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Do echolocation calls of wild colony-living Bechstein's bats (*Myotis bechsteinii*) provide individual-specific signatures?

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Abstract Social animals often use vocal communication signals that contain individual signatures. As bats emit echolocation calls several times per second to orient in space, these might seem ideal candidates for conveying the caller's individual identity as a free by-product. From a proximate perspective, however, coding of caller identity is hampered by the simple acoustic structure of echolocation signals, by their task-specific design and by propagation loss. We investigated the occurrence of individual signatures in echolocation calls in individually marked, free-living Bechstein's bats (*Myotis bechsteinii*) in a situation with defined social context in the field. The bats belonged to two different colonies, for both of which genetic data on relatedness structure was available. While our data clearly demonstrate situation specificity of call structure, the evidence for individual-specific signatures was relatively weak. We could not identify a robust and simple parameter that would convey the caller's identity despite the situation-specific call variability. Discriminant function analysis assigned calls to call sequences with good performance, but worsened drastically when tested with other sequences from the same bats. Therefore, we caution against concluding from a satisfactory discrimination performance with identical training and test sequences that individual bats can reliably be told apart by echolocation calls. At least the information contained in a single call sequence seems not to be sufficient for that purpose. Starting frequencies did give the best discrimination between individuals, and it was

also this parameter that was correlated with genetic relatedness in one of our two study colonies. Echolocation calls could serve as an additional source of information for individual recognition in Bechstein's bats societies, while it is unlikely that a large number of individuals could be reliably identified in different situations based on echolocation alone.

Keywords Vocal signature · Individual recognition · Echolocation · Vespertilionidae · Relatedness

Introduction

Social animals use communication to coordinate their activities (Milton 2000; Boinski 2000) and to recognise group members in situations when confusing between individuals has fitness costs (Searby and Jouventin 2003; Searby et al. 2004). Vocal communication signals in many social mammals and birds contain individual signatures used by conspecifics to individually recognise the caller (e.g. Reby et al. 1998; Searby and Jouventin 2003; Charrier et al. 2003; Searby et al. 2004). Bats are highly gregarious and produce a wide range of vocal signals with purely communicative function (Fenton 1985; Kanwal et al. 1994; Pfalzer and Kusch 2003). Their calls often have a complex acoustic structure and thereby offer the possibility to encode individual or context-specific information in details of time and frequency parameters (Searby and Jouventin 2004; Siemers 2005). Individual recognition using social calls in bats occurs in different behavioural contexts (e.g. pup recognition; Balcombe 1990).

In addition to social calls, which occur in equivalent forms in many other animals, bats produce echolocation calls that generate echoes permitting a bat to negotiate its three-dimensional environment (Schnitzler et al. 2003). Echolocation is an active sensory system that involves emission of loud calls at short-time intervals. These signals (and perhaps some echoes) are available to any animal that can hear them; i.e. echolocation inevitably has an additional communicative function. Bats eavesdrop on

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other bats (Barclay 1982; Fenton 2003) and often will be able to determine species identity from echolocation calls (e.g. Parsons and Jones 2000; Russo and Jones 2002; Biscardi et al. 2004; Obrist et al. 2004). Only few bat species show sexual dimorphism or age differences (mainly first year vs older) in echolocation signals (e.g. Neuweiler et al. 1987; Russo et al. 2001). Kazial and Masters (2004) recently reported laboratory behavioural evidence that big brown bats (*Eptesicus fuscus*) can distinguish sex of conspecifics by their echolocation calls. However, it is unknown so far whether bats can use echolocation calls to distinguish between individuals in a social context. Classical laboratory observations suggested that greater horseshoe bats (*Rhinolophus ferrumequinum*) might be able to do so (Möhres 1967).

Given the long lifespans and the high stability of colonies in many bat species, we can expect cooperation among colony members (Kerth et al. 2000), which may depend upon individual recognition (Kerth et al. 2002). As bats anyway have to broadcast echolocation calls several times per second to orient in space, these might seem to be ideal candidates for conveying the individual identity of the caller to colony members as a free by-product. For a bat, acoustic signatures would be perceivable over greater distance than olfactory, tactile or visual individual-specific signatures. From an ultimate perspective, it therefore seems reasonable to hypothesise that there is strong selection pressure favouring the evolution of individual signatures in echolocation calls. Further, individually distinctive sonar signals might allow bats to recognise their own echoes to avoid sonar jamming (Masters et al. 1991, 1995; Ratcliffe et al. 2004; Ulanovsky et al. 2004).

From a proximate perspective, however, three inter-related lines of argument challenge this expectation (Siemers 2005). (1) The acoustic structure of echolocation calls is simple; for example in most vespertilionids they are downward-frequency-modulated sweeps. Unlike social calls, echolocation calls do not have complex acoustic structures that would readily encode individual variation. (2) Bats vary the structure of their echolocation calls depending on the current echolocation task or situation, optimising the information content of the echoes (Fenton 1990; Schnitzler et al. 2003), thereby setting the stage for situation specific rather than individual signatures. For example, vespertilionid bats flying in open spaces use long calls at long-pulse intervals with all energy concentrated in a narrow-frequency band to increase detection probability. When the same bat flies in cluttered spaces, it uses short, broadband calls at shorter pulse intervals. Echoes of these calls deliver short, precise time markers to the bat's hearing system and thereby increase localisation and ranging accuracy needed to fly through vegetation without collision. Signal structure thus is varied greatly in response to the current situation, and individual signatures would have to be very robust not to be masked by the situation-specific variability. Nevertheless, the situation specificity of echolocation calls implies that bats automatically communicate information on their current behaviour to both intra- and interspecific eavesdroppers (Barclay 1982; Fenton

2003). (3) Propagation loss will further blur possible individual signatures of echolocation calls, especially in the high-frequency range. Both sound emission (mouth or nose) and sound reception (ears) in bats are directional. Depending on the relative position of the sending and the receiving bat, especially high frequencies might be severely attenuated, because both atmospheric attenuation and directionality increase with frequency. Finally, the relative velocity of one bat to the other can cause Doppler shifts that change the call frequencies for the receiving bat in the range of several kilohertz.

Despite the situation specificity of echolocation call structure, there is evidence of individual-specific signatures in echolocation calls, both from field (Brigham et al. 1989; Obrist 1995; Fenton 2003; Fenton et al. 2004) and laboratory recordings (Masters et al. 1991, 1995; Burnett et al. 2001; Kazial et al. 2001). To disentangle the situation specificity of echolocation call structure from possible individual signatures and to measure the reliability of these signatures, it is necessary to include several call sequences for each individual into the analysis. This prerequisite can easily be met in the laboratory (Burnett et al. 2001; Siemers et al. 2001a), but it is more difficult in the field, and only Obrist (1995) has documented repeated measures of the echolocation calls of known individuals, but not in social context.

We investigated the occurrence of individual signatures in echolocation calls in free-living Bechstein's bats (*Myotis bechsteinii*) belonging to two different colonies. Bechstein's bats use their broadband, downward-frequency-modulated echolocation calls to orient in their three-dimensional forest habitat, while they rely on listening for prey generated sounds to find rustling arthropods that are gleaned from vegetation (Staden and Schnitzler 1995; Siemers and Swift 2005). Females live in maternity colonies that comprise about 15 to 40 adults; males are solitary (Kerth 1998, 2006). Colonies are closed societies, and individual females live together for many years and breed communally (Kerth et al. 2000). Colonies comprise closely related and genetically unrelated individuals (Kerth et al. 2002), and colony members cooperate by transferring information about day roosts among each other (Kerth and Reckardt 2003). Gas-chromatographic analyses revealed that facial secretions contain information about colony membership and individuality (Safi and Kerth 2003). Females of one colony selectively prevented members of a foreign colony from entering their roost during confrontation tests (Kerth et al. 2002). As colonies show fission-fusion behaviour (Kerth and König 1999), colony members probably have to identify each other to coordinate their regular roost switching and to be able to prevent the intrusion of foreigners.

To test whether echolocation calls could be used for identification of colony members, we chose a behaviourally meaningful situation: lactating females approaching and/or circling at the roost where they left their newborn young. If the bats' calls contain individual-specific information, one would expect it to show at least in this context for several reasons. First, because this way pups

could recognise their respective mothers and already wait at the entrance before those actually enter the box to nurse them (Elsasser 1995). Second, colony members could identify approaching conspecifics before those enter a roost, and this might reduce the chance for misidentification of colony members as foreigners and thus avoid potential fighting (Kerth et al. 2002). Third, when bats depart from roosts it might likewise be useful to signal one's identity, for example to allow other colony members to follow to a new roost site (Kerth and Reckardt 2003). At the same time, the approaches to the roost as well as circling around it provided well-defined, standardised situations for call recordings. The bats of the two study colonies were individually marked with transponders (PIT tags; Kerth and König 1996). With the help of automatic reading devices attached to the roosts, we identified our study animals (Kerth and Reckardt 2003) and recorded several call sequences from known wild bats in the same night at the same roost site as well as at a second site on a different night without interfering with the animals' natural behaviour.

The data set allowed us to test several hypotheses: first, that the bats' call structures differed between the two recording locations within the home range of one colony, depending on vegetation density; second, whether the calls could be reliably assigned to individuals regardless of situation variability and third, the potential for a genetic basis for individual call signatures of bats in either colony.

Methods

Field sites and animals

Fieldwork was carried out in July 2002 in the home range of the two Bechstein's bat colonies "Blutsee" and "Höchberg". Both colonies occur in deciduous forests near the city of Würzburg (northern Bavaria, Germany) and use bat boxes as day roosts ("2FN", Schwegler, Germany). The colony Blutsee has been continuously studied since 1993 and the colony Höchberg since 1996 (Kerth 1998, 2006). All adult females (Blutsee, 18; Höchberg, 19) were individually marked with PIT tags (Kerth and König 1996). We obtained sound recordings from 23 females (Blutsee, 10; Höchberg, 13). From a previous study (Kerth et al. 2002), degrees of pairwise relatedness obtained with the program "relatedness" (version 5.0; Queller and Goodnight 1989), and family pedigrees were available for 21 of the 23 study animals (Blutsee, 10; Höchberg, 11).

Recording set-up and identification of individual bats

We fixed the recording microphone to the tree trunk at about 1 m below the box currently used by the bats and pointed it upwards at about 45 degrees relative to the ground. In this way, we obtained rather standardised recordings of bats approaching the box or circling around

the roost tree. The roost and surroundings were surveyed, and bat activity recorded by video (Sanyo VC 1950 CCD video cameras and Orion Combi 600 LCD recorders) under stroboscopic infrared illumination in temporal synchrony with sound recordings. Synchronisation was achieved by writing a video-time code (VITC code) into the digital sound file and the video frames (electronics custom-made; Department of Animal Physiology, University of Tübingen). At the Blutsee colony, we additionally monitored and recorded the interior of the box using infrared illumination and an infrared video camera (Ikegami-47E) attached to a recorder (Panasonic AG 6720A-E; Kerth and König 1996). Each roost was equipped with an automatic reading device for identifying the arriving individuals. Readers recorded and stored PIT-tag numbers and departure or arrival times of bats when they passed the antenna in the box's entrance area (Kerth and Reckardt 2003). Members of the Blutsee colony were also identified on videotapes by their plastic split rings (each animal carried an individual combination of ring colours; Kerth and König 1996). We precisely synchronised the clocks of the transponder reader and of the computer controlling the VITC code for sound and video recordings. We only included call sequences into the analysis where we could unequivocally identify the individual bat by a close match between the times of transponder reading and sound recording as well as a video observation of a circling bat just prior to entering or just after leaving the roost.

As the bats had given birth few days prior to our recordings, they repeatedly returned to the roost during the night to nurse their young (Kerth 2006). We therefore were able to obtain at least two recordings per night for most individuals. All call sequences contained between 4 and 27 calls.

Recording sites

We obtained recordings from the Blutsee colony from two consecutive nights from two different boxes. One roost tree was located in a rather open situation on the bank of a swampy pond. The respective bat box (154) was hanging 2.8 m above ground, and there were only four other trees within 5 m of the box tree. None of these trees had branches in the same height as the box above ground. The other roost tree stood in a rather closed beech forest matrix. The box (95) was hanging 2.9 m above ground, and there were nine other trees within 5 m of the box tree, seven of these trees had branches in the height of the box above ground and some of those branches almost touched the box entrance (Fig. 1). We obtained recordings with satisfactory quality from both boxes for seven individuals. Three additional bats were recorded only at the box 154 in the more open situation. Recordings from the Höchberg colony were obtained from a box (2) that was placed at a tree 3.5 m above ground with four other trees within 5 m of the box tree. There, we were able to record 13 unequivocally identified individuals.

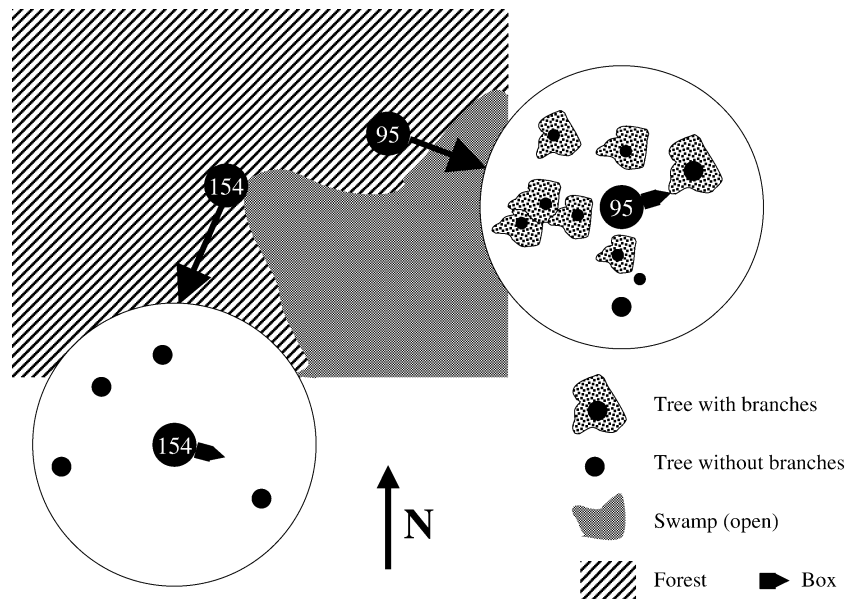


Fig. 1 Schematic drawing of the locations of the two boxes at which we obtained recordings in the home range of the Blutsee colony. Around each box, circles with a radius of 5 m are shown in detail to illustrate the clutter situation (*insets*). The black circles in the centre of each inset represent the tree where the box was suspended. The squares indicate the box positions on the tree, and the small arrows on top of them represent the directions the

entrances were facing. The box 154, which was hanging in a relatively open situation, had only four trees within a radius of 5 m from it, and none of these trees had branches in the height of the box above ground. In contrast, box 95 was surrounded by nine trees within a radius of 5 m, and seven of these trees had branches in the height of the box

Sound recording and analysis

We used a custom built ultrasonic microphone [flat-frequency response (± 3 dB) in the range of 30–120 kHz and ± 6 dB in the range of 15–160 kHz; Department of Animal Physiology, University of Tübingen]. The signals were A/D converted with custom built hardware and software (Department of Animal Physiology, University of Tübingen, and PCTape, Menne BioMed) at a sampling rate of 480 kHz with 16-bit depth (additional eight-times oversampling for digital anti-aliasing filtering), fed into a laptop computer via the USB port and stored on the hard disk.

Digital signal analysis was performed with custom written software (Selena, Department of Animal Physiology, University of Tübingen). Calls were displayed as spectrograms (frequency vs. time vs intensity plots) with frequency range from 0 to 240 kHz using a fast Fourier transform (FFT) with Hann window and 256 sample points. Each FFT window was enlarged to a 1,024-point window by zero padding to improve reading accuracy in the frequency domain. Spectrograms were calculated with 95% overlap (i.e. 13 new points of 256 per FFT) to improve reading accuracy in the time domain. The resulting spectrograms had 0.47 kHz frequency reading accuracy (240 kHz bandwidth/512 frequency lines that resulted from the 1,024-point FFT) and 0.03 ms temporal reading accuracy (13 new FFT points/480 kHz sampling rate). The frequency–time course of the first harmonic of each signal was tracked automatically by selecting the screen pixel with the highest amplitude value in the first harmonic

for each FFT. The automatically selected pixels were overlaid on the spectrogram, checked visually and corrected manually, if necessary. Time, frequency and amplitude values for each pixel (40 pixels per millisecond call duration) were exported to an Excel (Microsoft) spreadsheet. From these values we calculated starting frequency (SF) and terminal frequency (TF) relative to the pixel with highest amplitude (Fig. 2). SF-20 gives the frequency at which the signal first reaches an amplitude 20 dB below maximum amplitude. TF-20 gives the frequency where the signal again drops below an amplitude 20 dB below maximum amplitude. PD-20 gives the pulse duration (PD) as the interval between the points in time corresponding to SF-20 and TF-20. Accordingly, we measured SF-10, SF-6, SF-3, TF-3, TF-6, TF-10 and PD-10, PD-6, PD-3 (Fig. 2). We defined the modulation rate MR-20 as $(SF-20 - TF-20) / PD-20$, assuming a linear downward-frequency modulation. MR-10, MR-6 and MR3 were calculated accordingly. In total, we measured 16 call parameters that will not be completely independent from each other. However, individual signatures might be encoded in the frequency–amplitude relation as well as the frequency–time relation, and, therefore, in one of the different starting or terminal frequencies, pulse durations or modulation rates we measured. We therefore decided to run our analysis using all parameters. Based on the main outcome of the study that evidence for individual signature is weak, we argue that the use of many potentially intercorrelated parameters is a conservative approach to back up this finding.

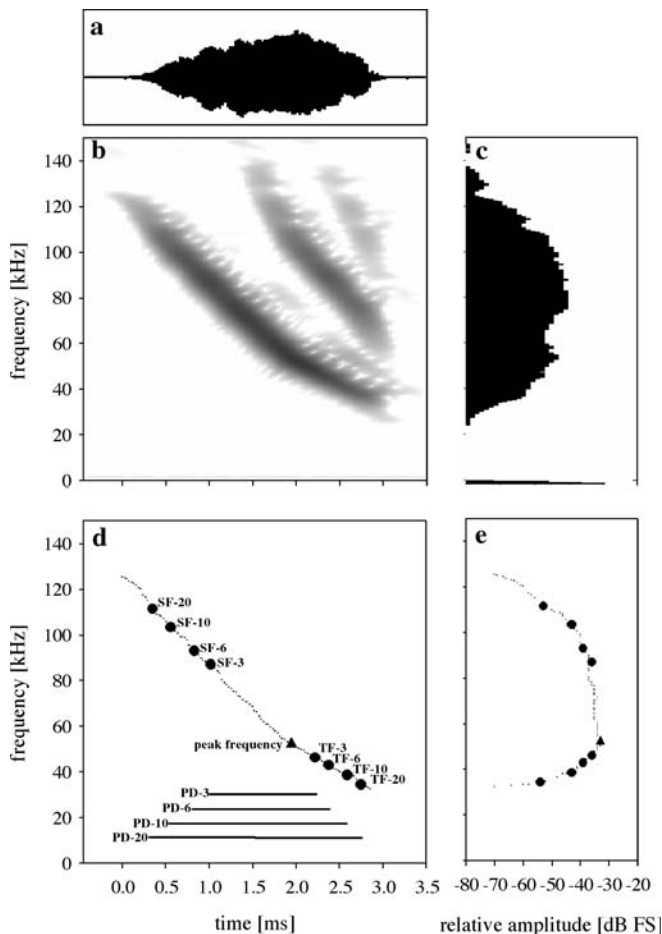


Fig. 2 Example illustration of parameter extraction from the call of a Bechstein's bat: **a** time signal, **b** spectrogram representation (256-point FFT, zero padding, to 1,024-point FFT, 95% overlap), **c** averaged power spectrum over the entire call. **d** Frequency–time course of the first harmonic of the call. The *small dots* show the pixels with highest amplitude that were automatically extracted. The *bold black dots and lines (bottom)* illustrate how starting (*SF*) and terminal frequencies (*TF*) and pulse duration (*PD*) were computed relative to the highest instantaneous amplitude (*black triangle*) in the call. **e** Power spectrum for the first harmonic, showing the amplitude values of the automatically extracted pixels with highest amplitude; note that the highest amplitude is at a different frequency than in **c**, because the power spectrum in **c** averages over all harmonics (three in this example)

Statistics

To compare pairwise degrees of relatedness to pairwise differences in call parameters, Mantel tests were performed with FSTAT (version 2.9.3 updated from Goudet 1995), using 20,000 randomisations for each test. All other analyses were done with Excel 2000, Systat 10 (SPSS) and Jump 4 (SAS). We used discriminant function analysis (DFA) to assign calls to individual bats in the Blutsee colony, for which we had a larger sample size of repeatedly recorded individuals from two different locations. As our data set comprises several sequences for most bats, we could test how well the discriminant function performed when tested with (1) the data set it was built on, with (2)

single calls that were excluded in turns from the computation of the respective discriminant function (jackknifing or “leave-one-out”) and with (3) different call sequences from the same bat from the same or from another recording location (cross validation).

Results

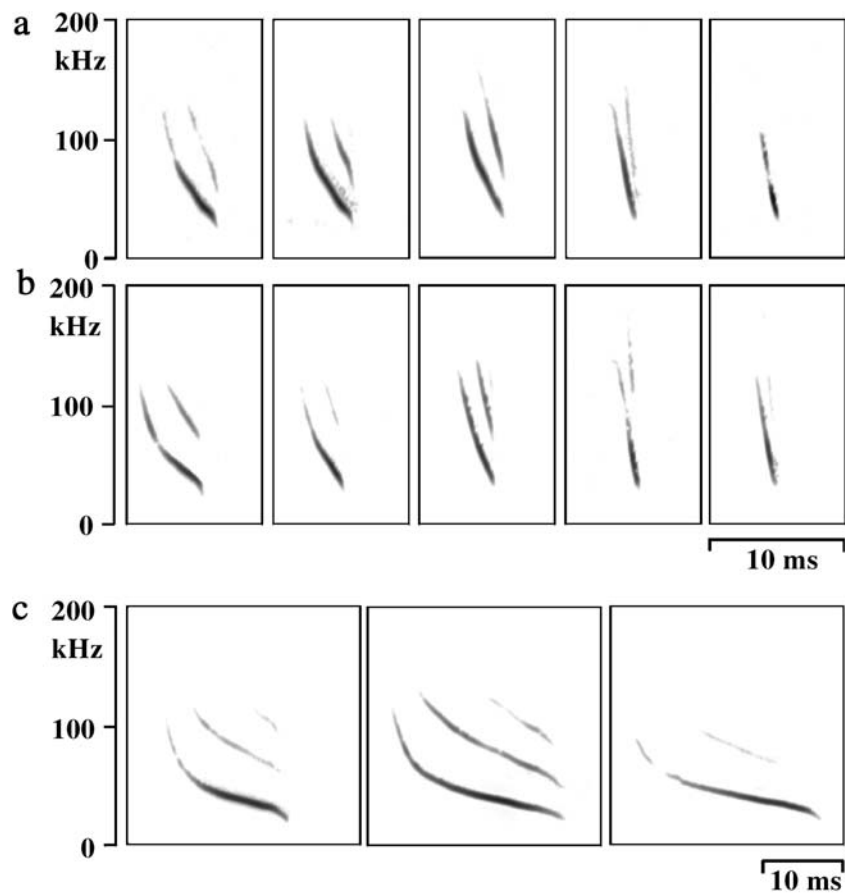
All Bechstein's bats emitted broadband, downward-frequency-modulated echolocation signals with most energy in the first harmonic (example calls in Fig. 3) and call durations of 1 to 6 ms. On few occasions, longer calls were interspersed singly or in pairs into a sequence of shorter echolocation calls (Fig. 3). We excluded signals with PD-20 > 6 ms from the analysis of echolocation calls as it is not clear whether these long calls primarily serve an echolocation function or are social calls, known to be individual specific in some bat species (Pfalzer and Kusch 2003).

Hypothesis I: situation-specific call structure

We recorded seven individuals from the Blutsee colony at both the box in the more open and the box in the closed forest situation (total of 182 calls). Separately for each of the two locations, we pooled all calls obtained within individuals and calculated a multivariate generalised linear model (GLM) for all call parameters measured (individual and location as independent variables, all call parameters as dependent variables; constant included). We found a significant effect of both individuals (Wilks' Lambda, 0.0921; F , 2.81; df , 96, 516; $p < 0.0001$) and locations (Wilks' Lambda, 0.4753; F , 6.20; df , 16, 90; $p < 0.0001$). For the time (PD) and frequency–time (MR) parameters, a much larger amount of variation could be explained by the different recording locations than by the different individuals (F test computed within GLM model; compare F values in Table 1, example call repertoires in Fig. 3). In contrast, individuality explained more variation than the two recording sites for some frequency parameters (TF-3, SF-20, SF-10, SF-6, SF-3). The four starting frequency measures differed only between individuals, but not between sites.

For a meaningful comparison of call parameters, it is necessary to cover intra-individual variability and therefore we included all calls with sufficient signal-to-noise ratio into the GLM (F tests, left side of Table 1). From a statistical viewpoint, however, taking several calls per individual can be regarded as pseudo-replication. We therefore ran additional analyses that only included one data point per individual and recording situation to corroborate our findings. To assess situation specificity of call parameters, we compared second order means of the call parameters between the two recording sites in a matched sample test (Table 1, centre). After Bonferroni correction for multiple testing, only PD-10 differed between sites and was, in accordance with the F tests, shorter when the bats flew around the box in closed

Fig. 3 Spectrogram of representative examples of Bechstein's bats' calls. **a** and **b** depict echolocation calls of two individuals from the Blutsee colony ordered from long (*left*, broadcast in more open situation) to short (*right*, broadcast in cluttered situation or shortly before landing at the roost), exemplifying that echolocation call repertoires comprise a continuum of calls with varying parameters and overlap strongly among individual bats. The notches in the call spectrograms are presumably caused by two-wave-front interference (Kalko and Schnitzler 1989) and should not be regarded as individual or situation specific. **c** Examples of long calls that were broadcast interspersed into echolocation sequences (first and second call in **c** from two different Blutsee bats, third call from a Höchberg bat; echolocation calls in **b** and the second long call in **c** stem from the same individual)



vegetation as compared to the more open situation (Table 1, centre; without correction all time and frequency-time parameters differ, but only one frequency parameter). To further assess individual specificity of call parameters, we correlated the individual means from one site with the means for the same individuals from the second site. Assuming robust individual signatures, one could predict a good correlation of the individual means between sites (e.g. some bats might always have relatively high starting frequencies or relatively long calls compared to the other individuals). However, the correlations were not significant for any of the parameters tested (Table 1, right). Likewise, maximum starting frequencies recorded for the seven individuals at both sites were not correlated (SF-20, $P=0.3280$; SF-10, $P=0.5584$; SF-6, $P=0.3309$; SF-3, $P=0.7340$).

Variability of call parameters was high within individuals and within colonies (Fig. 4). The parameter space used by the Blutsee and the Höchberg colony overlapped almost completely (Fig. 4). We refrained from a statistical comparison of the two colonies, because they could not of course be recorded at the same location and therefore it would not have been possible to distinguish situation-specific variability from possible colony differences (compare Pearl and Fenton 1996).

Hypothesis II: individual-specific signature

We used DFA on the frequency parameters to assign calls to individual bats for the Blutsee colony. When using the calls from just one sequence per individual to both compute and test the discriminant function, the performance of the algorithm was high above chance level (Table 2). When applying a jackknife procedure (removing one case at a time, calculating a discriminant function and then assigning this removed case), performance was lower, but still clearly above chance level. When we cross-validated the discriminant function with a test sample consisting of a different call sequence from the same night and location for each bat, the performance dropped dramatically. A similar picture showed when we took all calls recorded (from several sequences per bat, where available) at one of the two Blutsee colony roosts to compute a discriminant analysis. The discriminant function performed reasonably when tested on the learning sample (with and without jackknifing), but worsened drastically when tested with the calls recorded at the other Blutsee roost. The performance was not increased markedly when we included time parameters in addition to the frequency parameters (lower half of Table 2).

Table 1 Individual and site differences in call parameters from the Blutssee colony (see text for further explanations)

Parameter	GLM		Paired <i>t</i> test on site differences		Correlation of parameters means within individuals between sites
	Factor individual <i>F</i> and <i>P</i> value	Factor recording site <i>F</i> _{df} and <i>P</i> value	Mean (2nd order)	<i>t</i> _{df} and <i>P</i> value	<i>R</i> ² and <i>P</i> value
SF-20	<i>F</i> _{6,105} =5.59 <i>P</i><0.0001	<i>F</i> _{1,105} =0.001 <i>P</i> =0.9728	C, 113.0 kHz O, 113.9 kHz	<i>t</i> ₆ =-0.26 <i>P</i> =0.8004	<i>R</i> ² =0.21 <i>P</i> =0.3045
SF-10	<i>F</i> _{6,105} =4.93 <i>P</i>=0.0002	<i>F</i> _{1,105} =0.69 <i>P</i> =0.4089	C, 98.5 kHz O, 100.8 kHz	<i>t</i> ₆ =-0.68 <i>P</i> =0.5229	<i>R</i> ² =0.00 <i>P</i> =0.9526
SF-6	<i>F</i> _{6,105} =3.83 <i>P</i>=0.0017	<i>F</i> _{1,105} =1.36 <i>P</i> =0.2462	C, 92.3 kHz O, 94.0 kHz	<i>t</i> ₆ =-0.45 <i>P</i> =0.6420	<i>R</i> ² =0.00 <i>P</i> =0.8918
SF-3	<i>F</i> _{6,105} =3.96 <i>P</i>=0.0013	<i>F</i> _{1,105} =0.04 <i>P</i> =0.8373	C, 86.8 kHz O, 85.07 kHz	<i>t</i> ₆ =0.37 <i>P</i> =0.7263	<i>R</i> ² =0.14 <i>P</i> =0.4023
TF-20	<i>F</i> _{6,105} =5.47 <i>P</i>=0.0001	<i>F</i> _{1,105} =28.52 <i>P</i><0.0001	C, 43.4 kHz O, 40.2 kHz	<i>t</i> ₆ =4.05 <i>P</i> =0.0067	<i>R</i> ² =0.47 <i>P</i> =0.0898
TF-10	<i>F</i> _{6,105} =5.95 <i>P</i><0.0001	<i>F</i> _{1,105} =47.98 <i>P</i><0.0001	C, 49.3 kHz O, 43.9 kHz	<i>t</i> ₆ =2.23 <i>P</i> =0.0672	<i>R</i> ² =0.10 <i>P</i> =0.4814
TF-6	<i>F</i> _{6,105} =5.63 <i>P</i><0.0001	<i>F</i> _{1,105} =11.02 <i>P</i>=0.0012	C, 54.4 kHz O, 49.3 kHz	<i>t</i> ₆ =1.46 <i>P</i> =0.1935	<i>R</i> ² =0.03 <i>P</i> =0.6958
TF-3	<i>F</i> _{6,105} =7.32 <i>P</i><0.0001	<i>F</i> _{1,105} =5.52 <i>P</i> =0.0205	C, 60.4 kHz O, 54.1 kHz	<i>t</i> ₆ =2.00 <i>P</i> =0.0922	<i>R</i> ² =0.20 <i>P</i> =0.3126
PD-20	<i>F</i> _{6,105} =3.31 <i>P</i> =0.0051	<i>F</i> _{1,105} =40.78 <i>P</i><0.0001	C, 1.67 ms O, 2.44 ms	<i>t</i> ₆ =-4.28 <i>P</i> =0.0052	<i>R</i> ² =0.02 <i>P</i> =0.7745
PD-10	<i>F</i> _{6,105} =3.79 <i>P</i><0.0019	<i>F</i> _{1,105} =49.49 <i>P</i><0.0001	C, 1.16 ms O, 1.94 ms	<i>t</i> ₆ =-5.14 <i>P</i>=0.0021	<i>R</i> ² =0.10 <i>P</i> =0.2515
PD-6	<i>F</i> _{6,105} =4.11 <i>P</i>=0.0010	<i>F</i> _{1,105} =41.50 <i>P</i><0.0001	C, 0.89 ms O, 1.57 ms	<i>t</i> ₆ =-4.13 <i>P</i> =0.0061	<i>R</i> ² =0.16 <i>P</i> =0.3769
PD-3	<i>F</i> _{6,105} =4.03 <i>P</i>=0.0011	<i>F</i> _{1,105} =22.67 <i>P</i><0.0001	C, 0.62 ms O, 1.12 ms	<i>t</i> ₆ =-3.36 <i>P</i> =0.0152	<i>R</i> ² =0.14 <i>P</i> =0.4118
MR-20	<i>F</i> _{6,105} =2.37 <i>P</i> =0.0349	<i>F</i> _{1,105} =26.10 <i>P</i><0.0001	C, 42.5 kHz/ms O, 32.4 kHz/ms	<i>t</i> ₆ =3.26 <i>P</i> =0.0172	<i>R</i> ² =0.01 <i>P</i> =0.7972
MR-10	<i>F</i> _{6,105} =2.27 <i>P</i> =0.0426	<i>F</i> _{1,105} =20.46 <i>P</i><0.0001	C, 42.4 kHz/ms O, 32.3 kHz/ms	<i>t</i> ₆ =3.46 <i>P</i> =0.0134	<i>R</i> ² =0.00 <i>P</i> =0.9072
MR-6	<i>F</i> _{6,105} =1.85 <i>P</i> =0.0962	<i>F</i> _{1,105} =17.00 <i>P</i><0.0001	C, 41.9 kHz/ms O, 31.8 kHz/ms	<i>t</i> ₆ =3.16 <i>P</i> =0.0195	<i>R</i> ² =0.01 <i>P</i> =0.8288
MR-3	<i>F</i> _{6,105} =1.76 <i>P</i> =0.1147	<i>F</i> _{1,105} =15.70 <i>P</i><0.0001	C, 41.6 kHz/ms O, 30.8 kHz/ms	<i>t</i> ₆ =2.63 <i>P</i> =0.0389	<i>R</i> ² =0.26 <i>P</i> =0.2331
TPM <i>P</i>	<i>P</i> <0.0001	<i>P</i> <0.0001		<i>P</i> <0.0001	<i>P</i> =0.8607

As this table includes multiple testing, a more conservative alpha level than 0.05 has to be used for assigning significance. With 16 individual tests per column, $\alpha=0.05/16=0.0031$ results from a classical, rather conservative Bonferroni approach. Accordingly, *P* values below this level are highlighted in bold, and only these should be regarded as statistically significant. In addition, we follow Neuhauser (2004) in presenting individual *P* values in combination with the summary *P* value from the truncated product method (TPM *P*, last row of table). TPM *P*s were calculated using a program (tpm.exe) provided at <ftp://statgen.ncsu.edu/pub/zaykin/tpm> (see Zaykin et al. 2002) to test whether any of the tests with *P*<0.05 in the table are indeed significant (Neuhauser 2004).

SF Staring frequency, TF terminal frequency, PD pulse duration, MR modulation rate, C closed site, O open site, TPM truncated product method, GLM generalised linear model, *df* degrees of freedom

Hypothesis III: genetic basis of call structure

We used a Mantel test to investigate possible similarities in call structure between relatives within each of the two colonies. As calls differed markedly between the two Blutssee recording locations, we used data from one location only (open site, box 154) to minimise the confounding effect of

situation-specific call structure that might mask possible relatedness effects. For the same reason we focussed on frequency parameters and disregarded the highly site-specific time parameters. In a first step, we compared our two different measures of relatedness. As expected, pairwise degrees of relatedness obtained with a pedigree analysis correlated significantly positive with pairwise estimates of

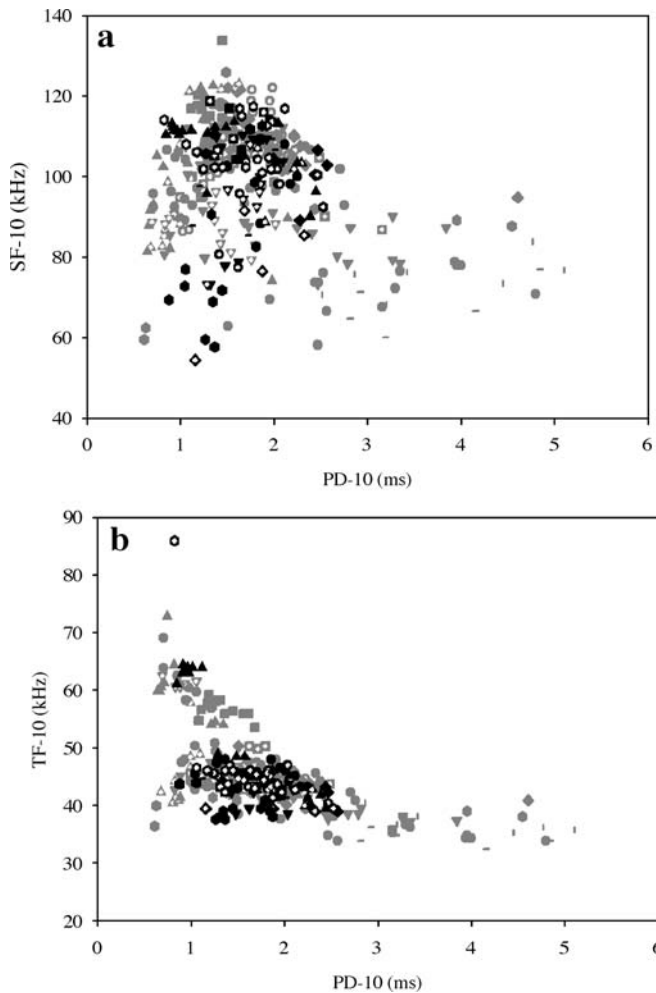


Fig. 4 Relation between frequency and time parameters for echolocation calls of bats from both study colonies. Starting (*SF-10*, **a**) and terminal frequency (*TF-10*, **b**) are plotted over pulse duration (*PD-10*) for 13 female Bechstein's bats from the Höchberg colony (*black symbols*; total of 112 calls from one roost location and one night) and for 10 female bats from the Blutsee colony (*grey symbols*; total of 238 calls from two boxes and two nights, seven bats recorded at both locations, see "Methods"). Calls of different individuals are represented by different symbols

genetic relatedness obtained with the program "relatedness" (Blutsee, $R^2=0.34$, $P=0.002$; Höchberg, $R^2=0.17$, $P=0.02$). For each colony, we compared the degree of relatedness among colony members with differences in their individual call structure. We focussed on two frequency parameters (*SF-20*, *TF-20*) because in both colonies all four starting frequency parameters (*SF-20* to *SF-3*) were significantly correlated with each other, and the same was true for all four terminal frequency parameters (*TF-20* to *TF-3*; data not shown). Among the members of the Blutsee colony, genetic relatedness, but not relatedness obtained by pedigree analysis, was significantly negatively correlated with inter-individual differences in *SF-20*. In contrast, we observed no such correlation for *TF-20*. In the

colony Höchberg, none of the two call parameters were significantly correlated with relatedness (Table 3).

Discussion

Our data support the hypothesis that the bats' echolocation calls structures differed between two recording locations. The most prominent effect was on call duration and, as expected, bats used shorter calls in more cluttered situations and longer calls in the more open ones. Short calls are thought to improve target separation and localisation (Simmons and Stein 1980; Schnitzler and Kalko 2001). The influence of habitat density and distance to acoustic clutter on call duration is well documented for other bat species both in the field and in the laboratory (Kalko and Schnitzler 1993; Siemers and Schnitzler 2000; Jensen and Miller 1999; Siemers et al. 2001b). Our study on the Bechstein's bat shows that this effect is robust on the basis of identified individuals all recorded at the same two sites in the field.

In contrast to their effect on time parameters, the recording sites and their different habitat density had a negligible effect on the frequency parameters we studied. Terminal frequency was a few kilohertz lower, and bandwidth therefore was higher, at the more open site (also compare Fig. 4b). At first, this broadening in bandwidth seems astonishing because many vespertilionids, including some *Myotis* species, switch from broadband to narrow-band signals when moving from cluttered to open space (e.g. Surlykke et al. 1993; Kalko and Schnitzler 1993; Jensen and Miller 1999; Siemers et al. 2001b). Narrowband calls are thought to improve prey detection probability (Schnitzler and Kalko 2001). Cluttered habitat is a typical situation for Bechstein's bats (Kerth et al. 2001), and our data suggest that both recording situations were cluttered enough for the bats to use broadband signals. These are beneficial for a good description of the echo-reflecting objects surrounding the bats (Neuweiler 1989, 1990, 2003; Fenton 1990; Schnitzler and Kalko 2001; Schnitzler et al. 2003; Siemers and Schnitzler 2004). Even *Myotis* species that detect their prey predominantly by echolocation but habitually forage in cluttered habitats maintain considerable call bandwidth when flying in rather open space (*Myotis nattereri*, Siemers and Schnitzler 2000; *Myotis brandtii*, Siemers et al. 2001b). It is reasonable to assume that this is even more the case for *M. bechsteinii* that detects its prey by listening for prey sounds (Staden and Schnitzler 1995; Siemers and Swift 2005) and therefore can adapt its call structure for spatial orientation instead of prey detection.

We found significant differences between individuals in the GLM computed for the Blutsee colony. However, the proportion of variation explained by the factor individuality was relatively small for most call parameters (compare *F* values in Table 1). Furthermore, the lack of correlation between sites for any individual's call parameter means

Table 2 Discriminant function analysis assigning single calls to individuals; Blutsee colony (see text for further explanations)

Parameters used	Learning sample	Test sample	Percent correct classification (means and range)		
			Learning sample	Learning sample jackknifed	Test sample
SF, TF	Open site 1st sequence	Open site 2nd sequence	72% (43–100)	50% (0–100)	17% (0–57)
	Open site all calls	Closed site all calls	54% (27–100)	45% (9–100)	26% (0–89)
	Closed site all calls	Open site all calls	83% (50–100)	60% (0–100)	8% (0–18)
SF, TF, PD	Open site 1st sequence	Open site 2nd sequence	83% (67–100)	52% (0–100)	24% (0–100)
	Open site all calls	Closed site all calls	65% (40–100)	50% (27–76)	26% (0–100)
	Closed site all calls	Open site all calls	91% (78–100)	63% (0–100)	12% (0–20)

The discriminant functions were computed based on the learning sample. Their classification performance was tested with the learning sample (without and with jackknifing) and cross-validated with a different set of calls, the test sample. $P < 0.0001$ for all discriminant functions

1st sequence, first call sequence recorded for every individual bat in a given night; 2nd sequence, second call sequence recorded for these individuals in the same night at the same location

Calls from seven bats are included in the discriminant analysis. Therefore, the chance level for correctly assigning a call to an individual bat can be assumed at 14.3%

SF Starting frequency, SF-3, SF-6, SF-10, SF-20 included; TF terminal frequency, TF-3, TF-6, TF-10, TF-20 included, PD pulse duration, PD-3, PD-6, PD-10, PD-20 included

does not support a simple and robust individual signature; for example encoded in starting and/or terminal frequency. In contrast to our study on forest bats, marked inter-individual differences in echolocation call frequencies were reported for some open space bats by Fenton et al. (2004). In sheep, playback experiments yielded support for a correspondingly simple frequency coding of individuality in mother–offspring communication (Searby and Jouventin 2003).

We tested the feasibility of assigning single calls to individual bats using DFA. Like other field studies that used a similar approach (e.g. Fenton et al. 2004), we found that assignment was satisfactory when using one call sequence per bat to both compute (“train”) and test the discriminant function [correct assignment, >70% (Fenton et al. 2004) vs. 72 and 83% (present study)]. We also could test the utility of DFA with different sequences from known individuals. We found that DFA performance dropped drastically when tested with other than the training sequences from the same bats (Table 2). Hence, correct assignment of single calls to individual call sequences was reliable, but not to individual bats. The drastic drop in assignment performance indicates that calls are rather uniform within a sequence, but differ considerably between sequences. Based on this finding, we caution against concluding from a satisfactory discrimination performance with identical training and test sequences that individ-

ual bats can reliably be told apart by echolocation call structures.

Despite the limitation of sample size, the DFA performance usually was above chance level even in the cross-validated tests. This suggests that echolocation calls have some potential to allow bats to discriminate between colony members in a natural situation. However, Bechstein’s bat colonies often comprise 20 or more individuals and are frequently neighboured by several other colonies (Kerth et al. 2002; Kerth 2006). If there are no colony-specific signatures in *M. bechsteinii* (see Pearl and Fenton 1996 for data on *Myotis lucifugus*), a powerful signal for individual recognition should therefore be variable enough to code about 100 different individuals. Based on the observed relatively low levels of correct assignments for only seven individuals in the cross-validated approach (Table 2), coding many more individuals seems a difficult task to achieve with echolocation calls alone.

On the other hand, bats may benefit from hearing many trains of many calls from roost mates, and they may be able to use a collective template to recognise conspecifics at the group or even individual level. Given bats’ high abilities to process acoustic information, it is possible that they can achieve a much better classification result from a sequential procedure on a call series and its patterning than single-call classification can achieve. Through such a process, even single-call classification levels that are only slightly above

Table 3 Correlation obtained by Mantel tests between two measures of pairwise relatedness and individual differences in call parameters obtained from members of the Blutsee and the Höchberg colony (see text for further explanations)

Parameter (individual means)	Blutsee (10 females)		Höchberg (11 females)	
	Genetic relatedness	Pedigree	Genetic relatedness	Pedigree
SF-20	$R_2=0.15$ $P < 0.01$	$R_2=0.02$ $P = 0.37$	$R_2=0.00$ $P = 0.68$	$R_2=0.01$ $P = 0.45$
TF-20	$R_2=0.00$ $P=0.81$	$R_2=0.01$ $P = 0.67$	$R_2=0.03$ $P = 0.22$	$R_2=0.04$ $P = 0.12$

chance level, as found in our study, could add up to identification levels useful for reliable identification of conspecifics. Or else, bats might recognise the situation another bat is in by its call characteristics and then be able to apply species-specific rules to separate the situation-specific call features from individual-specific ones. For comparison, while an acoustic analysis found no sexual dimorphism in the echolocation calls of laboratory-kept *E. fuscus* (Kazial et al. 2001), Kazial and Masters (2004) provided behavioural evidence that captive *E. fuscus* were able to recognise sex from echolocation calls.

There is preliminary behavioural evidence from the field indicating that *M. bechsteinii* pups recognise their mother when she is in approach flight towards the roost, but prior to landing (Elsasser 1995). This situation excludes visual or olfactory recognition cues and leaves auditory information as the only feasible cues indicating the bats' identity. But this information could be encoded in echolocation or social calls. Pfalzer and Kusch (2003) described long, downward-frequency-modulated calls as mother–infant contact signals in *M. bechsteinii* (C type calls; see also Hill and Greenaway 2005). Similar calls have been described for the Blutsee colony in situations when a mother enters a roost at night to nurse her young (Elsasser 1995). The parameters of these signals correspond well to those of the long calls interspersed into sequences of shorter echolocation calls that we recorded from female *M. bechsteinii* approaching the roosts (examples in Fig. 3c). Unfortunately, we did not obtain enough recordings of this call type to explore for individual signatures, but they clearly deserve further attention. Surely, these long calls would be better suited to carry information over some distance to conspecifics than the typical *M. bechsteinii* echolocation calls, because the former concentrate energy in lower frequencies for relatively long time and are therefore less affected by atmospheric attenuation loss (e.g. Wiley and Richards 1978).

Members of the Blutsee colony that were genetically similar to each other had similar starting frequencies (SF-20), allowing for the possibility that starting frequencies have a genetic basis. We cannot, however, exclude the alternative that daughters may copy or learn starting frequencies from their mothers instead of genetically inheriting them (Jones and Ransome 1993). Isolation calls used by bat pups to attract their mothers can have heritable signatures (Scherrer and Wilkinson 1993), but also may be matched to the mothers' individual call structure through vocal learning (Esser and Schmidt 1989; Esser 1994). The absence of a significant correlation between relatedness and call structure in the H6chberg colony suggests that, in *M. bechsteinii*, the relationship between call structure and relatedness is rather weak. This may also be a statistical problem in our data set. In both colonies we recorded only few closely related individuals. In the Blutsee colony, our data included two mother–daughter pairs and a pair of half-sisters, while in the H6chberg colony there was only one mother–daughter and one half-sisters pair. However, given the observed effects (*R* values), samples sizes would have to be larger than the

colony size of Bechstein's bat colonies to reach significance (Eq. 19.3, p 379, Zar 1999).

Conclusions

Our field data from individually marked, free-flying bats demonstrate situation-specific call structure, but the evidence for individual-specific signatures is relatively weak. We could not identify a robust and simple parameter that would convey the caller's identity despite the situation-specific call variability. Starting frequencies did give the best discrimination between individuals, and it was also this parameter that was correlated with genetic relatedness in one of our two study colonies. Our study suggests that echolocation signals could serve as an additional source of information for individual recognition in Bechstein's bats societies. Although it is unlikely that a large number of individuals could be reliably identified in different situations based on echolocation alone, echolocation calls may play an important role in the context of detecting foreigners. Because bats have to use echolocation calls to orient in space, this type of calls cannot be easily concealed unlike social calls that have a purely communicative function. In a next research step, definitive evidence for the role of echolocation signals for individual recognition has to come from behavioural experiments. The communicative potential of the long calls interspersed into echolocation sequences of homing bats deserves special attention in the context of encoding individuality.

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