedish coasts. *Zeitschrift für Säugetierkunde*, 62: 375–380.
- ALTRINGHAM, J. D., and M. B. FENTON. 2003. Sensory ecology and communication in the Chiroptera. Pp. 90–127, in *Bat ecology* (T. H. KUNZ and M. B. FENTON, eds.). The University of Chicago Press, Chicago, 779 pp.
- ARNOLD, A., A. SCHOLZ, V. STORCH, and M. BRAUN. 1996. The Nathusius' bats in flood plain forests in Nordbaden (SW Germany). *Carolinea*, 54: 149–158.
- BALCOMBE, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Animal Behaviour*, 39: 960–966.
- BALCOMBE, J. P., and G. F. MCCracken. 1992. Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Animal Behaviour*, 43: 79–87.
- BARLOW, K. E., and G. JONES. 1996. *Pipistrellus nathusii* (Chiroptera: Vespertilionidae) in Britain in mating season. *Journal of Zoology (London)*, 240: 767–773.
- BARLOW, K. E., and G. JONES. 1997a. Differences in songflight calls and social calls between two phonic types of the vespertilionid bat *Pipistrellus pipistrellus*. *Journal of Zoology (London)*, 241: 315–324.
- BARLOW, K. E., and G. JONES. 1997b. Function of pipistrelle social calls: field data and playback experiment. *Animal Behaviour*, 53: 991–999.
- BEHR, O., and O. VON HELVERSEN. 2004. Bat serenades-complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behavioral Ecology and Sociobiology*, 56: 106–115.
- BEHR, O., O. VON HELVERSEN, G. HECKEL, M. NAGY, C. C. VOIGT, and F. MAYER. 2006. Territorial songs indicate male quality in the sac-winged bat *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behavioral Ecology*, 17: 810–817.
- BOHN, K. M., G. S. WILKINSON, and C. F. MOSS. 2007. Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Animal Behaviour*, 73: 423–432.
- CIECHANOWSKI, M., and T. JARZEMBOWSKI. 2004. The size and number of harems in a polygynous bat *Pipistrellus nathusii* (Keyserling and Blasius, 1839) (Chiroptera: Vespertilionidae). *Mammalian Biology*, 69: 277–280.
- DAVIDSON, S. M., and G. S. WILKINSON. 2004. Function of male song in the greater white-lined bat, *Saccopteryx bilineata*. *Animal Behaviour*, 67: 883–891.
- DE FANIS, E., and G. JONES. 1995. Post-natal growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *Journal of Zoology (London)*, 235: 85–97.
- DE FANIS, E., and G. JONES. 1996. Allomaternal care and recognition between mothers and young pipistrelle bats (*Pipistrellus pipistrellus*). *Journal of Zoology (London)*, 240: 781–787.
- DE SAUSSURE, F. 1986. *Course in general linguistics*. Open Court Publishing, La Salle, Illinois, 236 pp. [original work published in 1916].
- ESSER, K. H., and U. SCHMIDT. 1989. Mother infant communication in the lesser spear-nosed bat *Phyllostomus discolor* (Chiroptera, Phyllostomidae) — evidence for acoustic learning. *Ethology*, 82: 156–168.
- FENTON, M. B. 1985. *Communication in the Chiroptera*. Indiana University Press, Bloomington, 161 pp.
- FENTON, M. B., D. S. JACOBS, E. J. RICHARDSON, P. J. TAYLOR, and W. WHITE. 2004. Individual signatures in the frequency-modulated sweep calls of large African large-eared free-tailed bats *Otomops martiensseni* (Chiroptera: Molossidae). *Journal of Zoology (London)*, 262: 11–19.
- FURMANKIEWICZ, J. 2003. The vocal activity of *Pipistrellus nathusii* (Vespertilionidae) in SW Poland. *Acta Chiropterologica*, 5: 97–105.
- GAISLER, J., V. HANÁK, V. HANZAL, and V. JARSKÝ. 2003. Výsledky kroužkování v České republice a na Slovensku, 1948–2000. [Results of bat banding in the Czech and Slovak Republics, 1948–2000]. *Vespertilio*, 7: 3–61.
- GEBHARD, J., and W. BOGDANOWICZ. 2004. *Nyctalus noctula* — Großer Abendsegler. Pp. 607–694, in *Handbuch der Säugetiere Europas*, Vol. 4/II, Fledertiere II (F. KRAPP, ed.). Aula-Verlag, Wiebelsheim, 1186 pp.
- GERELL-LUNDBERG, K., and R. GERRELL. 1994. The mating behaviour of pipistrelle and the Nathusius' pipistrelle (Chiroptera) — a comparison. *Folia Zoologica*, 43: 315–324.
- GRIFFIN, D. 1958. *Listening in the dark*. Yale University Press, New Haven, Connecticut, 413 pp.
- HÄUSSLER, U., E. MÖLLER, and U. SCHMIDT. 1981. Zur Haltung und Jugendentwicklung von *Molossus molossus* (Chiroptera). *Zeitschrift für Säugetierkunde*, 46: 337–351.
- HEISE, G. 1982. Zur Vorkommen, Phänologie, Ökologie und Altersstruktur der Raauhautfledermaus (*Pipistrellus nathusii*) in der Umgebung von Prenzlau (Uckermark), Bezirk Neubrandenburg. *Nyctalus*, 1: 281–300.
- HELVENSEN, O. VON, and D. VON HELVERSEN. 1994. The 'advertisement song' of the lesser noctule bat (*Nyctalus leisleri*). *Folia Zoologica*, 43: 331–338.
- HOOFFER, S. R., and R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica*, 5 (Supplement): 1–63.
- JAHELKOVÁ, H. 2003. Přehled a srovnání echolokačních a sociálních druhů čtyř evropských druhů netopýrů rodu *Pipistrellus* (Chiroptera: Vespertilionidae). [Review and comparison of the echolocation and social calls in four European bat species of the genus *Pipistrellus* (Chiroptera: Vespertilionidae)]. *Lynx (N.S.)*, 34: 13–28.
- JAHELKOVÁ, H., R. LUČAN, and V. HANÁK. 2000. Nové údaje o netopýru parkovém (*Pipistrellus nathusii*) v jižních Čechách. [New data about Nathusius's bat (*Pipistrellus nathusii*) in southern Bohemia (Czech Republic)]. *Lynx (N.S.)*, 31: 41–51.
- JONES, G. 1997. Acoustic signals and speciation: the roles of natural and sexual selection in the evolution of cryptic species. *Advances in the Study of Behaviour*, 26: 317–354.
- JONES, G., and S. M. VAN PARIJS. 1993. Bimodal echolocation in pipistrelle bats: are cryptic species present? *Proceedings of Royal Society of London*, 251B: 119–125.

- KALKO, E. K. V., and H.-U. SCHNITZLER. 1998. How echolocating bats approach and acquire food. Pp. 197–204, in *Bat biology and conservation* (T. H. KUNZ and P. A. RACEY, eds.). Smithsonian Institution Press, Washington D.C., 365 pp.
- KAZIAL, K. A., and W. M. MASTERS. 2004. Female big brown bats, *Eptesicus fuscus*, recognize sex from a caller's echolocation signals. *Animal Behaviour*, 67: 855–863.
- KOZHURINA, E. I. 1996. What may the 'songs' of noctule disclose to an observer? *Myotis*, 34: 5–15.
- KRAPP, F. (ed.). 2004. *Handbuch der Säugetiere Europas*, Vol. 4/II, Fledertiere II. Aula-Verlag, Wiebelsheim, x + 605–1186.
- LINA, P. H. C. 1990. Verre Terugmeldingen van den Nathusius's Dwergvleermuizen *Pipistrellus nathusii*, gevonden of geringd in Nederland. [Long-distance recoveries of Nathusius' pipistrelles *Pipistrellus nathusii* found or banded in The Netherlands]. *Lutra*, 33: 315–324.
- LUNDBERG, K. 1989. Social organisation and survival of the pipistrelle bat (*Pipistrellus pipistrellus*), and a comparison of advertisement behaviour in three polygynous bat. Ph.D. Thesis, Lund University, Lund, 88 pp.
- MASING, M. 1988. Long-distance flights of *Pipistrellus nathusii* banded or recaptured in Estonia. *Myotis*, 26: 150–158.
- MASTERS, W. M., K. A. S. RAVEN, and K. A. KAZIAL. 1995. Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. *Animal Behaviour*, 50: 1243–1260.
- MAYER, F., and O. VON HELVERSEN. 2001. Cryptic diversity in European bats. *Proceedings of the Royal Society of London*, 268B: 1825–1832.
- PEARL, D. L., and M. B. FENTON. 1996. Can echolocation calls provide information about group identity in the little brown bat (*Myotis lucifugus*)? *Canadian Journal of Zoology*, 74: 2184–2192.
- PETERSONS, G. 2004. Seasonal migrations of north eastern populations of nathusius bat, *Pipistrellus nathusii* (Chiroptera). *Nyctalus*, 3: 81–98.
- PFALZER, G., and J. KUSCH. 2003. Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology (London)*, 261: 21–33.
- PORTER, F. 1979. Social behavior in the leaf-nosed bat, *Carollia perspicillata*, I. Social organization. *Zeitschrift Tierpsychologie*, 49: 406–417.
- RACHWALD, A. 1992. Social organization, recovery frequency and body weight of the bat *Pipistrellus nathusii* from northern Poland. *Myotis*, 30: 109–118.
- RUSS, J. M., and P. A. RACEY. 2007. Species-specificity and individual variation in the song of male Nathusius' pipistrelles (*Pipistrellus nathusii*). *Behavioral Ecology and Sociobiology*, 61: 669–677.
- RUSS, J. M., J. K. O'NEILL, and W. I. MONTGOMERY. 1998. Nathusius' pipistrelle bats (*Pipistrellus nathusii*, Keyserling et Blasius 1839) breeding in Ireland. *Journal of Zoology (London)*, 245: 345–349.
- RUSS, J. M., G. JONES, I. J. MACKIE, and P. A. RACEY. 2004. Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae) a function for convergence in call design? *Animal Behaviour*, 67: 1005–1014.
- RUSSO, D., and G. JONES. 1999. The social calls of Kuhl's pipistrelle *Pipistrellus kuhli* (Kuhl, 1819): structure and variation (Chiroptera: Vespertilionidae). *Journal of Zoology (London)*, 249: 476–481.
- SCHMIDT, A. 1994a. Phänologisches Verhalten und Populations-eigenschaften der Rauhhautfledermaus, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), in Ostbrandenburg. Teil 1. *Nyctalus*, 5: 77–100.
- SCHMIDT, A. 1994b. Phänologisches Verhalten und Populations-eigenschaften der Rauhhautfledermaus, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), in Ostbrandenburg. Teil 2. *Nyctalus*, 5: 123–148.
- SIEMERS, B. M. 2006. Bats: communication by ultrasound. Pp. 699–704, in *The encyclopedia of language and linguistics*, Volume 1, 2nd edition (K. BROWN, ed.). Elsevier, Oxford.
- SKIBA, R. 2003. Europäische Fledermäuse — Kennzeichen, Echoortung und Detektoranwendung. Neue Brehm-Bücherei 648. Westarp Wissenschaften, Hohenwarsleben, 212 pp.
- STRELKOV, P. P. 2000. Seasonal distribution of migratory bat species (Chiroptera, Vespertilionidae) in the eastern Europe and adjacent territories: nursing area. *Myotis*, 37: 7–25.
- SZKUDLAREK, R., and R. PASZKIEWICZ. 2000. Sygnały godowe krajowych gatunków nietoperzy. [Mating social calls of Polish bat species]. *Nietoperze*, 1: 55–62.
- TAAKE, K. H., and H. VIERHAUS. 2004. *Pipistrellus pipistrellus* (Schreber, 1774) — Zwergfledermaus. Pp. 761–814, in *Handbuch der Säugetiere Europas*, Vol. 4/II, Fledertiere II. (F. KRAPP, ed.). Aula-Verlag, Wiebelsheim, x + 605–1186.
- VIERHAUS, H. 2004. *Pipistrellus nathusii* (Keyserling und Blasius, 1839) — Rauhhautfledermaus. Pp. 825–873, in *Handbuch der Säugetiere Europas*, Vol. 4/II, Fledertiere II. (F. KRAPP, ed.). Aula-Verlag, Wiebelsheim, x + 605–1186.
- WEID, R. 1994. Socialrufe männlicher Abendsegler (*Nyctalus noctula*). *Bonner zoologische Beiträge*, 45: 33–38.
- WILKINSON, G. S., and J. W. BOUGHMAN. 1998. Social calls coordinate foraging in greater spear-nosed bats. *Animal Behaviour*, 55: 337–350.
- ZAHN, A., and B. DIPPEL. 1997. Male roosting habits and mating behaviour of *Myotis myotis*. *Journal of Zoology (London)*, 243: 659–674.

Received 11 September 2007, accepted 15 January 2008

APPENDIX

A list of terms and abbreviations

Term		Explanation
1. Basic terms		
Call		A complex acoustic series composed of one or more clusters of structurally variegated elements, presumably a semantic unit of the display
Note		An elementary acoustic pulse, a continuous sound bordered by a silent interval, particular notes within a motif are denoted here with small caps and numbers in a sequence (e.g. a1, c3 etc.)
Syllable		A term referring to the acoustic design of a note as it differs from the design of other notes. Individual syllables are mostly denoted with the letters resembling their spectrographic characteristics (L, I, V, U, M)
Motif		A single note or a cluster of notes composed of a single syllable or a series of structurally related syllables (A, B, C, D, E)
Syntax		A way of sequencing of particular notes or motifs within a call
Syntagma		A fixed sequence of motifs or syllables representing a structural subunit of a call design (ABC, ABCBD, etc.)
Phonology		Acoustic design of particular elements of a call (notes, motifs)
Syntagmatics		Structural design of a call, syntax variation and repertoire of syntagms
2. Phonologic variables examined in this study		
Temporal (in ms)	Duration (dur)	of a note (ndur), motif or submotif (A1dur, Bdur, etc.), syntagma (ABCdur, ABdur, BCdur, etc.), a quasi-constant part of syllable (qCF)
	Intervals (ii)	between-motifs (a0-B ii, B-C ii), between syntagms containing motif A (Aii, measured from the first A note of the previous syntagma to the first A note of next syntagma), silence intervals between notes
Frequency (in kHz)	Basic variables	Frequency with maximal energy (fmaxe), start (stf) and end (enf) (both measured 40 dB below the level of fmaxe), the lowest (lof), the upper (upf)
	Supplementary	middle frequency of a syllable in the quasi-constant part or a frequency modulated part limited by different frequencies (mif), frequency modulated parts at the start of syllables (in motifs A, C, D) sweeping up (hook) and at the end of syllables sweeping down (tail)
Structural	Basic variables	presence and specificities of particular motifs (A, B, C, D, E), subseries of main motifs (A1, A2, D1-Dx), subseries of motif E (either following the motif C (cE), or B (bE)), number of notes in motifs and submotifs (A1nnote, Cnnote, etc.), syntagmatic strings composing a call (a sequence of motifs, e.g. ABC)
	Syllabic design of motif D	besides apparent differences in shape of the sonographic record, the syllables are defined by the relation between mif and enf: L (FM-qCF; qCF part > 2 ms, mif-enf < 4 kHz), N (FM-qCF-FM; qCF part > 2 ms, mif-enf > 4 kHz), U (FM-qCF-FM, qCF part > 2 ms, enf-mif > 4 kHz), (clear interruption between FM and qCF; originates from L, N, V), I (steep FM sweeping down), W, M (letter shape resembles the characteristic frequency course).
3. Contextual variables		
	Songflight	Advertising display performed on wings (SF), typically with echolocation calls
	Sedentary display	Advertising display performed from the roost (SD)
	Other	Presence of another individual (OI)