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Morphological correlates of echolocation frequency in the endemic Cape horseshoe bat, *Rhinolophus capensis* (Chiroptera: Rhinolophidae)

Lizelle J. Odendaal · David S. Jacobs

Abstract We investigated intraspecific variation in echolocation calls of the Cape horseshoe bat, *Rhinolophus capensis*, by comparing echolocation and associated morphological parameters among individuals from three populations of this species. The populations were situated in the center and at the western and eastern limits of the distribution of *R. capensis*. The latter two populations were situated in ecotones between vegetation biomes. Ecotone populations deviated slightly from the allometric relationship between body size and peak frequency for the genus, and there was no relationship between these variables within *R. capensis*. Nasal chamber length was the best predictor of peak frequency but not correlated with body size. The evolution of echolocation thus appears to have been uncoupled from body size in *R. capensis*. Furthermore, females used higher frequencies than males, which imply a potential social role for peak frequency. The differences in peak frequency may have originated from random founder effects and then compounded by genetic drift and/or natural selection. The latter may have acted directly on peak frequency altering skull parameters involved in echolocation independently of body size, resulting in the evolution of local acoustic signatures.

Keywords Echolocation · Allometry · Nasal chamber · Selection · Decoupling

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Introduction

Variation in acoustic signals used by animals for communication could lead to adaptive and/or reproductive divergence and potentially speciation. Geographic variation in acoustic signals have been reported in various taxa such as birds (Shieh 2004), anurans (Wilczynski and Ryan 1999) and mammals including echolocating bats (Barclay et al. 1999; Guillén et al. 2000; Gillam and McCracken 2007). Although most of these taxa use acoustic signals purely for communication, bats use echolocation for foraging, orientation (Simmons and Stein 1980) and communication (Fenton 1985). Thus, bats are ideal for studying factors affecting variation in acoustic signals.

Bats in the families Rhinolophidae (horseshoe bats) and Hipposideridae (leaf-nosed bats) produce medium to long calls with high duty cycle (HDC). The calls have a prominent constant frequency component followed by a brief frequency-modulated component with most energy contained in the second harmonic (Neuweiler 1984). During flight, these bats compensate for flight-induced Doppler shifts to ensure that the returning echo falls within the narrow frequency range of the "acoustic fovea"—a region of the cochlea with an over-representation of neurons sensitive to a unique frequency (Schuller and Pollak 1979). The returning echo is very similar in frequency to the calls they emit when stationary, often referred to as the 'resting frequency' (RF) (Schuller and Pollak 1979).

The HDC bats are ideal for investigating variation in echolocation because the RF recorded from handheld individuals can be used. Recording methods for different individuals can thus be standardized, and measurement error due to variations in flight speed and the direction of the bat relative to the microphone can be reduced. Our

study focused on patterns of intraspecific frequency variation in rhinolophids.

Body size is a major influence on echolocation variation, and it can cause variation at both the population and species levels. It scales negatively with call frequency in at least five bat families including the Rhinolophidae and Hipposideridae (Jones 1996); larger bat species produce lower frequency calls than smaller species. This relationship is also found within some species such as *Rhinolophus philippinensis* (Kingston and Rossiter 2004).

Selection could, however, produce variation in echolocation frequency by acting on echolocation independently of body size. If so, one would expect no correlation between body size and echolocation frequency. Instead, there should be a stronger correlation between call frequency and morphological features associated with call emission and production, and processing of echoes from those calls (Francis and Habersetzer 1998; Armstrong and Coles 2007). For example, the "noseleaves" of horseshoe bats may control signal direction and improve signal resonance (Zhuang and Müller 2007). A significant negative correlation exists between noseleaf width and peak frequency in horseshoe bats (Robinson 1996).

Features of the skull directly involved in signal production and reception of rhinolophid bats include the dorsal nasal chambers which have been shown to act as resonance chambers (Hartley and Suthers 1988; Suthers et al. 1988), and the cochlea. There is some support for the uncoupling of echolocation from body size. For example, nasal chamber size was the only morphological feature showing significant geographic variation in *Rhinonicteris aurantia*, and these differences were linked to echolocation frequency variation (Armstrong and Coles 2007). Similarly, rostrum size (which houses the nasal chambers) was the best predictor of echolocation frequency among rhinolophids (Stoffberg 2007). A significant negative correlation between call frequency and cochlear diameter for 15 rhinolophid and 10 hipposiderid species has also been reported (Francis and Habersetzer 1998). Body size was correlated with call frequency, and morphological features directly associated with echolocation in both of the latter two studies.

Although echolocation is largely used for prey detection and orientation, it may also play a role in communication (e.g. Russo et al. 2001; Ma et al. 2006; Jones 2008; Kazial et al. 2008; Grilliot et al. 2009; Yovel et al. 2009; Schuchmann and Siemers 2010). Echolocation calls may convey information about age and sex (Kazial and Masters 2004; Siemers et al. 2005). Juvenile rhinolophids produce lower frequency calls than adults in *R. euryale*, *R. mehelyi* (Russo et al. 2001), *R. blasii* (Siemers et al. 2005) and *R. ferrumequinum* (Jones and Ransome 1993). Similarly, call frequency differences may be related to sex

independently of body size. Females emit higher frequency calls than males in *R. rouxi* (Neuweiler et al. 1987), *R. hipposideros* (Jones et al. 1992) and *R. blasii* (Siemers et al. 2005), whereas males produce higher frequency calls in *Hipposideros speoris* (Jones et al. 1994). No apparent sexual dimorphism in body size occurs in any of these species, suggesting that the evolution of echolocation may have been uncoupled from body size. However, this can only be confirmed through uncorrelated differences in both body size and echolocation frequency (Armstrong and Coles 2007).

The Cape horseshoe bat, *Rhinolophus capensis*, is a medium-sized rhinolophid (forearm length 46–51.8 mm; Jacobs et al. 2007) endemic to the south western cape of South Africa. They are found in a variety of habitats along the coast of the Western and Eastern Cape Provinces. This species is thus ideal for teasing apart the factors that contribute to intraspecific variation in echolocation. We evaluated the factors causing the variation in the echolocation calls of *R. capensis* by investigating the relationship between echolocation calls and body size and the morphological characters directly associated with the production and reception of echolocation in this species.

Materials and methods

Study sites

Adult *R. capensis* individuals were sampled from three populations across its distribution during spring and summer 2007–2008. The three sites were De Hoop Nature Reserve (34°26′S, 20°25′E) in the Western Cape Province, Table Farm (33°17′S, 26°25′E) in the Eastern Cape Province and Steenkampskraal (30°58′S, 18°37′E) situated in the Namaqualand region of the Western Cape Province of South Africa (Fig. 1).

De Hoop is situated in the winter rainfall region of South Africa. There are two main roosting colonies of *R. capensis* within De Hoop: De Hoop Guano Cave and Hot Hole. The former is situated in a limestone cliff at the northern part of the land-locked De Hoop Vlei (shallow body of water that is periodically dry), while the latter is a sink hole 7 km from the Guano Cave. The dominant vegetation surrounding both colonies is coastal fynbos, characterized by evergreen, sclerophyllous shrubs, and dominated by restios. Table Farm is situated on the ecotone (area of environmental transition: Kark et al. 2002) between thicket in the east and Nama-Karoo in the west (Dold 2003). This region receives rain throughout the year, but predominantly from spring to summer (Stone et al. 1998). The colony we studied roosted in an abandoned tunnel excavated for a water pipeline. Steenkampskraal is
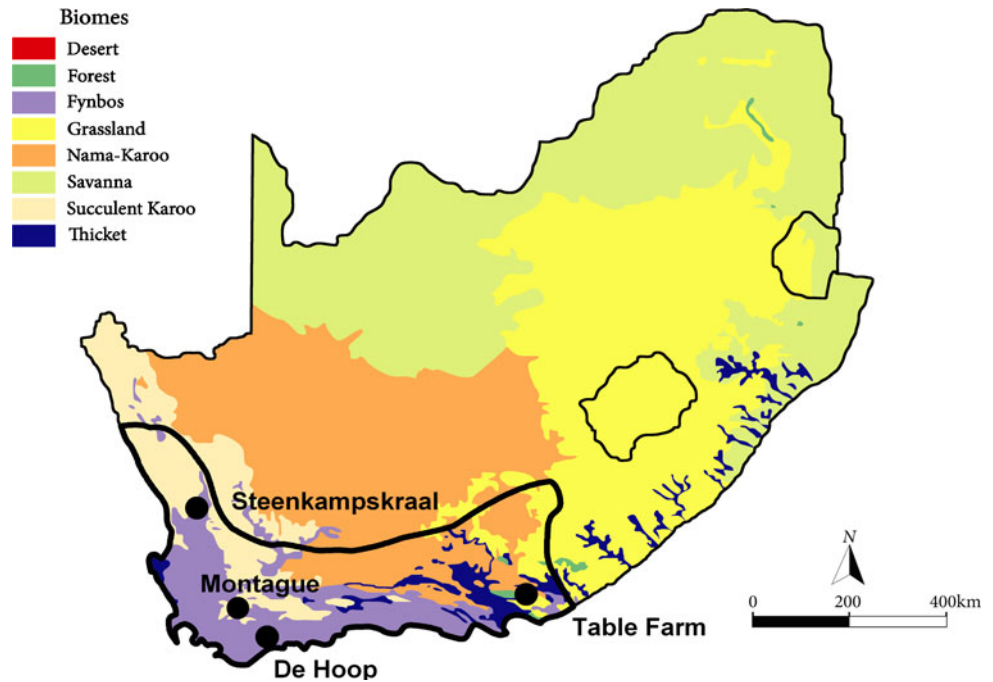
situated in the Succulent Karoo biome in the western part
of South Africa, known as Namaqualand. Although within
a winter rainfall region, this area is arid and is character-
ized by <150 mm of rain per annum (Cowling et al. 1999).
The major vegetation type of Namaqualand is Lowland
Succulent Karoo characterized by sparse, dwarf (ca. 30 cm
in height) succulents. Steenkampskraal is also situated
within an ecotone between Lowland Succulent Karoo,
Strandveld Succulent Karoo and Sandplain Fynbos
(Cowling et al. 1999).

Morphology

Bats were captured using mist nets and/or a harp trap
(Austbat Harp Trap, Faunatech, Mount Taylor, VIC, Aus-
tralia) placed near the entrance of the roosts. Hand-nets
were also used to capture roosting bats during the day. The
age and sex of each bat was recorded. Juveniles were
distinguished from adults by the presence of cartilaginous
epiphyseal plates in their finger bones (Anthony 1988).
Only adults were used in subsequent analyses. For each
bat, we measured body mass (to the nearest 0.01 g) using a
portable electronic balance (Ohaus Corporation, Pine
Brook, NJ, USA), and forearm length and noseleaf width
(to the nearest 0.01 mm) using dial calipers. To control for
seasonal variation in body mass and echolocation call
frequency, all sampling took place during the months of
late spring and early summer from 2007 to 2008. Body
mass may be influenced by various factors such as repro-
ductive status or whether the bat had been foraging. In an
attempt to eliminate this variation, only non-pregnant

females were used, and bats were measured late in the
afternoon when their guts were empty.

A photograph of each bat, right wing and tail extended,
was taken with a Canon Powershot S60 PC1088 digital
camera (Canon Inc., Japan) positioned 90° above the wing
to prevent angular distortion (Jacobs et al. 2007). The wing
was extended on graph paper to provide a reference for the
calibration of SigmaScan Pro 5 software (version 3.20,
SPSS Inc.), used to measure wingspan and wing area to
calculate wing measurements such as wing loading fol-
lowing Norberg and Rayner (1987).

Echolocation

Echolocation calls were recorded from hand-held bats
positioned 10 cm in front of an Avisoft Ultrasound Gate
416 (Avisoft Bioacoustics, Berlin, Germany) microphone.
Calls were recorded directly onto a HP Compaq nx7010
notebook computer with Avisoft SasLab Pro software.
Recordings were slowed down by ten and were analyzed
using BatSound Pro software (Version 3.20, Pettersson
Elektronik AB, Uppsala, Sweden) using a sampling rate of
50,000 Hz. The following parameters were measured: peak
frequency (kHz)—the frequency of maximum intensity
determined from the fast Fourier transformation (FFT)
power spectrum (size 1024; frequency resolution 684 Hz);
duration (ms)—the time from beginning to end of call,
determined from the oscillogram; and minimum frequency
of the frequency-modulated tail (kHz)—determined from
the spectrogram. Due to the random occurrence of back-
ground noise, a Hanning window was used. Bandwidth

(kHz) was calculated by subtracting the minimum frequency from the peak frequency.

The mean values of these echolocation parameters were calculated from ten, randomly selected, high-quality calls (calls with a high signal-to-noise ratio) for each bat. The amplitude of the signal was at least three times higher than that of the background noise as displayed on the log (dB) scale of the oscillogram. Because using the means from each parameter results in an echolocation call that is "constructed", and therefore not "real", the parameters of an original call that most closely resembled the calculated mean parameters were chosen for all subsequent analyses.

Skull morphology

Skulls of *R. capensis* ($n = 22$) were obtained from the Northern Flagship Institute and Iziko Museum in South Africa (Appendix). These included skulls from an additional population: Montague (33°47′S, 20°7′E). Voucher specimens of ten bats (five males and five females) from both Table Farm and Steenkampskraal were also collected after they were measured and their echolocation calls recorded so that their skulls could be removed. Skulls were also obtained from four specimens previously collected from De Hoop, and echolocation data for three of these were available. Thus, we had post-cranial morphological measurements and echolocation calls for 23 out of the 46 skulls. We took X-ray radiographs of the 46 skulls from above (transverse view) and from the side (sagittal view) (Fig. 2) following Armstrong and Coles (2007). Radiographs were taken with a custom-made Mamex prototype high-resolution scanner at 28 kV and 100 mA (S. Steiner: MRC/UCT Medical Imaging Research Unit). A scale was included with the radiographs for later calibration of measurements using Sigma Scan Pro 5 software. The following skull parameters were measured from the radiographs following Armstrong and Coles (2007): cochlear width (CW); cochlear area (CA); nasal width (NW); transverse area of the nasal capsule (TA); nasal capsule length (X–Y); nasal capsule height (Z–W) and sagittal area of nasal capsule (SA). For all radiograph measurements, the mean value of three measurements for each skull parameter was calculated and used in all analyses. Measurement error was determined by taking repeated measures ($n = 10$) of those characters that were difficult to measure.

Statistical analyses

Many studies have found significant variations in body size between populations of rhinolophid and hipposiderid bats (Francis and Habersetzer 1998; Thabah et al. 2006; Russo et al. 2007). These studies often used forearm length as an
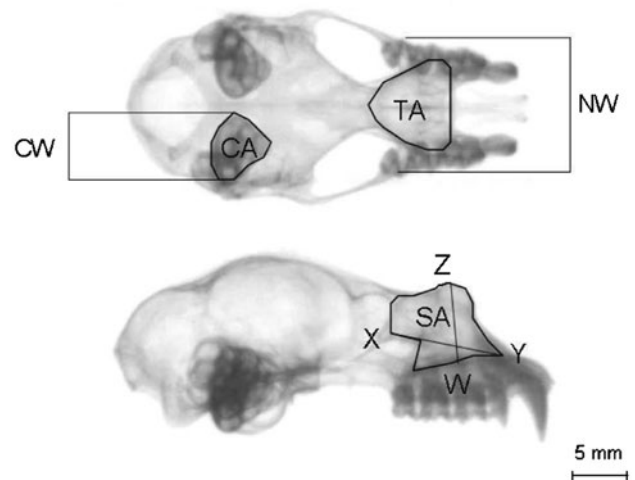

Fig. 2 Skull radiographs of a female *R. capensis* specimen showing the skull parameters measured: cochlear width (*CW*), cochlear area (*CA*), nasal width (*NW*), transverse area of the nasal capsule (*TA*), nasal capsule length (*X–Y*), nasal capsule height (*Z–W*) and sagittal area of nasal capsule (*SA*)

indicator of body size instead of body mass because the latter may be influenced diurnally by a range of factors and may be an unreliable measurement for comparative purposes. In doing so, intraspecific variation in body mass for rhinolophid bats has largely been ignored. However, in this study, regression analysis revealed that body mass and forearm length were strongly correlated in *R. capensis* ($r^2 = 0.346$, $F_{(1,102)} = 54.01$, $P < 0.00001$), and variation in body mass between populations due to differences in sampling method and season was controlled for as much as possible (see above). Most importantly, analyses incorporating forearm length yielded the same results as for body mass, and therefore, analyses based on the latter is reported here. There were also no significant differences in wingspan, wing area, body size or peak frequency between bats caught at Hot Hole [mean ± SD ($n = 16$): 30.3 ± 1 cm, 151 ± 9.1 cm^2, 10.5 ± 1.14 g, and 84.3 ± 0.8 kHz, respectively] and the Guano Cave [mean ± SD ($n = 28$): 30.5 ± 1 cm, 156.5 ± 9.8 cm^2, 10.12 ± 1.07 g, and 84.7 ± 0.76 kHz, respectively] at De Hoop (Tukey $P > 0.05$), i.e. there were no colony effects on these variables. Thus, data for the two colonies were pooled and used in all analyses as representing the entire De Hoop population. Analyses were based on data from equal numbers of males and females for each population, chosen randomly from our data set (Table 1). For the analyses of skull morphology, data for males and females were pooled to maximize sample size. The data used in all analyses did not depart from normality.

We used regression to establish the allometric relationship between body size and peak frequency for the African clade of the Rhinolophidae, one of the two clades identified

Table 1 Morphological and echolocation call parameters (mean ± SD) for three *R. capensis* populations

Variable	De Hoop			Table Farm			Steenkampskraal		
	Male (n = 23)	Female (n = 23)	All individuals (n = 46)	Male (n = 18)	Female (n = 18)	All individuals (n = 36)	Male (n = 11)	Female (n = 11)	All individuals (n = 22)
Body mass (g)	9.71 ± 0.60 (8.6–10.5)	10.85 ± 1.16 (9.0–12.9)	10.28 ± 1.08 (8.6–12.9)	13.58 ± 0.73 (12.5–14.7)	14.18 ± 0.91 (12.6–15.8)	13.88 ± 0.87 (12.5–15.8)	12.60 ± 0.72 (11.2–13.8)	13.69 ± 0.86 (12.5–15.1)	13.15 ± 0.95 (11.26–15.1)
Forearm length (mm)	48.33 ± 0.84 (47.1–50.6)	49.29 ± 0.86 (47.9–50.9)	48.84 ± 0.97 (47.1–50.9)	49.56 ± 0.91 (47.6–50.8)	50.67 ± 1.14 (48.6–52.7)	50.11 ± 1.16 (47.6–52.7)	50.75 ± 1.10 (48.5–53.0)	51.45 ± 0.99 (50.0–53.2)	51.45 ± 0.99 (48.5–53.2)
Wingspan (cm)	30.60 ± 0.89 (29.1–32.7)	30.42 ± 1.23 (28.0–32.4)	30.52 ± 1.07 (28.0–32.7)	29.06 ± 0.95 (27.5–30.3)	30.64 ± 1.26 (28.8–34.4)	29.85 ± 1.36 (27.5–34.1)	29.63 ± 0.79 (28.5–31.1)	29.95 ± 0.81 (28.3–30.7)	29.79 ± 0.805 (28.3–31.1)
Wing area (cm^2)	154.11 ± 9.62 (137.9–181.2)	156.4 ± 11.33 (134.9–175.6)	155.26 ± 10.46 (134.9–181.2)	153.93 ± 9.67 (137.5–170.6)	167.15 ± 14.56 (142.6–212.5)	160.54 ± 13.90 (137.5–212.5)	162.37 ± 8.41 (149.8–172.7)	161.45 ± 6.58 (147.8–169.3)	161.91 ± 7.38 (147.8–172.7)
Wing loading (Nm^2)	6.32 ± 0.51 (5.6–7.2)	6.97 ± 0.89 (5.7–8.7)	6.64 ± 0.79 (5.6–8.7)	8.85 ± 0.61 (7.9–9.9)	8.52 ± 0.70 (6.7–9.4)	8.68 ± 0.67 (6.7–9.5)	7.78 ± 0.60 (7.0–9.0)	8.49 ± 0.64 (7.4–9.6)	8.14 ± 0.71 (6.9–9.6)
Noseleaf width (mm)	8.05 ± 0.38 (7.5–9.1)	7.87 ± 0.35 (7.1–8.5)	7.96 ± 0.37 (7.1–9.1)	8.50 ± 0.16 (8.1–8.8)	8.37 ± 0.23 (8.0–9.0)	8.44 ± 0.21 (8.0–8.9)	8.22 ± 0.16 (7.9–8.4)	8.22 ± 0.28 (7.8–8.7)	8.22 ± 0.23 (7.8–8.7)
Peak frequency (kHz)	84.11 ± 0.74 (82.7–85.4)	85.08 ± 0.59 (84.1–86.3)	84.60 ± 0.82 (82.7–86.3)	85.44 ± 0.65 (83.7–86.3)	86.26 ± 0.54 (85.5–87.1)	85.84 ± 0.73 (83.7–87.1)	80.47 ± 0.57 (79.3–81.4)	80.85 ± 0.35 (80.4–81.4)	80.66 ± 0.50 (79.3–81.4)
Duration (ms)	40.56 ± 7.85 (29.0–58.0)	42.39 ± 6.23 (32.0–55.0)	41.48 ± 7.07 (29.0–58.0)	43.05 ± 5.53 (33.0–57.0)	44.33 ± 6.20 (34.0–54.0)	43.69 ± 5.83 (33.0–57.0)	45.54 ± 6.12 (38.0–58.0)	45.81 ± 7.73 (35.0–56.0)	45.68 ± 6.80 (35.0–58.0)
Lowest frequency (kHz)	72.35 ± 2.27 (67.9–76.7)	72.19 ± 2.76 (67.0–76.7)	72.27 ± 2.50 (67.0–76.7)	74.59 ± 2.60 (70.4–76.8)	74.59 ± 2.62 (70.7–81.10)	73.97 ± 2.28 (70.4–81.1)	70.18 ± 2.31 (66.0–74.0)	69.45 ± 1.91 (66.0–73.0)	69.45 ± 2.10 (66.0–74.0)
Bandwidth (kHz)	11.76 ± 2.52 (7.1–15.7)	12.89 ± 2.73 (9.1–17.6)	12.32 ± 2.66 (7.1–17.6)	12.06 ± 1.97 (8.7–15.9)	11.67 ± 2.69 (4.7–15.1)	11.87 ± 2.33 (4.7–15.9)	10.29 ± 2.03 (7.4–14.4)	11.40 ± 1.74 (8.4–14.4)	10.84 ± 1.93 (7.4–14.4)

Ranges provided in parentheses

by Stoffberg et al. (2010) as comprising the global Rhinolophidae, the other clade was the Asian clade. We then plotted the mean body size and mean peak frequency for each *R. capensis* population separately on this regression to determine whether populations diverged from this allometric relationship. Data for African rhinolophids were obtained from Jacobs et al. (2007). To ensure that variances were equal and data were normally distributed, mean peak frequency and mean body mass were log transformed. Peak frequency was also regressed against body mass, noseleaf width, cochlear width and cochlear area for all individuals across populations to determine the relationship between peak frequency and these variables in *R. capensis*.

Forward stepwise discriminant function analysis (DFA) was used to determine which variables (body mass, noseleaf width, wing morphology, echolocation call parameters and skull morphology) best discriminate between individuals from the three populations in this study. We used analysis of variance (ANOVA) to determine whether peak frequency differed among populations of *R. capensis*. Multivariate analysis of variance (MANOVA) and post-hoc Tukey tests were used to determine interpopulation and sex-linked differences in those morphological and echolocation call variables which the DFA identified as contributing most to the discrimination of the populations. 'Sex' and 'population' were used as categorical predictors. A Kolmogorov–Smirnov test for normality and Levene's test for homogeneity of variances were used to ensure that data met the assumptions of DFA and ANOVA/MANOVA (Zar 1999).

Forward stepwise multiple regressions were used to determine which skull parameter measured from the radiographs of the 23 voucher specimens for which we had echolocation data was the best predictor of peak frequency. Peak frequency was the dependent variable, and those skull parameters which DFA revealed as contributing most to differences among individuals from the different populations were independent variables. All variables were log transformed to ensure linearity. To test the normality of the residuals of all regressions, residuals were plotted against their normal scores. We used Statistica (version 8.0, StatSoft Inc., Tulsa, OK, USA) for all analyses.

Results

Divergence of *R. capensis* populations

There was an inverse correlation between body mass and peak frequency ($r^2 = 0.59$; $F_{1,10} = 14.51$, $P < 0.05$) for the African clade of rhinolophids, and all three populations of *R. capensis* fell within the 95% confidence limits of the relationship for the clade (Fig. 3). The De Hoop population fell on the line of allometry, but bats from Table Farm and

Steenkampskraal deviated, albeit slightly, from this line (Fig. 3). This suggests that not all the variation in peak frequency is explained by body mass in *R. capensis*.

Variation in body mass, wing morphology and echolocation

The three populations of *R. capensis* were significantly separated by DFA on body mass, noseleaf width, wingspan and peak frequency (Wilk's Lambda = 0.020, $P < 0.001$; Fig. 4; Table 2). All individuals within each population were classified correctly (100%). These analyses were supported by multivariate ANOVA with Tukey post hoc tests on the variables extracted from the DFA ($F_{10,188} = 152.2$, $P < 0.001$). Bats from Table Farm were heavier and used significantly higher peak frequencies than both De Hoop and Steenkampskraal (Tukey test, $P < 0.005$; Table 1). Steenkampskraal bats echolocated at the lowest frequencies, and bats from De Hoop had the lowest average body mass (Tukey test, $P < 0.005$; Table 1).

Sexual differences in body size and echolocation

Peak frequency differed significantly between sexes ($F_{5,94} = 16.9$, $P < 0.005$). Females echolocated at significantly higher frequencies than males at De Hoop and Table Farm (Tukey test, $P < 0.001$) but not at Steenkampskraal (Tukey test, $P > 0.05$; Table 1). Body mass also differed significantly between sexes at De Hoop and
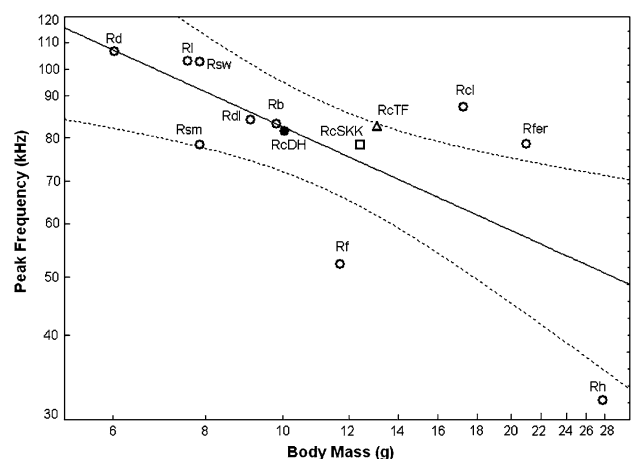
Fig. 3 The regression of log mass (g) and log peak frequency (kHz) for rhinolophids belonging to the African clade together with three populations of *R. capensis* [Steenkampskraal (RcSKK *open square*), Table Farm (RcTF *open triangle*) and De Hoop (RcDH *filled circle*)], *R. denti* (*Rd*), *R. landeri* (*Rl*), *R. swinnyi* (*Rsw*), *R. darlingi* (*Rdl*), *R. ferrumequinum* (*Rfer*), *R. blasii* (*Rb*), *R. simulator* (*Rsm*), *R. clivosus* (*Rcl*), *R. fumigatus* (*Rf*) and *R. hilderbrandti* (*Rh*). Data for South African rhinolophids were obtained from Jacobs et al. (2007). *Dashed lines* represent the 95% confidence limits. *Solid line* represents the best fit, where log peak frequency = 2.4122 – 0.4806 × log mass

Steenkampskraal where females were heavier than males (Tukey test, $P < 0.001$; Table 1). The interaction between population and sex was significant ($F_{10,188} = 3$, $P < 0.05$) suggesting that the degree of sexual dimorphism in peak frequency and body mass is significantly different between populations. We therefore compared males and females separately between populations for all of the above variables using one-way ANOVA's to control for sexual differences. Results indicated that there were still significant differences among populations (females: $F_{10,90} = 74.8$, $P < 0.01$; males: $F_{10,90} = 82.1$, $P < 0.01$).

Relationship between peak frequency, body size and the echolocation apparatus

There was no relationship between body mass and peak frequency within R. capensis ($r^2 = 0.0002$; $F_{1,102} = 0.002$, $P > 0.05$) such that the two populations in the ecotones had the highest body masses but one used the highest call
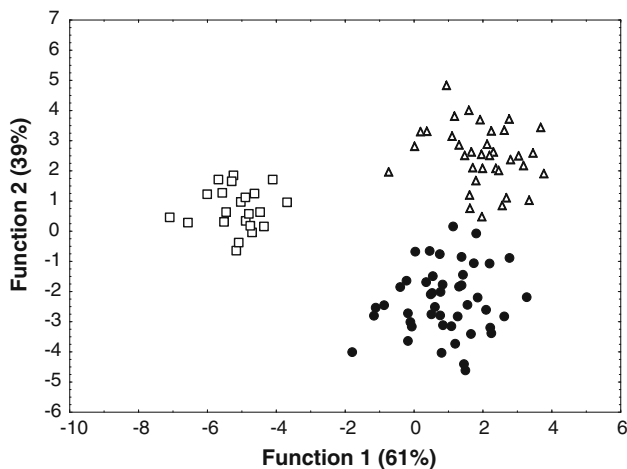

Fig. 4 Plot of canonical scores from discriminant function analysis on morphological and echolocation variables from three R. capensis populations: Steenkampskraal (*open squares*), Table Farm (*open triangles*) and De Hoop (*filled circles*)

frequency (Table Farm) while the other used the lowest (Steenkampskraal). Furthermore, although females across populations were larger, they used higher echolocation frequencies than males (Table 1) which is contrary to the inverse relationship between body size and peak frequency at the genus level.

The four populations of R. capensis for which we had skull morphology data (Table 3) were significantly separated by DFAs on greatest skull length and nasal capsule length, height and area (Wilk's Lambda = 0.1123, $P < 0.001$; Table 4). Individuals from these populations were classified correctly in 40 out of 44 cases (91%). ANOVA comparing these parameters confirmed the DFA results ($F_{21,89} = 5.44$, $P < 0.001$). We did not find significant differences among populations with respect to cochlear area (Tukey test, $P > 0.05$), and there were no significant correlations between peak frequency and cochlear width ($r^2 = 0.095$; $F_{1,21} = 2.208$, $P > 0.05$) and cochlear area ($r^2 = 0.07$; $F_{1,21} = 1.7$, $P > 0.05$) across individuals. Forward stepwise regression of peak frequency against those skull parameters identified by DFAs as being important in discriminating between populations (skull length, nasal capsule length, nasal capsule area, nasal capsule height and rostrum width) yielded a model ($r = 0.715$; $F_{4,18} = 4.7$, $P < 0.001$) of which only nasal capsule length was significant ($t = -2.16$, $P < 0.05$). Thus, nasal capsule length was the best predictor of peak echolocation frequency. In addition, nasal capsule length was not correlated with body mass across individuals ($r^2 = 0.022$; $F_{1,20} = 0.45$, $P > 0.05$). We also found no relationship between peak frequency and noseleaf width across individuals ($r^2 = 0.002$; $F_{1,102} = 0.25$, $P > 0.05$).

Discussion

Decoupling of echolocation from body size

We found no relationship between peak frequency and body mass within R. capensis. Previous within species

Table 2 Results of discriminant function analysis on morphological variables and echolocation variables		Function 1	Function 2	Wilks' Lambda	F to remove	P value
	Peak frequency	1.004	0.139	0.155	322.104	<0.001
	Body mass	−0.164	0.821	0.044	58.766	<0.001
	Noseleaf width	0.184	0.434	0.024	10.557	<0.001
	Wingspan	0.291	−0.925	0.025	11.849	<0.001
	Wing area	−0.294	0.742	0.023	7.387	<0.001
	Eigenvalue	7.506	4.78			
	Cumulative %	61	100			
	Wilks' Lambda	0.020	0.173			
	χ^2	385.62	173.68			
Only results of those parameters that contributed significantly to the model are shown	df	10	4			
	P value	<0.001	<0.001			

Table 3 Mean ± SD of skull parameters (mm) taken from radiographs of *Rhinolophus capensis*

Skull parameter	De Hoop	Steenkampskraal	Table Farm	Montague
Sagittal skull length	19.97 ± 0.49 (19.34–21.04)	20.73 ± 0.22 (20.33–20.99)	20.32 ± 0.34 (19.88–20.79)	20.07 ± 0.19 (19.78–20.43)
	n = 15	n = 10	n = 10	n = 10
Nasal capsule length (X–Y)	4.94 ± 0.24 (4.52–5.32)	5.57 ± 0.31 (5.08–6.23)	5.07 ± 0.18 (4.72–5.30)	5.29 ± 0.09 (5.11–5.42)
	n = 15	n = 10	n = 10	n = 10
Nasal capsule height (Z–W)	3.66 ± 0.09 (3.46–3.81)	3.80 ± 0.11 (3.61–3.94)	3.65 ± 0.09 (3.55–3.78)	3.49 ± 0.08 (3.46–3.71)
	n = 15	n = 10	n = 10	n = 10
Sagittal nasal capsule area (SA)	12.58 ± 0.52 (11.93–13.55)	13.93 ± 0.62 (13.04–14.88)	13.15 ± 0.45 (12.23–13.93)	12.70 ± 0.45 (12.13–13.46)
	n = 15	n = 10	n = 10	n = 10
Transverse skull length	19.92 ± 0.49 (18.92–20.82)	20.43 ± 0.20 (20.12–20.77)	20.48 ± 0.39 (20.03–21.03)	20.33 ± 0.43 (19.68–20.97)
	n = 14	n = 10	n = 10	n = 10
Nasal width across rostrum (NW)	7.37 ± 0.12 (7.11–7.52)	7.74 ± 0.14 (7.47–7.88)	7.58 ± 0.18 (7.35–7.86)	7.39 ± 0.24 (7.01–7.84)
	n = 14	n = 10	n = 10	n = 10
Transverse nasal capsule area (TA)	17.25 ± 0.63 (16.36–18.73)	18.21 ± 0.62 (17.50–19.21)	17.51 ± 0.77 (16.45–18.58)	17.51 ± 0.70 (16.19–18.71)
	n = 14	n = 10	n = 10	n = 10
Cochlear area (CA)	8.12 ± 0.27 (7.62–8.53)	8.38 ± 0.36 (7.66–8.84)	8.17 ± 0.46 (7.57–8.86)	7.99 ± 0.33 (7.25–8.30)
	n = 13	n = 10	n = 10	n = 8

Ranges are provided in parentheses. Missing values were excluded when calculating the mean

n sample size

Table 4 Results of discriminant function analysis on six skull parameters

	Function 1	Function 2	Function 3	Wilks' Lambda	F to remove	P value
Nasal capsule length	0.297	1.174	0.236	0.191	8.270	<0.001
Nasal capsule area	0.608	−1.061	−0.513	0.153	4.270	<0.05
Rostrum width	0.684	−0.197	−0.557	0.145	3.440	<0.05
Nasal capsule height	−0.790	0.485	1.439	0.168	5.842	<0.05
Greater skull length	0.644	0.037	−0.280	0.143	3.188	<0.05
Cochlear width	0.015	−0.480	0.302	0.123	1.095	>0.05
Eigenvalue	2.540	0.909	0.316			
Cumulative %	67	91	100			
Wilks' Lambda	0.112	0.397	0.759			
χ^2	83.088	35.041	10.461			
df	18	10	4			
P value	<0.0001	<0.001	<0.05			

comparisons have also found no such relationship in rhinolophid bats, but reasons for the apparent decoupling were not always clear or investigated (e.g. Jones et al. 1992; Jones and Ransome 1993). However, some studies have proposed that sonar partitioning to facilitate intraspecific communication (acoustic communication hypothesis) may explain why some species deviate from the allometric relationship between peak frequency and body size in rhinolophid bats [e.g. *R. euryale* and *R. hipposideros* (Russo et al. 2007); *R. clivosus* (Jacobs et al. 2007)]. Another recent study also found no relationship between body size and peak frequency between isolated populations of *Rhinonicteris aurantia*, but differences in skull morphological features directly involved in echolocation

production was apparent (Armstrong and Coles 2007). Thus, it was suggested that a decoupling of the echolocation apparatus from body size may have resulted in the observed peak frequency differences between populations of *R. aurantia*. However, this hypothesis can only really be confirmed in situations where both body size and peak frequency differ (Armstrong and Coles 2007), such as is the case in our study.

Skull parameters measured from radiographs of *R. capensis* individuals showed considerable differentiation (Table 3). Bats from Steenkampskraal had larger dimensions and greater areas for all skull parameters which correspond to their use of lower frequency calls (Armstrong and Coles 2007). Similarly, the higher frequencies used by

bats at De Hoop correspond to the smaller dimensions of their skull parameters (Armstrong and Coles 2007). Skulls from De Hoop and Montague were very similar in size, possibly because De Hoop is geographically much closer to Montague (Fig. 1) and they may form a single population. Nasal chamber length was the best predictor of peak frequency for *R. capensis*, and there was no relationship between this variable and body size. This, together with the absence of a relationship between peak frequency and body size, implies that selection may have acted directly on peak frequency altering skull parameters directly involved in echolocation independently of body size. These results are in agreement with previous studies which investigated the relationship between skull morphology and peak frequency in rhinolophids (Stoffberg 2007) and hipposiderids (Armstrong and Coles 2007) despite these studies not using calls and skull parameters from the same individuals as was done in our study. These results suggest that factors other than body size may be responsible for the observed intraspecific variation in peak frequency within *R. capensis*.

Despite interspecific correlations between peak frequency and other morphological characters associated with echolocation [e.g. noseleaf width (Stoffberg 2007) and cochlea (Francis and Habersetzer 1998)], no such correlations were found within *R. capensis*. Similarly, only a moderate relationship between noseleaf width and peak frequency was found in *Rhinonicteris aurantia* (Armstrong and Coles 2007), and there was considerable overlap in cochlear width despite divergence in peak frequency between populations of *H. cervinus* (Francis and Habersetzer 1998). Cochlear size also did not differ greatly between geographic isolates of *R. aurantia* (Armstrong and Coles 2007). Thus, results from this study support the view that the relationship between peak frequency and cochlear size is moderately plastic (Francis and Habersetzer 1998). The overall dimensions of cochlea may not be influenced by small changes in foveal frequency which involves fine tuning of the frequency sensitivity of the basilar membrane (Vater et al. 1985; Huffman and Henson 1993a, b).

Role of communication

Sexual dimorphism in peak frequency and body size was evident in all populations. Females were larger and echolocated at higher frequencies than males, a result which Chen et al. (2009) also found for *R. monoceros*. Previous studies have also found differences in peak frequency related to sex (Neuweiler et al. 1987; Jones et al. 1994; Russo et al. 2001, 2007; Chen et al. 2009), but differences between sexes were often higher than those reported here for *R. capensis*. For example, females echolocate ca. 4 and 3 kHz higher than males in *R. hipposideros* (Russo et al. 2007) and *H. speoris* (Jones et al. 1994), respectively.

Nonetheless, the observed sexual differences in peak frequency may have important implications for intraspecific communication. Individuals may be able to recognize the sex of echolocating conspecifics despite the partial overlap in CF frequency between sexes because the acoustic fovea of horseshoe bats allows for very fine frequency resolution. In addition, a recent study has shown that *R. euryale* and *R. mehelyi* are able to discriminate echolocation calls of their own species from those of three syntopic, congeneric species, despite considerable frequency overlap between species' (Schuchmann and Siemers 2010).

Population divergence in *R. capensis*

This study indicates that echolocation variation in *R. capensis* is not a by-product of body size variation which implies that selection may have acted on echolocation independently of body size. However, it is feasible that genetic drift acting on its own may explain the small but significant differences in peak frequency observed among the three populations of *R. capensis* because all are separated by large geographic distances. De Hoop is situated in the center of the species distribution, and it also shows the least divergence from the general allometry between body size and peak frequency for the clade. Thus, it is likely that the two ecotone populations diverged from the ancestral frequency of *R. capensis*. Preliminary genetic analyses (Rowen Van Eeden, personal communication) suggest that historic gene flow occurred from De Hoop toward the populations at Steenkampskraal and Table Farm with no return migrants. These are conditions under which genetic drift could exert substantial influence. However, there is no clear pattern between the degree of divergence and the distance between populations. De Hoop and Table Farm are furthest apart yet are most similar in echolocation frequency, and De Hoop and Steenkampskraal are closest but display greater divergence in echolocation. These patterns are in contrast to those found for *R. monoceros* where call frequency and genetic distance co-varied with geographical distance among populations (Chen et al. 2009). The patterns we report here suggest a potential role for natural selection. For example, differences in foraging habitat structure may influence call frequency variation among populations because of the proposed "adaptive complex" between wing morphology and echolocation frequency (foraging habitat hypothesis: Jones and Barlow 2004; Jacobs et al. 2007). Steenkampskraal is characterized by sparse, dwarf vegetation, and the vegetation in the other two habitats is dense and can reach heights of 2 m and more. Therefore, the lower echolocation frequencies of Steenkampskraal bats, combined with their higher wing loading, may be an adaptation for faster flight in the relatively open habitat at Steenkampskraal. However, at De Hoop, *R. capensis* foraged

close to the ground (Jacobs et al. 2007), and if *R. capensis* at Steenkampskraal does the same, then they may nevertheless experience the habitat there as cluttered. Another selective force that could cause variation in echolocation frequency is environmental humidity because high frequency calls are heavily attenuated in humid conditions (Lawrence and Simmons 1982). Populations in wet, humid environments should have lower call frequencies than populations in drier environments to maximize detection distances. It is also possible that differences in climate between sampling sites could strongly influence body size which may cause that slight correlations between body size and peak frequency are undetected. However, to adequately test the influence of climate and the relative roles of selection and drift require thorough analyses of the ecological and genetic variation within and among populations. This would require samples from more populations across the species distribution, not just representatives from the central, eastern and western limits (e.g. Chen et al. 2009).

Nonetheless, it is likely that some other factors, such as random founder effects, may have caused the initial divergence among *R. capensis* populations, which was then compounded by genetic drift and/or natural selection. Once acoustic divergence between populations occurs, the different frequencies between populations may be maintained by cultural transmission. While species-specific echolocation call structure is genetically determined, cultural transmission may also influence call frequency divergence between geographically separated populations of horseshoe bats (Russo et al. 2007) because the frequency used by juvenile rhinolophids is partly dependent on the frequency of its mother (Matsumura 1979; Jones and Ransome 1993) and therefore may have a learned component. A recent study investigated the possible role of maternal cultural transmission in maintaining the bimodal distribution of regional mean peak frequency differences within an island population of *Rhinolophus cornutus pumilus* (Yoshino et al. 2008). The acoustic difference of 5–8 kHz between the north and south regions of the island did not result in differences in habitat use or prey detection between regions, and this divergence was maintained despite sufficient nuclear gene flow. However, there was evidence for female philopatry, and divergence in peak frequency may result from random cultural drift and maintained by mother-offspring transmission because of the limited dispersal of females. Previous studies have documented the occurrence of vocal dialects or colony-specific calls in bats (e.g. Masters et al. 1995; Boughman and Wilkinson 1998; Esser and Schubert 1998). Thus, it is possible that the observed peak frequency differences among populations of *R. capensis* may be maintained through cultural transmission of local acoustic signatures. Demonstration

of this would require an investigation of gene flow and female philopatry.

Appendix

Museum codes for *Rhinolophus capensis* skulls used in this study:

Northern Flagship Institute: TM 27066, TM 29081, TM 29064, TM 29067, TM 29080, TM 29070, TM 29077, TM 29065, TM 29063.

Iziko Museum: ZM 14560H, ZM 14559, ZM 14560I, ZM 14561, ZM 35665, ZM 14560E, ZM 14560C, ZM 14560F, ZM 14560B, ZM 14560, ZM 14560G.

References

Anthony EL (1988) Age determination in bats. In: Kunz TH (ed) Ecological and behavioural methods for the study of bats. Smithsonian Institute Press, Washington, DC, pp 47–58

Armstrong KN, Coles RB (2007) Echolocation call frequency differences between geographic isolates of *Rhinonicteris aurantia* (Chiroptera: Hipposideridae): implications of nasal chamber size. J Mammal 88:94–104

Barclay RMR, Fullard JH, Jacobs DS (1999) Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Can J Zool 77:530–534

Boughman JW, Wilkinson GS (1998) Greater spear-nosed bats discriminate group mates by vocalizations. Anim Behav 55:1717–1732

Chen S-F, Jones G, Rossiter SJ (2009) Determinants of echolocation call frequency variation in the Formosan lesser horseshoe bat (*Rhinolophus monoceros*). Proc Biol Sci 276:3901–3909

Cowling RM, Esler KJ, Rundel PW (1999) Namaqualand, South Africa—overview of a unique winter rainfall desert ecosystem. Plant Ecol 142:3–21

Dold T (2003) The rediscovery of *Ornithogalum britteniae* (Hyacinthaceae) and an amendment to the description. S Afr J Bot 69:500–504

Esser K-H, Schubert J (1998) Vocal dialects in the lesser spear nosed bat, *Phyllostomus discolour*. Naturwissenschaften 85:347–349

Fenton MB (1985) Communication in the Chiroptera. Indiana University Press, Bloomington

Francis CM, Habersetzer J (1998) Interspecific and intraspecific variation in echolocation call frequency and morphology of horseshoe bats, *Rhinolophus* and *Hipposideros*. In: Kunz TH, Racey PA (eds) Bat biology and conservation. Smithsonian Institute Press, Washington, DC, pp 169–179

Gillam EH, McCracken GF (2007) Variability in echolocation of *Tadarida brasiliensis*: effects of geography and local acoustic environment. Anim Behav 74:277–286

Grilliot ME, Burnett SC, Mendonca MT (2009) Sexual dimorphism in big brown bat (*Eptesicus fuscus*) ultrasonic vocalizations is context dependent. J Mammal 90:203–209

Guillén A, Juste BJ, Ibáñez C (2000) Variation in the frequency of echolocation calls of *Hipposideros ruber* in the Gulf of Guinea: an exploration of the adaptive meaning of the constant frequency value in Rhinolophoid CF bats. J Evol Biol 13:70–80

Hartley DJ, Suthers RA (1988) The acoustics of the vocal tract in the horseshoe bat *Rhinolophus hildebrandti*. J Acoust Soc Am 84:1201–1213

Huffman RF, Henson OW Jr (1993a) Labile cochlear tuning in the mustached bat. I. Concomitant shifts in biosonar emission frequency. J Comp Physiol A 171:725–734

Huffman RF, Henson OW Jr (1993b) Labile cochlear tuning in the mustached bat. I. Concomitant shifts in neural tuning. J Comp Physiol A 171:735–748

Jacobs DS, Barclay RMR, Walker MH (2007) The allometry of echolocation call frequencies of insectivorous bats: why do some species deviate from the pattern? Oecologia 152:583–594

Jones G (1996) Does echolocation constrain the evolution of body size in bats? Symp Zool Soc Lond 69:111–128

Jones G (2008) Sensory ecology: echolocation calls are used for communication. Curr Biol 18(1):R34–R35

Jones G, Barlow KE (2004) Cryptic species of echolocating bats. In: Thomas JA, Moss CF, Vater M (eds) Echolocation in bats and dolphins. The University of Chicago Press, Chicago, pp 345–349

Jones G, Ransome RD (1993) Echolocation calls of bats are influenced by maternal effects and change over a lifetime. Proc Biol Sci 252:125–128

Jones G, Gordon T, Nightingale J (1992) Sex and age differences in the echolocation calls of the lesser horseshoe bat, *Rhinolophus hipposideros*. Mammalia 56:189–193

Jones G, Sripathi K, Waters DA, Marimuthu G (1994) Individual variation in the echolocation calls of three sympatric Indian hipposiderid bats, and an experimental attempt to jam bat echolocation. Folia Zool 43:347–362

Kark S, Mukerji T, Safriel UN, Noy-Meir I, Nissani R, Darvasi A (2002) Peak morphological diversity in an ecotone unveiled in the chukar partridge by a novel Estimator in a Dependent Sample (EDS). J Anim Ecol 71:1015–1029

Kazial KA, Masters WM (2004) Female big brown bats, *Eptesicus fuscus*, recognise sex from a caller's echolocation signals. Anim Behav 67:855–863

Kazial KA, Pacheco S, Zielinski KN (2008) Information content of sonar calls of little brown bats (*Myotis lucifugus*): potential for communication. J Mammal 89:25–33

Kingston T, Rossiter SJ (2004) Harmonic-hopping in Wallacea's bats. Nature 429:654–657

Lawrence BD, Simmons JA (1982) Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. J Acoust Soc Am 71:585–590

Ma J, Kobayasi K, Zhang S, Metzner W (2006) Vocal communication in adult greater horseshoe bats, *Rhinolophus ferrumequinum*. J Comp Physiol A 192:535–550

Masters WM, Raver KA, Kazial KA (1995) Sonar signals of the big brown bat, *Eptesicus fuscus*, contain information about individual identity, age and family affiliation. Anim Behav 50:1243–1260

Matsumura S (1979) Mother-infant communication in the horseshoe bat (*Rhinolophus ferrumequinum nippon*): development of vocalization. J Mammal 60:76–84

Mucina L, Rutherford MC (2006) The vegetation of South Africa, Lesotho and Swaziland. National Biodiversity Institute, Pretoria

Neuweiler G (1984) Foraging, echolocation and audition in bats. Naturwissenschaften 71:446–455

Neuweiler G, Metzner W, Heilmann U, Rübsamen R, Eckrich M, Costa HH (1987) Foraging behaviour and echolocation in the rufous horseshoe bat (*Rhinolophus rouxi*) of Sri Lanka. Behav Ecol Sociobiol 20:53–67

Norberg UM, Rayner JMV (1987) Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Phil Trans R Soc Lond B 316:335–427

Robinson MF (1996) A relationship between echolocation calls and noseleaf widths in bats of the genera *Rhinolophus* and *Hipposideros*. J Zool Lond 239:389–393

Russo D, Jones G, Mucedda M (2001) Influence of age, sex and body size on echolocation calls of Mediterranean and Mehely's horseshoe bats, *Rhinolophus euryale* and *R. mehelyi* (Chiroptera: Rhinolophidae). Mammalia 65:429–436

Russo D, Mucedda M, Bello M, Biscardi S, Pidinchedda E, Jones G (2007) Divergent echolocation call frequencies in insular rhinolophids (Chiroptera): a case of character displacement? J Biogeogr 34:2129–2138

Schuchmann M, Siemers BM (2010) Behavioral evidence for community-wide species discrimination from echolocation calls in bats. Am Nat 176:72–82

Schuller G, Pollak G (1979) Disproportionate frequency representation in the inferior colliculus of doppler-compensating greater horseshoe bats: evidence for an acoustic fovea. J Comp Physiol A 132:47–54

Shieh B-S (2004) Song structure and microgeographic variation in a population of the grey-cheeked fulvetta (*Alcippe morrisonia*) at Shoushan Nature Park, southern Taiwan. Zool Stud 43:132–141

Siemers BM, Beedholm K, Dietz C, Dietz I, Ivanova T (2005) Is species identity, sex, age or individual quality conveyed by echolocation call frequency in European horseshoe bats? Acta Chiropterol 7:259–274

Simmons JA, Stein RA (1980) Acoustic imaging in bat sonar: echolocation signals and the evolution of echolocation. J Comp Physiol A 135:61–84

Stoffberg S (2007) Molecular phylogenetics and the evolution of high frequency echolocation in horseshoe bats (Genus *Rhinolophus*). Dissertation, University of Cape Town, Cape Town

Stoffberg S, Jacobs DS, Mackie IJ, Matthee CA (2010) Molecular phylogenetics and historical biogeography of *Rhinolophus* bats. Mol Phylogenet Evol 54:1–9

Stone AW, Weaver AB, West WO (1998) Climate and weather. In: Lubke RA, de Moor I (eds) Field guide to the Eastern and Southern Cape Coasts. University of Cape Town Press, Cape Town, pp 41–49

Suthers RA, Hartley DJ, Wenstrup JJ (1988) The acoustic role of tracheal chambers and nasal cavities in the production of sonar pulses by the horseshoe bat, *Rhinolophus hildebrandti*. J Acoust Soc Am 162:799–813

Taylor PJ (2000) Bats of Southern Africa. University of Natal Press, Pietermaritzburg

Thabah A, Rossiter SJ, Kingston T, Zhang S, Parsons S, Mya KM, Zubaid A, Jones G (2006) Genetic divergence and echolocation call frequency in cryptic species of *Hipposideros larvatus s.l.*

(Chiroptera: Hipposideridae) from the Indo-Malayan region. Biol J Linn Soc 88:119–130

Vater M, Feng AS, Betz M (1985) An HRP-study of the frequency-place map of the horseshoe bat cochlea: morphological correlates of the sharp tuning to a narrow frequency band. J Comp Physiol A 157:671–686

Wilczynski W, Ryan MJ (1999) Geographic variation in animal communication systems. In: Foster SA, Endler JA (eds) Geographic variation in behaviour: perspectives on evolutionary mechanisms. Oxford University Press, Oxford, pp 234–261

Yoshino H, Armstrong KN, Izawa M, Yokoyama J, Kawata M (2008) Genetic and acoustic population structuring in the Okinawa least horseshoe bat: are intercolony acoustic differences maintained by vertical maternal transmission? Mol Ecol 17:4978–4991

Yovel Y, Melcon ML, Franz MO, Denzinger A, Schnitzler H-U (2009) The voice of bats: how greater mouse-eared bats recognize individuals based on their echolocation calls. PLoS Comput Biol 5:e1000400. doi:10.1371/journal.pcbi.1000400

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, New Jersey

Zhuang Q, Müller R (2007) Numerical study of the effect of the noseleaf on biosonar beamforming in a horseshoe bat. Phys Rev E 76:051902. doi:10.1103/PhysRevE.75.051902