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ORIGINAL ARTICLE

Social calls are subject to stabilizing selection in insular bats

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ABSTRACT

Aim Bats communicate by emitting social calls, and these often elicit reactions in conspecifics. Many such vocalizations are species-specific so that unambiguous signals can be transmitted and interpreted by conspecifics. In species-rich assemblages, evolutionary pressures might prompt interspecific diversification of call structure so that communication with heterospecifics is avoided. In species-poor island communities, where no risk of miscommunication occurs, stabilizing selection should prevail and preserve call structure and function. Call structure in island bats might be inherited from colonizers from the mainland and be maintained with little change in the absence of selection from heterospecifics. To test this hypothesis we studied *Pipistrellus maderensis*, an insular taxon occurring on the Madeira Archipelago, the Canary Islands and the Azores. It is closely related to one of the most widespread European pipistrelles, *Pipistrellus kuhlii*. *Pipistrellus maderensis* most probably evolved from a common ancestor shared with *P. kuhlii*, or from founders of that taxon that colonized the islands. We hypothesized that on Madeira Island, where no risk of ambiguous communication with heterospecifics exists, the structure and function of social calls should have been preserved by stabilizing selection. Echolocation calls, subject to different selection pressures, may instead show more pronounced differences between *P. maderensis* and *P. kuhlii*.

Location Madeira Island (Portugal, Atlantic Ocean), central and southern Italy.

Methods We recorded social and echolocation calls from allopatric populations of the two pipistrelles and explored interspecific differences in time and frequency characteristics. We also conducted playback experiments by broadcasting recordings of social calls from *P. kuhlii* and *P. maderensis* (taken respectively in peninsular Italy and on Madeira) and monitoring the bats' responses.

Results Social call structure showed a strong similarity between species, whereas echolocation calls were markedly different and exhibited a mean divergence of over 6 kHz in their frequency of maximum energy. On Madeira, *P. maderensis* significantly reduced flight activity when we broadcast *P. kuhlii* signals, as did *P. kuhlii* in Italy in response to *P. maderensis* calls.

Main conclusions Reliable interpretation of social calls provides benefits to both the signaller and the receiver because signals help to optimize food exploitation at foraging sites. In the absence of closely related species that can emit similar calls, this advantage may have acted as a strong evolutionary pressure, stabilizing social call structure in *P. maderensis* in insular ecosystems with limited foraging resources.

Keywords

Allopatry, bioacoustics, communication, echolocation, evolution, island evolution, Italy, Madeira, *Pipistrellus kuhlii, Pipistrellus maderensis.*

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INTRODUCTION

Specialized selective forces associated with island environments prompt the appearance of new characters that may differ markedly from those found in the ancestral colonizers (Mayr, 1967). An analysis of changes in the morphology of mammals demonstrated that islands may in fact accelerate evolutionary processes, promoting faster diversification (Millien, 2006). However, in the absence of fitness-dependent environmental or ecological change, traits that prove crucial for individual fitness will be preserved in their original states by stabilizing selection, a form of natural selection that eliminates the extremes of a character and favours intermediates (Stearns & Hoekstra, 2005). Stabilizing selection is therefore a key process in maintaining character stasis over prolonged periods (Estes & Arnold, 2007).

The structure of communication signals in animals may be stabilized whenever divergence from average signal properties results in decreased effectiveness of information transfer. Stability of signals allows for the fine-tuning between signaller and receiver that is necessary for mutual recognition and unambiguous communication (Ritchie, 1996). For example, signals that convey species identity for mate recognition can be maintained by stabilizing selection exerted by receivers to decrease the risk of attracting individuals from other species (e.g. Paterson, 1985; Ryan & Rand, 1993; Greenfield, 1997). In certain cases it can happen that only some variables of the signal are stabilized, while others are subject to different forms of selection (e.g., flash rate and length in fireflies, which evolved by directional and stabilizing selection, respectively; Greenfield, 1997). Many acoustic signals are subject to stabilizing selection, especially those involved in mate recognition (e.g. Gerhardt & Huber, 2002; Hunt *et al.*, 2007). Moreover, stabilization may be equally important for other forms of acoustic signalling, and here too structural divergence can be selected against so that communication is effective.

In addition to producing echolocation pulses, many echolocating bats broadcast a range of social calls exclusively for communication (Fenton, 1977, 1985, 2003; Russ *et al.*, 1998; Pfalzer & Kusch, 2003). Echolocation and social calls exhibit different degrees of intraspecific variation. In fact, the structure of echolocation calls is flexible, and is modified by bats in flight to optimize performance in tasks such as target detection, localization and classification in different habitats (Obrist, 1995; Vaughan *et al.*, 1997; Russo & Jones, 2002; Berger-Tal *et al.*, 2007). To ensure unambiguous communication, social calls with different functions often differ in structure (Fenton, 1985), each strucutre being highly stereotyped within species. Moreover, signals having the same function in different species typically differ among species so that undesired communication with individuals from other species is avoided. Such differences are maintained even among closely related taxa (Barlow & Jones, 1997a,b). Stereotyped social calls result from the suppression by stabilizing selection of random divergence from a species-specific signal structure that is clearly recognizable and interpretable.

In this study we hypothesize that the structure and function of frequently used social calls persist as a result of stabilizing selection in newly founded insular populations provided that no overlap occurs with calls of other island species. Indeed, calls may even be inherited in the same form by a newly evolved insular taxon deriving from the original colonizers. Stabilizing evolutionary pressures may preserve features of key signals, 'protecting' them against drift effects. The presence of species emitting similar calls might counter this process by promoting divergence from the original signal structure, thereby minimizing communication overlap between species and maintaining 'private communication channels' (Kingston *et al.*, 2001; Thabah *et al.*, 2006; Russo *et al.*, 2007). This phenomenon has been described for several taxa, including orthopteran insects (Higgins & Waugaman, 2004; Honda-Sumi, 2005), frogs (Höbel & Gerhardt, 2003; Pröhl *et al.*, 2006), birds (Irwin *et al.*, 2001; de Kort *et al.*, 2002) and, more recently, nocturnal primates (Braune *et al.*, 2008).

However, such pressures appear less likely to occur on islands than in mainland regions since islands harbour fewer species (as pointed out for bats by Findley, 1993). In the absence of these selective forces, call structure is presumably stabilized by natural selection.

To test our hypothesis we selected an insular bat, *Pipistrellus maderensis* (Dobson, 1878). Like other pipistrelle bats (Barlow & Jones, 1997a,b; Russo & Jones, 1999), *P. maderensis* emits social calls that contain multiple components when foraging. Social calls are emitted more frequently at lower ambient temperatures or later at night (D. Russo & S. Teixeira, pers. obs.), when reduced prey density increases competition for insect prey (Barlow & Jones, 1997b). As in other pipistrelle bats (Barlow & Jones, 1997b), these signals presumably function to repel conspecifics at foraging sites, given that they are often emitted during the pursuit of conspecifics. *Pipistrellus maderensis* is a strongly sedentary bat (Hutterer *et al.*, 2005), and is endemic to Madeira, the Canary Islands (but see Simmons, 2005) and possibly the Azores (Rainho *et al.*, 2002). It probably evolved from its closest relative *Pipistrellus kuhlii* (Kuhl, 1817) (Pestano *et al.*, 2003), a Western Palaearctic and Afrotropical bat that is widespread in much of southern Europe (Vernier & Bogdanowicz, 1999), or from ancestral forms common to both lineages. Ongoing work on local *P. maderensis* populations suggests that, assuming a molecular clock for cytochrome *b* of *c.* 4% divergence per million years (Myr^{-1}) (as employed for other *Pipistrellus* species; Hulva *et al.*, 2004), *P. maderensis* may have diverged from *P. kuhlii c.* 1.2 Ma (J. Jesus, unpublished data).

Pipistrellus kuhlii also emits social calls at foraging sites (Russo & Jones, 1999). Barlow & Jones (1997b) established that pipistrelle social calls are structurally and functionally species-specific; that is, they differ between species and do not elicit heterospecific reactions. Such calls may have evolved because they offer advantages both for the signaller and for the receiver. The call works to the advantage of the signaller because, by calling, the signaller repels newcomers and decreases competition for food (Barlow & Jones, 1997b).

Hearing the calls is advantageous for the receiver too, because it is warned that other conspecifics are already depleting the foraging patch and hence it may decide to feed elsewhere. These social calls are likely to be especially important in insular habitats, where, compared with the mainland, key selective pressures are more closely associated with intraspecific competition for limited resources (Lomolino, 2005).

Assuming that any divergence from the original social call structure would result in decreased communication performance (and, in turn, in decreased individual fitness) in *P. maderensis,* social calls may have been retained in their original form (presumably similar to those of *P. kuhlii*) by stabilizing selection.

We made the following predictions: (1) social call structure will be very similar between *P. maderensis* and *P. kuhlii*; (2) social calls may elicit heterospecific responses; and (3) echolocation calls, not being subject to the same conservative evolutionary pressures, will exhibit more pronounced divergence or variation.

MATERIALS AND METHODS

Structure of social and echolocation calls

We recorded social and echolocation calls from *P. maderensis* at foraging sites on the island of Madeira, Portugal (32°44′ N, 16°58′ W) and from *P. kuhlii* in three regions of central and southern Italy (Umbria, 43°12′ N, 12°04′ E; Abruzzo, 41°44′ N, 12°02′ E; and Lazio, 41°37′ N, 13°50′ E and 41°29′ N, 13°54′ E). In all areas, foraging sites were very similar (street lamps close to trees), and generally at least two bats were observed foraging (i.e. recording situations were similar). Species identity could be ascertained unambiguously. Of the three bats known to occur currently on Madeira (Jesus *et al.*, in press) – *P. maderensis*, *Plecotus austriacus* (J. Fischer, 1929) and *Nyctalus leisleri* (Kuhl, 1817) – only the former produces social calls of the type studied. Social calls emitted by *P. kuhlii* are also easily distinguished from those of other sympatric pipistrelle bats (Russo & Jones, 1999, 2000). In addition to the agonistic social calls comprising two or more components that we studied, both species emitted structurally different single-component calls (D. Russo, pers. obs.), and we excluded these from our analysis. To avoid pseudo-replication (Hurlbert, 1984), we selected only one call per site.

We recorded ultrasound at a high sampling rate (350 kHz) with the real-time mode of a D1000X bat detector (Pettersson Elektronik AB, Uppsala, Sweden). This device is equipped with a built-in 16-bit recording system and stores sound as WAV files onto a Compact Flash card. When a bat pass – a series of clicks heard in frequency division as a bat flew within range (Fenton, 1970) – was detected, we triggered the recording device manually. Analysis was performed with the software BatSound ver. 3.31 (Pettersson Elektronik AB).

We chose random calls for analysis, but discarded those with a poor signal-to-noise ratio. For each call, we generated a spectrogram and an oscillogram; a power spectrum was also obtained for each call component. Spectrograms and power spectra were obtained by applying a Fast Fourier Transform (FFT) with a Hamming window (window size 1048 samples, 98% overlap, frequency resolution 488 Hz). To ensure consistency in spectrogram measurements, we selected the same threshold (17) in the BatSound settings. For each social call, we considered the following variables: number of components in the call (*n comps*); maximum (*fmax*) and minimum (*fmin*) frequencies taken from the fundamental component (social calls are typically multi-harmonic); mean frequency of maximum energy (*freq*), calculated as the mean value of frequencies of highest energy taken from all call components; total call duration (*totdur*); and mean inter-component interval (*ici*), the average time interval between consecutive call components (Russo & Jones, 1999; Pfalzer & Kusch, 2003). For each echolocation call, we measured the start frequency, end frequency, frequency of maximum energy, and duration (Russo & Jones, 2002). Time variables (expressed in ms) were taken from oscillograms, and frequency variables (in kHz) from spectrograms, except for the frequency of maximum energy, which was measured from power spectra.

Differences in echolocation call frequencies may reflect different body size across species (Jones, 1999). Therefore, we also compared forearm length between 36 individuals of *P. maderensis* (15 females, 21 males) from Madeira, and 40 of *P. kuhlii* (20 males, 20 females) from Italy. Bats were captured in mistnets set at foraging sites, and forearm length was measured to the nearest 0.1 mm using dial calipers.

Testing social call function and interspecific reaction to signals

We tested whether foraging individuals of *P. maderensis* react to playbacks of *P. kuhlii* social calls on Madeira, and vice versa at the study sites in Italy. We also broadcast pre-recorded social calls of *P. kuhlii* at foraging sites of this species to compare heterospecific and conspecific reactions. Twelve sites (all near street lamps) were selected in each geographical area. A 'response' to social calls was represented by a difference in activity – measured as the number of bat passes during 3-min playback sessions – between experimental trials (social call playback) and 'control' (playback of recordings of background noise). The food patch defence hypothesis (Barlow & Jones, 1997b) was verified when activity (number of bat passes of target species) measured during experimental trials was lower than that during playback of control sound.

Experimental trial sequences were generated following Barlow & Jones (1997b). In the BatSound software, we applied the functions 'copy' and 'paste' to create sequences of social calls (previously recorded in the field in real time with a D-1000X bat detector) within a blank sound (WAV) file. Social calls, arranged in pairs, were repeated every 10 s in 3-min sequences. Control sequences contained only background noise. In this way we generated 36 sequences – 12 with *P. kuhlii* calls, 12 with *P. maderensis*, and 12 control files. Sound was played back by connecting the D1000X to an L60 ultrasound loudspeaker (Pettersson Elektronik AB); the maximum sound pressure level of signals broadcast by the device is *c.* 95 dB at 1 m (L. Pettersson, pers. comm.). The intensity of playback sequences was calibrated by detecting the sound broadcast with a U30 bat detector (Ultra Sound Advice, London, UK) at a distance of 15–20 m (i.e. approximately equal to the distance between the loudspeaker and bats in the field). Signals heard on the bat detector that were of a similar loudness to calls emitted by bats in flight at a comparable distance were used in the experiment. When needed, the sound pressure level was adjusted with the 'edit volume' option in the BatSound software. All sequences used for experiments had similar sound pressure levels.

The identity of bats under investigation at the experimental sites was confirmed by examining time-expanded or real-time recordings taken from foraging bats immediately before and after (and, in many cases, during) the experiment (for the criteria applied to identify *P. kuhlii* see Russo & Jones, 1999, 2002). All selected sites were well-known foraging areas of the two species under investigation, so there was no risk of accidentally counting passes by species other than those of interest. The number of passes was counted with a bat detector (D240; Pettersson Elektronik AB, U30; Ultra Sound Advice) in the heterodyne mode set to 45 and 40 kHz on Madeira and in Italy, respectively, corresponding to peak frequencies of the echolocation calls emitted by the study species (Russo & Jones, 1999, 2002; S. Teixeira & D. Russo, pers. obs.). Because sites were illuminated by street lights, bats were often observed during foraging, and their silhouette and flight style could be determined.

Activity was measured by counting the number of passes at each foraging site during playback sessions. The loudspeaker was positioned at a height of *c.* 2.5 m and directed upwards. We randomized both the order in which sites were visited and the sequence (social calls or control) broadcast. At all sites, a silent period of 3 min was used between tests to minimize any potential carry-over effects from the previous treatment.

Statistical analysis

We used an ANOVA to carry out univariate comparisons between species for all call variables. Most variables showed a normal distribution of residuals, as ascertained by a Ryan–Joiner test of normality ($P > 0.05$), and variance homogeneity. When departure from normality occurred, data were log-normalized before applying the ANOVA. Following Barlow & Jones (1997a) and Russo & Jones (2000), we also tested whether continuous call variables allowed for a multivariate discrimination between species. We applied a quadratic discriminant function analysis (DFA) with cross-validation including variables *fmax*, *fmin*, *freq*, *totdur* and *ici*. We generated Wilk's λ values with a MANOVA to test for statistical significance of the DFA model and to assess the discrimination power of each variable (Parsons & Jones, 2000; Russo & Jones, 2002). Forearm lengths were compared between species using a general linear model ANOVA with species and gender as main factors.

The numbers of passes by bats recorded respectively during playback of social calls and control sequences broadcast at each site were compared with a sign-test (Barlow & Jones, 1997b; Dytham, 1999). All tests were carried out with Minitab release 13 (Minitab Inc., State College, PA, USA). Statistical significance was set at $P = 0.05$.

RESULTS

Social calls had a very similar structure between species (Fig. 1). In both species, calls most frequently consisted of three components. Calls selected for analysis had two to five components. One *P. maderensis* call (outside the random sample used for quantitative analysis) consisted of six components. We found no significant differences between species for all temporal and spectral variables except *fmin*, which was slightly (on average less than 1 kHz) higher in *P. maderensis* (Table 1). For this factor too, frequency ranges largely overlapped between species.

DFA corroborated these results because limited discrimination was achieved. The best discriminating model included all variables, and although it correctly classified 80% of *P. kuhlii* calls (i.e. 40 out of 50), it did so for only 58% (29 out of 50) of *P. maderensis* calls, providing an overall discrimination performance of 69%. This was only 19% higher than random classification (50%). The model was significant (MANOVA, Wilk's $\lambda = 0.799$, $P < 0.002$). Wilk's λ values were identical (1.00) for all factors except *fmin*, which showed a slightly smaller value (0.83), corresponding to a better discrimination performance. When *fmin* was removed from the DFA, classification performance dropped to only 60% (i.e. only 10% above random classification).

Unlike social calls, echolocation calls differed markedly between species (Table 1). No overlap occurred between end frequency or frequency of maximum energy between species. Start frequency showed a borderline ($P = 0.07$), non-signifi-

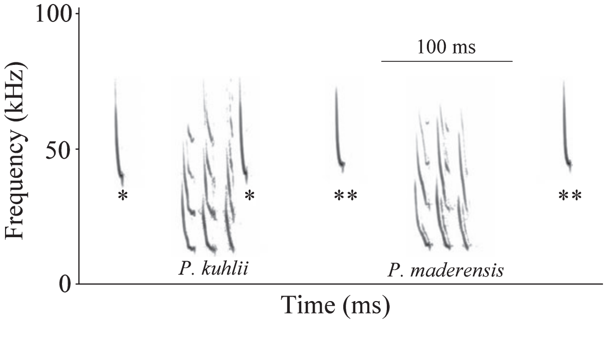

Figure 1 Spectrograms of three-component social calls of *Pipistrellus kuhlii* and *Pipistrellus maderensis* and corresponding echolocation pulses (marked with asterisks: *denotes *P. kuhlii*; **denotes *P. maderensis*).

Table 1 Descriptive statistics for parameters of *Pipistrellus maderensis* and *Pipistrellus kuhlii* social and echolocation calls ($n = 50$ for each species) and ANOVA levels of significance for differences between species.

Parameter	*P. maderensis*	*P. kuhlii*	*P*
Social calls			
n comps	3.1 ± 0.8	3.1 ± 0.8	*n.s.*
	2–5	2–5	
fmin (kHz)	12.0 ± 0.9	11.3 ± 0.6	< 0.001
	10.2–13.6	9.5–13.6	
fmax (kHz)	38.7 ± 8.5	40.0 ± 8.8	*n.s.*
	26.6–70.8	25.2–63.2	
freq (kHz)	17.8 ± 2.9	17.6 ± 3.9*	*n.s.*
	14.9–28.9	12.9–29.0	
totdur (ms)	37.1 ± 8.7	37.0 ± 7.4*	*n.s.*
	21–57	21–58	
ici (ms)	14.9 ± 2.8	14.7 ± 2.9	*n.s.*
	9.7–23.0	10.0–21.0	
Echolocation calls			
Start frequency (kHz)	65.8 ± 10.9	62.1 ± 8.9	*n.s.*
	45.7–95.3	44.3–82.4	
End frequency (kHz)	44.8 ± 1.2	38.7 ± 1.3	†
	42.2–47.7	35.4–41.5	
Frequency of maximum energy (kHz)	47.0 ± 2.0	40.7 ± 1.2	†
	43.7–54.9	36.5–42.4	
Duration (ms)	5.2 ± 1.0	6.2 ± 1.0*	< 0.001
	3.4–8.1	4.8–9.5	

n comps, number of social call components; *fmin*, minimum frequency; *fmax*, maximum frequency; *freq*, frequency of maximum energy; *totdur*, total call duration; *ici*, mean time interval between call components; *n.s.*, not significant.

*ANOVA carried out on log-transformed values.

†Parameters showing no range overlap between species were not tested with ANOVA. Values shown are means ± SDs, and ranges are also presented.

cant trend towards higher values in *P. maderensis*, and duration was significantly shorter for the latter.

The higher frequencies used in echolocation by *P. maderensis* are paralleled by its smaller size. Both gender ($F_{1,1,72} = 15.56$) and species ($F_{1,1,72} = 17.19$) had significant effects on forearm length ($P < 0.005$), whereas the interaction between them did not. In *P. maderensis*, males had forearm lengths averaging 32.7 mm (0.9 mm SD), and females averaged 33.3 mm (0.7). In *P. kuhlii*, males had forearm lengths averaging 33.3 mm (0.7), and females averaged 34.2 mm (1.0).

In all playback tests except one (on *P. maderensis*), social calls induced a significantly reduced bat activity relative to control sequences (Fig. 2). *Pipistrellus kuhlii* showed similar responses both to its own recordings (sign test, $n = 12$, median = −7.5, $P < 0.001$) and to those of *P. maderensis* ($n = 12$, median = −12.0, $P < 0.001$). Likewise, *P. maderensis* showed a significant decrease in activity when *P. kuhlii* social calls were broadcast ($n = 12$, median = −4.5, $P < 0.01$).

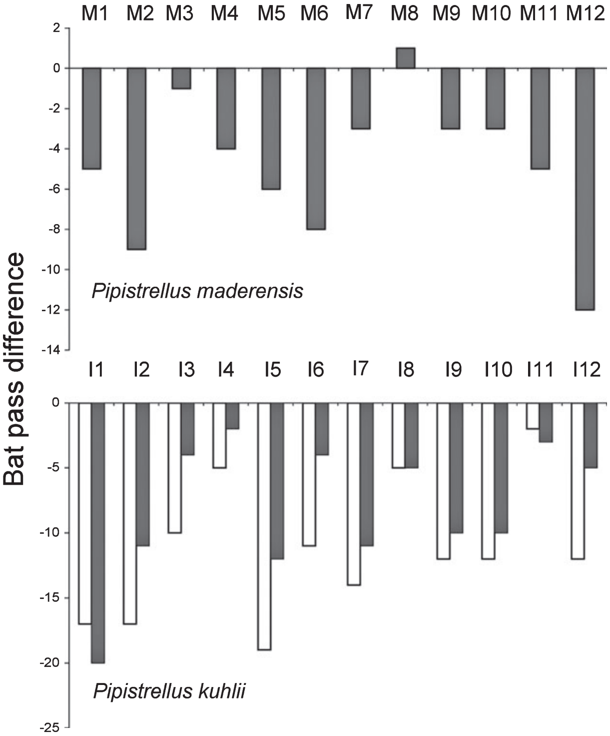

Figure 2 The response of *Pipistrellus maderensis* and *P. kuhlii* to playback experiments at 24 foraging sites (12 on Madeira, 12 in Italy). Each bar represents the difference in bat activity, measured as the difference in the number of bat passes between experimental trials and control trials for one site. Grey bars illustrate responses to *P. kuhlii* calls, and white bars illustrate responses to *P. maderensis* calls. M 1–12: sites on Madeira. I 1–12: sites in Italy.

DISCUSSION

Social and echolocation calls in *P. maderensis* and *P. kuhlii*

Our study demonstrated a strong similarity of social call structure in the two bat species. Although *fmin* showed a statistically detectable difference between the species and was the main contributor to the multivariate discrimination of *P. kuhlii* (but not of *P. maderensis*) social calls, this variable showed considerable overlap between taxa, suggesting that the observed difference is of little biological significance. Moreover, the number of call components showed no consistent differences between species, unlike the situation in sympatric *P. pipistrellus* and *P. pygmaeus*, which typically show differences in the numbers of components, frequency values, and even in the spectrogram 'shape' of the call components (Barlow & Jones, 1997a).

Although the number of call components also showed an identical modal value in both species (three), calls with a different number of components were sometimes recorded. As in a previous study (Russo & Jones, 1999), the maximum number of components recorded for *P. kuhlii* in this study was five. Six components can occur in *P. maderensis*, albeit very rarely. Further playback experiments would be needed to ascertain the role of the single-component calls recorded from both pipistrelle bat species at foraging sites.

The functional value of the different number of components is debatable. In both species, the same bat may consecutively emit calls with different numbers of components (D. Russo, pers. obs.), thus ruling out the hypothesis that this variable conveys 'stable' information regarding the emitter with respect to gender, age, rank or body size. Varying the number of components in the calls may instead offer a simple, unambiguous way to express some aspect of motivation in the signaller (e.g. it might correspond to a more or less 'intense' message during chases).

Given the similarity in the social calls, we predicted that each species would respond to social calls emitted by the other species. This was confirmed by the reactions recorded in our experiments; that is, social calls were so similar between species that they overcame species communication barriers, and each species responded to the social calls of the other. This result differs from that in the cryptic species *P. pipistrellus* and *P. pygmaeus*, where responses are species-specific (Barlow & Jones, 1997b).

Although social calls have retained the same structure (and function) as in *P. kuhlii*, echolocation calls in *P. maderensis* showed major structural differences, with frequency of maximum energy and end frequency being markedly higher in *P. maderensis*, which also exhibited a significantly if only slightly (*c.* 1 ms) shorter call duration. The only parameter that did not differ significantly between species (start frequency) still showed a trend towards higher values in *P. maderensis*. Start frequency is affected substantially by atmospheric attenuation (Lawrence & Simmons, 1982), and is hence strongly dependent upon the distance that the bat was recorded at. Start frequency therefore shows considerable variability within species, which makes the detection of interspecific differences difficult. Moreover, *P. maderensis* had a slightly, yet significantly, smaller body size than *P. kuhlii*. We propose that the observed differences in echolocation call frequencies arise from the smaller body size of *P. maderensis*, which gives it a narrower vocal tract, causing the production of higher frequencies (Heller & Helversen, 1989; Jones, 1999). Call duration also typically scales negatively with body mass across species that emit calls containing narrowband components (Jones, 1999). Body size reduction is a widespread effect of insularity reported for many bat species, and is probably related to limited food resources (Krzanowski, 1967). Correlated evolution between morphological (e.g. body mass, bill size) and acoustic (i.e. song frequency and temporal patterning) traits has also been proposed for some bird groups (Podos, 2000; Seddon, 2004).

An alternative hypothesis is that higher frequencies allow *P. maderensis* to exploit prey of different size, as by adopting higher call frequencies a bat may detect smaller prey. The wavelength of sound gives an estimate of prey size below which echo strength rapidly decreases (Houston *et al.*, 2004). Wavelength (in mm) may be calculated as 1000× (speed of sound in air at 25°C and 80% relative humidity, equalling 347.65 m s^{-1}) per call frequency of maximum energy (Hz). Using the mean values recorded in this study, we obtain wavelength values of 7.4 and 8.5 mm for *P. maderensis* and *P. kuhlii*, respectively, values that might allow *P. maderensis* to better detect smaller prey.

A case of stabilizing selection?

In species-rich assemblages, similarity in social calls may result in ambiguous communication and, consequently, in decreased fitness. This situation may prompt acoustic character displacement, resulting in different call structures and distinct communication channels between species. In rhinolophoid bats, the constant frequency component of echolocation pulses may convey social information such as species identity (Kingston *et al.*, 2001; Thabah *et al.*, 2006; Russo *et al.*, 2007). This hypothesis may explain the divergence in echolocation call frequencies recently reported for Sardinian populations of *Rhinolophus hipposideros* and *R. euryale*, which do not overlap with those for *R. mehelyi* (Russo *et al.*, 2007). In that case, geographic isolation prevents gene flow from allopatric, mainland populations, which would otherwise mask the observed pattern.

Pressures leading to social call divergence are also evident in the sympatric pipistrelles *P. pipistrellus* and *P. pygmaeus* (Barlow & Jones, 1997a; Russo & Jones, 2000). Such differences are effective in allowing unambiguous communication with conspecifics and may have evolved partly by acoustic character displacement (Barlow & Jones, 1997b; Jones, 1997). Both *P. pipistrellus* and *P. pygmaeus* emit social calls that are more distinctive than in the species we studied: this may reflect both a longer time since they diverged from a common ancestor (possibly *c.* 5 Ma; Barratt *et al.*, 1997) and their frequent occurrence in sympatry (Mayer & von Helversen, 2001).

In a simplified bat community such as that of Madeira, selective pressures for structural shifts such as those imposed by the presence of species broadcasting similar signals will be absent, and stabilizing selection instead appears the most parsimonious hypothesis to explain structural and functional preservation of social calls. Although the outcome of character stabilization should be an increase in individual fitness, experimental assessments that estimate fitness are unlikely to be feasible, and hence the only conceivable approach to understanding the consequences of signal stability is that of comparative structural and functional analyses, as carried out in this study.

Although produced at different rates, calls identical to those produced at foraging sites to repel conspecifics are also employed in the mating season by males of *P. pipistrellus* and *P. pygmaeus* to attract females (Barlow & Jones, 1997a). In this case, evolution has been parsimonious, as the same signal structure plays a different, context-dependent communication function. It is unknown whether the same situation occurs in mating individuals of *P. maderensis* and *P. kuhlii.* Should this be the case, partner recognition would represent a further evolutionary pressure for maintaining social call structure in these bats.

No information on social call structure is available for Tenerife, where *P. maderensis* and *P. kuhlii* are sympatric according to Pestano *et al.* (2003). In that case, call structure may diverge as a result of selection for effective communication barriers, and studies similar to ours set in areas of sympatry would therefore be of great interest. Habitat segregation might also play a role in preserving distinct communication at foraging sites, so this aspect would be worthwhile to investigate too. However, the phylogenetic and taxonomic status of the Canarian *P. kuhlii* appears uncertain, and bats on the Canary Islands may be quite distinct from those of mainland populations: this should be taken into account for future behavioural and bioacoustical comparisons. On the Canary Islands, *P. maderensis* and *P. kuhlii* show pronounced morphological similarities. Pestano *et al.* (2003) found that the Canarian *P. maderensis* is more closely related to the local *P. kuhlii* clade than the latter is to *P. kuhlii* from continental populations. Pestano *et al.* (2003) suggest that the two lineages may interbreed, and hence their status as separate species is questionable. Finally, Simmons (2005) reports that Canary Island populations referred to as *P. kuhlii* by Pestano *et al.* (2003) may in fact consist of the African *Pipistrellus hesperidus*.

Bats may easily cross short stretches of sea: *Miniopterus schreibersii* and *Myotis capaccinii* can move between the islands of Mallorca and Menorca, a distance of at least 40 km (Amengual *et al.*, 2007). It is unknown whether gene flow exists among insular populations of *P. maderensis*, but given the large distances among islands, gene flow will probably be extremely infrequent or absent. The geographical isolation of the Madeira Archipelago presents strong barriers to gene flow: it lies *c.* 650 km off the closest mainland (African coast), and is far enough from other islands harbouring *P. maderensis* to make frequent contact with allopatric populations very unlikely (Canary Islands, *c.* 450 km; Azores, *c.* 850 km).

Inheritance of social calls: genes or memes?

In this study we recorded rates of social call production of up to 0.8 calls s^{-1}, that is, almost a call per second (D. Russo, *pers. obs.*). Because two or more bats are typically found at sites where social calls are emitted, hundreds of such calls may be heard in a few hours at a single foraging site. Such signals are certainly among the most frequently emitted social calls in echolocating bats. We propose that this frequent use may account for the faithful inheritance of call structure across generations and, ultimately, across species.

An interesting analogy is offered by the cultural evolution of human languages. This follows trajectories analogous to those of biological evolution by natural selection (descent with modification; Pagel *et al.*, 2007). In human language, words used frequently are less susceptible to change (Ellis, 2002). Modelling suggests that the number of times a word is used alters the rate at which new word forms arise, since fewer accidental errors occur for highly expressed words (Pagel *et al.*, 2007). In an analogy with natural selection, this signifies that frequently used words are subject to stabilizing selection.

We have assumed that social call structure is shaped mainly by natural selection processes. The possible influence of social learning in determining at least some features of social calls in echolocating bats is actually unknown. In many birds, social learning plays a major role in structuring songs (e.g. Zeigler & Marler, 2008; but see Seddon, 2004). Vocal learning is much rarer in mammals. For instance, alarm call structure in rodents is heritable, hybrids showing calls of intermediate structure (Nikol'skii *et al.*, 1984; Nikol'skii & Starikov, 1997) or signal structure resembling that of one parent more than the other (Koeppl *et al.*, 1978).

However, evidence for vocal learning of social calls exists for only a few bat species. Female greater spear-nosed bats, *Phyllostomus hastatus*, form social groups that cooperate in foraging. Although they are not closely related, group mates produce similar social calls that function to signal group membership and facilitate cooperation (Wilkinson & Boughman, 1998, 1999). Bats transferred experimentally between groups adjust call structure to match that of the group they join, confirming that vocal learning occurs in this species (Boughman, 1998). *Hipposideros terasensis* emits echolocation calls that are dominated by a constant frequency component and that are probably used in communication as well as in echolocation (Thabah *et al.*, 2006; Russo *et al.*, 2007). Bats transplanted into new social groups apparently shift this frequency value to match that of existing colony members (Hiryu *et al.*, 2006). These experiments prove that some form of socially induced (i.e. learnt) 'tuning' mechanism is operating at least in some species, even though the physiological and behavioural bases for performing the adjustment must of course be genetically inherited. Therefore, stabilization of pipistrelle social calls might be driven by either genetic or cultural factors, or possibly by a combination of both: in all cases, the frequent use of such signals in communicating bats might have favoured the process. However, it is debatable whether cultural learning could be so persistent as to preserve signal structure over many generations, and to cope with the long time-frame and the dramatic changes of speciation.

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