



## Research Article

## A flexible communicator: Social call repertoire of Savi's pipistrelle, *Hypsugo savii*

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### Abstract

Bat social calls are specifically tailored for communication and play different roles according to their structure. Their structure is stereotyped and species-specific so they may aid acoustic identification of bats. To provide the first quantitative description of social call repertoire of *Hypsugo savii* and offer a way to identify this bat in free flight we made audio recordings in four areas of Central and Southern Italy in summers 2011–2015. We identified single and multiple-component social calls and categorized them into five structure types. Within each structure type, call frequency and / or duration differed between single and multiple-component calls, the latter being shorter and showing higher frequencies. In multiple-component calls both the number of syllables and the way they were associated were highly variable, making it difficult to recognize patterns. Some motifs, however, showed the same first component type and final sequence. We also recorded trill-like calls and two complex sequences of multiple-component social calls (songs) lacking repeated motifs. The complex association of syllables, the rarity of recurrent motifs and the significant structural flexibility suggest that social calls serve a range of scopes and that they might convey acoustic signatures and other individual-specific features.

## Introduction

Many terrestrial and aquatic mammals use vocalisations to communicate with conspecifics, especially over long distances (Poole et al., 1988; Tyack and Clark, 2000; Reby and McComb, 2003). Bats are highly vocal mammals, i.e. they make extensive use of vocalizations, not only for navigation and foraging (echolocation calls), but also to communicate with conspecifics in a wide range of circumstances (Jones and Holderied, 2007). Typically, bat social calls have lower frequencies and longer duration than echolocation calls, being tailored to convey information over longer distances (Lawrence and Simmons, 1982; Fenton, 2003). Unlike echolocation calls, whose structure changes to match the different sensory tasks required to orientate, navigate or detect targets (e.g., Kalko and Schnitzler, 1993; Obrist, 1995), social calls typically show little intraspecific variation to maintain separate intraspecific communication channels and convey unambiguous information (Barlow and Jones, 1997a,b; Russo et al., 2009).

Social calls are often diversified within the vocal repertoire of a bat species, i.e. they vary in structure according to the type of information conveyed (Fenton, 1994) and are broadcast in many different contexts (Fenton, 1985; Pfalser and Kusch, 2003) such as e.g. presence of disturbance or predators (distress calls; Russ et al., 1998), courtship (e.g., male songflights aimed to defend a territory or attract females; Barlow and Jones, 1997b) and maintenance of contact with conspecific members (e.g., isolation calls and directive calls between mother and infant; Balcombe and McCracken, 1992). However, the same call structure may have different functions among species and/or contexts in which it is emitted (Fenton, 1985). In the study of pipistrelle bats in Europe

(*Pipistrellus* spp.) social calls provided key clues to demonstrate the existence of cryptic species (Barlow and Jones, 1997a,b) as well as important insights into behavioural, ecological and evolutionary aspects (Kolb, 1981; Gustin and McCracken, 1987; Barlow and Jones, 1997a,b; Russo et al., 2009; Georgiakakis and Russo, 2012).

The Savi's pipistrelle *Hypsugo savii* (Bonaparte, 1837) is a small vespertilionid bat with a body mass of 5–9 g and forearm length 31–37 mm (Dietz and Kiefer, 2014). A Palearctic species, it occurs from South Europe and North Africa through the Middle East and the Caucasus to Central Asia and Northern India (Horáček and Benda, 2004; Wilson and Reeder, 2005; Hutson et al., 2008). Recently, a northward expansion of Mediterranean populations of *H. savii* was documented, so that this bat is now present also in Central and Eastern Europe (reviewed in Uhrin et al., 2015). The species has quite generalist roosting and foraging requirements, so it may occur in a broad range of habitats (Russo and Jones, 2003; Hutson et al., 2008; Di Salvo et al., 2009; Dietz et al., 2009; Ancillotto et al., 2014). In this study, we present the first quantitative description of *H. savii* social calls. Besides contributing to a better understanding of the species' behavioural repertoire, we aim to offer a further approach to acoustic identification of bats in flight, which might complement that based on echolocation where *H. savii* co-occurs with species broadcasting similar echolocation pulses. *H. savii* emits echolocation calls made of a frequency-modulated (FM) component followed by a constant-frequency (CF) portion lasting ca. 8 ms and peaking at 31–38 kHz (Russo and Jones, 2002). The species is sympatric with *Pipistrellus kuhlii* over much of its range and in some regions also co-occurs with *P. nathusii* (Mitchell-Jones et al., 1999). Sympatry with these pipistrelles is likely to increase in the future since both *H. savii* Uhrin et al. (2015) and *P. kuhlii* (Ancillotto et al., 2016) are expanding their ranges. Echolocation calls of these three bats show

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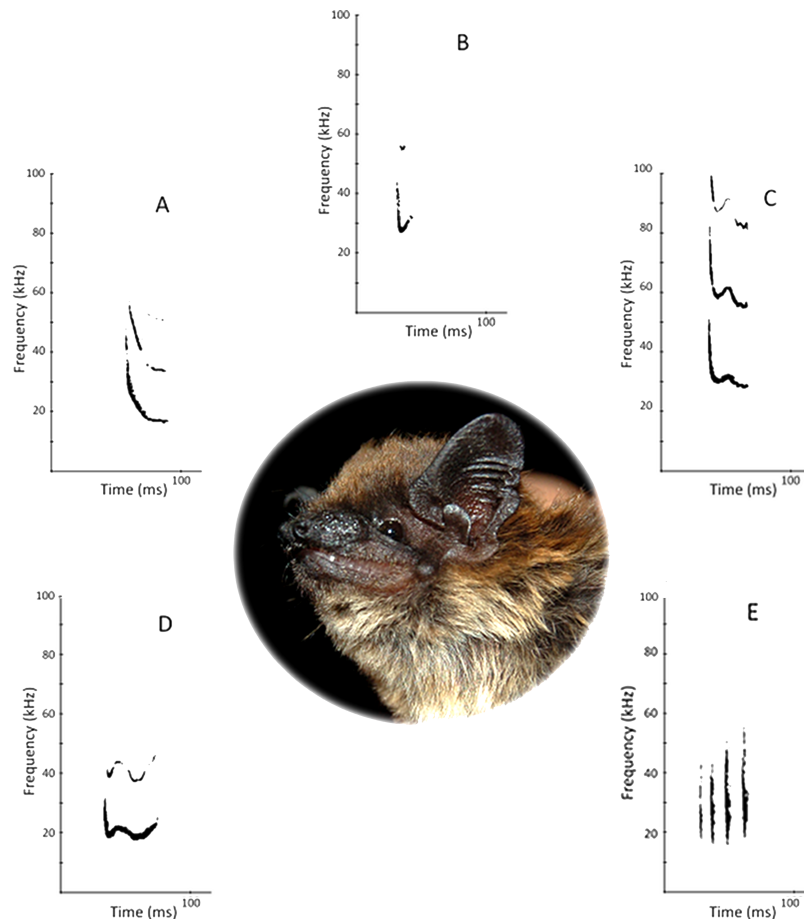


Figure 1 – Spectrograms of representative call components (syllables) of *Hypsugo savii* social calls: A-E. (bat image courtesy of L. Cistrone).

partial overlap in spectral and temporal features, making species identification ambiguous at least within certain value ranges (e.g. Russo and Jones, 2002; Russo and Voigt, 2016) under sympatric conditions. In such cases, species-specific social calls may inform acoustic identification of this bat.

## Materials and methods

We recorded social calls of *H. savii* in four areas of Central and Southern Italy in summer 2011–2015. Study areas were located in the Abruzzo, Lazio and Molise National Park (41°48' N 13°46' E), the Vesuvius National Park (40°49' N, 14°24' E), the metropolitan area of Rome (41°53' N, 12°31' E) and the island of Capri (40°33' N, 14°14' E). Recording sites within the study areas were visited by bats for drinking (cattle troughs) and foraging (cliffs, street lights, woodland edges). We established the presence of *H. savii* in the study area by mist-netting and by examining recordings of echolocation calls (following Russo and Jones, 2002). Sequences containing echolocation calls whose values overlapped with those of other pipistrelles were discarded (Russo and Jones, 1999). Calls were recorded using manually-operated Pettersson D1000X bat detectors and unattended D500X recording units (Pettersson Elektronik AB, Uppsala, Sweden) at a 500 kHz sampling rate. The D1000X was kept in the heterodyne mode to detect the presence of bats. When a bat pass was heard, the operator activated direct sampling manually and stored 2–5 s of sound onto the device's flash card. D500X were secured to rocks or trees and directed towards open space or water surface with an upward angle of 45°.

Sound analysis was performed with the software BatSound ver. 4.1.4 (Pettersson Elektronik AB). From each call we measured the following variables: start (*fstart*) and end (*fend*) frequencies taken from the fundamental component (social calls are typically multi-harmonic); frequency of maximum energy (*fmaxe*); syllable duration (*duration*); number of call components (for multiple-component calls, this was the

number of syllables in motif). Frequency values, expressed in kHz, were taken from spectrograms except *fmaxe* which was taken from the power spectrum; time measurements, expressed in ms, were taken from oscillograms. Calls were categorized according to recording site and season (lactation: July; mating: late August–September).

Preliminary tests done on a subset of calls recorded by using the two recording device models at the same time showed no significant difference in the values of the variables measured, so further recordings were pooled together for the analysis regardless of the bat detector used. Social calls of *H. savii* were made of either single or multiple components (syllables), which we classified in “types” according to the structure of their spectrograms. We applied an analysis of variance (GLM-ANOVA) followed by Tukey's post-hoc comparisons to explore differences in sound parameters between single and multiple components within each type. We validated statistically type classification by performing a multivariate analysis of variance (GLM-MANOVA) and a multivariate discriminant function analysis (DFA) with cross-validation. Such analyses were restricted to single-component calls only since components within multiple structures were not statistically independent from each other. To test whether syllable structure varied according to the multiple component sequence syllables were part of, we carried out a GLM for each sound parameter entering syllable type and individual social call identity as treatments. Normality of residuals and homogeneity of variance were checked with Ryan-Joiner and Levene's tests respectively. All tests were performed with MINITAB release 9.2 and R package (R Core Team, 2016); values of  $p < 0.05$  were considered significant.

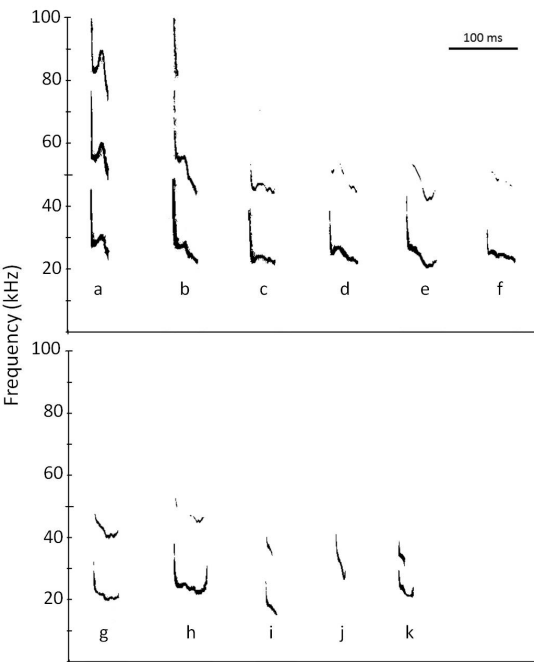
## Results

We recorded 118 social calls of *H. savii*, either emitted as single units or combined into variable associations of 2–9 syllables, totalling 221 components (Tab. 1). We typified syllables visually according to the

**Table 1** – Descriptive statistics (mean±SD) for time and frequency variables of single- and multiple-component social calls in *Hypsugo savii*. *n* = number of syllables; *fstart* = start frequency; *fend* = end frequency; *fmaxe* = frequency of maximum; *duration* = duration of component; *ncmc* = number of components in multiple calls. Frequencies are given in kHz, time variable in ms.

Type	<i>n</i>	<i>fstart</i>	<i>fend</i>	<i>fmaxe</i>	<i>duration</i>	<i>ncmc</i>
A	23	37.6±6.1	19.8±3.7	24.2±4.3	11.0±6.1	
single		28.8–47.7	13.4–26.3	14.7–29.8	4.7–29.5	
A	7	49.0±11.7	27.5±5.0	33.1±6.4	7.3±1.2	5.3±1.7
multiple		31.4–61.5	21.5–34.8	24.5–41.0	5.9–9.6	4.0–8.0
B	22	40.0±7.1	26.0±4.7	25.3±4.2	9.4±1.7	
single		29.5–51.2	15.7–32.1	15.5–31.3	6.7–12.8	
B	16	43.9±7.6	28.5±3.2	28.0±2.0	10.3±3.4	2.5±1.2
multiple		28.3–56.7	22.3–35.8	22.3–30.8	6.0–17.8	2.0–6.0
C	15	43.7±7.7	23.8±5.3	25.2±3.7	26.2±8.3	
single		33.5–61.5	14.7–38.3	16.3–29.6	11.9–44.9	
C	33	45.4±8.3	31.3±6.6	31.7±5.7	15.8±7.4	6.0±2.2
multiple		30.4–61.9	14.6–40.4	19.2–40.2	7.7–44.7	2.0–9.0
D	10	32.8±5.1	23.7±2.5	20.9±3.8	36.8±9.4	
single		23.4–41.0	18.0–26.9	10.8–24.5	24.5–54.0	
D	18	35.9±8.8	29.1±5.9	26.1±4.5	21.1±9.7	4.1±2.1
multiple		24.0–48.4	21.6–40.0	19.8–35.0	11.0–49.8	2.0–9.0
E	77	52.8±16.6	24.2±7.8	33.2±7.6	3.2±1.1	5.9±2.2
multiple		26.7–85.7	9.3–40.0	17.2–51.5	1.4–5.4	3.0–9.0

shape of their spectrogram, obtaining five categories (hereafter labelled as A-E; Fig. 1, Tab. 2). Type C and D showed an especially diverse range of structures (Fig. 2). Within types, single calls vs. syllables of multiple calls showed significant structural differences (GLM-ANOVA, Tab. 3). In general, within each structure type, call frequency and / or duration differed between single and multiple-component calls, the latter being shorter and showing higher frequencies. A DFA carried out on single-component calls including *fstart*, *fend*, *fmaxe* and *dura-*



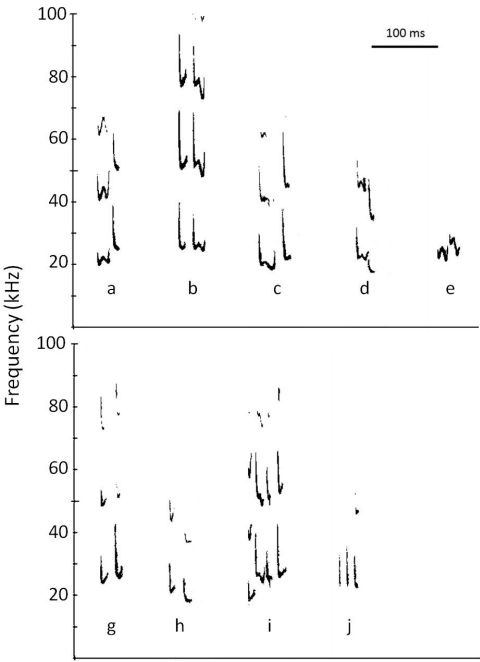
**Figure 2** – Comparison of spectrograms showing the structural variation (a-k) of syllables in C and D types of *Hypsugo savii* social calls.

**Table 2** – Description of structural categories recognized for spectrograms of *Hypsugo savii* social calls. Spectrograms are given in Figs. 1 and 2.

Type	Description
A	Steep downward frequency sweep (FM) followed by a quasi-constant frequency (QCF) part
B	Steep downward frequency sweep (FM) followed by a rapidly ascending frequency-modulated final part
C	Steep downward frequency sweep (FM) followed by a frequency modulated, highly variable final part
D	Narrow downward frequency sweep (FM) followed by a frequency modulated part with a rapidly ascending frequency-modulated final portion
E	Steep downward frequency sweep (FM): this structure type only occurred in multiple calls

*tion* correctly classified calls to types with an overall classification rate of 77.1% (*n*=70) and the model was significant (MANOVA's Wilk's  $\lambda$ =0.09817,  $F_{12,166}$ =19.5,  $p<0.005$ ).

Syllables greatly differed in numbers and types within multiple social calls (Fig. 3). Moreover, their structure was influenced by the individual multiple call they belonged to as shown by GLM analysis (Tab. 4). However, a recurring motif was made of a type-D first component followed by a variable number of type A, B or C components and one or more type-E syllables ending the sequence (Fig. 4). An example of this motif type is illustrated in Fig. 5. Multiple-component calls only made of E-type syllables were also recorded during the mating period (trill-like, Fig. 1). For completeness we also illustrate two complex sequences of multiple-component calls (an example song is shown in Fig. 6) recorded at a drinking site in the Vesuvius National Park. These were made of an irregular succession of social calls not assignable to any of the categories described above, in which repeated motifs did not occur. In such sequences the observed minimum frequencies were in the audible range and lower than those recorded for the same syllable structures in both single- and multiple-component social calls.



**Figure 3** – Spectrograms of different syllable combinations in multiple social calls of *Hypsugo savii* (a-j).

## Discussion

Our study provides a first quantitative description of social call repertoire of *H. savii* and shows that the latter is complex and highly flexible. This complexity is much higher than that recorded for other pipistrelloid bats (Barlow and Jones, 1997a,b; Russo and Jones, 1999; Russo et al., 2009; Georgiakakis and Russo, 2012), within this group only comparable to that observed in *Pipistrellus nathusii* (Furmankiewicz, 2003; Jahelková et al., 2008; Jahelková, 2011). To our best knowledge, no previous study has quantitatively described social calls for any of the ca. 20 species of the genus *Hypsugo* (Simmons, 2005).

Although single-component social calls are broadcast occasionally by other pipistrelles (e.g., Russo et al., 2009; Skiba, 2009), these seem to be frequently used by *H. savii* in flight (A-D). The structure of single calls resembled that of multiple call syllables so that we could use the same categories to classify them, but the observed differences in frequency and durations are perhaps due to the fact that they encode different information and / or likely play a different role.

In our study we could not link call structure to their function, our prime objective being to provide a picture of the species' vocal repertoire to be applied to field identification. However, the complex association of syllables, the rarity of recurrent motifs and the significant flexibility in call component structure suggest that in this bat social calls serve a broad range of scopes and that their complexity may be useful to convey acoustic signatures and other individual-specific features, as found in other pipistrelle species (Jahelková et al., 2008). Within this context it is worth noting that the structure of type C calls was very similar to a motif syllable broadcast by *Pipistrellus nathusii* to com-

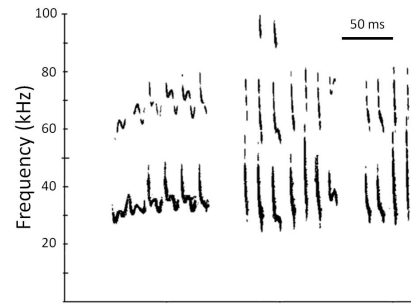


Figure 5 – Spectrogram showing an example of motif variation in *Hypsugo savii* social calls.

municate individual identity of males in a colony in the mating period (Jahelková et al., 2008). Moreover in our selection procedure, which implied excluding social calls recorded where *H. savii* and *P. kuhlii* co-occurred, we may have erroneously discarded a few social calls belonging to the former species, yet we believe this risk was negligible.

In our study, trill-like calls (i.e. repeated type E elements) were recorded only during the mating period: such calls are likely to be linked to reproduction. Trill-like calls assume different functions in different species: distress calls to attract conspecifics in stressful contexts (Fenton et al., 1976; Russ et al., 1998); used at maternity roosts during mother-pups interactions (Kolb, 1981); or advertisement calls produced by males in songflight in the mating season to attract females to mating roosts (Gerell-Lundberg and Gerell, 1994; Barlow and Jones, 1997b; Furmankiewicz, 2003).

Our findings may facilitate acoustic identification for *H. savii* in areas where this bat is sympatric with other pipistrelle species broadcasting similar echolocation calls, particularly in the recently colonized areas of eastern Europe, where the presence of this newcomer might be overlooked in bat surveys. However, our study only considered Italian populations and did not encompass potential geographic variation. The vocal plasticity shown by *H. savii* would merit further investigation, also employing playback experiments, to explore functions as well as geographic, individual and seasonal effects. Moreover, the taxonomic position of *Hypsugo* spp. is currently unclear, with the occurrence of a range of cryptic taxa possibly making the correct identification of these species a complex task (Veith et al., 2011). In this scenario, an extensive and comparative description of social calls emitted by bats in the genus *Hypsugo* may facilitate the identification of cryptic species occurring in sympatry across the Mediterranean basin (Veith et al., 2011), e.g. *H. darwinii* in Sardinia and Sicily, thus favouring the study of biogeographical processes (Russo et al. 2009) and informing management. ☞

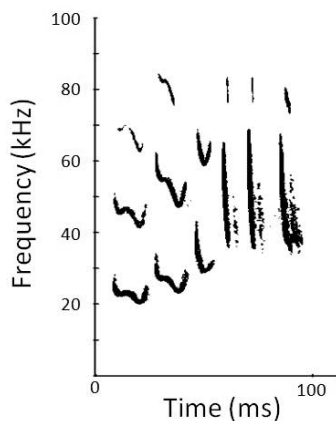


Figure 4 – *Hypsugo savii* motif spectrogram showing typical start and end portions.

Table 3 – Results of GLM ANOVA on *Hypsugo savii* social call used to make comparisons between single and multiple syllables bearing the same structure (A-D).

	Single vs. Multiple							
	A (n=30)		B (n=38)		C (n=48)		D (n=28)	
	F	p	F	p	F	p	F	p
fstart	11.8	<0.005	2.6	n.s.	0.5	n.s.	1.0	n.s.
fend	20.3	<0.005	3.5	n.s.	15.1	<0.005	7.5	<0.05
fmaxe	18.2	<0.005	5.6	<0.05	15.9	<0.005	9.9	<0.005
duration	2.6	n.s.	1.2	n.s.	18.9	<0.005	17.1	<0.005

Table 4 – GLM ANOVA showing the effects of individual call (Call Id.), call type and their interaction on syllable variables of multiple social calls in *Hypsugo savii* (n = 154).

	Call Id.		Type		Call Id. × Type	
	F	p	F	p	F	p
fstart	39.0	<0.005	5.6	<0.005	0.3	n.s.
fend	13.3	<0.005	11.5	<0.005	2.8	n.s.
fmaxe	26.2	<0.005	6.0	<0.05	0.2	n.s.
duration	0.1	n.s.	33.2	<0.005	0.0	n.s.

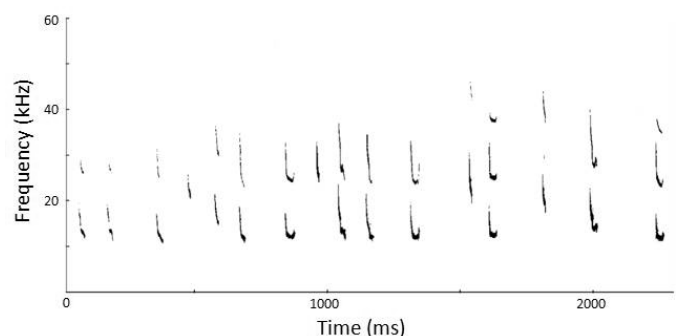


Figure 6 – Spectrogram of *Hypsugo savii* song recorded during the mating period at a drinking site, Vesuvius National Park. Note that the minimum frequencies are audible and lower than those observed in the same syllables belonging to single or multiple social calls.

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