ECOMORPHOLOGICAL DIFFERENCES BETWEEN SISTER SPECIES, *RHINOLOPHUS CAPENSIS* AND *RHINOLOPHUS SWINNYI*.

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Dissertation presented for the degree of Master of Science in the Department of Zoology, University of Cape Town
February 2008

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DECLARATION

I, Elizabeth Jane Kelly, hereby declare that the work contained in this dissertation is the result of my own research and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. I empower the University of Cape Town to reproduce any part of the dissertation for research purposes. The text does not exceed 50 000 words and no part has been submitted in the past, or is being submitted, to any other university in fulfilment of a degree.

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ABSTRACT

Phenotypic analyses of sibling species provide the opportunity to examine divergence that is caused by adaptation rather than phylogenetic history. *Rhinolophus capensis* and *Rhinolophus swinnyi* diverged from a common ancestor between 15 and 20 million years ago. The Fynbos biome of the south-western Cape (South Africa) arose around the same time, and its distribution is coincident with that of *R. capensis*. Since this event probably influenced the speciation of these species, I examine differences in the ecomorphology of these bats in their current distributions. *R. capensis* is bigger than *R. swinnyi,* with corresponding differences in echolocation call signatures and wing morphology. Individuals from populations on the edge of the distributions of both *R. capensis* and *R. swinnyi* are larger, and have larger wing loadings, than those further towards the centre of their ranges. The edge populations also occur with a major transition zone where a number of biomes meet. The larger body size of *R. capensis,* and of the populations at the ecotone, is probably an adaptation that provides identical benefit in both cases. Larger body sizes allow them to widen their niche width, and an increased wing loading makes commuting flights to adequate resources more energetically efficient. Body size was the major difference between *R. capensis* and *R. swinnyi* with allometric responses in echolocation calls, as well as wing and skull morphology.

ACKNOWLEDGEMENTS

This dissertation could not have been completed without the guidance, academic support and understanding of A/Prof David Jacobs; help in the field from Maryalice Walker, Mandy Mason, Robyn Verrinder, Zandi Zwane, Lizelle Odendaal and Brenda Kelly. The assistance of Prof Ric Bernard, Mr and Mrs White of Table Farm, Charlene April, the staff of the Amatole Museum and Teresa Kearney from the Transvaal Museum, and the generous contribution of the late Lloyd Wingate, was essential to the completion of this research.

All my thanks and love to Darren Taylor; my love and life, for giving me both and for being the warmest, most life affirming and most gorgeous diversion ever; Brenda and Patrick Kelly for support of every kind in the face of any challenge, I am so lucky to have you; Rob Kelly, Maria Wobben, Anne and Jonathan for love and money; Dominic Riordan for critical support and reviews and for much understanding; and Samantha Stoffberg for so much help and for reviewing my work. For support, acceptance and love, I thank Dr David Kibel, Bianca Greeff, Gillian Rennie, Mandy Mason, Robyn Verrinder and Benjamin Taylor (the only black dog I want in my life). And a special thank you to the people who keep taking me in when I have no where else to go: Sonja and the Niederhumers, Mdzananda Animal Clinic, and Lewis Nieburg and Darren Taylor (again).

This research was funded by a grant to David Jacobs from the National Research Foundation (GUN 2053611).

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CHAPTER 1

INTRODUCTION

A central question in evolutionary theory is how species form, i.e. how speciation occurs (Cracraft and Prum 1988, Grant 2001). Historically, speciation has been thought to occur predominantly in allopatric populations, which are separated geographically and are exposed to different sets of selection pressures. However, modern views on speciation call for the elimination of geographic classifications, and the analysis of evolutionary mechanisms instead (Via 2001, Kirkpatrick and Ravigne 2002).

Speciation remains a controversial subject because of the time frame and the complexity of factors that cause it, which makes empirical evidence difficult to attain. Only multi-factor evidence consisting of fossils, genetics and phenotypic data that agree with the organism's biogeography can give a full explanation of a species' origin. As this is a mostly elusive ideal, models have mushroomed as a replacement for understanding how organisms speciate.

Whilst becoming more encompassing, models are by their nature restrictive and cannot include all factors likely to influence a speciation event. There are many models that describe how speciation may occur. The most widely accepted is allopatric speciation which can be promoted by factors that enhance reproductive isolation such as migration (Church and Taylor 2002). Other models show the possibility of parapatric speciation (Gavrilets 2000) and increasingly more convincing mechanisms of sympatric speciation under various conditions have been described (Dieckman and Doebeli 1999, Drossel and McKane 2000, Doorn *et al.* 2004, Bhattacharyay and Drossel 2005, Bolnick

2006). Thus, the theoretical development of speciation has advanced with very little empirical support.

Where reproductive isolation results from divergent natural selection in different environments, ecological speciation occurs. Such speciation has been widely accepted since the emergence of the Biological Species Concept but there are still few empirical examples in support of it (Schluter 2001). Some empirical evidence for ecological speciation has been found in studies of host shifts in phytophagus insects (Via and Hawthorne 2002, Thomas *et al.* 2003) and in Stickleback fish (Hatfield and Schluter 1999). When frequency dependent selection acts on an ecologically relevant character, the resultant disruption produces two characters which become more dissimilar over time (Geritz and Kisdi 2000). Such ecological adaptation may lead to the origin of diverse species (Kruuk 1999). One of the consequences of ecological speciation, since selection acts on the phenotype, is phenotypic divergence (for a full review of phenotypic divergence from disruptive selection see Rueffler *et al.* 2006). Thus by examining phenotypic divergence and the environment in which it arose, it may be possible to infer the causal selection forces.

Ecomorphology examines the covariation of organisms' morphological differences with their environment and habits (Mullany and Gale 1996). It provides a framework on which to examine phenotypic divergence where speciation is the result of ecological divergence. A match between morphology and ecology has been found in turtles (Claude *et al.* 2004), fish (Hulsey and Garcia de Leon 2005), bears (Sacco and Van Valkenburgh 2004) and birds (Lack 1945). In bats, a correlation between morphology and ecology has been found repeatedly (e.g. Findley and Black 1983,

Norberg and Rayner 1987, Fenton and Bogdanowicz 2002). Matches in morphology and ecology in divergent populations have been found in the presence of impermeable (Jacobs 1996) and permeable barriers (Miller-Butterworth *et al.* 2003).

Resource partitioning has, however, been found in morphologically similar bats (Saunders and Barclay 1992, Barlow *et al.* 1997, Arlettaz 1999), suggesting that morphology is not the only factor determining resource use. Niche partitioning in morphologically similar species may be the result of differences in prey availability (Saunders and Barclay 1992, Siemers and Schnitzler 2004). Even in cases where there is little morphological difference, there can be divergence in echolocation (Thabah *et al.* 2006) which may affect the foraging ecology of bats (Siemers and Schnitzler 2004). Morphology can give a good estimation of what bats are capable of eating, but not necessarily what they actually do eat (Freeman 1981) and, as such, morphological studies should always be understood in the context of an animal's ecology and biology. Investigation into the life history of an animal can, therefore, aid the understanding of the link between morphology and ecology (Dechmann *et al.* 2006). Furthermore, for studies of speciation, it is most appropriate to examine sister taxa since differences in ancestors, ancestral selection pressures and consequently the phylogenetic history can be eliminated as the cause of differences (Chesser and Zink 1994, Panhuis *et al.* 2001, Arlettaz 1999). Differences in phenotype are rather the result of adaptive divergence or genetic drift.

Wing morphology and echolocation call characteristics are usually correlated (Jacobs 1999), forming what is known as an adaptive complex (Aldridge and Rautenbach 1987, Arita and Fenton 1997, but see Jacobs *et al.* 2007). An adaptive complex exists in bats because some combinations of wing morphology, echolocation call design, foraging

site and behaviour may have a negative adaptive value. For example, bats that forage in cluttered areas have wings with a low aspect ratio and wing-loading, as well as calls dominated by a frequency-modulated (FM) component because this allows them to manoeuvre easily and to discern prey from background noise. However, bats with high aspect ratio wings and high wing loading, which provide little manoeuvrability or agility, would be maladapted in a cluttered habitat.

A combination of adaptations allows bats to operate optimally in their nocturnal environment (Schnitzler and Kalko 1998). These include physiological (using torpor to conserve energy), behavioural (hunting style and diet), sensory (echolocation) and morphological (wing shape and body size) adaptations. The analysis of locomotive and trophic characters shows the ecological importance of morphological differences (Hespenheide 1973, Campbell *et al.* 2007). For this reason, it is relevant to analyse wing morphology, echolocation call design, skull structure and diet in bats. Body size can be affected by prey size, reproductive rate, metabolism, social dominance and is one of the most important factors to consider in eco-morphological analyses (Hespenheide 1973). The relationship between these factors and body size works in both directions, they affect each other to produce complex interactions.

Echolocation call characteristics are important for considering divergence in bats since selection acting on sensory systems may initiate speciation (Jacobs *et al.* 2006). Rhinolophids emit constant frequency calls with the majority of energy placed on the second of a series of harmonics. Morphs of Wallacea's bats that have recently diverged echolocate at different harmonics of the same fundamental frequency. In rhinolophids, the swapping of the dominant harmonic of calls within those of a fundamental frequency

alters the availability of prey items and is also involved in communication. It can, therefore, initiate divergence in a population via assortative mating and eventual reproductive isolation and speciation (Kingston and Rossiter 2004). Different echolocation call parameters are optimal for perceiving different types of objects in different environments (Schnitzler and Kalko 1998). Each species has a dominant type of call, which is defined by the call shape, duty-cycle, peak frequency, harmonic structure, duration and bandwidth (Schnizler and Kalko 1998). The calls can be dominated by either a constant frequency or frequency-modulated component. Bats using high duty-cycle calls have interpulse intervals that are shorter than their calls (Fenton *et al.* 1995). These calls are usually dominated by a constant frequency (CF) component and high duty-cycle bats shift the frequency of their emitted calls to counter the Doppler Effect that results from their own movement. This Doppler Shift Compensation (Schnitzler 1968) generates an echo at a frequency that falls within the acoustic fovea (Schuller and Pollak 1979) of the bat.

The use of high duty-cycle, narrow bandwidth calls allows bats to detect 'acoustic glints'. These are high amplitude regions of the echo resulting from an insect beating its wings. Long duration calls and constant frequency components increase the likelihood of detecting weak echoes and acoustic glints (Kingston *et al.* 2003). The likelihood of detecting a glint increases with increasing duty-cycle and bats are able to gain information on the wing size and beat frequency of the prey (Schnitzler and Kalko 1998, Kober and Schnitzler 1990).

A broader bandwidth call enables the bat to get three-dimensional information about the location of the prey and clutter and provides greater resolution of objects

(Kober and Schnitzler 1990). Frequency-modulated calls are not good for detecting weak echoes and detecting prey at long range. Constant frequency (narrow bandwidth) calls are good for the detection of prey but not for localising it. Prey detection distance decreases with increasing bandwidth of calls and capture success increases with increasing distance of the prey from clutter (Siemers and Schnizler 2004). Rhinolophids use calls with a long constant frequency component that end with a small frequency modulated tail (Aldridge and Rautenbach 1987), which provides the bat with the advantages from both call component types (Schnitzler and Kalko 1998). High duty-cycle bats occurring in dryer climates have higher call frequencies than those from tropical forests because of the attenuating effects of humidity (Heller and von Helversen 1989).

Even though each species uses calls with a specific suite of characters, echolocation calls are used flexibly by bats to accommodate changing requirements in different situations. Echolocation behaviour changes are evident in the difference between calls while bats are searching for, and when they are approaching, prey (Kalko and Schnitzler 1998). The duration and pulse rate decrease and the bandwidth increases on approaching prey (Kalko 1995, Macias and Mora 2003). Pipistrelle bats even change their calls depending on the amount of clutter that is in the area in which they are flying (Kalko 1995).

Body size affects most aspects of an animal's biology. It can determine the basal metabolic rate, reproductive rate, prey availability, flight capabilities and echolocation frequency. For example, the size of vocal and nasal chambers affects call frequency such that larger bats with larger sound producing organs usually produce lower frequency calls (Barclay and Brigham 1991, Heller and von Helversen 1989). Call frequency is thus

indirectly proportional to body size (Barclay and Brigham 1991, Jones 1999). Since bigger bats have lower frequency calls, their calls are also of longer wave length because of the inverse relationship between frequency and wavelength (Pye 1993). Since sound waves are only affected by objects equal to or larger than its wavelength, bats with low frequency sound are supposedly unable to detect small prey (Houston *et al.* 2004). Furthermore, larger bats are less manoeuvrable because they have to move faster to stay aloft (Norberg and Rayner 1987). This has implications for the types of habitats in which they can forage efficiently (open rather than cluttered habitat), which would in turn affect their diet and ecology in general (Arlettaz 1996, Aldridge and Rautenbach 1987). Thus the inverse allometric relationship between body and peak echolocation frequency, and deviations from this relationship, can give an indication of the presence of specific adaptations (Armstrong and Coles 2007, Jacobs *et al.* 2007).

Morphology is not only influenced by habitat and diet, but also by migration and reproduction (Norberg 1995). As in most biological systems, the relationship between variables is complex and these factors also act on each other in the other direction. For example, a high aspect ratio and bigger body size is generally advantageous for migration. However, some migratory species couple low aspect ratio with low wing loading. Similarly, some species with high aspect ratio and high wing loading are not migratory (Norberg and Rayner 1987). It is therefore invalid to make presumptions about the adaptive significance of morphological characters without knowledge of the animal's ecology and life history (Dechmann *et al.* 2006).

There are five basic parameters which are used when examining the wing morphology of bats. These are wing-loading (the ratio of body weight to wing area),

aspect ratio (the square of the wing span divided by the wing area), wing area, wingspan and wing tip shape (The ratio of the area of the arm wing to hand wing, T_s, divided by the difference between the ratio of the length of the hand wing to arm wing, T_l, and T_s). A high value for the wing tip shape, implying a rounded wing tip is beneficial to bats flying amongst high clutter and is correlated with short wings. Pointed wings confer increased agility and flight speeds to the bats. Wing tip shapes are not confounded by overall body size (Norberg and Rayner 1987). Wing-loading is a measure of a bat's manoeuvrability. High wing loading is beneficial for fast flight and turning agility and large wing areas are good for carrying loads. A small wingspan and low aspect ratio (short, wide wings) is optimal for flying in cluttered areas and through small spaces since they provide greater manoeuvrability (Norberg and Rayner 1987). For a full review of wing morphology and flight performance, see Norberg and Rayner (1987).

Even though differences in foraging habitat correlate with the size and wing morphology of bats (Aldridge and Rautenbach 1987, Norberg and Rayner 1987), foraging behaviour is highly flexible (Arlettaz 1996), but within the limits of physical constraints (Aldridge and Rautenbach 1987, Norberg and Rayner 1987, Kalko and Schnitzler 1998). It follows that by examining wing morphology, the limits of a bat's resource use capabilities should be revealed.

The analysis of skull morphology is important in evolutionary studies because they hold the eating apparatus, the brain and the sense organs (D'Anatro and Lessa 2006). Analysis of the size of various parts of the skull and dentary in relation to the rest of the skull gives clues to the gape, masticatory power and crushing ability of bat jaws (Freeman 1979, Freeman 1981). Bats are, in general, opportunistic feeders (Arlettaz

1996) but some generalisations have been noted about correlations between morphology and diet. Bigger jaws correspond with larger prey sizes (Barlow *et al.* 1997). Bats capable of eating hard prey have skulls that are more robust with thick jaws, fewer larger teeth and large cranial crests for muscle attachment (Freeman 1979). Bats with more gracile skulls, and thinner jaws hold more small teeth, are restricted to soft bodied prey e.g. Lepidoptera and Diptera (Freeman 1979, Freeman 1981, Jacobs 1996). Thus larger bats may have more variable diets because they can take hard or soft prey (Freeman 1981). Likewise, large bats eat a range of prey sizes but small bats are limited to eating small prey (Aldridge and Rautenbach 1987, Barclay and Brigham 1991). The ability of larger bats to take smaller prey may, however, be limited by their relatively low frequency calls. Jacobs *et al.* (2007) did not find any difference in the range of insect sizes taken by *R. capensis* and *R. clivosus* despite the latter being bigger than the former.

Sibling species provide an opportunity to investigate evolutionary divergence in a situation where any differences between the sibling species are likely to be due to adaptive divergence rather than differences in their phylogenetic history; i.e. time before the speciation of these two bats.

Rhinolophus capensis and *Rhinolophus swinnyi* belong to the family Rhinolophidae, a group of insectivorous bats that are distinguished by the horseshoe shape of the nose leaf which is used during the emission of echolocation calls. They are clutter foragers with low aspect ratio wings, allowing the necessary manoeuvrability to avoid obstacles (Norberg and Rayner 1987). They have high-frequency, high duty-cycle, calls dominated by a CF component with a short frequency-modulated tail at the end

(Fenton *et al.* 1995). The two species differ in mean body mass by 4.03g and in peak echolocation frequency by 24.1 kHz (Jacobs *et al.* 2007).

Rhinolophus capensis is the larger bat (11.1g) with the lower mean frequency (83.9 kHz, Jacobs *et al.* 2007) echolocation call. *R. swinnyi* has an average mass of 6.8g and peak frequency of 107 kHz (Jacobs *et al.* 2007). In a phenetic analysis of the Rhinolophidae, *R. denti* and *R. swinnyi* were found to group together with *R. capensis* (Bogdanowicz 1992), reflecting their high degree of similarity. A phylogeny based on a supermatrix comprising mitochondrial cytochrome *b* and three nuclear introns suggests a similar scenario. *Rhinolophus capensis* and *R. swinnyi* diverged 16.8 ± 7.1 mya, and the *R. swinnyi* lineage diverged further; giving rise to *R. denti* and *R. simulator* (Stoffberg 2007). A relaxed Bayesian clock was used to estimate the time of divergence. A phylogeny, created by Guillén *et al.* (2003) using mitochondrial cytochrome *b*, also shows *R. swinnyi* and *R. capensis* as sister species.

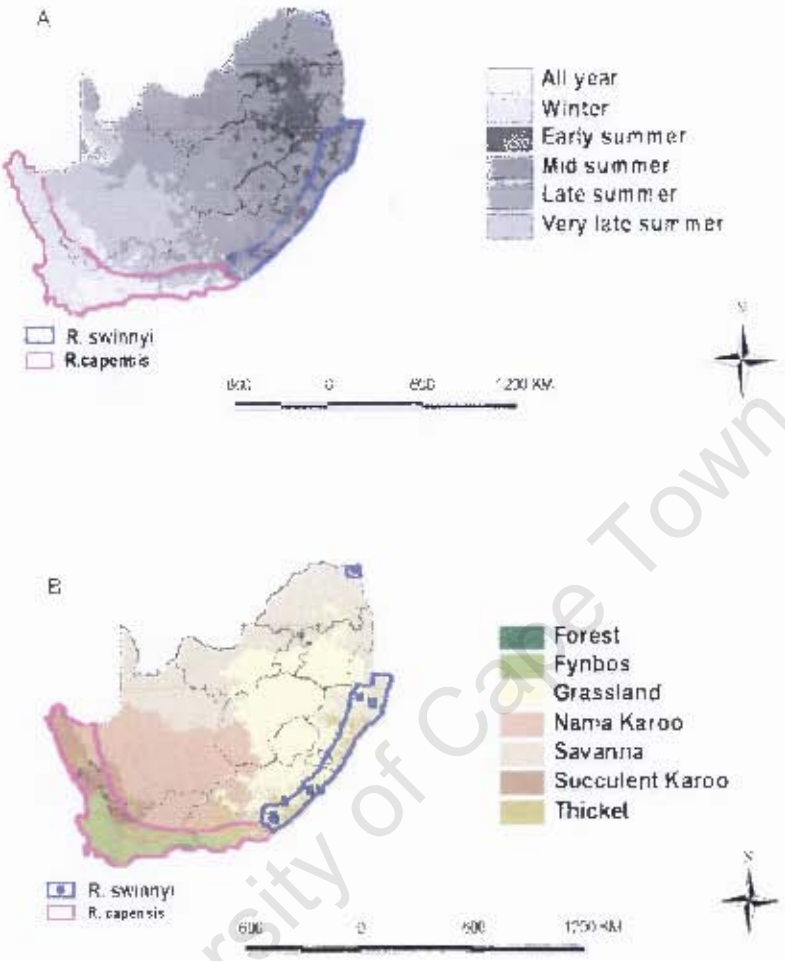

Figure 1.1. The distributions of *Rhinolophus swinnyi* and *Rhinolophus capensis* in South Africa. (A) shows the species' distributions on a map of the seasonality of rainfall in South Africa and (B) shows the species' distributions on a map of South African biomes, where individual populations of *R. swinnyi* that have been sampled are shown in blue squares. Maps are derived from Rutherford and Westfall (1986) and courtesy of Samantha Stoffberg and the distributions derived from Taylor (2000).

Rhinolophus capensis is endemic to the Fynbos biome. Its distribution stretches down the West Coast and along the South Coast of South Africa (Taylor 2000). Its distribution ends at the Kafffrarian Transition Zone in the South-Eastern Cape (Figure 1.1) The Cape Floristic Region (CFR) is one of the most plant-species-rich regions in the

world and is dominated by sclerophyllous vegetation and characteristically low in nutrients (Wright and Samways 1998). The transition zone is a region of high climatic and geographic complexity where four biomes (the thicket, Fynbos, grassland and Nama Karoo) meet. *Rhinolophus swinnyi* has a distribution stretching from the Kaffrarian Transition Zone, along the east coast of South Africa and extending into Zambia in the north.

Pollen assemblages and evidence inferred from the leg bones of bovid fossils indicate that the Fynbos biome was subtropical forest in the early Neogene (*ca* 20 million years ago) (Coetzee and Muller 1984, DeGusta and Vrba 2003). The Fynbos was fully established by the mid-Miocene (15 mya, Partridge 1997) and radiation in the Plio-Pleistocene (3-5mya) is thought to be largely responsible for the current species richness in the Fynbos region (Pennington *et al.* 2004). Mediterranean climates are especially diverse; they contain almost 20% of vascular plant species but cover only 5% of continents. The Fynbos, for example, covers only a fraction of land area covered by tropical rain forests, but has about half as many species (Cowling *et al.* 1996). Despite high plant diversity, Giliomee (2003) found that floral species diversity is not reflected in the diversity of insect species. However, this was refuted by Proches and Cowling (2006) who found that insect diversity in the Fynbos is comparable to that of other biomes, especially when galling insects are taken into consideration.

The change in biome from forest to Fynbos between 15 and 20 mya coincides with the estimated time of divergence of *R. swinnyi* and *R. capensis* and may have been the cause of their phenotypic divergence and their subsequent speciation.

Hoffman and Baker (2003) investigated speciation in a number of species of *Carollia* by correlating age of divergence (estimated using molecular markers) with the geological and geographical events that caused their divergence. In the case of *R. capensis* and *R. swinnyi*, we know the time of divergence and that it coincides with a major shift in vegetation from tropical forest to Fynbos.

I investigated the ecomorphological differences between these two species and between geographically separated populations within each species. My aim was to document divergence in their phenotypes so as to gain insight into possible causes for their divergence. The phenotypic characters that I will consider include echolocation calls, wing morphology, body size, skull morphology and diet.

I predict that the species in the dryer, resource deficient Fynbos biome will be larger, with correspondingly low echolocation call frequency and larger wing loadings than the grassland species. The bigger size would be advantageous in a resource limited habitat because of a wider niche width and ability to fly larger distances efficiently.

CHAPTER 2

METHODS

Bats were captured at two locations. *Rhinolophus swinnyi* was captured at Sandile's Cave in the Pirie Forest (32°43´S, 27°17´E) near King Williams Town in the Eastern Cape Province, South Africa. Sampling was done in February and April 2007. Sandile's Cave is positioned near the top of a mountain in the Amatole Mountain range, surrounded by extensive indigenous thicket and yellow-wood forest, exotic plantation forests and agricultural lands.

Rhinolophus capensis was sampled from Table Farm, a few kilometres from Grahamstown (33°17´S, 26°25´E), in the Eastern Cape Province, South Africa, in April 2007 (Figure 2.1). At Table Farm, *R. capensis* roosts in a rock shaft that was dug through a hill to house a water pipe. Grahamstown is bordered by four different biomes; Succulent Karoo, Grassland, subtropical Thicket and Fynbos. It is at the centre of the Kaffrarian transition zone which makes it a region of great complexity both geographically and climatically (Goldblati 1978, Cowling *et al.* 1999).

Figure 2.1. The sampling sites in South Africa. *Rhinolophus capensis* was sampled from De Hoop (purple) and Table Farm (pink). *Rhinolophus swinnyi* was sampled from Sandile's Cave KWT (dark blue) and Kokstad (light blue).

Data from *R. capensis* sampled in July 2005 at De Hoop Guano Cave (34°26′S, 20°25′E) in the De Hoop Nature Reserve, Western Cape, South Africa (Walker 2006) and data from *R. swinnyi* from Kokstad (30°31′S, 29°29′E) in July 2004 in KwaZulu Natal, South Africa were also included (Collected by D. Jacobs). Inclusion of these data sets allowed a comparison of populations from within and at the edge of the respective species' distributions.

Kokstad is situated in the Grassland biome in the south of KwaZulu Natal and is at the foot of the Drakensberg Mountains. De Hoop Nature Reserve is located within the Fynbos biome. The vegetation is stunted, scrubby and sclerophyllous, typical of coastal Fynbos. There is a Milkwood (*Sideroxylon inerme*) forest on the northern side of the large vlei (McDonald *et al.* 1990); a shallow seasonal lake, which forms part of the south-western boundary of the reserve. *Rhinolophus capensis* roosts with *R. clivosus, Myotis tricolor, Nycteris thebaic*a and *Miniopterus natalensis* in the De Hoop Guano Cave, located in a cliff just above the Milkwood forests. The bat population in this cave has been estimated to be up to 300 000 individuals (McDonald *et al.* 1990).

The western side of the Fynbos biome receives most of its rainfall in the austral winter (May to September). De Hoop, which is included in the western part of the Fynbos gets most of its rainfall between May and September with average daily temperature maxima of 28° C in summer and 17° C in winter (McDonald *et al.* 1990). The eastern side of the Fynbos has aseasonal rainfall so the maxima are spread across the year, but is highest in austral spring (August-November) and autumn (March-May). At the ecotone where the Fynbos meets the Grassland and Thicket biomes, a summer rainfall pattern

becomes predominant. The daily average temperature maxima in this region are 25° C in summer and 20°C in winter (South African Weather Service).

Rhinolophus swinnyi individuals were caught at Sandile's Cave using a harp trap (Austbat Harp Trap, Faunatech, Mount Taylor, Victoria, Australia) erected outside the lower entrance of the cave about 2 hours after emergence. This ensured that the bats had foraged prior to capture. The bats at Table Farm were hand netted in the morning once the bats were in torpor, and those at De Hoop Guano Cave were caught through the night with mist nets and a harp trap. Hand netting could not be used in Sandile's Cave because the bats roosted out of the reach of hand nets. A combination of echolocation call frequency, size (forearm length and mass) and dentition was used to identify the bats to species using the dichotomous key in Taylor (2000). Bernard (1985) also identified the rhinolophids at Table Farm as *Rhinolophus capensis*.

The body masses of captured bats were measured with a digital electronic scale (Ohaus Corporation, Pine Brook, New Jersey, USA) to the nearest 0.01g. This was done after at least five hours from capture to ensure that the bats had voided their digestive contents and faecal pellets were collected at the same time. The body and extended right wing of each bat (Saunders and Barclay 1992, Figure 2.2) was photographed with a Canon Powershot A50 digital camera (Canon Inc. Lake Success, New York, USA). These images were used to measure wingspan, wing area, body length, tail length, arm wing length and area, hand wing length and area, and shoulder length using ImageJ free software (United States National Institutes of Health, Bethesda, Maryland). This program has been used successfully by Armstrong and Coles (2007) for measurements from radiographs of bat skulls and for other biological measurements (Ho *et al.* 2003, Nugochi

et al. 2003). Each measurement taken on ImageJ was repeated three times (not consecutively) and then averaged to reduce measurement errors. These measurements were subsequently used to calculate total wingspan, wing area, aspect ratio, wing loading and wing tip shape index of the wings according to Norberg and Rayner (1987).

The adult status of the bats was established by visually checking the ossification of the gap between the diaphysis of the metacarpal and proximal phalanx (Kunz and Anthony 1982). Forearm lengths were measured to one decimal place using dial callipers.

The sex of the bats was recorded and the reproductive state determined visually for the males by the size of the testes. For the females, pregnancy was inferred using a combination of factors including the time of year, a protruding abdomen that persisted after the digestive system had been voided, light palpation and enlarged nipples. A reproductive state of lactation was inferred if there was an obvious hairless ring around enlarged nipples and if milk was expressed on light pressing of the nipples (Racey 1969). Pregnant and lactating bats were not included in analyses as their increased body mass could skew results.

Figure 2.2. Photograph of a bat with extended wing from which morphological measurements were taken.

Rhinolophus swinnyi and *R. capensis* skulls were obtained on loan from the Amatole Museum in King Williams Town and from the Transvaal Museum (TVL Museum) in Pretoria. The skulls were taken from bat specimens collected from De Hoop Nature Reserve and Het Kruis (*R. capensis*) and well as from King Williams Town and the Kruger National Park (*R. swinnyi*). Het Kruis is a town on the West Coast of South Africa, close to Citrusdal (32° 38' 58" S 19° 24' 31" E). Photographs showing dorsal, ventral, and left and right lateral views of the jaw and braincase (Figure 2.3) were taken using a Canon Powershot A50 Camera (Canon Inc. Lake Success, New York, USA). A list of specimen codes, sample sizes and sexes is included in the appendix.

Figure 2.3 Photographs of the lateral view of the skull and dentary.

Linear measurements were taken in millimetres to one decimal place from the photographs of the skulls using ImageJ free software. Bat skull measurements from digital images have been done previously by Gannon and Racz (2006). Photos were standardised by calibrating each picture against a 10mm object. Each measurement was repeated three times, non-consecutively, and then averaged. The standard error was

calculated ($\delta/\sqrt{n}$) for each parameter to ensure that significant results are not the result of measurement error. This approach to skull morphology analysis i.e. the parameters used, was established by Freeman (1979) and used subsequently by Jacobs (1999) and more recently by Schoeman and Jacobs (2003).

The following nine measurements were taken from the jaw according to Jacobs (1996) (Figure 2.4):

- the distance from the anterior surface of the mandibular fossa to the origin of the masseter muscle (bottom of the left angular process) [a],
- the distance from the top of the left condyle to the insertion of the masseter muscle (bottom of the left angular process) [b],
- the ratio of a to b [a:b],
- the height of the condyle (top of the left condyle to the plane of the alveoli of the left first and second molar) [d],
- the height of the coronoid process (top of the left coronoid process to the plane of the alveoli of the left first and second molar) [e],
- the length of skull (from the occipital to the alveolus of the canine) [f],
- the length of the dentary (from the back of the left condyle to the epiphysis of the dentaries) [g],
- the length of the left maxillary tooth row (from the front of the left fourth premolar to the back of the left third molar) [h]
- the dentary thickness (from the plane of the alveoli of the left first and second molar to the bottom of the left dentary) [i].

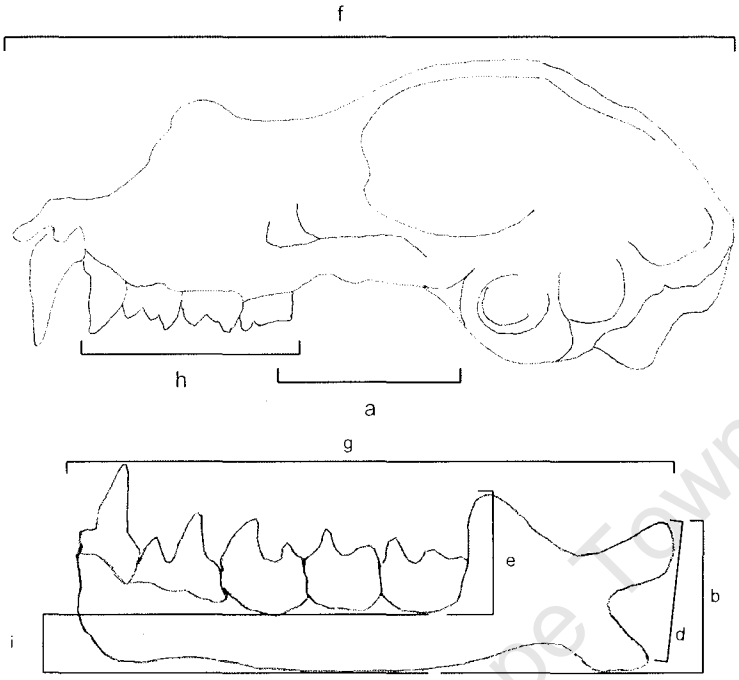

Figure 2.4. The measurements taken of the skull and dentary. A description of the measurements and their abbreviations are provided in the text.

Bats captured at night were kept overnight in breathable cotton bats to allow time for them to void their stomach contents. I waited at least five hours before processing the bats that were caught in the morning. Faecal pellets were collected from each bag before the bats were released in the evening.

A minimum of five and maximum of 15 pellets were analysed from each bat depending on the number of pellets present. Each of these pellets was submerged in a small amount of water and teased apart under a dissection microscope. All intact insect parts were mounted on a slide and identified to order using a dichotomous taxonomic key (Scholtz and Holm 1985) and reference collection from insect collections at the site (see below). The percentage volume that each insect order contributed to the total volume of the pellet was estimated to the closest 5-10%. Due to the highly masticated nature of the

pieces of insects in the samples, only eight suitable claws of beetles from *R. capensis* and seven claws from *R. swinnyi* were extracted for an analysis of prey size.

This method of analysing diet has been used extensively for bats (e.g. Schoeman and Jacobs 2003) but is subject to bias, especially towards hard bodied insects like Coleopterans and against soft bodied insects (Rabinowitz and Tuttle 1982). The method has been justified, however, through a blind test by Kunz and Whitaker (1983), which showed it to reasonably represent the diet of bats.

A reference collection of insects was collected from inside the forest at Sandile's Cave and about 500m from the tunnel at Table Farm using a black light insect light trap (BioQuip Products, Rancho Dominguez, CA, USA). At both sites the light trap was suspended from a tree over a pitfall trap. This was powered by a car battery and run for the duration of the trapping night. There was one trapping night at Table Farm, three at Sandile's Cave in February and two at Sandile's Cave in April.

Echolocation calls from hand-held bats (Russo *et al.* 2001, Salsamendi *et al.* 2005) were recorded directly onto a Fujitsu Siemens notebook computer (Fujitsu-Siemens Computers GmbH, China) using the Avisoft UltraSoundGate 416 with an UltraSoundGate CM16 microphone and Avisoft-RECORDER software (all from Avisoft Bioacoustics, Berlin, Germany). Calls were recorded in real time at a sampling rate of 250 000 Hz (16bits, mono). Ten calls were analyzed for each bat using BatSoundPro software version 3.20 (Pettersson Elektronik AB, Uppsala, Sweden) on a Fujitsu-Siemens Amilo notebook computer. The calls were selected on the basis of their large signal to noise ratio and even spacing. One call out of the measured 10 was used for analysis, chosen by its close resemblance to the mean of all 10 calls. This ensured that an actual

call that was representative of the calls for that bat was used, and avoided pseudoreplication.

Call duration was measured from the oscillogram and the peak echolocation frequency and bandwidth of the frequency modulated tails were measured from a power spectrum with a Fast Fourier Transformation of 512. The bandwidth of the frequency modulated tail was measured from the power spectrum at 20dB below the peak frequency according to Macías *et al.* (2005). Call duration was measured from the start of the constant frequency component to the end of the frequency modulated tail.

Statistical Analysis

Statistica (v. 7.0. StatSoft Inc. 2300 East 14th St. Tusa OK 74104 USA) was used for all analyses except for the Cluster, Simper, Anosim and MDS analyses of the diet which were done in Primer software version 5 (Plymoth Marine Laboratory, Plymoth, UK).

Data from February for *R. swinnyi* and April for *R. capensis* (Table Farm) were used for morphological analyses since they had the largest sample size and the risk of pseudoreplication (due to sampling the same bat twice) was avoided by only using data from one time at a single place. Data from February and April for *R. swinnyi* and from April for *R. capensis* were used in the dietary analysis.

To uncover phenotypic divergence in *R. capensis* and *R. swinnyi*, the mean and standard deviations of echolocation, morphological and ecological parameters were calculated. A one-way ANOVA was used to find differences in echolocation and morphology of adult and sub-adult members of *R. capensis* and *R. swinnyi*. Tukey Post

Hoc tests were used for pairwise comparisons. A Factorial ANOVA was used to identify sexual and geographic dimorphism in the two species.

Data were checked for normality and homogeneity of variances, and outliers removed when clearly indicative of measurement error. Small deviations from normal were allowed to accommodate the nature of biological data, and to which I considered ANOVA robust.

Deviations from allometric relationships for members of a clade can be indicative of evolutionary adaptation (e.g. Sweet 1980). Log10 values were used in regressions of body mass against wing area and peak echolocation frequency as well as wing loading against peak echolocation frequency for the four populations of the two species together with the other South African members of the Rhinolophidae. Data for other South African rhinolophids were collected by D.S. Jacobs.

A separate regression of mass against wing loading and mass against wing area was done for *R. capensis* and *R. swinnyi*. An ANCOVA for the homogeneity of slopes was done to test whether the slopes were significantly different for the two species. The ANCOVA compares the confidence limits of the two slopes and finds no difference if the confidence limits overlap. An ANOVA was done on the residuals of wing loading to identify any difference in the vertical position of the data between the two species. This was done according to Dietz *et al.* (2006).

Multivariate statistics were used to manage the number of interacting parameters that form a bat's phenotype. Multivariate statistics are effective for ecological analyses where variables seldom act alone but rather form a complex of interactions, especially in light of the adaptive complex identified in bats (Aldridge and Rautenbach 1987).

Principal Component Analyses (PCA) are robust to explore auto-correlated character states which are common in morphological data. For example, the aspect ratio of wings is correlated with wingspan and wing area. Discriminant Function Analyses (DFA) are, however, sensitive to autocorrelation. I overcame the problem of autocorrelation by first performing a PCA and then using the principal component scores for each individual in the DFA for the skull data and by only using mass and two measures of wing size in the morphological PCA.

PCAs summarise a number of variables into a few which can then be viewed graphically. It finds an axis through the data which explains the majority of the variation. It generates an Eigenvalue for each component, which indicates which of the principle components carry the most variation. It also generates factor loadings that show which variables dominate each principle component. It is then possible to see which parameters account for most for the differences between groups.

By using principal component scores in the DFA, the criterion of independent variables is satisfied. The DFA fits a model to the data and identifies which variables, or principle components in this case, best discriminate between species. Squared Mahalanobis distances give a measure of how far different groups are from each other in morpho-space by measuring the distance between the centroids of the groups. This is particularly useful when more than one discriminant function is revealed since it can be used to see which groups are more similar to each other in a quantitative manner.

A PCA was done for mass, wing span and wing area for both populations of *R. capensis* together with the two populations of *R. swinnyi*. The same analysis was done for the skull and dentary measurements of *R. capensis* (De Hoop and Het Kruis) and *R.*

swinnyi (King Williams Town and Kruger National Park), in addition to a DFA on these principal components.

Primer software was used for analysing the composition of the diet of the two species and between two months in *R. swinnyi*. This program has predominantly been used in marine and eco-toxicology (e.g. Clarke 1999, Nero and Sealy 2005) studies but is increasingly used in studies of bats (e.g. Castro-Luna *et al.* 2007). Arcsine transformed proportions of prey items in the diet were used to create similarity matrices. The Bray-Curtis measure of similarity was used for these matrices and from it cluster analyses were done to see which bats have their dietary composition in common with each other. A group average sorting method was chosen so that groups of bats with the most similar diet cluster together on the dendrograms.

Multidimensional scaling (MDS) plots were created from the similarity matrices. The MDS plot gives a stress value that represents how much distortion occurred when the data were compressed into two dimensions. A low stress value indicates fidelity in the plot to the real distances, or similarity, between bats. A one-way analysis of similarity (ANOSIM) was done to determine the significance of the similarities between assemblages. A similarity percentage analysis (SIMPER) was also performed to determine which prey items accounted for intra-group similarities and inter-group differences and by how much they differed/were similar.

A backward stepwise regression analysis was done to establish which phenotypic variable was the best predictor of the incidence of moths and beetles in the bats' diets. The proportions of moths and beetles in the diet were arcsine transformed according to Schoeman and Jacobs (2003).

CHAPTER 3

RESULTS

Age difference

A significant difference was found between the adult and sub-adult phenotypes of *R. swinnyi* (One-way ANOVA $F_{(10,37)} = 15.3$, $P < 0.00001$; Table A1). Tukey post hoc tests revealed a significant difference in both peak frequency ($df = 46$, $P < 0.001$) and bandwidth ($df = 46$, $P < 0.001$) of the echolocation calls. The adults had a higher peak frequency (107.8 kHz ± 0.8, n= 30) than sub-adults (106.5 kHz ± 0.9, n= 21), which corroborates results found by Russo *et al.* (2001). There were also significant differences in mass ($df = 46$, $P < 0.001$), forearm length ($df = 46$, $P < 0.02$), wing loading ($df = 46$, $P < 0.02$) and wing area ($df = 46$, $P < 0.003$).

No significant differences were found between the phenotypes of *R. capensis* adults and sub-adults (One-way ANOVA $F_{(10,22)} = 1.6$, $P = 0.2$, Table A1). A Tukey post hoc test, however, showed a significant difference only in wing loading ($df = 31$, $P < 0.04$). The lack of difference may be due to the small sample size of sub-adults in this species.

Sexual and geographic dimorphism

While there were significant differences between species (Factorial ANOVA Wilks' Lambda = 0.02, $F_{(6,154)} = 145.9$, $P<0.000001$) and between sexes of the two species (Factorial ANOVA Wilks' Lambda = 0.7, $F_{(3, 77)} = 9.8$, $P<0.00001$), there was no significant difference within species (Factorial Anova, species*sex, $F_{(6, 154)} = 1.8$, $P >$

0.1). Sexual dimorphism was found in the forearm, wingspan and mass of *R. capensis* Table Farm, ($P < 0.03$). For forearm and wingspan, the values for females were larger. Larger females were also found by Dietz *et al.* (2006) in an analysis of the five European rhinolophids. There was no sexual dimorphism in the mass of *R. swinnyi* (KWT) or *R. capensis* (De Hoop) (P's > 0.2; Table A2).

Only adults were used in further analyses. Sexual dimorphism within both species was accounted for in subsequent analyses by including equal numbers of males and females. I used 15 male and 15 female *R. swinnyi* from King Williams Town (KWT), three of each sex from *R. swinnyi* from Kokstad (KOK), 14 male and 14 female *R. capensis* from Table Farm (TF) and 13 individuals of each sex for *R. capensis* from De Hoop (DH).

Echolocation

Echolocation calls were taken from hand-held bats (examples shown in figure 3.1). The same technique was used for both species but due to the nature of hand held calls, minimal functional information should be inferred from parameters with a time dimension. The peak echolocation frequencies of the two species differ by 22 kHz (Table 3.1). Both species place most energy in the second harmonic. Bearing in mind the misgivings of hand-held calls, but that both species were sampled in the same manner, it is notable that *R. capensis* calls are significantly longer in duration and the FM tails have a narrower bandwidth than those of *R. swinnyi* (One-way ANOVA $F_{(6,110)}= 280.6$, $P<0.01$). The mean peak frequency of *R. capensis* at Table Farm is almost 3 kHz higher than that at De Hoop.

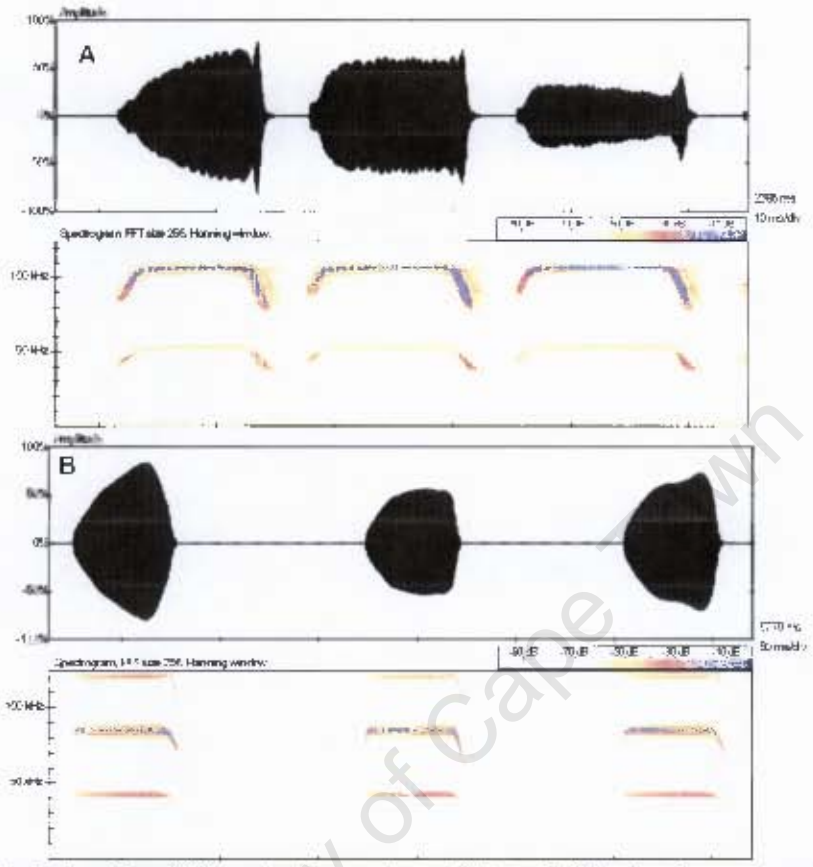

Figure 3.1. Typical calls of *Rhinolophus swinnyi* (A) and *Rhinolophus capensis* (B). *R. capensis* calls have lower peak frequency and a frequency modulated tail of narrower bandwidth than the calls of *R. swinnyi*. Both species place most energy in the second harmonic of the call.

Morphological divergence

When compared with other members of the family Rhinolophidae, *Rhinolophus capensis* (De Hoop) and *R. swinnyi* (KWT and Kokstad) scale allometrically within the 95% confidence limits for peak echolocation frequency against mass (Figure 3.3). *R. capensis* from Table Farm, however, falls outside of the allometric relationship for the family. It has a higher mass and a slightly higher echolocation frequency than *R. capensis* from De Hoop (Table 3.1). Selection appears to have acted mostly on the body size of *R.*

capensis at Table Farm, while its echolocation frequency is not greatly divergent from the De Hoop population (Table 3.1).

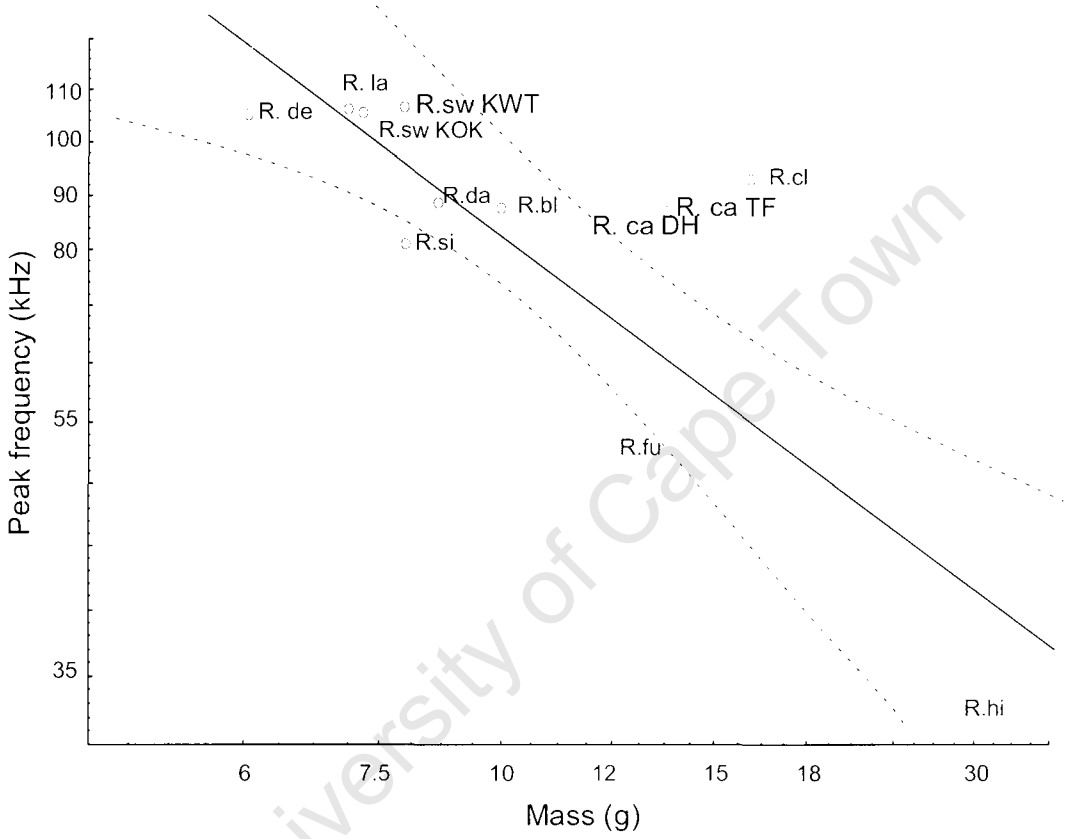

Figure 3.2. The allometric relationship between mass and peak echolocation frequency in the Rhinolophid bats of South Africa, including *R. swinnyi* from KWT and Kokstad as well as *R. capensis* from Table Farm and De Hoop. The regression line (y = 2.5695 - 0.6354x) explains 63% of variation in the data (R^2 = 0.63). The following abbreviations are used for the species: *Rhinolophus denti* (R.de), *R. landeri* (R.la), *R. swinnyi* (Kokstad) (R.sw KOK), *R. swinnyi* (King Williams Town) (R.sw KWT), *R. simulator* (R.si), *R. darlingi* (R.da), *R. blasii* (R.bl), *R. capensis* (De Hoop) (R.ca DH), *R. capensis* (Table Farm) (R.ca TF), *R. fumigatus* (R.fu), *R. clivosus* (R.cl) and *R. hildebrandti* (R. hi). All data except those from De Hoop and KWT were captured by D.S. Jacobs.

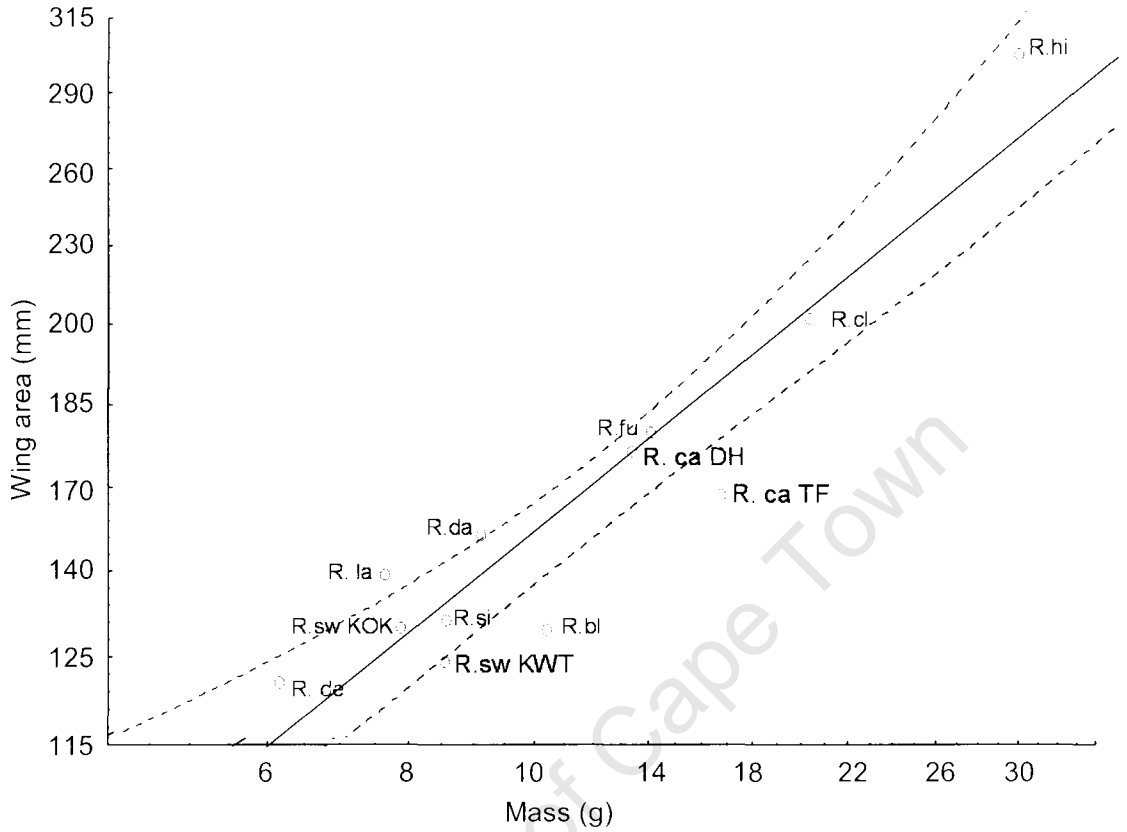

Figure 3.3. The allometric relationship between mass and wing area in the Rhinolophidae of South Africa. The regression line ($y = 1.6 + 0.5x$) shows a significant relationship between variables and explains 90 % of variation in the data ($R^2 = 0.90$). Abbreviations are the same as those used in figure 3.2.

Rhinolophus swinnyi (Kokstad) and *R. capensis* (De Hoop) both fall within the 95% confidence limits for the allometric relationship between mass and wing area in the South African Rhinolophidae (Figure 3.3). Neither of these populations is on the edge of the South African distributions for their species; De Hoop is in the centre of the distribution for *R. capensis* and Kokstad further into *R. swinnyi's* distribution than King Williams Town. *Rhinolophus swinnyi* (KWT) and *R. capensis* (TF) both fall just outside of the confidence limits (Figure 3.3). Both edge populations are bigger in size and smaller in wing area than their corresponding populations toward the centre of their respective distributions.

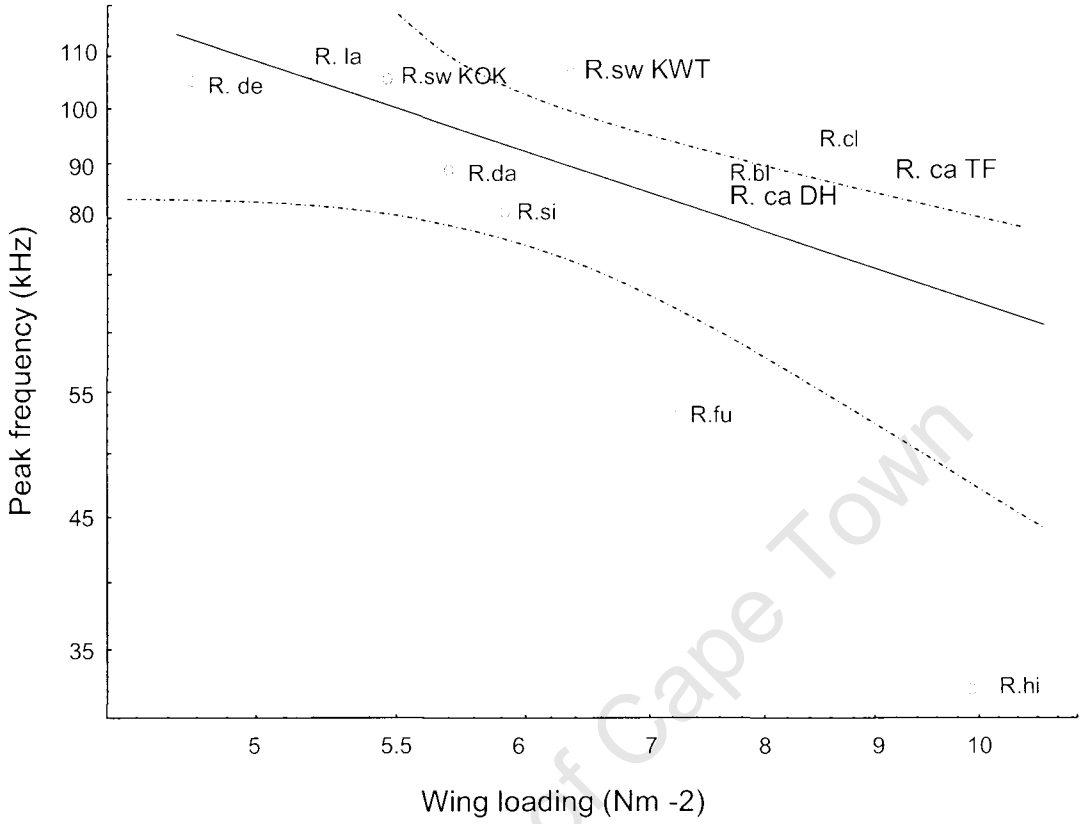

Figure 3.4. The allometric relationship between wing-loading and peak call frequency of the South African members of the Rhinolophidae. The relationship is significant with $P < 0.05$ and the regression line ($y = 2.7589 - 1.0128x$) explains only 43% of variation ($R^2 = 0.43$). Abbreviations are the same as those used in figure 3.2.

A similar pattern was observed in the allometry of wing-loading and peak echolocation frequency for the rhinolophids in South Africa (Figure 3.4). Populations from within the distributions fall within the confidence limits for the allometry of the family but the populations from the edge fall outside. The difference between populations appears larger in wing-loading than in peak frequency, suggesting that selection has acted on the body size and wing morphology of these bats specifically and that the differences in call frequency are a result of those changes.

Population differences aside, both species follow the allometry for the family in general. It appears that they are on the same evolutionary trajectory and that *R. capensis* is essentially a 'scaled up' version of *R. swinnyi*.

Divergence in mass and wing parameters

The mass and wing parameters of all four populations are significantly different (One-way ANOVA $F_{(12,154)}$= 46, P<0.01, Table 3.1). Tukey post hoc tests revealed a significant difference in mass between the populations of both species (df = 46, P < 0.0002). There was a significant difference in wing-loading between the two *R. capensis* populations (df = 27, P < 0.04; Table 3.1) but not between *R. swinnyi* and *R. capensis*.

Rhinolophus capensis (De Hoop) is significantly smaller (df = 27, P< 0.02) with a smaller wing loading than its counterpart population (df = 87, P < 0.0002). Similarly, *R. swinnyi* from Kokstad was significantly different from their counterparts from King Williams Town (df = 87, P < 0.0002). The small difference in call peak frequency between the two *R. capensis* populations translates to a 0.1mm difference in wavelength which probably does not translate into a functional difference (85.8 kHz = 4.0mm wavelength and 83.2 kHz = 4.1mm wavelength). In summary, there are marked differences in body mass with only slight differences in echolocation for both species (Table 3.1).

Table 3.1: Mean ± SD of body size, echolocation and wing parameters for *Rhinolophus capensis* (*R. ca*) from Table Farm and De Hoop and *Rhinolophus swinnyi* (*R. sw*) for KWT and Kokstad. Values which are significantly different ($p<0.01$) from each other within a variable are shown in bold. Where more than two differences occur in a variable, the pairs that differ significantly from each other are indicated with an asterisk (*). Exact values of statistical tests are given in the text.

Bat species and location	*R. ca* Table Farm	*R. ca* De Hoop	*R. sw* KWT	*R. sw* Kokstad
Number of bats	28	26	30	6
Body size				
Mass (g)	**14.1 ± 1.3**	**11.1 ± 0.8**	8.3 ± 0.3	7.8 ± 0.6
Echolocation parameters				
Peak frequency (kHz)	85.8 ± 0.7	83.2 ± 0.5	107.8 ± 0.8	106.8 ± 0.2
Bandwidth (kHz)	**11.1 ± 1.6**	No data	**19.4 ± 4.1**	No data
Duration (ms)	44.2 ± 7.6	No data	20 ± 4.8	21.7 ± 4.6
Wing parameters				
Forearm length (mm)	50.1 ± 1.2	49.6 ± 0.9	43.7 ± 0.9	43.6 ± 1.2
Wing area (cm^2)	155.8 ± 9.3	165.1 ± 18.9	124.6 ± 13.1	130.7 ± 3.2
Wingspan (cm)	30.0 ± 1.2	30.6 ± 1.5	25.9 ± 2	27.7 ± 0.6
Wing loading (Nm^{-2})	**8.8 ± 0.7** *	**6.7 ± 0.7** *	**6.6 ± 0.7** **	**5.6 ± 0.2****
Aspect ratio	5.7 ± 0.3	5.7 ± 0.3	5.4 ± 0.7	5.9 ± 0.2
Tip shape index	1.5 ± 0.3	1.4 ± 0.3	2.0 ± 0.5	1.5 ± 0.3

The regression of wing loading against mass in both populations of *R. capensis* and *R. swinnyi* (Figure 3.5) indicates that selection has acted on body size in the two populations of each species and in the two species. An ANCOVA for the homogeneity of slopes found that wing loading does increase with increasing mass ($F = 40.0$, $P < 0.000001$), but there is no significant difference in the relationship between wing loading and mass for the two species ($F = 0.41$, $P=0.52$). The slopes for *R. swinnyi* and *R. capensis* are not significantly different (interaction of mass and species $F = 1.8$, $P = 0.18$).

An ANOVA on the wing loading residuals showed that the regression lines are coincidental ($F = 0.00$, $P>0.05$).

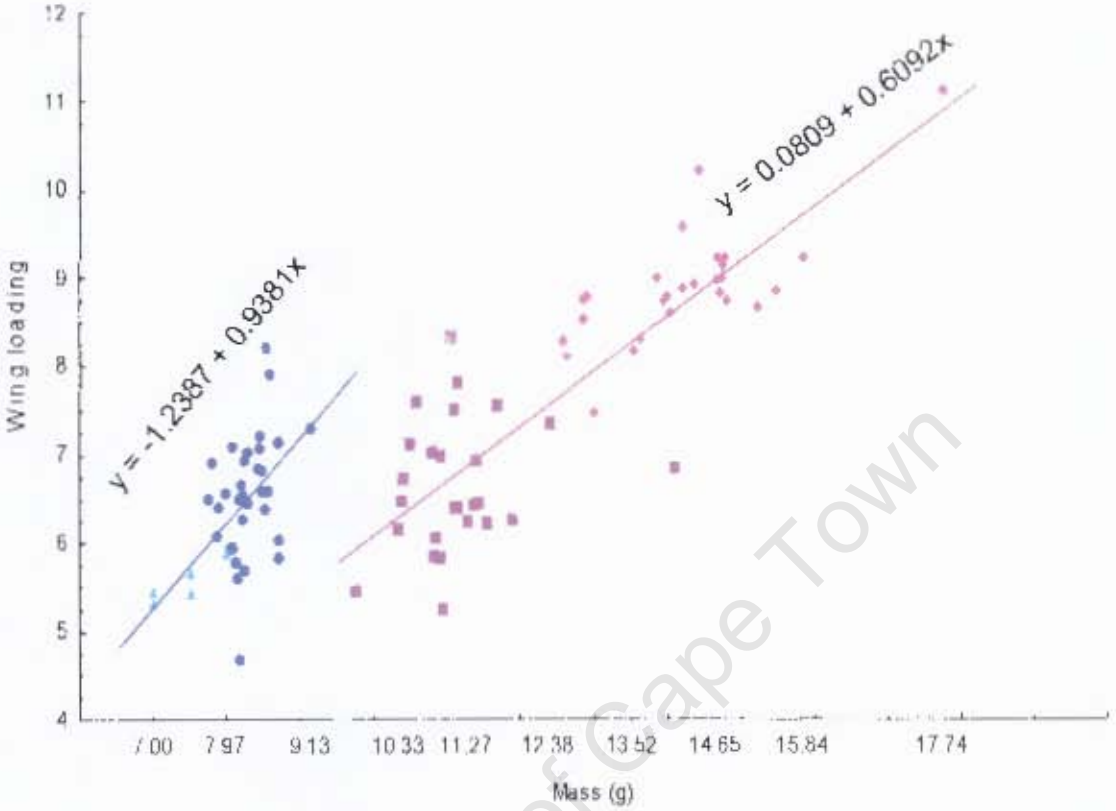

Figure 3.5. The relationship between mass and wing-loading in *Rhinolophus swinnyi* (y = -1.2387 + 0.9381x, $R^2 = 0.3$) and *Rhinolophus capensis* (y = 0.0809 + 0.6092x, $R^2 = 0.7$) The two populations of *R. capensis* are differentiated graphically by colour and shape. De Hoop in purple squares, Table Farm in pink diamonds. *R. swinnyi* KWT is shown in dark blue circles and the Kokstad population in light blue triangles.

There is no relationship between mass and wing area (figure 3.6) in *Rhinolophus capensis* ($r = 0.07$, $df = 52$, $P > 0.6$) or in *R. swinnyi* ($r = 0.007$, $df = 36$, $P > 0.9$). It appears that there has been a disruption in the usual relationship between wing area and body size (where wing area increases allometrically with body size).

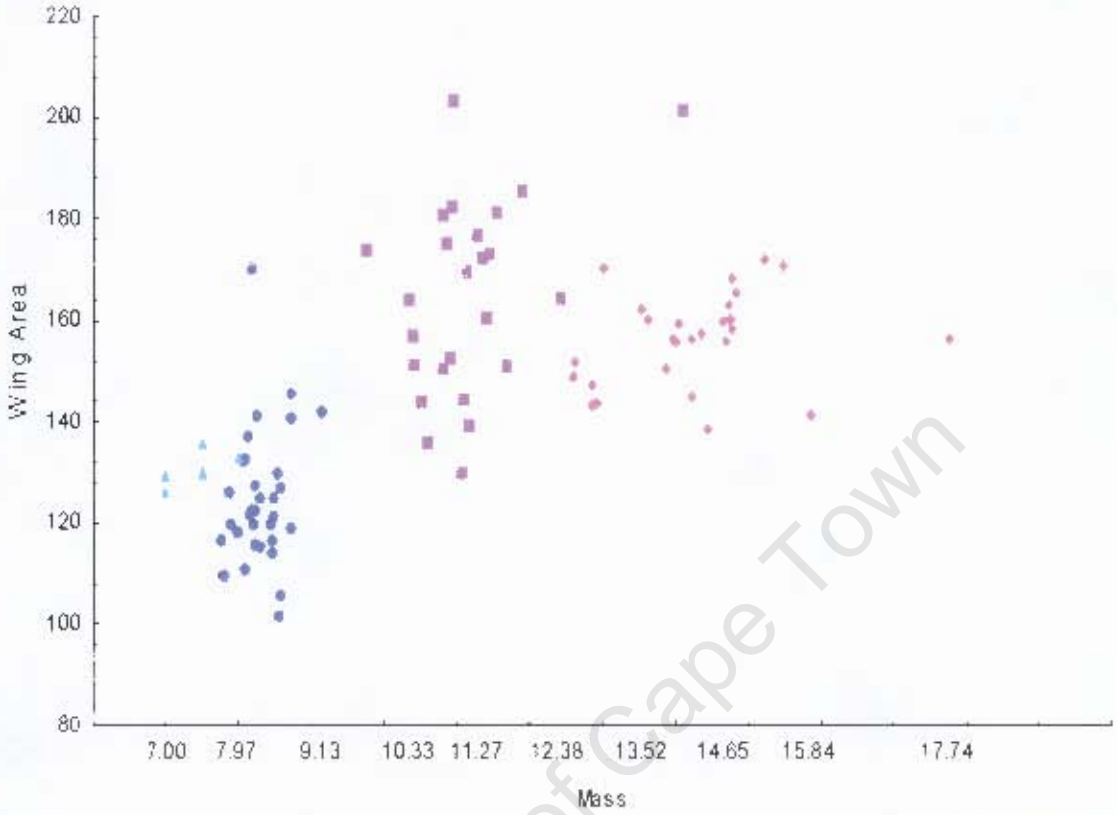

Figure 3.6. A plot of mass against wing area for *Rhinolophus swinnyi* (KWT population in dark blue circles, Kokstad population in light blue triangles) and *Rhinolophus capensis* (De Hoop population in purple squares, Table Farm population in pink diamonds). There was no relationship between the two parameters in either species.

A Principle Component Analysis that included mass, wing span and wing area as variables revealed three principal components (PCs). PC1 explains 81.4% of the variation in the data and is loaded most negatively by wingspan and wing area (Tables 3.2. and 3.3.). I have considered PC1 as a 'wing size' component. PC2, which explains 14.6% of the variation, is loaded highest, and positively, by mass and is thus considered to the "body mass" component. PC3 only accounts for 4.1% variation and is not dominated by any of the three variables.

Table 3.2. Eigenvalues and percent variation for the six Principal Components from the analysis of mass and wing parameters for *Rhinolophus swinnyi* (KWT and Kokstad) and *Rhinolophus capensis* (De Hoop and Table Farm).

	Eigenvalue	%Variation
PC1	2.4	81.4
PC2	0.4	14.6
PC3	0.1	4.1

Table 3.3. The variable loadings of each variable on the first four principal components from the PCA of mass and wing parameters of both populations of *Rhinolophus capensis* and *R. swinnyi*.

	PC1	PC2	PC3
Variables			
Mass	-0.8	0.6	-0.0
Wingspan	-0.9	-0.2	0.3
Wing area	-0.9	-0.3	-0.2

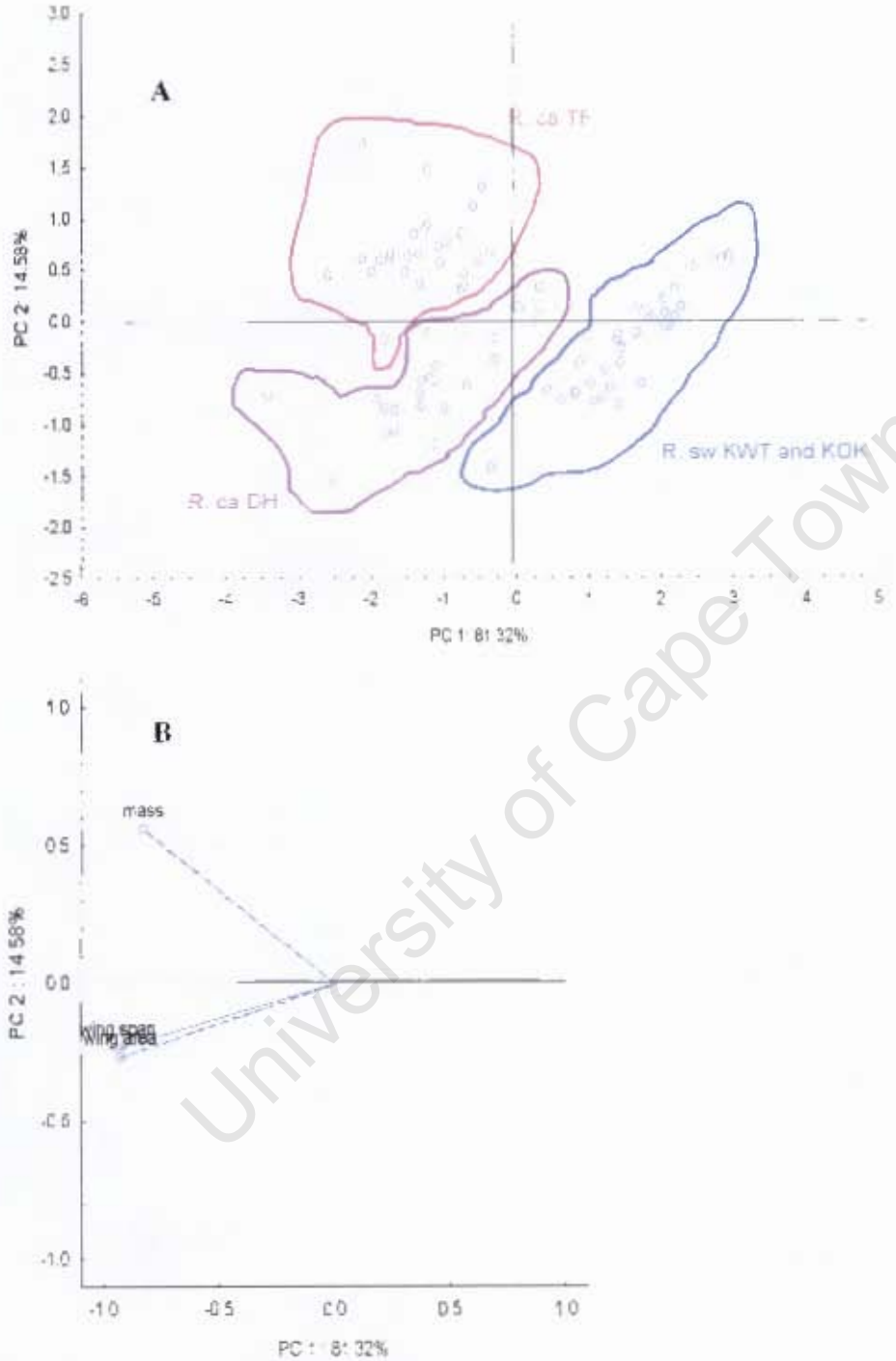

Figure 3.7. A). Case loadings on PC1 and PC2 for *Rhinolophus swinnyi* (R.sw KWT and Kokstad) and *Rhinolophus capensis* (R.ca De Hoop and Table Farm). *R. capensis* (Table Farm) is circled in pink, *R. capensis* (De Hoop) in purple and *R. swinnyi* (KWT and Kokstad) in blue. B) Plot of the variable loadings on PC1 against PC2. The variables include body mass, wing span and wing area.

The Principal Component Analysis shows a separation of the two *Rhinolophus capensis* populations along PC2; body size. *R. swinnyi* populations overlap each other completely along PC1 and PC2. However, *R. swinnyi* loads more positively on the wing size axis (PC2). Since *R. swinnyi* is smaller than *R. capensis*, wing size decreases along the x-axis in this figure.

Skull parameters

Rhinolophus capensis has a longer skull and dentary than *R. swinnyi* (Table 3.4). Despite differences in skull length, the height of the condyle and coronoid process is the same for both species. All parameters, except for the ratio of a:b, are significantly different and have negligible measurement errors. In the PCA and DFA below, the species are divided into their constituent populations to show trends in skull differences in different geographic areas. This was not done for significance testing due to very low sample sizes in some populations.

Table 3.4. Mean ± standard deviation for all skull parameters (cm) for *Rhinolophus capensis* (*R. ca*) from De Hoop and Het Kruis and *Rhinolophus swinnyi* (*R. sw*) from King Williams Town (KWT) and Kruger National Park (KNP). Significant results shown in bold.

Bat species	*R. sw*	*R. ca*	U* and significance	Standard error
Number of bats	16	18		
Skull parameters				
a [distance from the anterior surface of the mandibular fossa to the origin of the masseter muscle (bottom of the left angular process)]	0.4 ± 0.0	0.5 ± 0.0	48 **P<0.001**	0.001
b [distance from the top of the left condyle to the insertion of the masseter muscle (bottom of the left angular process)]	0.2 ± 0.0	0.3 ± 0.0	38 **P<0.001**	0.0009
a:b [ratio of a to b]	1.8 ± 0.4	1.6 ± 0.4	95 P=0.09	N/A
d [height of the condyle (top of the left condyle to the plane of the alveoli of the left first and second molar)]	0.3 ± 0.0	0.3 ± 0.0	0 **P<0.00001**	0.001
e [height of the coronoid process (top of the left coronoid process to the plane of the alveoli of the left first and second molar)]	0.2 ± 0.0	0.2 ± 0.0	10 **P<0.00001**	0.0008
f [length of skull (from the occipital to the alveolus of the canine)]	1.8 ± 0.0	2.1 ± 0.1	0 **P<0.00001**	0.0009
g [length of the dentary (from the back of the left condyle to the epiphysis of the dentary)]	1.1 ± 0.1	1.3 ± 0.1	49 **P<0.01**	0.0006
h [length of the left maxillary tooth row (from the front of the left fourth premolar to the back of the left third molar)]	0.5 ± 0.0	0.6 ± 0.0	0 **P<0.00001**	0.0009
i [dentary thickness (from the plane of the alveoli of the left first and second molar to the bottom of the left dentary)]	0.1 ± 0.0	0.2 ± 0.0	4 **P<0.00001**	0.001

*Mann-Whitney U test

In the Principal Component Analysis of skull parameters for all four populations of both species (Figure 3.8), PC 1 explains the most variation (64.9%) followed by PC2 (23.1%, Table 3.6.). PC3 only accounts for 4.9% of the variation. There is separation of the two species along PC1 but almost none along PC2. Skull length (f) and dentary thickness (h) loads highest on PC1 suggesting that this component is largely a size component with the largest species (*R. capensis*) having the larger skull. The fact that there is very little separation along PC2 separating the two species suggests that gape (a:b ratio loads highest on this component) and the general shapes of the skulls of the two species has not changed much. The a:b ratio is a measure of gape, such that a larger ratio of the distance from the mandibular fossa to the origin of the masseter muscle, to the distance of the condyle to masseter muscle insertion site, the larger the effective gape, and the larger the prey that can be taken (Freeman 1979). This is supported by the fact that the smaller *R. swinnyi* has the same sized parameters as the larger *R. capensis* (e.g. height of the condyle (d), height of the coronoid process (e)). Higher condyles are associated with greater masticatory power, whereas a lower coronoid process and larger condyle is found in carnivores where greater biting force is important. The maxillary tooth row length relates to the amount of prey struggling that the bat can cope with since thickening of bones is associated with greater amounts of stress on it (Freeman 1979). Thus it appears as if the skull has retained its function despite the change in size. The Het Kruis and De Hoop *R. capensis* populations are separated obliquely when PC1 and PC2 are considered, where the Het Kruis population has a larger gape. This separation is explored further below.

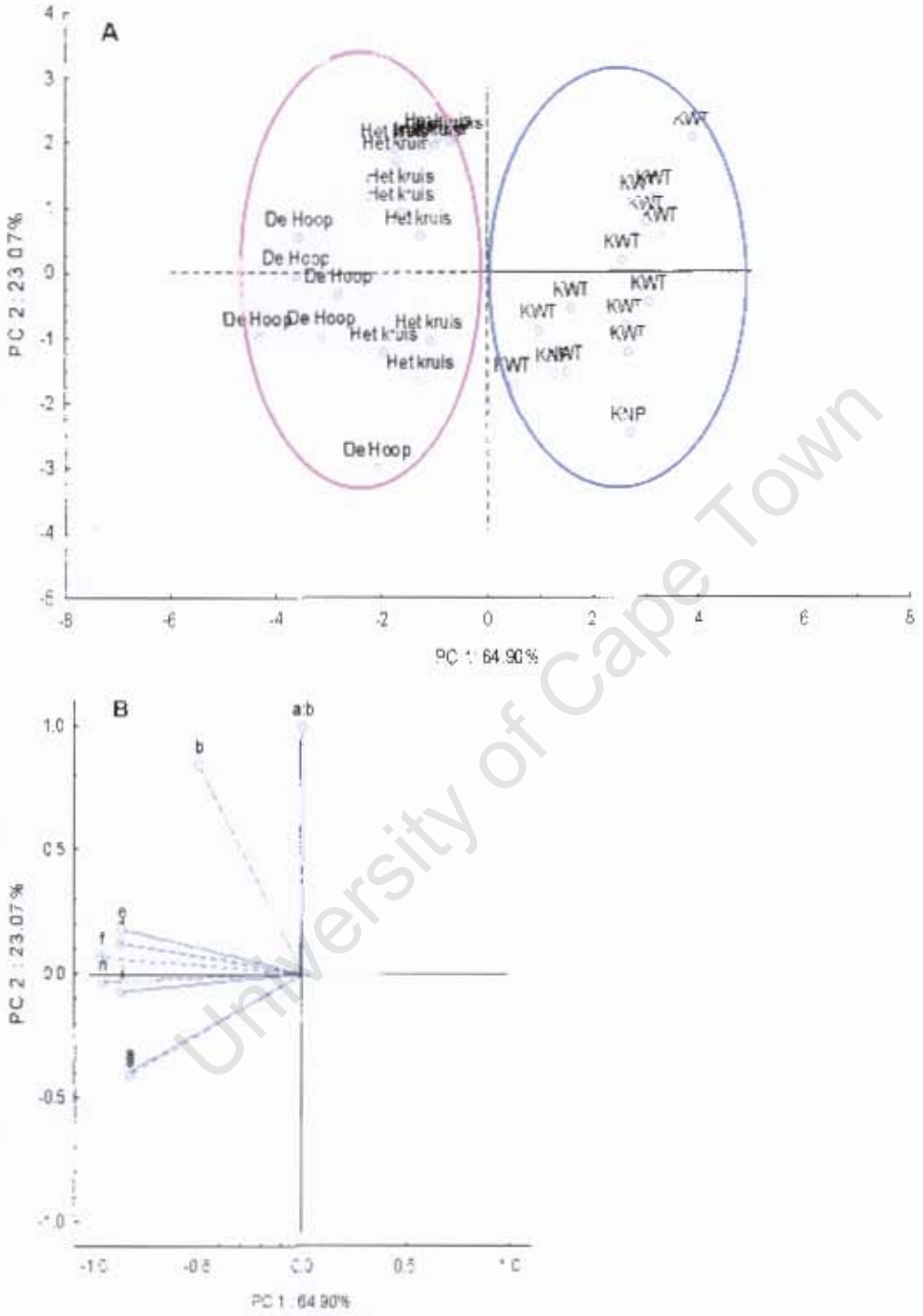

Figure 3.8. A) Case loadings on PC1 and PC2 for *Rhinolophus swinnyi* (KWT and Kruger National Park) and *Rhinolophus capensis* (De Hoop and Het Kruis). *R. capensis* is circled in pink and *R. swinnyi* in blue. The maxillary tooth row and skull length decrease along the x-axis.
B) Plot of the variable loadings on PC1 and PC2. Variables consist of eight skull parameters and one ratio of skull parameters.

A Prinicipal Component Analysis of the skull measurements from the two populations of *R. capensis* (Figure 3.9) showed that most of the variation in the skull data is contained in the first three Principal Components (PCs). PC1 accounts for 51% of variance, followed by PC 2 (25%) and PC3 (10.1%; Table 3.7).

Table 3.5. Eigenvalues and percent variation from the PCA of skull parameters for *Rhinolophus swinnyi* (KWT and KNP) and *R. capensis* (De Hoop and Het Kruis).

	Eigenvalue	%Variation
PC1	5.8	64.9
PC2	2.1	23.1
PC3	0.4	4.9
PC4	0.2	2.6
PC5	0.2	2.1
PC6	0.1	1.5
PC7	0.1	0.7
PC8	0.0	0.3
PC9	0.0	0.0

Table 3.6. Variable loadings on the nine Principle components from the PCA of skull parameters for *Rhinolophus capensis* and *R. swinnyi*.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Variables									
A	-0.8	-0.4	0.3	-0.1	-0.0	-0.1	-0.1	-0.0	-0.0
B	-0.5	0.8	0.1	-0.1	-0.1	0.0	-0.1	-0.0	0.0
a:b	-0.0	1.0	-0.1	-0.0	-0.1	0.0	0.0	0.0	-0.0
D	-0.9	0.1	-0.3	0.3	0.1	-0.0	-0.1	0.0	-0.0
E	-0.9	0.2	0.3	0.1	0.2	0.1	0.1	0.0	-0.0
F	-1.0	0.1	-0.0	-0.1	0.0	-0.1	0.1	-0.1	-0.0
G	-0.8	-0.4	-0.1	0.1	-0.3	0.2	0.0	-0.0	-0.0
H	-1.0	-0.0	-0.0	0.0	-0.1	-0.2	0.1	0.1	0.0
I	-0.9	-0.1	-0.3	-0.3	0.1	0.1	-0.0	0.0	-0.0

Parameters a, g and h load highest of PC1. These three parameters are concerned with the length of the skull and dentary and the distance from the mandibular fossa to the

origin of the masseter muscle and maxillary tooth row. A larger distance from the mandibular fossa to the origin of the masseter muscle relative to the distance from the condyle to the insertion site of the masseter muscle indicates a larger gape. Since both populations have the same values for the latter measurement (b), the increased gape in the Het Kruis population is a consequence of the increased distance of the former measurement (a). PC1 will be considered a 'size and gape' component.

Parameters b and e load highest on PC2. An increase in the height of the coronoid process (e) provides leverage for the masseter muscle and consequently greater crushing power of the jaw. The coronoid process is usually high in carnivores, which have a strong biting force for slicing through flesh. The distance from the condyle to the origin of the masseter muscle is proportional to the size of the gape such that a bat with a smaller distance has a larger gap (Freeman 1979, 1981). The size and hardness of the prey that a bat can process will be determined by this component since it is a measure of gape and crushing ability. This principal component will be called 'bite force and gape'.

R. capensis (De Hoop) has a relatively small gape for its size because of the small distance from the condyle to masseter muscle origin. Parameter d loads highest on PC3 and corresponds with the height of the condyle. Bats with a larger condyle usually have larger masseter muscles and thicker dentaries, making them better suited to deal with hard prey (Freeman 1979). The two populations of *R. capensis* are separated mostly on PC1 (Figure 3.8A), such that the Het Kruis population can take larger prey due to a wider gape and larger skull than the De Hoop population. The separation along PC2 is probably mostly due to the difference in gape as well.

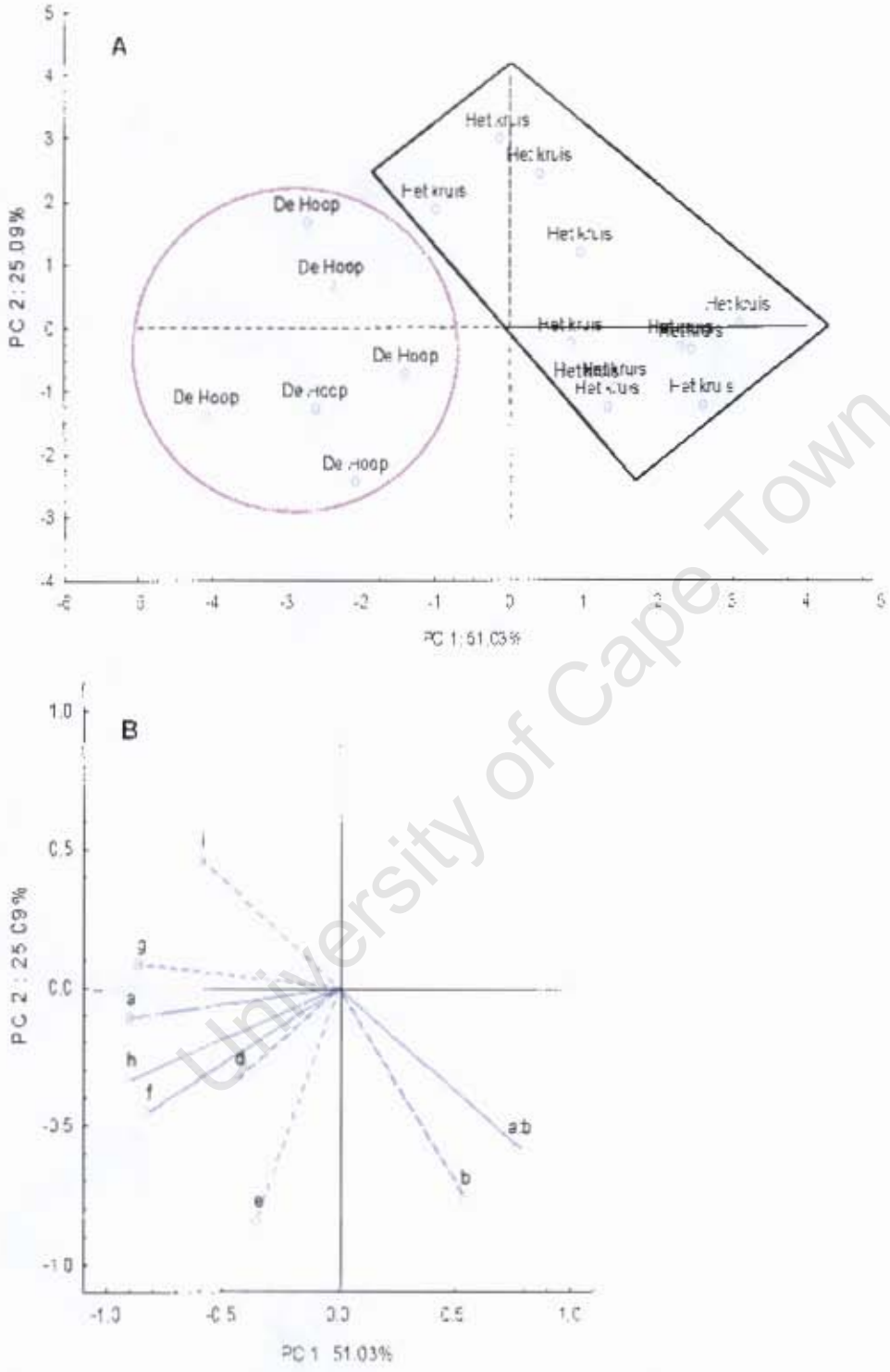

Figure 3.9. A) Case loadings on PC1 and PC2 for *Rhinolophus capensis* in De Hoop and Het Kruis. *R. capensis* (De Hoop) is circled in purple and *R. capensis* (Het Kruis) in black. B). Plot of the variable loadings on PC1 and PC2. Variables consist of eight skull parameters and one ratio of skull parameters

Table 3.7. Eigenvalues and percent variation for the nine Principal Components from the analysis of skull parameters for *Rhinolophus capensis* (De Hoop and Het Kruis).

	Eigenvalue	%Variation
PC1	4.6	51.0
PC2	2.3	25.1
PC3	0.9	10.1
PC4	0.5	5.9
PC5	0.4	4.1
PC6	0.2	2.3
PC7	0.1	1.0
PC8	0.1	0.7
PC9	0.0	0.0

Table 3.8. The loadings of each parameter on the nine Principle Components for the PCA of skull parameters for *Rhinolophus capensis* (De Hoop and Het Kruis).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Variables									
A	-0.9	-0.1	-0.3	-0.1	0.0	-0.2	-0.1	-0.1	-0.0
B	0.5	-0.8	-0.2	0.3	0.1	-0.1	-0.0	-0.1	0.0
a:b	0.8	-0.6	-0.0	0.2	0.1	-0.0	0.0	0.0	-0.0
D	-0.4	-0.3	0.8	0.2	-0.1	-0.0	-0.1	-0.0	-0.0
E	-0.4	-0.8	-0.2	-0.1	-0.1	0.3	-0.1	0.0	-0.0
F	-0.8	-0.5	0.0	0.0	-0.3	-0.0	0.2	-0.0	-0.0
G	-0.9	0.1	0.1	0.0	0.5	0.2	0.1	-0.0	-0.0
H	-0.9	-0.3	-0.0	-0.0	0.1	-0.2	-0.0	0.2	0.0
I	-0.6	0.5	-0.2	0.6	-0.1	0.1	-0.0	0.0	-0.0

A Discriminant Function Analysis for the principal component scores from the PCA of skull parameters of *Rhinolophus capensis* and *R. swinnyi* yielded a model with three discriminant functions (Table 3.10) with a Wilk's Lambda = 0.004 ($F_{(27,61)}$ = 13.4, P<0.0001).

Principle Component 1 differentiated best between groups with Wilk's Lambda = 0.1, $F_{(3,21)}$-to-remove = 263.3, P<0.000001, followed by PC6 (Wilk's Lambda = 0.01,

$F_{(3,21)}$-to-remove = 15.3, P<0.00001) and then by PC 2 (Wilk's Lambda = 0.01, $F_{(3,21)}$-to-remove = 9.9, P<0.001) and PC5 (Wilk's Lambda = 0.01, $F_{(3,21)}$-to-remove = 8.9, P<0.01). PC 7 had a Wilk's Lambda = 0.01, $F_{(3,21)}$-to-remove = 6.4 P<0.01 and PC 3 had a Wilk's Lambda = 0.01, $F_{(3,21)}$-to-remove = 4.7, P<0.01. PCs 4 and 8 were not significant contributors to the Discriminant Function model.

Figure 3.10 shows separation of the species on DF1 and separation of the populations of the species on DF2. The standardised coefficients for the discriminant functions show that PC1 loads highest on DF 1 and PC2 loads highest on DF2. The species are thus differentiated by jaw strength, *Rhinolophus* capensis having a more robust skull. *Rhinolophus swinnyi* KWT and *R. capensis* Het Kruis have larger gapes than their conspecific populations.

Table 3.9. Standardised coefficients for the three Discriminant Functions for the principal components of skull parameters.

	Discriminant Function 1	Discriminant Function 2	Discriminant Function 3
	Standardised coefficient	Standardised coefficient	Standardised coefficient
Variable			
PC1	1.9	0.1	-0.1
PC2	-0.3	-1.0	-0.3
PC3	0.2	0.8	0.1
PC4	0.4	0.1	0.5
PC5	0.6	-0.9	0.1
PC6	1.2	-0.6	0.4
PC7	-1.0	0.0	-0.6
PC8	-0.3	0.4	0.4
PC9	-0.4	0.3	-0.7
Eigenvalue	0.6	-0.9	0.1

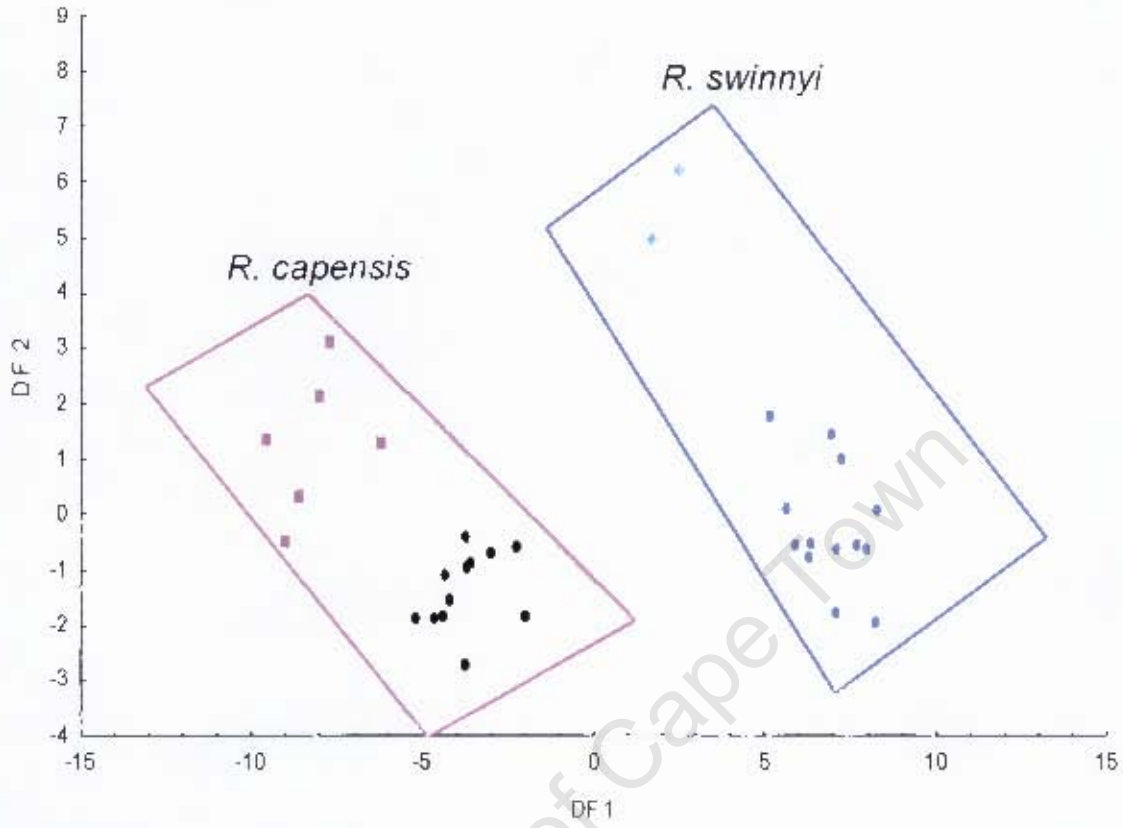

Figure 3.10. A plot of Discriminant Function 1 and 2 for *Rhinolophus capensis* (purple square) and *R. swinnyi* (blue square). The *R. capensis* (De Hoop) population is shown in purple squares and the Het Kruis population in black circles. The *R. swinnyi* (KWT) population is shown in dark blue circles and the KNP individuals in light blue diamonds.

The squared Mahalanobis distances showed that the *R. swinnyi* (KWT) population is more different from the *R. capensis* (De Hoop) population (D^2=230.8) than from the *R. capensis* Het Kruis population (D^2=115). The populations of *R. capensis* from Het Kruis and De Hoop are more similar to each other (D^2=29.4) than the two populations of *R. swinnyi* (D^2=60.45). *Rhinolophus swinnyi* (KNP) and *R. capensis* (Het Kruis) differed by (D^2=82.5) and *R. swinnyi* (KNP) and *R. capensis* (De Hoop) by (D^2=129).

Ecological Divergence

Rhinolophus swinnyi and *R. capensis* both have diets dominated by Lepidopterans, followed by Coleopterans. *Rhinolophus capensis* took no dipterans and *R. swinnyi* took no hemipterans. One *R. swinnyi* individual had almost its entire faecal volume composed of hymenopterans which was probably a single opportunistic event since this order was not evident in the faeces of any other individuals (Table 3.10).

Table 3.10. Mean ± SD percent volume of prey categories in the diet of *Rhinolophus swinnyi* and *Rhinolophus capensis* and the length of beetle claws found in the pellets.

Bat species and age	*R. swinnyi* (February)	*R. swinnyi* (April)	*R. capensis* (April)
Number of bats	20	16	26
Prey type			
Lepidoptera	89.3 ± 22.0	68.5 ± 36.3	95.8 ± 4.1
Coleoptera	5.6 ± 18.1	29.0 ± 33.02	3.9 ± 4.2
Diptera	2.3 ± 6.1	0	0
Hemiptera	0	0	0.007± 0.04
Hymenoptera	0	6.2 ± 24.67	0
Unknown	2.8 ± 8.5	0	0.3 ± 1.1
Beetle claw size			
Sample size		7	8
Mean (mm) ± SD (Minimum and maximum in brackets)		2 ± 1.3 (0.75-4mm)	2.8 ± 0.3 (2.25-3.25mm)

A dendrogram (Figure 3.11A) identifies three major groupings according to dietary composition. The two species show a large amount of overlap, which is illustrated by none of the groups being dominated by either species with the exception of the group to the left of the MDS plot (Figure 3.11B), depicted to the right of the dendrogram.

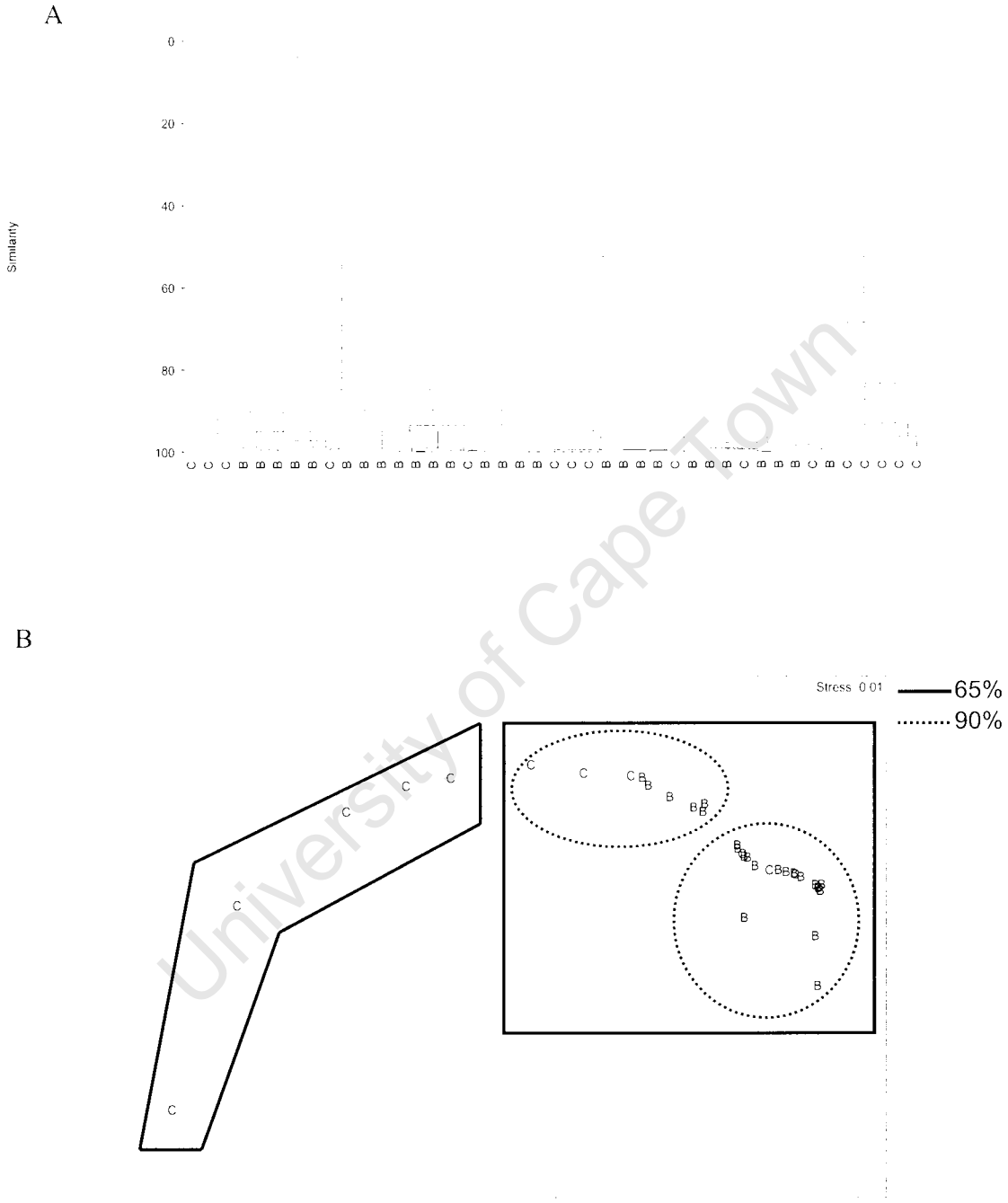

Figure 3.11. A dendrogram of the relationships in dietary composition between *Rhinolophus capensis* (B) and *Rhinolophus swinnyi* (C) in the same month and the Multi Dimensional Scaling plot (MDS) of similarities in dietary composition between *Rhinolophus capensis* (B) and *R. swinnyi* (C) in April. Two groups are blocked, which are composed of individuals with 65% similar in dietary composition (smooth line). One of the groups is made up of two sub-groups that are 90% similar (dotted line). This figure was created using the Bray-Curtis measure of similarity.

The MDS plot confirms the large amount of overlap in the diets of the two species. The stress level is very low at 0.01, indicating that the similarities and differences between the diets of the individuals have largely been maintained during the compression of the data into two dimensions. The groups identified in the MDS plots and which are mixed with respect to species, with the exception of the group to the left composed entirely of *R. swinnyi*, are significantly different (ANOSIM R statistic = 0.273 and P = 0.002). The individual that ate predominantly Hymenoptera was excluded from the MDS plots since the degree to which it polarised the data obscured the degree to which the other individuals are similar. It is, however, included in the dendrograms and is visible on the far left. These results suggest that inter-individual differences are more pronounced than interspecific differences.

The subgroup on the far right has individuals with diets dominated by Lepidopterans (Figure 3.11B). The centre subgroup has individuals with diets dominated by Lepidopterans but with some Coleopterans. The group on the left is composed of individuals with a high proportion of Coleopterans in their diet.

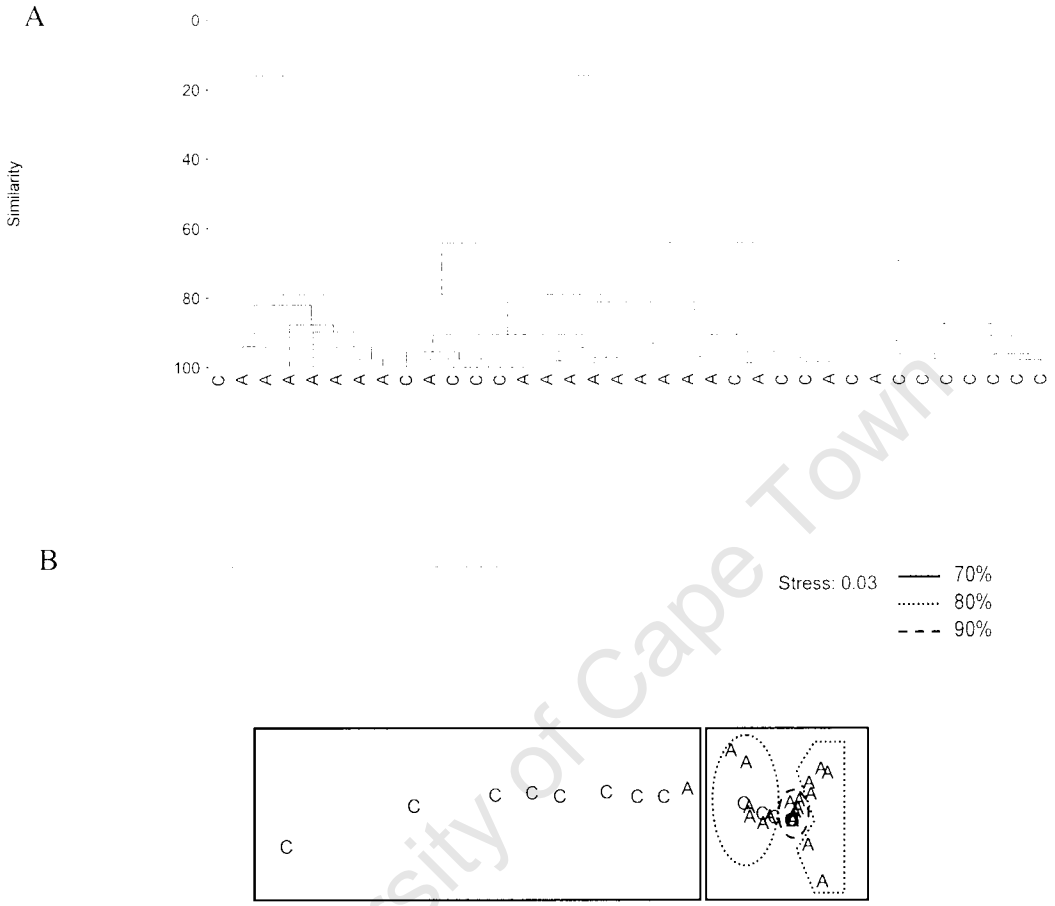

Figure 3.12. The Multi Dimensional Scaling plot (MDS) of similarities in dietary composition between *Rhinolophus swinnyi* February (A) and *R. swinnyi* April (C). Two groups are blocked, which are composed with individuals with 70% similar in dietary composition (smooth line). One of the groups is made up of three sub-groups that are 80% similar (dotted line) and 90% similar (dashed line).

For the MDS plot of the diet of *R. swinnyi* in February and April, the group on the left has individuals with diets that increase in the proportion of Lepidopterans to Coleopterans from left to right (Figure 3.12B). The group on the right is made up of individuals who also consumed orders other than Coleoptera and Lepidoptera. The

subgroups are divided according to the proportions of these orders that the bats took. This figure was calculated using the Bray-Curtis measure of similarity.

A Similarity Percentage (Simper) Analysis shows a large degree of similarity within the two species; 91.25% similarity in *R. swinnyi* individuals and 92.53% in *R. capensis*. There is only 9.04% dissimilarity between the two species (Table 3.12). Lepidopterans account for most of the intra-species similarity for both species and Coleopterans account for most of the dissimilarity (5.11%) between species. Only values for the highest contributing variables are shown.

Table 3.11. Results of the Simper Analysis of the dietary compositions of *Rhinolophus swinnyi* in February and in April.

	Rhinolophus swinnyi February	*Rhinolophus swinnyi* April	February-April
Average percent	Similarity=91.25	Similarity=63.16	Dissimilarity=29.77
Prey type			
Lepidoptera	96.82	81.82	30.70
Coleoptera	-	18.18	49.69
Hymenoptera	-	-	9.73

Table 3.12. Results of the Simper Analysis of the dietary compositions of *Rhinolophus swinnyi* in April and *R. capensis* in April.

	Rhinolophus swinnyi April	*Rhinolophus capensis* April	*R.swinnyi-R.capensis*
Average percent	Similarity=63.16	Similarity=92.53	Dissimilarity=27.09
Prey type			
Lepidoptera	81.82	95.27	33.75
Coleoptera	18.18	-	53.69
Hymenoptera	-	-	10.69

A backwards stepwise regression of the proportion of moths in the diet against wing parameters, peak echolocation frequency and mass was performed to see which was the best predictor of percentage moths in the diet. The regression yielded a model (r =

0.374390, F = 64.57932 and P < 0.01) which eliminated all variables except wing tip shape (t = -3.35384, P<0.01). Wing tip shape is negatively associated with the incidence of moths in the diet. The same analysis on proportion of beetles in the diet yielded the model with Wilks' Lambda =0.7 $F_{(7,54)}$ = 3.1, P<0.009. It eliminated all variables except wing loading, which was negatively associated with the percentage beetles in the diet (t = -2.1, P<0.05). Wing loading decreased with increased beetles in the diet and the wing tip shape became more pointed when more Lepidopterans were found in the diet. This indicates that slower flight speeds and greater manoeuvrability are advantageous for catching these insect orders.

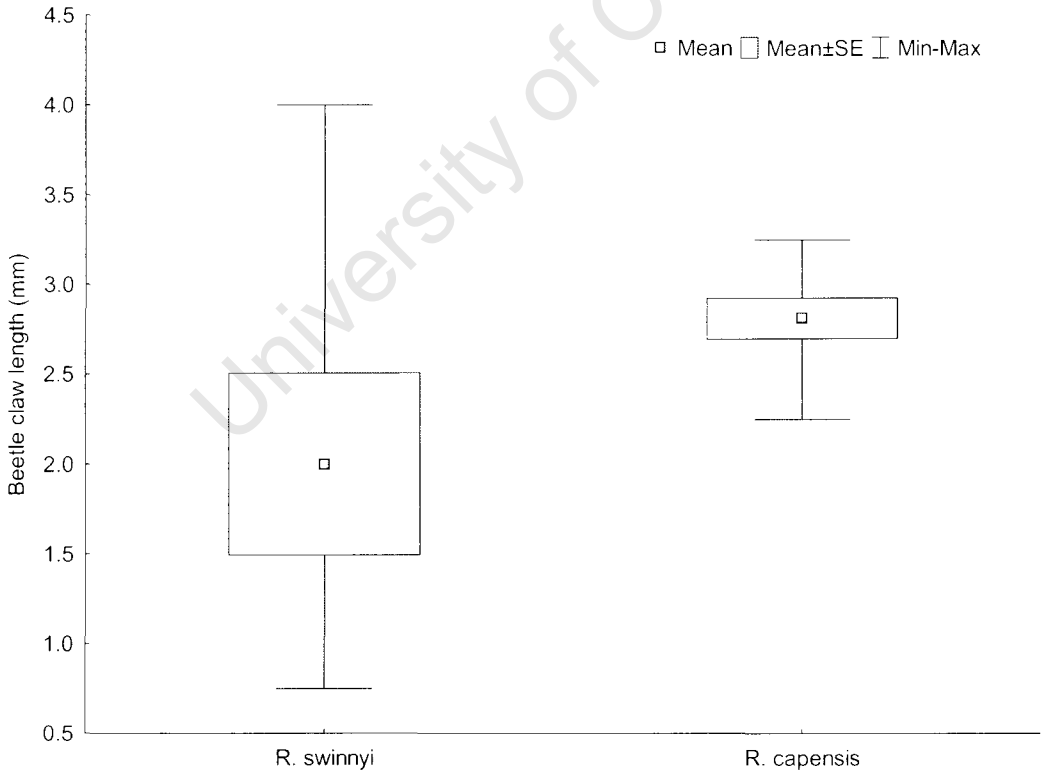

Figure 3.13. Box plot of the mean, standard error and minimum and maximum values of beetle claw lengths found in the faeces of *Rhinolophus swinnyi* and *Rhinolophus capensis*.

Although the median size prey taken by *Rhinolophus swinnyi* was slightly smaller than that taken by *R. capensis,* the median size of prey was not statistically different (Mann-Whitney U=17.5, P=0.22; Figure 3.13). However, *R. swinnyi* took a larger range of prey than *R. capensis* at both ends of the spectrum (Figure 3.13). The largest prey that *R. swinnyi* took was larger than that taken by *R. capensis* and the two species had significantly different variances (Levene's test $F_{1,13} = 21.2$, $P = 0.0005$). *Rhinolophus swinnyi* may have been able to detect smaller prey than because they have higher frequency and broader bandwidth calls, whilst large prey remained available to them because of their similarly sized gape (see PCA analyses on skull parameters above).

CHAPTER 4

DISCUSSION

There were marked ecomorphological differences between *Rhinolophus capensis* and *R. swinnyi*, and between populations within each species. *Rhinolophus capensis* is a bigger bat than *R. swinnyi* and, as predicted by the inverse relationship between mass and echolocation frequency (Jones 1999), it has a lower echolocation call frequency. Wing morphology and echolocation calls showed allometric responses to the change in mass. Although the skulls of *R. capensis* were bigger, the shape and therefore the function of the skulls appeared to remain the same. *R. capensis* is, therefore, essentially a scaled up version of *R. swinnyi*. I review the differences between species, the likely functional significance of these differences and outline their possible causes.

The differences in body mass between populations of the same species are not the result of pregnancy, the inclusion of sub-adult samples, or due to the sampling of another species by mistake. Table Farm bats were caught in April and King Williams Town bats in February, which is prior to fertilisation (Bernard 1985). In addition, no significant difference was found between the masses of males and females of both populations of *Rhinolophus capensis* (De Hoop) or *R. swinnyi* (KWT). The mass, forearm and call frequency together exclude the possibility of *R. capensis* bats being mistaken for *R. darlingi*. Furthermore, the anterior premolar was observed in the tooth row of *R. capensis* individuals.

There is a difference of 22 kHz in peak echolocation frequency between *Rhinolophus capensis* and *R. swinnyi*. Higher frequency calls, like that of *R. swinnyi*,

attenuate more in air but also enable the bats to resolve smaller objects (Vaughan 1972). Lawrence and Simmons (1982) show that bats with calls over 100 kHz (e.g. *R. swinnyi*) lose over 3dB/m to atmospheric attenuation, whereas *R. capensis* would lose between 2.3 and 2.7dB/m. They suggest that bats with echolocation frequencies over 100 kHz must have selection pressures acting on features that are of greater importance than prey detection range. The very high frequencies at which these bats are calling are unlikely to translate to a functional difference in terms of range and the resolution of small objects. The difference in call peak frequency between species equates to about a 1mm difference in wavelength. The dietary analysis showed that there is no significant difference in the size of the prey taken, which supports the assertion that the functional difference in frequency is negligible, although the sample size in the dietary analysis is relatively low and the mean prey size could change with a higher sample size or with changes in insect availability over the course of a year.

Since higher frequencies confer increased resolution, they would be advantageous for foraging in areas of increased clutter and structural complexity. *Rhinolophus capensis* calls are longer and have narrower bandwidths than those of *R. swinnyi*. According to predictions about the echolocation of bats flying in cluttered habitats (Schnitzler and Kalko 1998), *R. capensis* calls may be better suited to a relatively more open environment, albeit still cluttered. Despite these predictions, the difference in environmental attenuation and wavelength indicate that there is little functional difference in the calls of these two species as outlined above. Harmonic hopping in rhinolophids has been shown to be a basis for speciation, although this was in bats calling at relatively low frequencies (27.2-53.6 kHz, Kingston and Rossiter 2004). It is

consequently unlikely that the calls of *R. capensis* and *R. swinnyi* are a significant driving factor in the divergence of these species.

The Fynbos is up to 3m high (Rutherford and Westfall 1986) and forms dense clusters of plants which bats cannot fly through. *Rhinolophus capensis* flies predominantly within 1m above the vegetation (Jacobs *et al.* 2007) and probably forages in clutter as it negotiates the edge of the plants. Grassland, like the Fynbos, is fairly homogenous and less complex than savannah and forest environments. *R. swinnyi* in Grassland probably flies just above the vegetation in a similar manner to *R. capensis* in the Fynbos due to their gross similarity in form.

The forest, on the other hand, is highly cluttered with horizontal and vertical obstacles and presents structural complexity in 3 dimensions. The forest is composed of plant species of various heights but the majority are large and the tallest is tens of meters high. *R. swinnyi* in the forest flies within the canopy and therefore forages in a high degree of clutter. *R. swinnyi* is not restricted to forests, however, and pockets of tropical thicket up the east coast of South Africa may present a cluttered environment, to which *R. swinnyi* is best suited to exploit based on echolocation and wing morphology. However, since *R. swinnyi* inhabitats both forest and savannah/Grassland, it is unlikely that clutter alone is responsible for the difference in body size and frequency. Instead, it is more likely that selection acted on body size with allometric responses in echolocation frequency. This is supported by both species scaling allometrically with other members of the Rhinolophidae for call frequency.

Body size affects and is affected by all aspects of an animal's biology. The most important effects in this case may include foraging range and generality of diet. A small

body size confers certain advantages to bats in particular biomes. Smaller bats are more manoeuvrable, able to fly through small spaces (Norberg and Rayner 1987), and have jaws that are stronger relative to their size (since muscle power varies by the square of its linear measurements but volume varies by the cube, Freeman (1979)). Since *R. swinnyi* has a smaller body, it would be better adapted for forest and savannah biomes because these habitats are relatively cluttered.

There is a greater abundance of flying insects in the forest and savannah biomes (Proches and Cowling 2006) than in the Fynbos, which may be due to the higher annual rainfall in these biomes. The increased manoeuvrability of *R. swinnyi* would be advantageous for taking the available insects in clutter. In resource poor habitats, such as the Fynbos, a small size would be a distinct disadvantage. This is because of the ratio of surface area to volume, which makes metabolic running costs relatively high (McNab 1980). Since wing loadings are reduced in small bats, commuting flights for finding food would be energetically inefficient, as increased wing loading is good for higher flight speeds. Small body sizes, therefore, would not be suited to an environment where resources are sometimes limited or are highly dispersed.

On the other hand, a large body size would confer certain advantages in sub-optimal habitats where resources are sometimes in short supply. Insectivorous bats have relatively low basal metabolic rates, apparently to mitigate the energetic effects of the high seasonality of insect availability (McNab 1980). The use of torpor in insectivorous bats is an additional means to mitigate energetic costs (Barclay et al. 2001), although this is not addressed in this project. Bigger insectivorous bats have an added energetic advantage to being large in areas of high variability and unpredictability in that their

larger size would assist them in covering more ground in the search for food. Be this as it may, larger bats would have greater overall energy requirements.

In addition, feeding specialists are big or small in size but generalists are always big (Brown *et al.* 1993, Swartz *et al.* 2003). It follows that a generalist bat would be able to take a greater range of insects, whereas a small bat would be restricted to small prey and for which it is specialised. Larger body sizes would then be at a clear advantage when insects are difficult to find or when the preferred prey is unavailable. Bigger generalists also tolerate habitat fragmentation better than small ones and highly mobile mammals view habitat fragmentation on a landscape scale (Gehring and Swihart 2003), making it likely that *R. capensis* is at an advantage over *R. swinnyi* at the ecotone between Fynbos and Grassland/savannah. This may also account, in part, for the increased size of *R. swinnyi* in the population at the ecotone.

Commuting flight, which is beneficial for finding adequate food in a single night, requires faster flight speeds so that larger distances are covered. As a result, bats that perform commuting flights generally have higher wing loadings to achieve these higher flight speeds (Norberg and Rayner 1987). The explanation for the larger body mass in the Fynbos species and in the ecotone/edge populations may, therefore, also lie in the need for commuting flights to exploit less abundant resources more efficiently.

To summarise, the smaller body size of *Rhinolophus swinnyi* makes it well adapted for foraging with manoeuvrability in a high degree of clutter and for resolving small prey against a cluttered background. The relatively low wing loading conferred by lower mass makes commuting flights inefficient so this species would be better suited to an environment where resources are not limited or highly diffuse. *R. capensis*, which is

bigger in size, would be able to make energetically efficient commuting flights. I propose that the larger body size of *R. capensis,* and both populations at the respective range edges, provide a larger niche width (Jones 1996) which enhances survival in suboptimal environments. Significantly, the ecotone occurs at the edge of the range of both species. This would be a sub-optimal environment in comparison to the centre of the distribution. This edge effect may be a major reason for the increased body sizes found at the ecotone populations. Larger body sizes in both *R. capensis* and edge populations of both species could be for the same gains: wider niche widths and greater wing loadings for commuting flights.

The divergence of *Rhinolophus capensis* and *R. swinnyi* occurred about 16 million years ago (Stoffberg 2007). There was progressive desiccation from the Miocene (*ca* 20 mya) to the Plio-Pleistocene (*ca* 2-3 mya, Coetzee and Muller 1984). Grasses arose about 18mya and by the mid-Miocene (approximately 15 mya) the Fynbos was fully established (Partridge 1997). It is generally accepted that the current forest-fynbos distributions are controlled by fire (Manders 1990, Geldenhuys 1994) and that differences in soil nutrients are an effect rather than a cause of the current distribution of these two biomes. Cowling *et al.* (1999) emphasise that the eastern and western sides of the Fynbos biome are under fundamentally different controls as rainfall is less predictable and peaks in spring and autumn in the east. This difference in abiotic characters likely contributes to the differences observed in *R. capensis* populations.

The Fynbos is a nutrient poor biome with highly variable and frequent fires (Kruger and Bigalke 1984, Van Wilgen *et al.* 1985, Christian and Stanton 2004), strong winds and a high abundance of galling insects (Proches and Cowling 2006), which are

not available to bats as food. The abundance of Lepidopterans, however, is considered to be relatively high in the Fynbos (Rutherford and Westfall 1986). The flora is adapted for fire and many plant species require fire for propagation (Bond 1980). Insect abundance is, however, highly affected by fire (Swengel 2001). Moth abundance decreases in the dry season (Haber and Frankie 1989) and the dry summer months of the winter rainfall area are particularly harsh (van der Merwe *et al.* 1994). Since insect abundance is highest after first rains, the summer drought may be a time of particular resource dearth for the Fynbos bats. From the study of variation of fat stores in bats over time, it is clear that insectivorous bats experience the most variation in food availability, relative to other trophic groups (McNab 1976). This may be pronounced in the Fynbos because of fire and summer droughts. This short, diffuse supply of resources may be responsible for the increased body size of *R. capensis* as outlined above.

Ecotones represent areas of evolutionary novelty since the selection pressures are probably different to those in any one of the biomes that compose it (Smith *et al.* 1997). Furthermore, landscape fragmentation, one of the characteristics of ecotones, can affect the abundance of certain bees and beetles (Donaldson *et al.* 2002) and vegetation islands have one fifth the species of mainland patches of the same size. Insects are adversely affected by habitat fragmentation (Didham *et al.* 1996).

Populations of the two species at the periphery of their respective distributions both lie in the middle of the ecotone and are divergent from populations from within the distributions. This duality of factors that makes the environment at the transition zone/range edge is probably the main cause of differences between populations of both species. Wing area was lower in these peripheral populations and, in combination with

increased body mass, results in higher wing loadings. Differences in echolocation, wing parameters (other than wing area) and skull morphology did not deviate from allometry and could therefore be explained by the difference in size in the two edge populations in comparison to their counterpart populations within the biomes. The main difference, therefore, in the peripheral populations is the same as the difference between species; an increase in niche width and size and a decrease in wing area resulting in an increase in wing loading and consequently less manoeuvrability, but more efficient long distance flight.

Squared Mahalanobis distances indicate that *R. swinnyi* populations are more divergent from each other than *Rhinolophus capensis* populations. This is probably the result of their range covering more than one biome and occurring across latitudes, although these are likely not the only factors at play. This hypothesis was not addressed empirically in this study. Edge populations are often not highly adapted for their environment since gene flow from the rest of the distribution can have a homogenising effect and detract from local adaptation (Kawecki and Ebert 2004). The environment at the edge of the distribution may not, therefore, be as well suited to the ecophysiology and morphology of each species. The adaptive significance of phenotypic differences should thus be viewed with the dynamic relationship between natural selection and gene flow in mind.

This study may have been affected by the inclusion of data that was collected by other people, which may have introduced measurement error. As the sampling was done in two very different locations at different times of year (although within the same season), it is impossible to measure all the factors which are responsible for the

differences observed in the phenotype of these bats. Likewise, since the sampling sites where I collected data are controlled by very different climatic systems, it is possible that differences in body mass were affected by each site being at different points in the temporal fluctuation of insect abundance. This project also suffered from very low sample sizes in some populations from which the skulls were taken, and in the number of beetle claws found for the analysis of prey size. Due to the limitations that the scope of this project imposes, it was not possible to compare the complete suite of phenotypic characters that occur in an animal. Whether or not this is possible in any case is questionable. I do feel that the characters described in this project give a well rounded observation of the major characters of bats that most affect their survival: echolocation, wing morphology, body size, skull morphology and diet.

Conclusion

The major difference between *Rhinolophus capensis* and *R. swinnyi* is in body size and associated differences in echolocation peak frequency and skull and wing morphology. This difference is mirrored in the populations of the two species such that those on the edge of the distributions are larger in size than individuals from populations towards the centre of their respective distributions. *Rhinolophus capensis* prevails in a habitat that is by and large homogeneous but, due to a variety of rainfall and fire regimes and a general condition of nutrient poverty, resources are low and diffuse. Similarly, ecotones are regions of fragmentation and variability in resources. A larger body size imparts a wider niche (Jones 1996) for taking more prey types, larger wing loadings for more efficient commuting flights and minimised energetic constraints (McNab 1980).

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APPENDICES

Table 1. Mean ± SD for body size, echolocation and wing parameters for adults and sub-adults. Significance differences from one-way ANOVAs are indicated in bold type. *Rhinolophus swinnyi* is represented by the abbreviation *R.sw* and *R. capensis* by *R.ca*.

Bat species and age	*R.sw* adults	*R.sw* sub-adults	One-way ANOVA P-value	*R.ca* adults	*R.ca* sub-adults	One-way ANOVA P-value
Number of bats	30	21		28	5	
Body size						
Mass (g)	8.3 ± 0.3	6.9 ± 0.7	**0.0001**	15.1 ± 1.4	12.7 ± 1.9	0.1
Echolocation parameters						
Peak frequency (kHz)	107.8 ± 0.8	106.5 ± 0.9	**0.0001**	85.8 ± 0.7	86.3 ± 0.2	0.1
Band width	19.4 ± 4.1	14.5 ± 2.9	**0.0002**	11.1 ± 1.6	9.9 ± 1.5	0.1
Duration	19.9 ± 4.8	20.0 ± 3.9	0.9	44.2 ± 7.6	42.3 ± 12.4	0.6
Wing parameters	30	21		53	5	
Forearm length (cm)	43.7 ± 0.9	42.8 ± 1.6	**0.01**	49.9 ± 1.1	49.7 ± 1.3	0.5
Wing area (cm^2)	124.6 ± 13.1	112.4 ± 15.6	**0.002**	159.8 ± 15.1	151.2 ± 6.7	0.3
Wing span (cm)	25.9 ± 2.0	25.5 ± 2.3	0.6	30.3 ± 1.4	29.9 ± 0.8	0.9
Wing loading (Nm^{-2})	6.6 ± 0.7	6.1 ± 0.6	**0.01**	7.8 ± 1.3	9.8 ± 1.3	**0.03**
Aspect ratio	5.4 ± 0.7	5.8 ± 0.5	**0.004**	5.7 ± 0.3	5.9 ± 0.3	0.2
Tip shape index	2.0 ± 0.5	2.0 ± 0.3	0.4	1.5 ± 0.3	1.6 ± 0.2	0.6

Table 2: Mean ± SD echolocation and wing parameters for *Rhinolophus swinnyi* (*R.sw*) KWT and *Rhinolophus capensis* (*R.ca*) (De Hoop and Table Farm) adult males and females. P-values from the Factorial ANOVA are given.

Bat species and sex	*R.sw* ♀♀ KWT	*R.sw* ♂♂ KWT	P-value	*R.ca* ♀♀ TF	*R.ca* ♂♂ TF	P-value	*R.ca* ♀♀ DH	*R.ca* ♂♂ DH	P-value
Number of bats	19	20		13	15		12		
Body size									
Mass (g)	8.5±0.5	8.2±0.2	P>0.5	14.8±1.1	13.6±0.8	**P<0.01**	11.4±1	10.8±0.5	P>0.2
Wing parameters									
Forearm length (cm)	44.3±0.7	43.1±0.6	P>0.5	50.7±1.1	49.5±1	**P<0.006**	46±12.9	49±0.06	P>0.4
Wing span (cm)	27±1.2	25±2.2	P>0.2	30.8±1.1	29.3±0.6	**P<0.0002**	30.8±1.6	30.5±1.4	P>0.5
Wing loading (Nm^{-2})	6.5±0.7	6.7±0.6	P>0.5	8.9±0.7	85.6±0.6	P>0.7	7±0.7	6.4±0.7	P>0.1

Table 3. Museums and specimen information for the skulls used in the morphometric analysis.

Museum	Specimen no.	code	Location	Species	Sex
Transvaal	29081	No data	De Hoop	*R. capensis*	Male
Transvaal	29072	No data	De Hoop	*R. capensis*	Male
Transvaal	29069	No data	De Hoop	*R. capensis*	Male
Transvaal	29066	No data	De Hoop	*R. capensis*	Male
Transvaal	29064	No data	De Hoop	*R. capensis*	Male
Transvaal	40576	No data	De Hoop	*R. capensis*	Male
Transvaal	36938	No data	Pafuri	*R. swinnyi*	No data
Transvaal	1024	No data	Port St. Johns, Pondoland	*R. swinnyi*	No data
Transvaal	36101	No data	KNP Fig tree camp	*R. swinnyi*	Female
Transvaal	40506	No data	King Williams Town	*R. swinnyi*	Female
Transvaal	40503	No data	King Williams Town	*R. swinnyi*	Male
Transvaal	36584	No data	King Williams Town	*R. swinnyi*	No data
Transvaal	36580	No data	King Williams Town	*R. swinnyi*	No data
Amatole	24292	1978	King Williams Town	*R. swinnyi*	Female
Amatole	c3817	1762	King Williams Town	*R. swinnyi*	Male
Amatole	c3846	1763	King Williams Town	*R. swinnyi*	Female
Amatole	32610	No data	King Williams Town	*R. swinnyi*	No data
Amatole	24293	1978	King Williams Town	*R. swinnyi*	Male
Amatole	24299	1979	King Williams Town	*R. swinnyi*	Female
Amatole	24302	1979	King Williams Town	*R. swinnyi*	Male
Amatole	24303	1979	King Williams Town	*R. swinnyi*	Female
Amatole	127a	1760	King Williams Town	*R. swinnyi*	No data
Amatole	3658	1811	Het kruis NWCP	*R. capensis*	Female
Amatole	3654	1812	Het kruis NWCP	*R. capensis*	Female
Amatole	3655	1813	Het kruis NWCP	*R. capensis*	Female
Amatole	3662	1815	Het kruis NWCP	*R. capensis*	Male
Amatole	3663	1816	Het kruis NWCP	*R. capensis*	Female
Amatole	3674	1824	Het kruis NWCP	*R. capensis*	Female
Amatole	3675	1825	Het kruis NWCP	*R. capensis*	Female
Amatole	3676	1826	Het kruis NWCP	*R. capensis*	Female
Amatole	3679	1828	Het kruis NWCP	*R. capensis*	Female
Amatole	3680	1829	Het kruis NWCP	*R. capensis*	Female
Amatole	3681	1830	Het kruis NWCP	*R. capensis*	Female
Amatole	20185	3668	Het kruis NWCP	*R. capensis*	Female