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Chapter 6
Convergence as an Evolutionary Trade-off in the Evolution of Acoustic Signals: Echolocation in Horseshoe Bats as a Case Study

David S. Jacobs, Gregory L. Mutumi, Tinyiko Maluleke and Paul W. Webala

Abstract The evolution of novel acoustic signals that are optimal for a particular function or habitat may restrict distinct lineages to the same ecological niche resulting in convergence of phenotypic traits. Such convergence could represent an evolutionary trade-off. The evolution of flutter detection may have restricted horseshoe bats (*Rhinolophus*) to similar foraging modes resulting in the convergence of phenotypic traits across different lineages. We investigated convergence in African rhinolophids using several phenotypic features. There was pronounced convergence between distantly related lineages including *R. damarensis* and *R. darlingi*, and between *R. simulator* and *R. blasii*. However, phenotypic divergence, notably in body size and resting frequency, was also evident amongst close relatives of *R. damarensis* and *R. darlingi*. These relatives diverged from both the ancestral character state and *R. damarensis* and *R. darlingi*. Such divergence suggests that an evolutionary trade-off associated with flutter detection is probably not the cause of convergence in these bats.

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6.1 Introduction

Convergent evolution, or simply convergence, is the independent evolution of similar phenotypes amongst genetically distinct lineages (Losos 2011; Jacobs et al. 2013). When it involves fitness-enhancing traits, convergence is often attributed to adaptation to similar ecological niches (Colborn et al. 2001; Losos 2011) and is often used as evidence for natural selection. Some classical examples include the evolution of wings in pterosaurs, bats and birds and convergent evolution of mammals and marsupials (Futuyma 1998; Ridley 1996). However, besides natural selection, other processes such as random genetic drift or biological constraints can also lead to convergence (Stayton 2008). Thus, it has been recommended that pattern be separated from process when investigating convergence (Stayton 2015), allowing one to recognize convergence independently of the process that caused it.

Investigations of convergence have to be done within a phylogenetic framework for two reasons: firstly to ensure that lineages being investigated are in fact distinct in accordance with the definition of convergence and, secondly, to determine the character state of the putative ancestor of the lineages being compared. The ancestral character state is essential in determining the extent to which lineages have diverged from the common ancestor which allows the determination of whether convergence is the result of constraints or selection and/or drift.

Shared biological constraints can cause similarity in the phenotypes of distinct lineages even in the absence of selection or drift (Losos 2011) because of stasis, i.e. the lineages have diverged very little from the common ancestor. Such stasis can result from several factors. For example, the lineages have too little variation to respond to selection pressures, or because they have not experienced any selection pressure or selection for a particular trait prevents them from responding to other selection pressures, e.g. evolutionary trade-offs (Roff and Fairbairn 2007).

Evolutionary trade-offs occur when selection on one trait is opposed by the loss of fitness as a result of a concomitant change in another (Futuyma 1998; Resnick et al. 2000; Roff 2000; Roff and Fairbairn 2007). Such trade-offs may result in neither trait reaching its adaptive optimum. For example, functional trade-offs in the skulls of birds and bats between feeding and signal production has resulted in reduced song producing abilities in birds and reduced masticatory power in bats (Ballantine 2006; Jacobs et al. 2014). If such evolutionary trade-offs are shared amongst lineages, especially as a result of sharing a fitness-enhancing innovation, their phenotypes may converge as a result of the innovation preventing responses to other selection pressures. Sensory traits such as bird and frog song and echolocation may be particular susceptible to such trade-offs because slight deviations may render them ineffective. This could result in a high level of convergence across different lineages in these traits (e.g. Jacobs et al. 2013).

Bat echolocation is a unique sonar system used for orientation, foraging and communication (Thomas et al. 2004; Jones and Siemers 2010). Bat echolocation can be divided into two broad categories: low-duty-cycle (LDC) echolocation in which the period (time elapsed between the starting points of successive calls) is long relative to the duration of the calls and high-duty-cycle (HDC) echolocation in which the period is short relative to the duration of the calls. Duty cycle is expressed as a percentage and is the ratio of the duration of the call to the period (Fenton 1999).

HDC echolocation is an evolutionary innovation that occurs in three Old World families of bats, the Hipposideridae, the Rhinonycteridae and the Rhinolophidae and one American species *Pteronotus parnellii* (Mormoopidae). HDC allows these bats to overcome the masking effects of clutter (echoes from non-target objects), e.g. background vegetation that interferes with echoes from the target. HDC bats have a uniquely specialized system in which they couple an acoustic fovea with Doppler shift compensation (DSC). The acoustic fovea is a region of the auditory cortex that is sensitive to a very narrow range of frequencies. The frequency of the echolocation calls whilst the bat is at rest, called the resting frequency (RF), falls within the narrow range of frequency of the acoustic fovea. However, the returning echo for a bat in flight returns to the bat's ears at a higher frequency as a result of the Doppler shift of the returning echo due to the bats forward motion. This means that the returning echo would fall outside of the range of the fovea. To compensate for this during flight, hence DSC, the bat emits its calls at a lower frequency than its resting frequency so that the frequency of the returning echo falls within the range of the acoustic fovea (Schnitzler and Flieger 1983; Neuweiler 1984; Schnitzler and Denzinger 2011).

This echolocation system overcomes the masking effects of clutter through the generation of acoustic glints (Neuweiler 1984; Schnitzler and Kalko 2001). HDC bats emit calls of long duration (>30 ms) dominated by a constant-frequency (CF) component with short frequency-modulated (FM) components at the beginning and end of the CF component (Schnitzler and Flieger 1983; Neuweiler 1984). These bats use the long-duration CF components in combination with DSC to generate constant echoes from the background. The bats do so by adjusting the frequency of their emitted call to compensate for the changes in frequency resulting from the Doppler shift in frequency caused by its flight speed relative to the stationary background. The bat is then able to detect flying targets by the acoustic glints superimposed on this constant background echo. Acoustic glints are changes in frequency and amplitude generated when the bat's call is reflected off the fluttering wings of flying insects during the insect's wing beat cycle (Schnitzler and Flieger 1983; Neuweiler 1984). When the long CF signal impinges upon a wing, the amplitude and frequency of the echo are dependent on the position of the wing and whether the wing is moving towards the bat or away from it. The amplitude of an echo is dependent on the size of the object generating it. When the insect wing is perpendicular to the impinging echolocation call at the top or bottom of its wing beat cycle, all of its surface area reflects the call, producing an echo of high amplitude. When the wing is parallel to the bat, only its edge reflects its call and a weak echo is generated. Similarly when the wing is moving down and towards the bat during the first part of the power stroke or up and towards the bat during the recovery stroke, the frequency of the impinging echolocation call is Doppler shifted to a higher frequency. In the same way, when the wing is moving away from the impinging call towards the top or bottom of its beat cycle, the frequency of the echo is Doppler shifted lower than that of the bat's signal. These changes in the amplitude and frequencies of echoes from insect wings are perceived by the bat as amplitude and frequency glints against the constant echo from the background clutter (Schnitzler and Flieger 1983; Neuweiler 1984), and this allows it to detect flapping insect wings against background clutter.

The specialized echolocation system of HDC bats restricts them to foraging in dense vegetation where distances to background vegetation are short and atmospheric attenuation (Lawrence and Simmons 1982) is reduced allowing detectable acoustic glints. Flying in dense vegetation means that these bats have to fly slowly and manoeuvrably which requires short broad wings that generate lift at low flight speeds (e.g. Figure 6.1; Norberg and Rayner 1987). The wings and echolocation system of bats therefore form an adaptive complex (Aldridge and Rautenbach 1987; Norberg and Rayner 1987) which is constrained by the acoustic and aerodynamic requirements of their specialized ecological niche. This could result in particularly pronounced convergent morphology and echolocation in the family Rhinolophidae.

Morphology and echolocation in rhinolophids are indeed highly convergent throughout their Old World distribution. Despite differences in size, wing shapes are remarkably similar across rhinolophid species (Fig. 6.1). This similarity in morphology has resulted in the 78 or so recognized species being placed in a single genus (*Rhinolophus*) (Csorba et al. 2003). The genus consists of two major phylogenetic clades: an Afro-Palaearctic clade and an Asian clade (Stoffberg et al. 2011; Dool et al. 2016, Foley et al. 2015). Furthermore, these bats are known to use their habitats in the same way and are likely, therefore, to encounter similar prey. This may result in the convergence of skull morphology as well. However, it is evident that, although there might be evolutionary trade-offs that constrain some species causing convergence, given the wide range in body size and call parameters, these organisms are obviously responding to other selection pressures as well. How organisms respond to different selection pressures and the trade-offs involved as a result of responding to a suit of selection pressure is an interesting area of enquiry. African rhinolophids currently comprise 27 species (Happold and Cotterill 2013) with many of them having wide geographic distributions (Monadjem et al. 2010). Rhinolophids are thus ideal for investigating the role of evolutionary constraints on convergence.

Evidence for evolutionary constraints as the cause of convergence has to account for the role of shared ancestry as the cause of phenotypic similarity. We therefore investigated the role of evolutionary constraints in the evolution of phenotypic convergence in the Southern African rhinolophids in a phylogenetic context. We used the recent phylogeny reported in (Dool et al. 2016) to do so. If convergence stems from stasis as a result of evolutionary constraints, then the phenotypes of most or all of these species should converge on each other and the ancestral phenotype. Furthermore, there should be more extant species that echolocate at or above the ancestral frequency than below because higher frequencies facilitate DSC (Waters 2003).

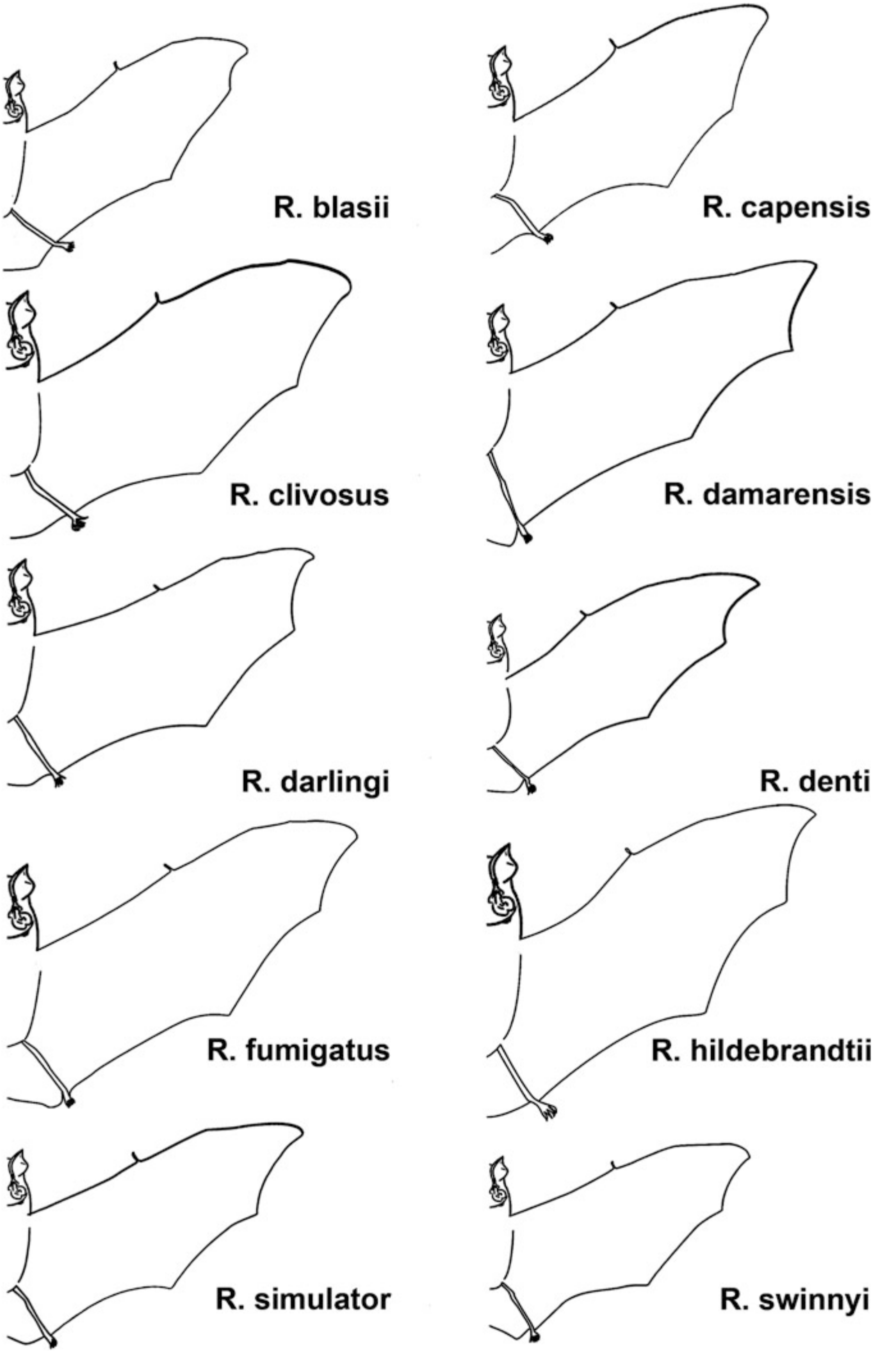

Fig. 6.1 Outline of the wings of several Southern African rhinolophids

6.2 Methods

6.2.1 *Taxonomic Notes*

We investigated phenotypic convergence in 12 species of African Rhinolophidae (Table 6.1). Lineages within the family Rhinolophidae are poorly resolved and are likely to contain several cryptic species (Dool et al. 2016). Although this may be true for several groups in this family, it is particularly evident in the *fumigatus* group which includes *Rhinolophus fumigatus*, *R. damarensis*, *R. darlingi*, *R. eloquens* and *R. hildebrandtii* (Csorba et al. 2003; Jacobs et al. 2013; Dool et al. 2016). In this study, we used the species designations of Dool et al. (2016). *R. fumigatus* from the western part of Southern Africa appears to be a distinct but sister lineage to *R. fumigatus* from the eastern half of Africa (Dool et al. 2016). We thus treat *R. fumigatus* from the eastern part of the continent as *R. fumigatus* sensu stricto since the type specimen for this species comes from Ethiopia in East Africa. We refer to the lineage from the west as *R. cf. fumigatus*. We place all *R. hildebrandtii* with resting call frequencies between 37 and 39 kHz in *R. cf. mossambicus* (Dool et al. 2016). We treated all other rhinolophids with resting frequencies of between 42 and 46 kHz as belonging to *R. hildebrandtii* for the following reasons: (1) there is currently no genetic data that allow us to place them in the species designations erected by Taylor et al. (2012), (2) the range in resting frequency is small and (3) we found similar ranges within the same roost, e.g. 42–44 at Mushandike (20°7′ S, 30°35′ E), Zimbabwe, and 44–46 kHz at big Baobab tree (22°30′ S, 30°37′ E), South Africa (Table 6.1). Furthermore, we used the designation of *R. cf. simulator* for a lineage that displayed genetic similarity to *R. simulator* (Dool et al. 2016) but echolocated at a much higher frequency (Table 6.1).

6.2.2 *Sampling*

Bats were caught from caves and disused mine shafts across their distributional ranges in Southern, Eastern and Central Africa using hand nets and continuously monitored harp traps and mist-nets. Captured bats were held individually in soft cotton bags. Sex and reproductive status were checked immediately following capture, and juveniles, pregnant or lactating bats were released immediately. Reproductive status was determined by examination of the nipples and palpation of the abdomen of female bats (Racey 1988). Juveniles were distinguished from adults by the presence of cartilaginous epiphyseal plates in their finger bones detected by trans-illuminating the bat's wings (Anthony 1988).

The forearm length (FA) to the nearest 0.1 mm and body mass to the nearest 0.1 g was measured using dial callipers and a portable electronic balance, respectively. We also measured the upper tooth-row length (CM^3), head height (HH), head width (HW), head length (HL), and nose-leaf width (NLW) to the nearest 0.1 mm using a dial callipers (Table 6.2).

Table 6.1 Means ± SD and ranges (in parentheses below the means) of phenotypic parameters of Southern African rhinolophids. Sample sizes are given below species names

Species	Mass (g)	Forearm (mm)	CM^3 (mm)	HH (mm)	HW (mm)	HL (mm)	NLW (mm)	RF (kHz)
Rbl (15)	9.3 ± 0.5 (8.4–10.5)	45.4 ± 0.7 (43.7–46.5)	6.6 ± 0.4 (6.0–7.0)	6.7 ± 0.2 (6.1–7.0)	9.3 ± 0.5 (8.4–10.1)	18.1 ± 1.3 (16.0–19.7)	8.2 ± 0.7 (7.1–9.3)	90.9 ± 1.1 (87.6–91.8)
Rca (62)	12.0 ± 1.1 (9.1–14.6)	50.4 ± 1.3 (47.4–53.5)	7.8 ± 0.7 (6.5–9.8)	9.2 ± 1.4 (6.1–12.7)	10.0 ± 1.2 (7.5–13.6)	19.8 ± 1.6 (17.2–22.1)	±8.50.5 (7.4–9.3)	81.8 ± 3.7 (75.0–86.8)
Rcl (54)	18.0 ± 2.0 (14.1–21.7)	54.8 ± 1.8 (52.3–59.4)	8.3 ± 0.7 (6.7–10.2)	9.8 ± 1.4 (7.6–13.8)	10.5 ± 1.3 (8.1–13.8)	21.8 ± 1.3 (19.8–24.1)	7.6 ± 0.5 (6.3–8.5)	91.6 ± 1.0 (88.2–93.5)
Rda (19)	10.7 ± 0.6 (9.3–11.5)	49.5 ± 2.0 (47.3–51.3)	7.2 ± 0.5 (6.2–8.2)	7.5 ± 0.4 (6.6–8.1)	9.1 ± 0.6 (7.5–9.8)	19.0 ± 1.3 (17.1–20.7)	8.1 ± 0.4 (7.2–8.8)	86.7 ± 1.4 (84.7–89.1)
Rdr (28)	8.9 ± 1.6 (6.2–13.8)	46.5 ± 1.3 (44.0–49.3)	7.2 ± 0.7 (5.8–8.2)	7.8 ± 0.7 (6.6–9.8)	9.7 ± 0.3 (9.1–10.0)	19.7 ± 1.4 (17.1–21.9)	8.4 ± 0.4 (7.7–8.9)	81.3 ± 2.5 (78.4–84.5)
Rde (20)	6.8 ± 1.2 (5.7–8.8)	42.7 ± 0.9 (41.5–44.3)	6.1 ± 0.4 (5.2–6.5)	6.0 ± 0.7 (5.0–7.2)	7.8 ± 0.7 (6.7–8.9)	15.7 ± 1.2 (13.5–17.5)	6.7 ± 0.4 (6.0–7.3)	111.8 ± 2.7 (107.5–115.6)
Rel (20)	19.8 ± 1.0 (18.0–21.5)	58.7 ± 1.9 (56.0–63.2)	–	–	–	–	12.5 ± 0.5 (12.0–13.0)	45.1 ± 0.6 (43.9–45.7)
Rfu (32)	13.1 ± 1.3 (10.9–16.8)	52.0 ± 1.4 (48.5–54.5)	8.2 ± 0.6 (7.1–8.9)	9.0 ± 0.6 (8.2–10.0)	11.1 ± 0.9 (9.9–12.6)	23.4 ± 2.1 (18.9–27.9)	10.6 ± 0.9 (8.1–12.3)	55.6 ± 1.4 (53.3–57.3)
R. cf. fu (38)	18.8 ± 1.4 (16.2–22.4)	58.6 ± 1.7 (55.1–62.0)	–	–	–	–	–	55.0 ± 0.9 (53.0–56.5)
Rhi (30)	28.7 ± 2.8 (25.0–33.5)	63.8 ± 1.6 (62.1–65.9)	9.5 ± 1.0 (8.3–11.9)	9.5 ± 1.0 (10.0–11.9)	11.3 ± 1.0 (11.1–16.2)	27.4 ± 1.4 (23.9–28.9)	12.3 ± 0.7 (11.2–13.9)	44.8 ± 1.4 (43.3–47.1)
Rmo (22)	27.7 ± 5.1 (23.6–38.8)	62.6 ± 1.5 (60.0–66.9)	10.2 ± 0.7 (8.6–11.3)	12.3 ± 0.9 (11.0–14.9)	13.3 ± 1.2 (11.1–14.6)	26.4 ± 1.4 (25.2–29.1)	13.4 ± 0.6 (12.3–14.4)	38.8 ± 0.7 (36.8–39.7)
Rsi (20)	7.3 ± 1.0 (6.2–10.3)	44.7 ± 1.3 (43.4–47.1)	6.7 ± 0.6 (6.1–7.9)	7.0 ± 0.4 (5.8–8.2)	9.1 ± 0.6 (7.8–9.9)	18.3 ± 1.4 (17.1–21.0)	7.9 ± 0.4 (7.3–8.8)	81.8 ± 2.3 (77.4–84.9)

(continued)

Table 6.1 (continued)

Species	Mass (g)	Forearm (mm)	CM^3 (mm)	HH (mm)	HW (mm)	HL (mm)	NLW (mm)	RF (kHz)
R. cf. si (24)	5.8 ± 0.7 (5.0–7.1)	41.5 ± 1.4 (39.1–44.3)	6.5 ± 0.4 (5.7–7.5)	6.6 ± 0.3 (6.0–7.3)	8.6 ± 0.3 (8.3–9.4)	17.4 ± 1.0 (15.6–19.1)	7.4 ± 0.4 (6.7–7.9)	103.5 ± 1.6 (100.5–106.5)
Rsw (22)	± 7.60.7 (7.0–9.8)	± 43.70.9 (42.5–44.8)	–	–	–	–	–	107.20.6 (106.5–108.1)

Abbreviations: *Rbl* = *R. blasii*; *Rca* = *R. capensis*, *Rcl* = *R. clivosus*; *Rda* = *R. damarensis*, Rdr = *R. darlingi*; *Rde* = *R. denti*; *Rel* = *R. eloquens*; *Rfu* = *R. fumigatus*; *R. cf. fu* = R. cf. fumigatus; *Rhi* = *R. hildebrandtii*; *Rmo* = *R. mossambicus*; *Rsi* = *R. simulator*, *R. cf. si* = *R. cf. simulator*; *Rsw* = *R. swinnyi*; CM^3 = length of the upper tooth row; HH = head height, HW = head width; HL = head length, NLW = nose-leaf width; RF = resting frequency

Table 6.2 Phenotypic parameters measured from live bats in the field

Abbreviation	Name	Description
NLW	Nose-leaf width	The broadest distance across the two leafs measured in millimetres
CM^3	Upper tooth-row length	Upper tooth-row length (measured in millimetres) from the end of the last molar to the front end of the incisor
HH	Head height	Head height measured (in millimetres) from beneath the jaw to the topmost tip of the head
HW	Head width	Head width measured (in millimetres) from behind the two ears
HL	Head length	Condylobasal length (measured in millimetres) from the nose tip to behind the nap

Echolocation calls from hand-held bats were recorded and analysed as in Mutumi et al. (2016). Hand-held calls allow the determination of resting frequency (RF; frequency of maximal energy when at rest) in rhinolophid bats (Siemers et al. 2005) and eliminate variations in frequency as a result of rhinolophid bats compensating for Doppler shifts during flight (Schnitzler 1987).

In addition to the RFs reported here, we also obtained the RFs from other species from Csorba et al. (2003) and Zhou et al. (2009) to compare the number of extant species with RFs equal to, above or below that of the reconstructed ancestral frequency (Dool et al. 2016).

6.2.3 *Statistical Analyses*

As far as possible, we kept sexes equal to account for potential sexual dimorphism. We conducted a principal component analysis (PCA) on 8 phenotypic variables (Table 6.1) to extract 8 independent and uncorrelated principal components from the original set of variables to meet the assumptions of discriminant function analysis (DFA). DFA was done on the factor scores of the first two principal components to examine instances of multivariate phenotypic convergence within African rhinolophids. Prior to PCA, variables were $\log_{10}$ transformed. All statistical analyses were done in Dell Statistica (version 13, Southern African Analytics Pty Ltd.).

6.3 Results

The first two principal components (PC) recovered from the PCA explained 87.4 % of the variation (PC 1—79.5 %; PC2—6.4 %). The two roots extracted by DFA on these two principal components explained 100 % of the variation (Table 6.3) PC 1

Table 6.3 Results of discriminant function analysis on principal component scores extracted by principal component analyses on 8 phenotypic variables (Table 6.1)

	Root 1	Root 2	Wilks' λ	$F_{(10,\ 305)}$	P
PC 1	−5.63	−0.21	0.15	968.0	<0.0001
PC2	0.47	−2.52	0.03	176.98	<0.0001
Eigenvalue	31.92	5.62			
Cumulative (%)	85.03	100			
Wilks' λ	0.004	0.15			
χ	1666.40	584.97			
df	20	9			
P	<0.0001	<0.0001			

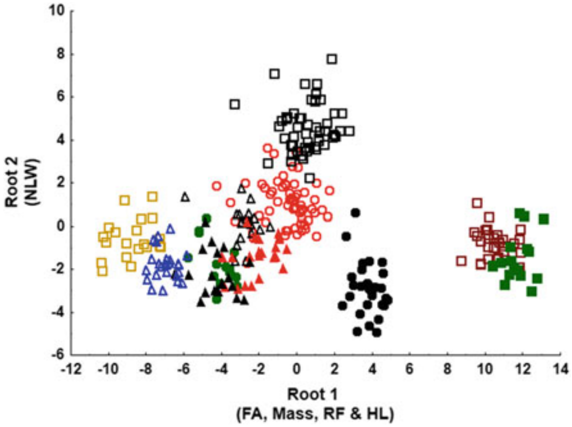

Fig. 6.2 Plot of canonical scores obtained from discriminant function analyses on phenotypic parameters (Table 6.1) for several Southern African rhinolophid species. Some of the phenoptypes with heaviest loadings are given below each root (abbreviations are given in Table 6.1). Key to species: *R. blasii—solid green circles*; *R. capensis*—open *red circles*; *R. clivosus*—open *black squares*; *R. damarensis*—open *black triangles*; *R. darlingi*—solid *red triangles*; *R. denti*—open *gold triangles*;; *R. fumigatus* (east)—solid *black circles*; *R. hildebrandtii*—open *brown squares*; *R. mossambicus*—solid *green squares*; *R. simulator—solid black triangles*; *R. cf. simulator*—open *blue triangles*

(associated mainly with FA, RF, mass and head length) loaded the highest on Root 1 and PC 2 (associated with NLW) loaded the highest on Root 2 (Table 6.3; Fig. 6.2).

All species were tightly clustered together with the exception of the three largest species using the lowest call frequencies, *R. fumigatus*, *R. hildebrandtii* and *R. mossambicus* (Fig. 6.2; Table 6.1). These species loaded highest on Root 1 whilst the rest loaded lowest on Root 1 (Fig. 6.2). However, the squared Mahalanobis distances between all species were significant ($F_{(2,\ 305)}$s > 14, Ps < 0.00001) with the exception of the mahalonobis distances between *R. blasii* and *R. simulator*

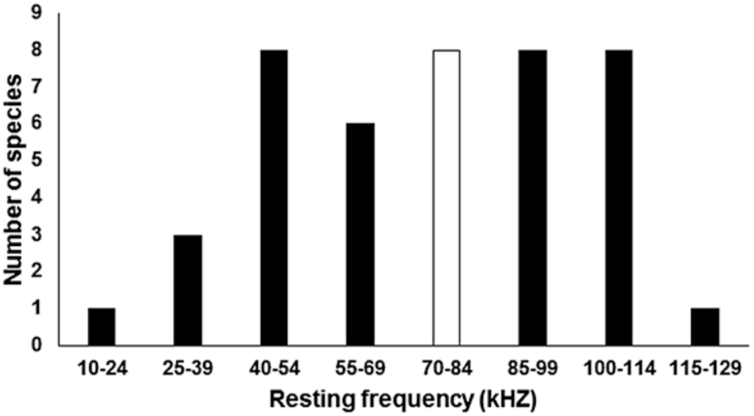

Fig. 6.3 Distribution of resting frequencies of extant rhinolophids (*black bars*) in relation to the ancestral frequency band. The *white bar* represents the ancestral frequency band and the extant bats that have resting frequencies within it

($F_{(2,\ 305)}$ = 1.14, P = 0.32; Fig. 6.2). Although the mahalonobis distance between *R. damarensis* and *R. darlingi* (mahalonobis distance = 0.27; $F_{(2,\ 305)}$ = 14.50, P < 0.00001; Fig. 6.2) and between *R. hildebrandtii* and *R. mossambicus* (mahalanobis distance = 1.92; $F_{(2,\ 305)}$ = 10.65, P < 0.0001; Fig. 6.2) were significant these two pairs of species had the lowest significant mahalanobis distances. These results reflect why *R. hildebrandtii* and *R. mossambicus* were formerly placed in a single species (Taylor et al. 2012). Lastly, there was complete overlap in the RFs of *R. fumigatus* and *R. cf. fumigatus* but almost no overlap in their masses and forearm lengths (Table 6.1).

There was little difference in the number of extant rhinolophid species that echolocated above or below the ancestral frequency band (Fig. 6.3). Similarly, the number of extant species echolocating within or above the ancestral resting frequency was slightly higher (24 vs. 17 species), but not significantly so (χ^2 = 1.19; df = 1, P > 0.1), than those echolocating below the ancestral frequency (Fig. 6.3).

6.3.1 Discussion

Most of the species considered converged on the 95 % confidence intervals of the ancestral character state calculated as 45.6–54.8 mm for forearm length and 72.6–81.8 kHz for resting frequency for all extant rhinolophids (Dool et al. 2016). The phenotypes of the species considered here ranged in forearm length and resting frequency from 39–54 mm and 75–92 kHz (Table 6.1), respectively. Convergence on the ancestral phenotype was particularly evident in the *capensis* group, but also for two species pairs, *R. damarensis*/*R. darlingi* and *R. hildebrandtii*/*R.*

mossambicus in the *fumigatus* group and *R. blasii* which is placed in a basal position to the Afro-Palaearctic clade (Fig. 6.2, Table 6.1; Dool et al. 2016). Mass did not carry any phylogenetic signal (Dool et al. 2016) and is not therefore considered in these comparisons.

Convergence is also evident between species pairs. There is pronounced convergence between *R. damarensis* and *R. darlingi* (Fig. 6.2), which occurs in separate clades within the *fumigatus* group (Jacobs et al. 2013; Dool et al. 2016) in support of the study by Jacobs et al. (2013). Similar phenotypic convergence is evident between *R. hildebrandtii* and *R. mossambicus* in the *fumigatus* group (Fig. 6.3) and between *R. simulator* of the *capensis* group and *R. blasii* (Fig. 6.2). The RFs of the two *fumigatus* lineages also converge but not their body sizes (Table 6.1). Thus, there is convergence in species that have overlapping distributions (*R. hildebrandtii* and *R. mossambicus*; *R. simulator* and *R. blasii*) and in species with disjunct distributions (*R. damarensis* and *R. darlingi*; *R. cf. fumigatus* and *R. fumigatus*) suggesting that in at least some cases local adaptation to the same habitats may not be the cause of the convergence.

The convergence described here may result from one or more of several processes such as inheritance from a common ancestor, adaptation to similar environments, random genetic drift and shared constraints (Losos 2011; Harmon et al. 2005). It is unlikely that the convergence we report here is the result of inheritance from a common ancestor. *R. mossambicus* is a sister lineage to *R. cf. fumigatus* and not to *R. hildebrandtii* (Dool et al. 2016) despite *R. mossambicus* and *R. hildebrandtii* being formerly placed in the same species (see Taylor et al. 2012). Similarly, *R. simulator* and *R. blasii* are also from different clades (Dool et al. 2016).

Furthermore, the fact that there are genetically closely related lineages within the *fumigatus* and *capensis* groups that are phenoptypically divergent probably excludes biological constraints, such as evolutionary trade-offs, as an explanation for convergence between several lineages. Such divergence indicates that despite their highly specialized echolocation system, rhinolophids are nevertheless able to respond evolutionarily to other forces in their environment. This is supported by the wide divergence from the ancestral body size (Table 6.1) and resting frequency (Fig. 6.3) observed amongst species of rhinolophids. The role of other processes (e.g. selection or drift) is supported by the absence of bias in the distribution of RFs amongst extant bats (Fig. 6.3), towards the ancestral RF or higher frequencies. This is so despite more pronounced Doppler shifts at higher frequencies which allow the acoustic fovea of these bats to encompass a wider range of frequencies. A wider range means that these bats can use less precise shifts in frequency (Waters 2003), facilitating DSC. It would appear that shifts towards lower frequencies, which would make DSC more difficult, would require substantial selection pressure.

Local adaptation also appears to be an unlikely explanation for the convergence at least between *R. damarensis* and *R. darlingi* which occur in different biomes. Random genetic drift may offer a better explanation for convergence between these two lineages (Jacobs et al. 2013). However, the two pairs of lineages *R. blasii*/*R. simulator* and *R. hildebrandtii*/*R. mossambicus* occur in the same biomes and some times in the same roost (DSJ, unpublished data) suggesting that adaptation to similar habitats could offer a valid explanation for the convergence in these pairs of lineages. However, one cannot exclude the possibility that both these species pairs have largely retained the character states of their closest common ancestor which had a FA length of 49.4 mm and a RF of 86.4 kHz (Stoffberg et al. 2011), with some divergence from the ancestral character state due to adaptation (e.g. for discrete frequency bands, see Mutumi et al. 2016) or genetic drift (Jacobs et al. 2013). Stasis may also explain convergence in RF between the two sibling lineages, *R. cf. fumigatus* and *R. fumigatus*. However, the divergence in their body sizes suggests that adaptation to local environments, at least in body size, may play a role in the evolution of these lineages. *R. cf. fumigatus* occurs in the more arid western half of Southern Africa whilst *R. fumigatus* is distributed in the more eastern half of Africa. The larger body size in *R. cf. fumigatus* could therefore be an advantage in the more arid and cooler conditions that prevail over its geographic range (Monadjem et al. 2010) in accordance with James' Rule (James 1970). Why there was not a concomitant allometric response in the RFs (see Jacobs et al. 2007) of these two lineages remains to be determined.

Phenotypic divergence in the African Rhinolophidae is also evident. It is particularly pronounced in the *fumigatus* group (Fig. 6.2). Although there is pronounced convergence between *R. damarensis* and *R. darlingi*, close relatives of these two species in the *fumigatus* group, *R. eloquens*, *R. fumigatus*, *R. cf. fumigatus*, *R. hildebrantii* and *R. mossambicus*, have diverged appreciably from both the ancestral character state and *R. damarensis* and *R. darlingi* (Fig. 6.2, Table 6.1) supporting convergence as a result of other processes besides stasis. Furthermore, there is a pronounced repeated pattern of divergence in RF amongst pairs of sibling species where one member of the pair retains the ancestral frequency and the other member of the pair diverges appreciably with RFs > 100 kHz. For example, the RF of *R. simulator* is similar to that of the ancestral frequency but its sibling lineages, *R. cf. simulator* and *R. denti*, have RFs > 100 kHz. The same is true for *R. capensis* and its sibling species *R. swinnyi* (Table 6.1). This suggests that RF may play an integral role in lineage diversification in this family of bats despite echolocation being primarily involved in orientation and prey detection. Its role in lineage diversification may be mediated by its secondary function in communication (Bastian and Jacobs 2015).

In conclusion, we have identified several instances of convergence amongst African rhinolophids. It is unlikely that the convergences reported here is the result of evolutionary trade-offs or some other kind of biological constraint. However, further investigation of other processes that may be responsible for convergence in the Rhinolophidae is likely to offer great insight into the evolution of convergence in bats in general and in other taxa as well. The success of such investigations is entirely dependent on robust pholygenies and accurate determination of ancestral character states.

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