ORIGINAL ARTICLE

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Bat serenades—complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*)

Abstract Vocalisations of many songbirds, anurans, and insects are shaped by sexual selection. Males acoustically compete for territories, and females choose their mates by means of male courtship songs. In courtship, richness and complexity of elements are often favoured characters. Only a few examples of complex songs are known in mammals. Males of the harem-polygynous sac-winged bat (*Saccopteryx bilineata*, Emballonuridae) have an uncommonly complex vocal repertoire, and different song types of males are used in the context of territorial defence and in courting females. We classified the daytime vocalisations of 16 male *S. bilineata* from a colony in Costa Rica, both on the basis of their acoustical properties and the social context in which they occurred. Seven vocalisation types were differentiated: echolocation pulses, barks, chatter, whistles, screeches, territorial songs and courtship songs. Territorial songs were short, rather stereotyped and not obviously directed towards a certain conspecific. They appear to be of importance in male competition for harem territories, in which females roost during the day. Courtship songs were exclusively observed when males displayed towards a female; they were long and complex, and consisted of highly variable elements ("calls"). We classified the calls in courtship songs of six males into call types, based on acoustical properties, mainly spectral purity and duration. Four call types are described in detail: trills, noise-bursts, "short tonal" calls, and "quasi constant frequency" calls. Twelve parameter values were extracted from the most common call type, the trill. Discriminant function analysis of trills showed that different males had different repertoires. This could allow females to use trill parameters for recognition of individual males and thus for mate choice.

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Introduction

In the context of sexual selection, acoustic signals play a role in male contest or female attraction (Andersson 1994). Territorial songs are found in many species and are commonly produced by competing males. Courtship songs are a usual component of male display towards females in birds, frogs, and insects (e.g. Searcy and Andersson 1986; Gerhardt and Huber 2002). The prevalence of either intra- or inter-sexual selection in the evolution of songs has been a topic of controversy since White (1789) and Darwin (1871). Proof for both functions has been found, sometimes for the same vocalisation (Searcy and Andersson 1986; Bradbury and Vehrencamp 1998). The majority of these studies have been performed in songbirds (see overview in Catchpole 1987; Kroodsma and Byers 1991; Andersson 1994).

In bats, there are several very detailed studies dealing with social vocalisations (e.g. Brown 1976; Fenton 1977; Matsumura 1979; Porter 1979; Brown et al. 1983; Balcombe 1990; Kanwal et al. 1994; Kingston et al. 2000; Davidson and Wilkinson 2002), but only a few focus on the significance of acoustical signals in the context of territorial behaviour or mate choice (examples are Wickler and Seibt 1976; Bradbury 1977; O'Shea 1980; Lundberg and Gerell 1986; McWilliam 1987; von Helversen and von Helversen 1994; Barlow and Jones 1997; Wilkinson and Boughman 1998; Boughman and Wilkinson 1998; Davidson and Wilkinson 2004). The scarcity of knowledge about the relevance of bat vocalisations in the context of sexual selection may be due to the fact that bats rarely show territorial or courtship behaviour in captivity. Direct observation of social interactions in the field is often impeded by the inaccessibility of roosts and the nocturnal activity of most bat species. Thus, in many cases, we lack contextual correlates of acoustic social signals, which can often give

indications of their social significance (Kodric-Brown and Brown 1984). In addition, quantitative data about the variability of vocalisations and the overlap of individual repertoires are rare. However, such data are crucial for testing hypotheses regarding the correlation of sound patterns and behaviour or fitness.

The sac-winged bat (*Saccopteryx bilineata*, Emballonuridae) is one of the few bats in which non-echolocation vocalisations have been described from field recordings (Bradbury and Emmons 1974; Davidson and Wilkinson 2002, 2004). Colonies of this harem-polygynous bat comprise up to 50 individuals (Bradbury and Vehrencamp 1976). Males defend harem territories against other males during the entire year, sometimes with vigorous fights. They may chase or hit each other with their folded wings, jam thumb claws in the rival's wing membrane or even, while biting and clutching each other, fall to the ground and lie there for several seconds, apparently unconscious (Tannenbaum 1975; own observations). The harem territories are used as day roosts by up to eight females. The composition of female groups in the male territories often is stable over years (Heckel et al. 1999). However, females may occasionally move between different harems (Bradbury and Emmons 1974; Tannenbaum 1975; Heckel et al., unpublished data), and a genetic parentage study revealed that about 70% of the young born in a harem territory were not sired by the harem owner, but by other males of the colony or even by males from outside the roost (Heckel et al. 1999; Heckel and von Helversen 2003). This finding implies that males are not able to monopolise copulations within their harem, and indicates a high potential for female choice.

Correspondingly, males strongly differ in their reproductive success, indicating an intense sexual selection pressure (Heckel et al. 1999; Heckel and von Helversen 2003). This, on the other hand, may explain the efforts (acoustical and other) males invest in mate attraction: unlike other harem-polygynous bat species, *S. bilineata* males perform a great variety of optical, olfactorial, and acoustical display behaviours that are believed to attract and retain females (Bradbury and Vehrencamp 1977). For example, they perform energetically costly hovering flights, which serve to fan male scents towards the female (Voigt and von Helversen 1999).

The courtship songs described in this paper represent an acoustical aspect of the male's complex behavioural display. We describe male *S. bilineata* vocalisations and characterise their behavioural context and acoustical structure. Two types of vocalisations were found that seem to play a role in sexual selection and are therefore studied in more detail: territorial and courtship songs.

Methods

Study site

We recorded 40 h of daytime vocalisations from 16 male *S. bilineata* in a colony at the Organisation for Tropical Studies field station La Selva in Costa Rica (10°20′N, 84°10′W; province Heredia). Data acquisition took place from 10 February to 21 May 1999 and from 15 January to 20 April 2000. The latter period of days overlapped with the second half of the mating season, which lasts from December to January (Voigt and von Helversen 2001).

Recordings were performed during the main activity periods of the day; about 2 h before dusk and after dawn. Additional vocalisation samples were taken to control for effects of daytime. Recordings included a minimum of ten territorial songs from each of 16 males, acquired by scan sampling, and a minimum of 10 min of courtship song from 12 males, acquired by focus sampling.

The colony of *S. bilineata* studied here has been the subject of investigations since 1994. It is located in an abandoned hut and surrounded by secondary forest and plantations. About 50 *S. bilineata* roost mainly on the vertical walls inside the building. All animals in the colony have been captured, their sex determined, measured, and individually marked with colour and numbered rings (Heckel et al. 1999; Voigt and von Helversen 2001). Bats have gradually been accustomed to observers inside the hut so we encountered optimal observation and recording conditions in an undisturbed field population. During the study period a census of animals in the colony was taken every 3 days, yielding an average number of females for each male territory. Territory ownership was stable during periods of field work, but three territories were taken over by other males between 1999 and 2000.

Sound recording and processing

Recordings were performed with an analogue video recorder adapted for ultrasound recording (Panasonic NV-100). We used a microphone capsule BT 1759 (Knowles), a microphone amplifier OP 37, and a two step NF amplifier LF 356 for signal acquisition. The microphone set-up had a free field response of ±10 dB from 0.5 to 32 kHz and of ±18 dB from 0.5 to 90 kHz. The directional characteristic of the microphone was focused using an oval parabolic reflector (diameters 30.0 and 33.5 cm), which increased the sensitivity at the focus point. For better control of the mi-crophone direction, we fixed an infrared pointer to the microphone; the infrared spot could be located with a night vision device (Litton Monocular M911). This set-up permitted recordings of single individuals even during the main activity periods at dusk and dawn when many males vocalised at the same time. Attributing signals to individuals was ensured by visual observations spoken on the comment track of the video tape. To permit a detailed analysis of the temporal relationship between acoustics and behaviour we videotaped an animal's behaviour during 20% of the recordings of vocalisations (Sony Digital Handycam DCR-TRV10E PAL).

Recordings were digitised with a DSP card at 500 kHz sample rate with 16 bit depth (DSP 56301 PCI A/D D/A Rev. 1.0.8.7.96). The DSP card was also used to perform playback experiments in the colony. Signals were amplified with a 12 V bridge power amplifier and fed into a hi-fi Dome Tweeter (Dynaudio D21/2). The loudspeaker had a frequency response of ±10 dB from 2 to 50 kHz and of ±19 dB from 2 to 100 kHz. The peak to peak amplitude of the playbacks was matched to natural signals recorded at the same distance and with the same microphone. For visualisation, extraction and fundamental frequency measurement of the recorded signals we used Avisoft-SASLab Pro (Specht 2002). Spectrograms were generated using a 1024 point FFT and a Hamming window with 75% overlap. Parameter extractions from the measured fundamental frequency were conducted with a routine in dBase IV (Ashton-Tate 1989). Discriminant function analysis was performed in SYSTAT (Systat Software 2002). Other statistical analyses and tests were conducted in SPSS 11.5 (SPSS 2002). Measurements are reported as mean ± standard deviation and additionally as median and quartile distance when the skewness (defined as mean / median) was bigger than 1.1 or smaller than 0.9. Samples for measures were from six individuals, ten measurements each, if not stated differently.

Table 1 Mean, standard deviation, median, and inter-quartile-distance of parameters measured in trill calls of six randomly chosen sac-winged bat (*Saccopteryx bilineata*) males (*n*=1,830)

Parameter	Mean	Standard deviation	Median	Inter-quartile distance
Total length (ms)[b]	64.6	19.8	63.0	26.0
Discontinuity = length of pauses / total length (%)[a]	0.050	0.079	0	0.077
Longest pause at beginning of call (ms)[a]	0.001	0.003	0	0
Longest pause at end of call (ms)[a] [b]	0.001	0.002	0	0.002
Main frequency (in mean spectrum kHz)[b]	19.7	7.6	17.3	4.6
Mean fundamental frequency (kHz)[b]	17.5	2.1	17.5	3.1
Initial frequency (kHz)[b]	17.2	3.8	17.6	6.0
End frequency (kHz)	15.6	3.4	15.5	5.3
Difference between initial and end frequency (kHz)[b]	1.6	4.7	1.7	6.6
Number of modulations[b]	7.0	2.5	7.0	4.0
Medium frequency stroke in modulations (kHz)[b]	2.7	0.8	2.6	0.7
Modulation frequency = number of modulations / (total length – length of pauses) (Hz)	116	26	118	29

[a] Asymmetric parameters with mean/median smaller than 0.9 or larger than 1.1
[b] Parameters which were included in the stepwise DFA

Terminology

The following terminology was used to describe non-echolocation vocalisations. The smallest vocalisation units were named syllables. We defined syllables as vocalisation bouts surrounded by at least 2 ms of silence, similar to other authors (Catchpole 1980; Kanwal et al. 1994). Syllables were combined to calls when silent intercepts neither lasted longer than the mean length of the two neighbouring syllables, nor longer than 10 ms. Thus, calls consisted of at least one to several syllables.

In the spectrum of harmonically structured signals we distinguished fundamental frequency, 1st harmonic, 2nd harmonic etc. Signals showing a defined fundamental frequency and harmonic structure were termed tonal, when not containing more than five harmonics below 60 kHz. Signals with more than five harmonics below 60 kHz were called multiharmonic. Signals having no discernible fundamental frequency and harmonics structure were called noise-bursts or noisy.

Analysis of vocalisation types

Vocalisations were classified into seven vocalisation types (echolocation pulses, barks, chatter, screeches, whistles, territorial and courtship songs) containing one to several calls. These classifications were based on the acoustical structure of the vocalisations as well as on the social context in which they appeared. In the further analysis we focussed mainly on two vocalisation types, which seemed to be of significance in sexual selection, namely territorial and courtship songs. We distinguished these two song types by their measurable acoustical properties (see Table 2) as well as by the fact that, during courtship song, males always pointed their head towards a specific female. We use the term "song" following the definition for birds by Pettingill (1970) in Baylis (1982): "Song is a vocal display in which one or more sounds are consistently repeated in a specific pattern. It is produced mainly by males, usually during the breeding season." Additionally we defined single songs as being separated from each other by at least 10 s of silence or non-song vocalisations (e.g. echolocation pulses).

Time for each territorial song from 15 males was annotated during two entire days in March and April 1999. Data on frequency of occurrence of courtship songs were kindly provided from unpublished data by S. Meister from 40 entire-day video recordings in eight male territories. Courtship songs were acoustically analysed in six randomly chosen males. From the entire courtship song recordings of these males, we randomly chose 20 fragments, each of 30-s duration, and used these 10 min of song for further analysis. The analysed song bouts contained between 203 and 1,492 (836.5±440.5) calls per male, resulting in a total of 5,019 analysed courtship song calls. The calls could be categorised into seven call types, which were distinguished by solely considering their acoustical structure, since no differences in behavioural context could be determined.

The most common call type was the trill; trills were recorded exclusively in courtship songs. We parameterised trills using 12 parameter values indicated in Table 1. To quantify male song variability and repertoire overlap, a linear stepwise discriminant function analysis (DFA) was performed for a total of 1,830 trills from six randomly chosen males. We used a randomly chosen half of the data set in the DFA and cross-validated the results with the second half. Prior probabilities were adjusted to the sample size per male. The DFA also extracted the parameters that best predicted the affiliation of trills to the respective male.

Results

Vocalisations of *Saccopteryx bilineata* males

We grouped male vocalisations recorded in the colony of sac-winged bats into seven vocalisation types (Fig. 1).

Echolocation pulse

Echolocation pulses were emitted by flying as well as perched animals. Pulses were mainly triggered by disturbances in the colony or by other colony members flying around.

Echolocation pulses typically contained a constant frequency (CF) or slightly upwards modulated part with a fundamental frequency of 23.4±0.70 kHz (Fig. 1A). The 1st harmonic, with a CF part around 44–48 kHz, was the loudest. Usually pulses started with an upward frequency modulated (FM) hook at the beginning and had a downward FM hook at the end. The pulse duration was 7.4±1.9 ms.

Bark

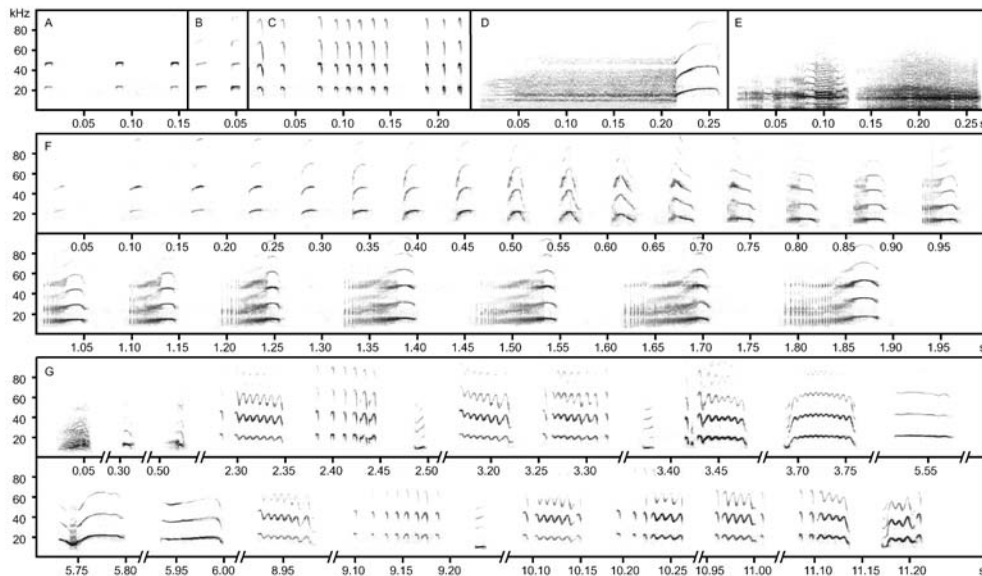We recorded barks mainly from males that were not evidently interacting with other colony members. In some

Fig. 1A–G Sonagrams of the seven vocalisation types emitted by sac-winged bat (*Saccopteryx bilineata*) males in the day roost. Frequency is plotted as a function of time (parameters of the FFT are given in the Methods section). Removed silent intervals between calls are indicated as a gap in the time axis. **A** Three echolocation pulses; **B** two barks; **C** chatter sequence; **D** whistle from a male performing a hovering flight in front of a female; **E** two screeches vocalised during an agonistic encounter between two males; **F** a complete territorial song; **G** a 12-s long fraction from a courtship song of 4 min 25 s total duration: a multiharmonic signal is shown at the beginning; short tonal calls at 0.30, 0.51, 2.48, 3.37, and 9.92 s; trill calls at 2.28, 2.38, 3.16, 3.25, 3.42, 3.69, 8.92, 9.10, 10.09, 10.19, 10.96, 11.08, and 11.17 s; quasi-CF calls at 5.52, 5.73, and 5.94 s

cases, they were also uttered by males in agonistic conflicts.

Barks resembled the previously described echolocation pulses in respect to frequency range and modulation (Fig. 1B), but had their spectral maximum in the fundamental frequency. Moreover, barks tended to be longer than typical echolocation pulses (10.5±1.9 ms).

Chatter

This vocalisation type was frequently emitted by males throughout the day at their roost sites. Similar to barks, chatters were not evidently accompanied by interactions between individuals.

Chatters occurred in sequences or trains consisting of up to 50 calls with a duration of 5.5±2.6 ms each (Fig. 1C). The sequences could reach a duration of up to several seconds. Single chatter calls resembled echolocation pulses, but usually had a higher degree of modulation. Distances between single calls were regular within one train, lasting 22.3±21.5 ms (median 13.6, quartile distance 19.7). Longer pauses were often interspersed between chatter trains containing shorter pauses (Fig. 1C). In courtship songs, a continuous shortening of pauses between chatter calls could be observed, which resulted in a fusion of the single calls. Such syncopated syllable trains represented a transition of chatter calls to trill calls, the most common component of courtship songs (Fig. 1G). In general, other vocalisation types, like territorial songs or components of courtship songs, were often embedded in trains of chatter.

Whistle

Whistles were exclusively performed during hovering displays of males in front of females (see Fig. 3A). In these flight performances males and females show remarkable interactions. The males exhibit specialised wing strokes, in order to fan their odour towards the females (Voigt and von Helversen 1999). Both individuals often emitted social calls during the flight display, opening their mouths synchronously to the odour-fanning movement of the male. In this situation the females articulated a noisy screech, which could last up to approximately 300 ms. The males at the same time emitted a very loud and tonal whistle (Fig. 1D).

Whistles were among the most stereotyped and loudest vocalisations recorded in the colony. They were audible to human ears, although only a minor portion of their sound energy was allocated in the human hearing range. Whistles had a duration of 66.7±44.1 ms and mostly consisted of an FM upstroke with the fundamental frequency increasing to 19.9±1.9 kHz. Usually, the call ended in an FM downstroke. As the 1st harmonic was the loudest, the main frequency was 39.9±3.7 kHz for the CF part, a range also characteristic for echolocation pulses. Typically the whistle was preceded by a noisy screech of 74.2±51.1 ms duration, which sometimes included multiharmonic parts.

Screech

Screeches were the only vocalisation recorded from both males and females. In females, screech calls seemed to be the most common vocalisation outnumbered only by echolocation pulses. Screeches were emitted during agonistic encounters between individuals of both sexes, mainly during territorial conflicts. Screeches of females were common responses to male hover displays (Fig. 3A).

The duration of screeches was 97.0±82.6 (median 76.1, quartile distance 120.1), the sound energy was distributed over a broad spectrum (Fig. 1E). This evokes a noisy impression to the human listener. If discrete harmonics were discernible, at least five of them were located below 60 kHz. The degree of noisiness varied between and within different screech calls. Fig. 1E shows a screech recorded during a territorial encounter between two males. It includes both multiharmonic and noisy components.

Territorial song

This song type was the most conspicuous vocalisation in the colony. To human listeners it gave the impression of a high frequency whinny. Territorial songs consisted of 10–50 mostly tonal calls (Fig. 1F). Typically the first calls were mainly upward frequency modulated, merging into inverted V-shaped calls in the middle of the song. These inverted V calls covered a frequency range of c. 10 kHz and reached a highest frequency similar to the fundamental frequency of echolocation pulses (around 22 kHz). Calls at the end of the song where lower in fundamental frequency (down to about 6 kHz) and mostly headed by a noisy buzz. Call duration extended from about 10 ms at the beginning to up to 100 ms at the end of territorial songs.

The acoustical structure described here was typical for territorial songs emitted during the main activity periods in the morning and evening. Territorial songs recorded during the middle of the day usually deviated from this typical structure. The most striking difference was the omission of low frequency calls at the end of the song and the lack of buzzes at the beginning of these end-calls. These songs had been named "short songs" by Bradbury and Emmons (1974) in comparison to "long songs" emitted in the morning and evening. But since call types
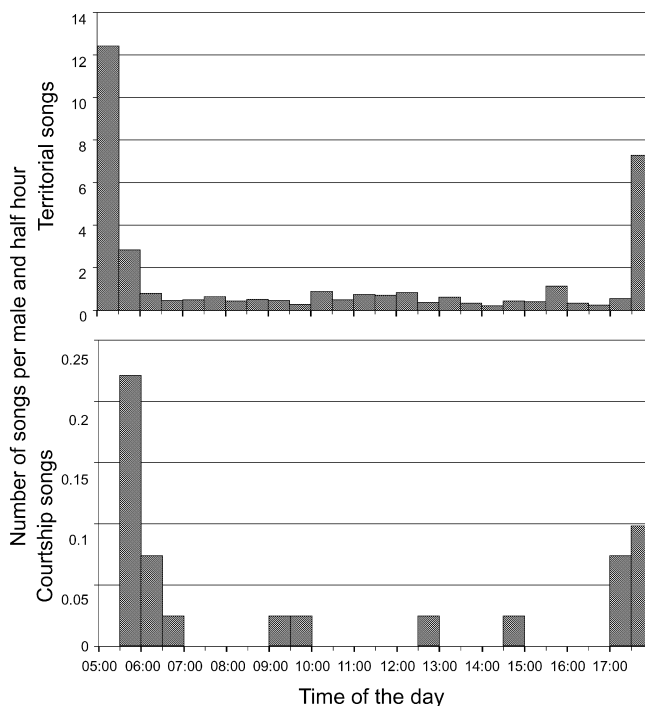

Fig. 2 Territorial and courtship song activity in the day roost. The number of songs per male and half hour is plotted against daytime. Data on territorial songs are from 15 territorial males that were observed during two entire days. Data on courtship song stems from eight territorial males that were videotaped over five entire days each (unpublished data kindly provided by S. Meister). Note that courtship songs start half an hour later than territorial songs, which dominate during the first half hour when males come back to the day roost from nightly foraging flights and females are still absent

of both were the same, and since there was a smooth transition between the two extremes, we did not follow this differentiation.

In comparison to courtship songs, territorial songs were shorter, less variable in duration and harmonic structure and contained more tonal calls (Table 2). Duration of tonal calls and pauses between them was smaller and less variable than in courtship songs. Moreover, tonal calls had less peaks in the fundamental frequency.

Territorial songs were exclusively emitted by males, mostly in the morning after returning to the roost, and in the evening before leaving (Fig. 2). Since females return to the roost later than the males and leave earlier in the

Table 2 Mean, standard deviation, median, and inter-quartile-distance of parameters measured in territorial and courtship songs. Median and inter-quartile-distance are given in parentheses and only for asymmetric parameters with mean/median smaller than 0.9 or larger than 1.1

Parameter	Territorial song	Courtship song
Total length (s)[a]	1.62±0.56	42.2±44.2 (30, 27.0)
Harmonic structure (% of tonal calls)[a]	82.1±6.1	57.4±14.1
Length of tonal calls (ms)[a]	36.8±16.3	59.1±27.0
Length of pauses between tonal calls (s)[a]	0.05±0.02	0.94±1.42 (0.42, 0.86)
Main frequency of tonal calls (kHz)	16.6±2.8	16.9±3.1
Modulation of tonal calls (% of calls with more than one peak)[a]	9.3±6.0	66.6±9.5

[a] Parameters which differed significantly between territorial and courtship songs (Mann-Whitney U-test, $P<0.001$, $n=20$)
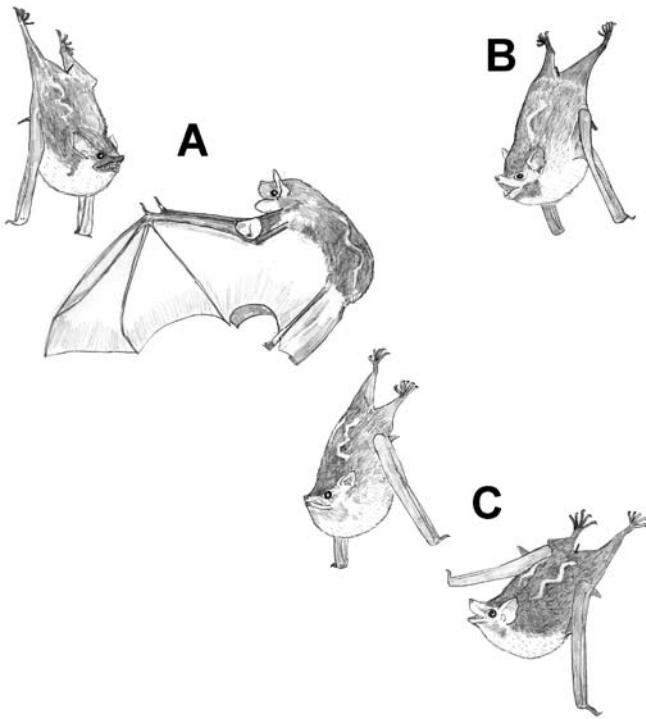
Fig. 3A–C Behavioural correlates of different male *S. bilineata* vocalisations. **A** The whistle emitted by a male in hovering flight is accompanied by a simultaneous female screech. **B** A male emits a territorial song while perched in his harem territory. **C** A male performing a courtship song towards a female. The male is perched below the female and sings with his head directed to her

evening (Bradbury and Emmons 1974; Tannenbaum 1975; Voigt and von Helversen 1999), the majority of territorial songs in the colony was vocalised in the absence of females. While singing, males were mostly perched in their harem territory (Fig. 3B), and did not obviously direct the song at a specific inhabitant of the colony.

However, the temporal distribution of territorial songs from different males was not random. During the main activity period between 0500 hours and 0700 hours, the number of songs per 20-s time intervals differed significantly from a random Poisson distribution (Kolmogorov-Smirnov goodness of fit test: $P<0.001$, $Z=3.58$, $n=251$). Song data were sampled for three days (number of males observed: $n_1=11$, $n_2=10$, $n_3=7$) and were pooled since the mean number of songs per 20-s interval did not differ significantly for the sample days (one-way ANOVA: $P>0.3$, $F=1.09$, $df=2$). The temporal distribution of songs was clumped: we observed more intervals without songs, less intervals with few songs and more intervals with high song numbers than expected in the random distribution. This was also confirmed by the dispersion index (dispersion index I = variance/mean: $P<0.001$, $I=2.63$, $z=13.9$, $n=251$).

Observations suggested that the clumped distribution could be explained by the fact that songs from different males were simultaneously triggered, either by external signals like colony members passing the territories on the wing, or by songs of other males. To test the latter possibility, we played back 13 different territorial songs recorded in the colony to one male. Territorial songs from that male were counted as answers when they fell into a 2-s interval after the playback. Pauses between playbacks were randomly varied between 20 and 30 s. We observed significantly more territorial songs during the answer-intervals than would be expected with a temporal random distribution of songs (binomial test: $P<0.001$, $n=15$).

Courtship song

Courtship songs like territorial songs occurred mainly during the peak activity periods in the morning and evening, but after territorial song activity had ceased in the morning, and before it began in the evening (Fig. 2). Our observations suggest that the number of courtship songs is highest during the mating season, and decreases at the end of January (S. Meister, personal communication; own observations).

Courtship songs were often accompanied by hover flights or salting. These are behavioural components, which have been attributed to the context of courtship behaviour (Voigt and von Helversen 1999). Singing males always aimed their head and display towards one certain female inside the male's territory at a time (Fig. 3C). However, singing males could focus on different females consecutively. Courtship song activity of one male continued for up to 1 h. We never observed a male performing a courtship song without a female sitting nearby. Females that came to rest in a male's range temporarily seemed to attract more courtship songs than females continually present in the harem territory.

During courtship songs, the singing male typically approached the perched female from above or the side. The approaching male moved horizontally in a fitful manner and poked its closed wing towards the female (Fig. 3C). The body movements seemed to be synchronised with the vocalisation of song calls. Most often, females terminated approaches by striking their wing against the male. Following the strike the male soared up and hovered in front of the female, then landed about 20–50 cm away from her and started a new approach.

In Fig. 1G we show a courtship song sequence. Most of the sound energy was located above 20 kHz and therefore recording and visualisation of these songs was only possible with an ultrasound recording system. Different courtship song calls were articulated more or less loosely with variable intervening distances. Often calls comprised several syllables. Males showed an exceptionally high variability in the calls they emitted in courtship songs.
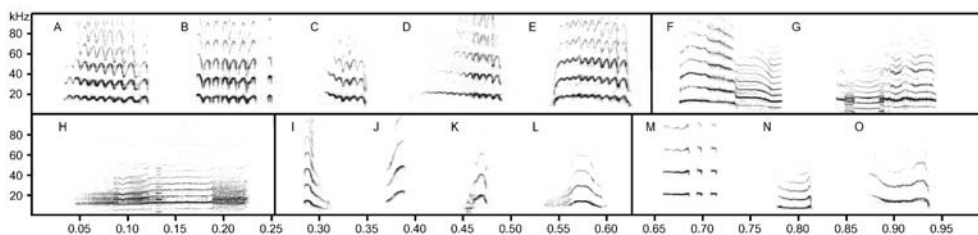
Fig. 4A–O Sonagrams of call types in courtship songs. **A–E** trill calls; **F–H** noise-burst calls; **I–L** short tonal calls; **M–O** quasi-CF calls. Calls are taken from different song bouts and had not been emitted in the order presented here

Elements of the courtship song

Trills accounted for 42% of all calls in courtship songs and were almost exclusively found in this song type (Fig. 4A–E). Trills included tonal calls with a rippled modulation (vibrato) of the fundamental frequency, a modulation frequency of at least 20 Hz and at least three modulation maxima. Frequency modulation minima were generally more tapered in the sonagram than maxima (Fig. 4A–D) resulting in a characteristic arc-like shape. Although the upstroke and duration of single modulations mostly increased within a trill call (Fig. 4A–E), the entire call usually showed a downward modulation (Fig. 4A,C,D). Trills contained up to seven recorded harmonics of the fundamental frequency.

Second most common (33%) were noise-burst calls (Fig. 4F–H). This group comprised all multiharmonic and noisy vocalisations with more than five harmonics below 60 kHz. Similar vocalisations were also observed in other behavioural contexts than courtship song, e.g. during agonistic encounters, as mentioned above. The fundamental frequency (if discernible) of noise-burst calls was very variable (Fig. 4F–H). Minimum frequencies could be as low as 4 kHz, resulting in good audibility for human listeners (Fig. 4G). Distances between harmonics were often very small (down to about 3 kHz). In many noise-burst calls no predominant frequencies could be identified, but the sound energy was blurred over a wide frequency range (Fig. 4H). Often calls contained different sections with a variable degree of noisiness (Fig. 4F–H).

Short tonal calls (Fig. 4 I-L) were the third most frequent call type (10%). Total length was below 40 ms with no more than two modulation maxima in the sonagram (trills contained more than two maxima). Short tonal calls differed in the range of their fundamental frequency (Fig. 4J,L) and their duration (Fig. 4K,L). Moreover fundamental frequencies were very variable in shape, down- or upwards modulated or a combination of both (Fig. 4I–L).

Fourth most common (6%) were quasi-constant-frequency calls (Fig. 4M–O). In this category we included all tonal calls varying less than 10% in the fundamental frequency during at least half of their length. Quasi-CF calls differed from the CF hover-flight whistles described above in having a higher discontinuity (Fig. 4M) and/or a lower fundamental frequency (Fig. 4N) and/or an alternate modulation form (Fig. 4O).

Further call types, not described in this paper, comprised up to 9% of the total number of analysed calls. Territorial songs, which in a few cases were interspersed in courtship songs, were not categorised as courtship song calls. The same applies to chatter, which occurred frequently both in and out of courtship songs.

Trills in courtship songs: repertoires of different males

Trills were the most common call type in courtship songs and exclusively observed during the performance of these songs. Thus, we considered trills to be the most appropriate call type to quantify an overlap of male courtship repertoires. Twelve acoustical parameters were measured in 1,700 trills from the six chosen males (parameter values in Table 1). Trills were highly variable in most of the measured parameters. This was for example true for the total number of modulations and the total length of the call (Fig. 4A,C), the total discontinuity (Fig. 4B,E), and the fundamental frequency at the onset and end of the call (Fig. 4 B,C,E).

We used DFA to quantify the overlap of male repertoires. The DFA assigned trills to males on the basis of the five discriminant functions extracted from the measured acoustical parameters. Eight of the 12 parameters were included during the stepwise procedure (see Table 1). Means of discriminant function values differed significantly between males (Wilks Lambda=0.37, $P<0.001$). Trill variability and differences between males were caused by the measured variables (Table 1) to a different degree. The main frequency and the end frequency of trill calls had the strongest influence (Fig. 5); they showed the highest within-group correlation with the first two discriminant axes (main frequency: $r_{axis1}=0.65$, $r_{axis2}=0.40$; end frequency: $r_{axis1}=-0.04$, $r_{axis2}=0.65$; difference between initial and end frequency: $r_{axis1}=0.47$, $r_{axis2}=-0.63$; initial frequency: $r_{axis1}=0.54$, $r_{axis2}=-0.14$; mean fundamental frequency: $r_{axis1}=0.44$, $r_{axis2}=-0.54$; other correlation coefficients $r<0.5$). Plots of discriminant function scores for each trill are presented in Fig. 5; axis 1 accounted for 58%, axis 2 for 24% of the total variance (axis 3:11%, axis 4:6 %, axis 5:1 %). Males 1, 3, 5, and 6 were mainly differentiated from the others
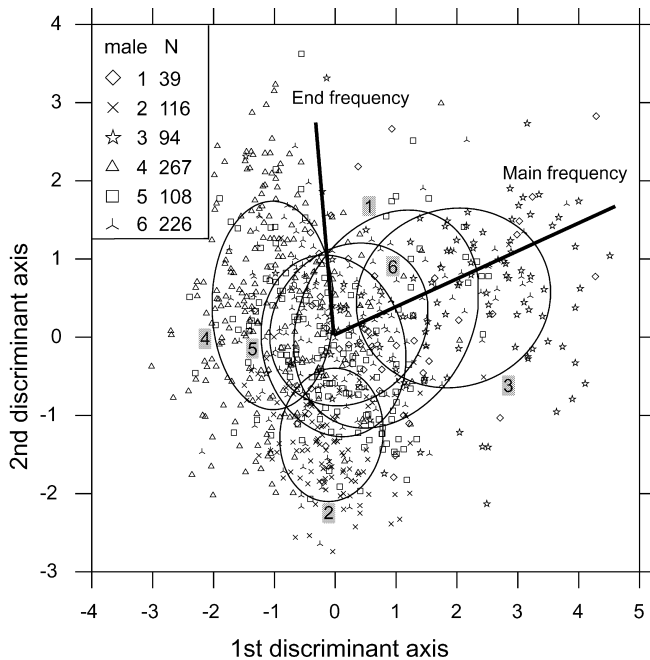
Fig. 5 Parameters accounting for trill variability and differences between males. 850 trill calls of six males were differently distributed on the first two axis extracted in a stepwise discriminant analysis, which included eight of the twelve measured variables (Table 1). Axis 1 accounted for 58% and axis 2 for 24% of the total variance of the data set. *Ellipses* enclose 49% of all trill calls from a respective male (49% percent were also correctly assigned in the DFA) and are labelled with male numbers. The strongest variables main frequency and end frequency are depicted as joint plot vectors (coordinates are proportional to the correlation of variables and axes). Males 1, 3, 5, and 6 were mainly differentiated from the others on *axis 1* with the main frequency as strongest variable (r_{axis1}=0.65). Males 2 and 4 differed on *axis 2*, which was highest correlated with the end frequency (r_{axis2}=0.65). *Scales* are scores of the canonical discriminant functions

on axis 1 with a difference in main frequency. Male 2 and 4 mainly differed on axis 2 and in end frequency. The extracted canonical functions allowed a correct assignment of trills to males in 49% of all cases in the half of the data-set randomly chosen for cross-validation (53% in the other half).

Discussion

Acoustic and behavioural features were also used to classify vocalisation types of *S. bilineata* males on Trinidad, by Bradbury and Emmons (1974); these authors did not mention courtship songs, possibly because their devices did not record these high frequency vocalisations at that time. In our classification we followed Bradbury and Emmons nomenclature. In a recent study on male *S. bilineata* vocalisations on Trinidad, Davidson and Wilkinson (2002) differentiated 21 call types (syllables). These authors assigned the syllables to four categories (syllables with most of their amplitude at 20 kHz, broadband noise or screech syllables, short FM

syllables, and long, tonal FM syllables), all of which also could be found in our recordings from Costa Rica.

Transitions between vocalisation types

As can be seen from the sonagrams (Fig. 1), most of the social vocalisations of *S bilineata* males, except the multiharmonic or noisy calls and screeches, consist of elements with a similar basic structure (named "chirp" by Bradbury and Emmons in 1974). Chirps resemble an "inverted V", similar to echolocation pulses, which in emballonurid bats have a characteristic "inverted U" structure. Hunting flight echolocation calls of *S. bilineata* are usually emitted as "doublets" with alternating frequencies of around 45.1 and 47.1 kHz (Kalko 1995; own observations). Frequency alternation can be used to decrease ambiguity in signal-echo correlation when the distances of sound-reflecting objects may be large, so that the travelling time of the echo exceeds the silent interval between calls. Within the roost we never recorded doublets; since the distance of objects inside the roost is very short and well known to the animals, alternating the frequencies may be unnecessary.

As echolocation pulses resemble many of the other vocalisation types in their temporal structure, they may be seen as a basis from which other vocalisation types have evolved. This is, for example, true for barks, which differed from echolocation pulses only in duration and in a stronger accentuation of the fundamental frequency. We named barks following the nomenclature of Bradbury and Emmons (1974). However, the observed length of barks in our colony (10.5±1.9 ms) was somewhat shorter than indicated in their paper (10–20 ms). Also the trills, typical for the courtship song, may be interpreted as continuous rows of connected elements of "inverted V"-structure.

The 1st harmonic, instead of the fundamental frequency, normally is the loudest frequency in emballonurid echolocation calls. This is not the case for many social vocalisations where the fundamental frequency is loudest. Compared to echolocation calls and barks, the fundamental frequency is still lowered in territorial songs, which therefore become audible to the human ear. As low frequencies are less attenuated in air, these calls may be adapted to be especially loud when perceived by a territory rival.

Potential of male *Saccopteryx* social calls for sexual selection

Social calls, especially songs, are often used for marking male territories and their main role may lie in intra-sexual competition, but they can also be used for female choice (Andersson 1994; Bradbury and Vehrencamp 1998). Most courtship songs have only the second function.

Theoretical considerations suggest that vocalisations used in the context of territorial competition should be short, simple, moderately loud, stereotyped, frequently

repeated and individually distinguishable. This may facilitate positional learning of neighbours, individual recognition and counter-singing (Kodric-Brown and Brown 1984; Bradbury and Vehrencamp 1998). These criteria apply to *S. bilineata* territorial songs when comparing them to courtship songs. Although territorial songs show less variability, both within and between individuals, songs from different males are distinguishable (Bradbury and Emmons 1974). However, we have not studied this question quantitatively.

Also the social context of territorial songs clearly indicates their function in male-male competition. When returning to their roost sites in the morning, *S. bilineata* males reoccupy their territories, which have been vacant during the night. Sorting out territories often entails territorial conflicts between males (Tannenbaum 1975). Voigt and Streich (2003) showed that experimentally emptied harem territories were occupied quickly by new males. These males are "queuing" for the position of the harem owner, probably for long time intervals. The vast majority of territorial songs were observed during the time when males came back to the roost in the early morning, when most territorial conflicts occurred, and females were still absent. Females return to the roost later than the males and leave earlier in the evening (Bradbury and Emmons 1974; Tannenbaum 1975; Voigt and von Helversen 1999). Playback of territorial songs that had been recorded in the colony before instantaneously caused a male to answer with territorial songs himself, in the same manner as males responded to real songs. Male counter-singing is typical for territorial vocalisations and may serve mutual assessment of physical conditions (Davies and Halliday 1978; Clutton-Brock and Albon 1979; Capp and Searcy 1991; Beletsky and Orians 1996; Gerhardt and Huber 2002).

This makes it very probable that the main function of territorial songs is to demarcate harem territories in the day roost. However, this does not exclude an additional role in mate attraction, as has been suggested by Bradbury and Emmons (1974) and Tannenbaum (1975), and as is suspected for many bird songs (Kroodsma 1977; Kodric-Brown and Brown 1984). Since most of the design features for territoriality and mate attraction are similar, there is no serious trade-off imposed when combining both functions in a song (Bradbury and Vehrencamp 1998).

Courtship songs differed from territorial songs mainly in having a longer duration, lower volume, higher diversity in the harmonic structure and duration of calls, and in being directed at a specific female. Thus, courtship songs satisfy theoretical design features for a vocalisation associated with intersexual selection (Bradbury and Vehrencamp 1998). Courtship song frequency seemed to decrease after the mating period, also indicating a context of female choice.

To our knowledge, courtship songs have not been described so far in *Saccopteryx*. This may be due to their relative low volume and high frequency range, and also due to the fact that they are vocalised less often than territorial songs and in a scattered manner during the whole day. Therefore they are less perceptible to human listeners. Once familiar with courtship songs, they were most easily detected by the associated male behaviour, such as fitful body movements or wing-poking. Thus, courtship songs were the acoustical part of a complex scheme of courtship behaviour, which also comprises visual and olfactory signals (Voigt and von Helversen 1999).

If females, at least in part, base their decision for a specific mate on the male's courtship songs, they require the ability to differentiate males on the basis of song parameters. Trills from the courtship songs of six males differed significantly in various acoustical traits. Main frequency and end frequency were the strongest parameters when differentiating males on the basis of their trills (Fig. 5). Thus these trill traits could serve as a basis for female choice.

Whistles were only recorded during the performance of male hover flights, where they were emitted synchronously to odour-fanning wing strokes (Voigt and von Helversen 1999). Hover flights impose high energy costs (Winter et al. 1998; Voigt and Winter 1999; Voigt and von Helversen 1999) and possibly strain the males to the limits of their respiratory abilities. The vocalisation of loud and lengthy whistles during hover flight may impose an additional respiratory and energetic cost on the males. Whistles could therefore function as an honest signal for female choice, as they are costly (Zahavi 1975; Maynard-Smith 1991). A recent study on Trinidad showed individual differences between *Saccopteryx* males in "screech-inverted-V calls", which resemble the whistles described in this paper. Males differed in peak frequency, duration and bandwidth of the tonal part of whistles (Davidson and Wilkinson 2002) and the number of females in a males territory were negatively correlated to the number and peak frequency of inverted-V elements and positively correlated to their duration (Davidson and Wilkinson 2004). Thus whistles may be regarded as "dynamic parameters" (Gerhardt and Huber 2002) and females might use them as measure of the male's physical abilities.

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