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**LOCAL ADAPTATION OF GEOFFROY'S  
HORSESHOE BAT, *Rhinolophus clivosus*, TO  
THE CAPE FLORISTIC REGION OF SOUTH  
AFRICA**

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## ABSTRACT

Populations of species adapt to the environment in which they live. This study investigated local adaptation in *Rhinolophus clivosus* (Chiroptera: Rhinolophidae) by comparing its phenotype with that of a co-occurring endemic species, *R. capensis*, in the Cape Floristic Region of South Africa. If *R. clivosus* has become locally adapted, its phenotype would be predicted to have diverged from *R. clivosus* populations elsewhere in the country while converging upon *R. capensis*. Evidence for local adaptation was found in *R. clivosus* at De Hoop Nature Reserve. The population has undergone a reduction in body size with correlated allometric responses in flight morphology. The wing shape of *R. clivosus* at De Hoop has not changed, resulting in a reduction in wing loading with a consequent increase in manoeuvrability. Thus *R. clivosus* at De Hoop is simply a scaled-down version of *R. clivosus* elsewhere and a scaled-up version of *R. capensis*. Factors such as competition and gene flow may have mitigated against local adaptation, however. Furthermore, whether phenotypic plasticity rather than natural selection may have been responsible for the apparent convergence between *R. capensis* and *R. clivosus* requires future research and advances in the study of evolutionary development and population genetics.

## DEDICATION

*Dedicated to my parents, Donald A. Walker, Jr. and Leslie J. Harkins, who took me for my first walk in the Maine woods; to my siblings, Don and Jen, Pam and Gaurav, and to my family: Mum, Aunt Florence, Aunt Gracia, Uncle Mike, Uncle Bernard, Aunt Peggy, Aunt Mary-Alice, Aunt Katie, Uncle Tony, Liptidy, Bop-Bop, Aunt Mary and Uncle Jim, Catherine, Heidi, Cedric, Aric, Aaron, Curtis, Brett, Laurencia, Steven, Christine, Cliff, Emily, Tom, Sarah, Nate, Wendy, Jim, Evelyn and Akash for their love and support.*

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# CHAPTER 1

## INTRODUCTION, RATIONALE AND RESEARCH AIMS.

### INTRODUCTION

In the process of evolution by natural selection (Darwin, [1859] 1978), organisms possessing traits best suited to their environment are more likely to survive to reproduce. These traits give the bearer a selective advantage over individuals with traits less suited to their environment, passing on a relatively greater proportion of their genes (Huxley, 1948) to the next generation. In this way, certain traits persist over evolutionary time.

A species' phenotype consists of a suite of behavioural, morphological and physiological adaptations. The bill shape of a bird, the call structure of a whale, and the wing design of a bat are all aspects of phenotype. Phenotype influences the way a species interacts with its environment as well as with other species in that environment. However, the phenotype best suited to a particular environment is usually unknown and it is simply assumed that the phenotype exhibited by a local population is the one best suited, i.e. locally adapted, to the environment where the individuals live. One instance in which this assumption is most likely to be correct is that of the phenotype of an endemic species, provided founder effect or genetic drift with subsequent reinforcement (Wright, 1932; Dobzhansky and Pavlovsky, 1957) played little or no role in shaping the endemic phenotype. Both founder effect and genetic drift may play a larger role in isolated populations where, in most circumstances, a few individuals established the endemic population. However, where founder populations may be larger (Clegg *et al.*, 2002), the phenotype of the endemic species may largely result from local selection pressures and

could be used as a model for local adaptation. An endemic species could thus serve as a control with which a closely related focal species could be compared to determine whether the focal species is locally adapted or not. If the focal species has become locally adapted to the environment, its phenotype should converge on that of the endemic species and diverge from that of populations of the same focal species in other localities with different selection pressures (Kaweki and Ebert, 2004). However, such changes in phenotype of the focal species must be analyzed in the context of multiple constraints. Phylogenetic history, ontogeny and basic structure, or *bauplan*, may constrain a species' phenotype to a greater degree than natural selection can shape it (Gould and Lewontin 1979) limiting convergence towards the endemic phenotype. Phenotypic convergence could also be constrained by resource competition between an endemic species and a phenotypically similar focal species, resulting in small phenotypic differences that allow these species to coexist (Elton, 1927; Levin, 1970). On the other hand, phenotypic divergence between populations of the focal species may be lessened by gene flow between localities (Kaweki and Ebert, 2004).

### **What Is Local Adaptation?**

Local adaptation (reviewed by Huxley, 1948; Kaweki and Ebert, 2004) to certain habitats, foods and climates can result in adaptive radiation over a geographical gradient. A species may extend its range into new geographical locations where individuals with the appropriate traits have a selective advantage and therefore produce relatively more offspring. Successive generations become more suited to their environment, diverging in one or more characteristics from populations elsewhere in the species' range. The species has thus become "locally adapted." Host plant range extension in some invertebrates

(Vanbergen *et al.*, 2003), host availability in the case of parasitic organisms (Gandon and van Zandt, 1998), and changes in resource availability (Lovette *et al.*, 2002; Stuart-Fox *et al.*, 2004) or climate (Bertness and Gaines, 1992; Ayres and Scriber, 1994; Dybdahl, 1995; Carol and Dingle, 1996; Angilletta *et al.*, 2004) may initiate local adaptation and, over time, species divergence. Darwin's finches (Lack, 1961) and Hawaiian honeycreepers (Carroll and Dingle, 1996; Lovette *et al.*, 2002) are well-known examples of adaptive radiation via local adaptation. On the small islands of the Galapagos, local adaptation produces a correlation between beak morphology and diet across subgenera in Darwin's finches (Grant, 1986). Ground-finches in the subgenus *Geospiza* have robust, finch-like beaks and forage for seeds whereas cactus ground-finches in the subgenus *Cactornis* eat leaves, fruits, and buds with their short, thick beaks. Similarly, Hawaiian honeycreepers descended from an ancestral colonizer with a finch-like beak adapted for seed-eating; they can be separated into three groups based on beak morphology which corresponds to a change in diet: seed-eaters with finch-like beaks, nectarivores with elongated beaks, and honeycreepers with intermediate beak morphology eating both nectar and insects. Ecomorphological studies such as these can help uncover selection pressures that limit or promote certain phenotypes.

One of the more common and informative experimental methods for investigating the relative magnitude of natural selection and the occurrence of local adaptation is reciprocal translocation. This involves measuring the fitness of local versus "foreign" populations of a given species in the respective local and foreign environments. The local species should have a greater survivorship or fitness in the local environment than in the environment of the foreign species, and vice-versa. In the case of the butterfly *Papilio*

*canadensis*, for example. Ayres and Scriber (1994) observed the growth patterns of butterflies from an Alaskan population and a northern Michigan population under Alaskan and northern Michigan climatic conditions to determine the role climatic gradients play in the local adaptation of Alaskan and northern Michigan butterfly populations. In an Alaskan climate, where summers are cooler and shorter than in northern Michigan, Alaskan butterflies had a higher fitness than butterflies from Northern Michigan did. Larval growth and moulting ability at low temperatures were important components of fitness in the Alaskan climate. Alaskan larvae, which had higher metabolic rates adapted to a cooler climate, grew slightly slower at the higher northern Michigan temperatures. On host plants of poor nutritional quality, Alaskan larvae could not assimilate plant matter efficiently enough to compensate for an elevated metabolic rate. Such a reciprocal transplantation approach has been successful with other insects in the field (e.g. armoured scale insects, Hanks and Denno, 1994; spiders, Riechert and Hall, 2000) and with parasites between hosts (Xia *et al.*, 1998). However, it is not a common approach to investigate local adaptation in more derived animals, e.g. vertebrates. An approach previously undocumented in the literature and possibly more suitable for testing local adaptation in higher order mammals might be to compare the morphology and ecology of an endemic species with that of a wide-ranging sympatric species. This method eliminates the need to “transplant” a focal species into the environment of the local species, as the two species already share the same environment. To proceed with this approach, however, both long- and short-term ecological processes inhibiting or confounding the detection of local adaptation must be considered.

### Processes Inhibiting or Confounding Local Adaptation

Detecting local adaptation is complicated due to several confounding factors such as gene flow, phenotypic plasticity and resource competition. Gene flow may inhibit local adaptation because genotypes suited to different environments cannot become fixed (Slatkin, 1987). Individuals with genotypes adapted to certain localities may disperse into localities in which their genotypes are not as fit. They may still mate with locally adapted individuals, however. Thus gene flow can maintain genetic variability within populations in different localities and thereby oppose local adaptation (Storfer *et al.*, 1999). In a reciprocal transplant study of the scale insect *Pseudaulacaspis pentagona*, Hanks and Denno (1994) found that survival was higher for scale insects on their own host mulberry trees, but that gene flow inhibited local adaptation between scale insects on closely spaced neighbouring mulberry trees. As a result of gene flow, phenotypic differences may be smaller between populations of the focal species and larger between the focal species and the local species (Kaweki and Ebert, 2004). However, in cases where mating success is lower for immigrating individuals than for the resident focal population, gene flow may not inhibit local adaptation (Lenormand, 2002).

Competition between the focal and local species may inhibit local adaptation. The adaptation of the focal species to a different niche than the local species would result in less phenotypic convergence, with small phenotypic differences enabling the two species to coexist. The "niche" (Grinnell, 1917; reviewed by Whitaker *et al.*, 1975), defined as the functional role of a species in a community (Elton, 1927; Gause, 1934; Hardin, 1960), includes the resources a species exploits, the method it uses to exploit them, and the time and place in which it does so. A niche can be visualized as a multi-dimensional region in

space, the axes of which consist of the environmental conditions a given species requires for survival (Hutchinson, 1957), or the place a species occupies in "morphospace" (Findley, 1976; Findley and Black, 1983) when morphological or other phenotypic parameters are plotted in multivariate statistical space.

The niche a species occupies is more or less differentiated from those of other species in a community, allowing many species to coexist in the same locality. "Gause's Principle" (Gause, 1934), or the Competitive Exclusion Principle (Hardin, 1960), postulates that no two species may share the same ecological niche when resources are limited because the species better adapted to exploit certain resources will eventually out-compete and exclude the less adapted species from that niche. Competitive exclusion may shape species diversity in a community by constraining niche breadth and therefore the number of species that can coexist in a certain area. Two species facing slightly different selection pressures might overlap in several other aspects of their respective niches, but small differences in the factors limiting how they use their environment may allow them to coexist (Elton, 1927; Levin, 1970). Thus a greater diversity of species may coexist in narrow, tightly packed niches at the centre of a community (Findley and Black, 1983). Furthermore, closely related generalist and specialist species may coexist because the more flexible generalist species can occupy the portion of its predicted niche that the specialist does not exploit quite so well (Hardin, 1960). Invasion of niches in a new locality depends upon the degree of competition between the invader and the local species. As a rule of thumb, the species exposed to local conditions the longest should be better adapted to them and is more likely to survive at the expense of any less suited potential invader (Grinnell, 1904). However, genetic variability in an invading species

seems to be a key factor determining the success of its establishment in an environment shared with a species similar in phenotype (Carroll and Dingle, 1996).

Niche adaptation may both confound and limit local adaptation. Once it successfully invades a novel niche, a population of a foreign, wide-ranging species may become adapted to a novel niche and under natural selection diverge in phenotype from conspecifics elsewhere in its range (Kaweki and Ebert, 2004). Thus the focal species may not be adapted to the locality it shares with the endemic species, but may rather be adapted to a novel niche. If the focal species shares a similar niche with the endemic species, the phenotype of the focal species may converge on that of the endemic species as a result of niche adaptation. Niche adaptation may limit local adaptation when the focal species occupies a similar niche to that of a population of the focal species elsewhere. This would result in less phenotypic divergence between the focal species in a particular locality and populations of the focal species elsewhere.

Phenotypic divergence between populations of a focal species and convergence between a focal and local species may result from phenotypic plasticity rather than local adaptation. In contrast to local adaptation, phenotypic plasticity occurs when a single genotype interacts with environmental factors during development to produce multiple phenotypes. Plasticity may be behavioural, morphological or physiological and may also manifest itself in a species' life history (reviewed by West-Eberhard, 1989). Phenotypic plasticity might be driven by habitat variation, food availability, or an environmental gradient (reviewed by Miner *et al.*, 2005). By producing phenotypes that are better suited to prevailing environmental conditions, phenotypic plasticity may facilitate speciation by divergent selection (West-Eberhard, 1989). Phenotypic plasticity may influence species

interactions such as competition, and can affect the ways in which multiple species in a community coexist. Thus plasticity may affect community structure and ecology (Miner *et al.*, 2005). Populations of the focal species may have phenotypes well suited to their respective localities as a result of interactions between a plastic genotype and the environment. In this way phenotypic plasticity may result in divergent phenotypes between populations of the focal species in different localities. Furthermore, phenotypic similarities between a local and focal population may be due to the fact that the phenotype of the focal population has become well suited to the local environment through plasticity rather than through local adaptation. Phenotypic plasticity may be advantageous to a wide-ranging species that encounters different environmental conditions across its geographic range (Via *et al.*, 1997). Plasticity should manifest itself as a generalist phenotype that is then subject to further modification under the particular selection pressures of each environment, and phenotypic differences between populations may be smaller than predicted under the local adaptation model (Kaweki and Ebert, 2004).

In the absence of genetic evidence or where it is not feasible to do reciprocal transplantations while controlling for genetic differences (e.g. Merckx and Van Dyck, 2006), it is not possible to determine whether phenotypic convergence between the endemic and the focal population is a result of local adaptation or plasticity in the phenotype of the focal population. Nor is it possible to determine which process is responsible for shaping phenotypic divergence between populations of the focal species in different localities. Teasing apart the role of local adaptation and phenotypic plasticity is therefore beyond the scope of this study.

## RATIONALE

### Local Adaptation in Bats

The strong correlation between the phenotype and ecology of bats make them close to ideal organisms for studying local adaptation. For bats, ecological correlates have been identified for wing morphology (Norberg and Rayner, 1987), external morphology (Fenton and Bogdanowicz, 2002), craniodental morphology (Freeman, 1979; Freeman, 1981; Dumont and Herrel, 2003) and echolocation (Fenton and Rautenbach, 1986; Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Kalko and Schnitzler 1998; Bowie *et al.*, 1999). The relationship between phenotype and ecology provides insight into mechanisms of niche differentiation (Findley and Black, 1983; Swift and Racey, 1983; McKenzie and Rolfe, 1986; Saunders and Barclay, 1992; Barlow *et al.*, 1997; Kalko, 1998; Bernard, 2001) and geographic variation in wide-ranging species (Bogdanowicz, 1990; Jacobs, 1996; Jacobs, 1999; Barclay *et al.*, 1999; Aspetsberger *et al.*, 2003).

Ecomorphological studies of bats mainly focus on wing morphology and echolocation call structure because these two factors are functionally important to a bat's survival. Flight morphology tends toward an optimal combination of parameters suited for flight tasks that bats must perform to navigate and capture prey in different environments (Norberg and Rayner, 1987; Norberg, 1990; Norberg, 1994). Wing shape and area constrains flight capability and consequently foraging strategy (Norberg and Rayner, 1987) as well as the prey a bat may successfully capture and manipulate (Kalko, 1995). Wing loading (the ratio of wing area to body mass) determines minimum flight speed. Minimum flight speed increases with higher wing loading while manoeuvrability

(the ability to make tight turns) and agility (the ability to turn rapidly) decrease with increasing wing loading (Norberg and Rayner, 1987). Thus bats with higher wing loading should be less efficient at manoeuvring in cluttered habitats. Clutter refers to obstacles in the environment such as dense vegetation that bats must detect and avoid (Norberg and Rayner, 1987). Small bats with short wingspans are less likely to collide with obstacles in cluttered environments and may navigate through narrower spaces than larger bats (Jacobs, 1999). Aspect ratio (a ratio of wingspan to wing area) describes wing shape. Wings of low aspect ratio are short and broad, effective for flight in narrow spaces and energetically efficient fast flight over short distances. Wingtip index measures wingtip shape – the higher the wingtip index the more rounded the wingtips. Rounded wingtips allow a hovering bat to achieve maximum possible lift and give bats greater manoeuvrability in cluttered habitats when combined with low aspect ratio and low wing loading (Norberg and Rayner, 1987).

Certain combinations of these morphological variables should be optimized for the habitats in which bats forage and the flight maneuvers required to capture their prey (Norberg and Rayner, 1987). For instance, bats foraging in edge-and-gap environments should have low aspect ratio, average to low wing loading, rounded wingtips and small body size for slow manoeuvring around the canopy at the forest edge but relatively faster flight in gaps. These features should be combined with a short wingspan to reduce the risk of their wings catching on vegetation. Gleaning bats in clutter should have low wing loading, low aspect ratio and short wingtips for slow flight and high manoeuvrability while searching the ground or vegetation for insect prey.

Echolocation may also be optimized for the types of acoustics bats use in the habitat in which they forage (Neuweiler, 1989). Echolocation operates over short ranges because of the atmospheric attenuation of high frequency sounds used by bats (Griffin, 1971). Attenuation and the fact that insects are small and produce low intensity echoes mean that insectivorous bats must emit high intensity calls of short wavelength to receive an echo from an insect. However, bats must also avoid deafening themselves with their own calls. Duty-cycle, or the amount of time taken up by calls in an echolocation sequence, is one indication of how bats avoid self-deafening (Fenton *et al.*, 1995). Bats can be divided into two groups: high duty-cycle and low duty-cycle bats (Fenton, 1999). High duty-cycle bats typically emit long, constant frequency (CF) calls with very short inter-pulse intervals. These bats separate their call from an incoming echo on the basis of frequency, avoiding self-deafening. Thus they can listen for echoes while they call and pulse-echo overlap is not problematic (Kalko and Schnitzler, 1998). To determine distance to prey, high duty-cycle bats lower their call frequency to compensate for their own movement toward their prey so that echoes return in a narrow frequency range which is higher than the emitted call. This is referred to as Doppler-shift compensation (Schnitzler and Kalko, 1998). Narrowband CF calls are well-suited to prey detection, whereas frequency modulated (FM) calls are better suited to prey localisation (Schnitzler and Kalko, 1998). To improve prey localisation, high duty-cycle bats typically add an FM component to one or both ends of the CF call (Altringham, 1996). The long duration, high frequency CF component allows high duty-cycle bats to detect “acoustic glints” from the wings of fluttering insects against complex background echoes from their environment (Schnitzler and Kalko, 1998). An acoustic glint is a returning echo of

relatively higher amplitude caused when a bat's emitted pulse strikes the raised wing of an insect at right angles. The emitted pulse contacts a larger reflective surface area when the insect's wing is at the top of its wing beat cycle than when it is mid-way through.

Species in the families Rhinolophidae and Hipposideridae, and the New World species *Pteronotus parnellii*, are the only high-duty cycle species (Jones and Rydell, 2003).

Low duty-cycle bats emit short pulses (0.5-30 ms) separated by long interpulse or listening intervals. This separation of calls in time prevents self-deafening (Schnitzler and Kalko, 1998). Echolocation calls of low duty-cycle bats are frequency modulated (FM), constant frequency (CF), or some combination of these components. Frequency-modulated calls are broadband (sweeping through an octave of frequencies in a short period of time) or narrowband (covering a narrow frequency range). Low duty-cycle bats tend to use FM calls or FM calls followed by a narrowband component (Jones and Rydell, 2003). Broadband FM calls are useful for localizing prey (Schnitzler and Kalko, 1998) and suit bats foraging in vegetation (Neuweiler, 1989). FM calls ending in a longer narrowband CF component or 'tail' of a lower frequency may increase detection range for bats foraging in open or edge environments (Neuweiler, 1989; Schnitzler and Kalko, 1998). When searching for prey, bats should emit frequencies that maximize the strength of returning echoes but minimize attenuation (Jones and Rydell, 2003). The strength of a returning echo will be greatest at wavelengths close to the same size as the insect prey (Vaughan, 1972). In open spaces, long, low frequency signals are effective for long-range detection of large insects because only the echo returning from a large insect will be strong enough to be detected at longer ranges. Short, high frequency signals are better for

detecting smaller insects at closer ranges, such as in cluttered environments (Neuweiler, 1989; Schnitzler and Kalko, 1998).

Geographic variation in wing morphology, echolocation and diet may either be due to phylogenetic history or evolutionary convergence in which bats that are not closely related evolve similar morphology as a result of local adaptation to similar foraging habitat or prey. Within the genus *Myotis*, similar feeding strategies have evolved independently several times and have resulted in similarities in external morphology between species in different regions of the globe (Ruedi and Mayer, 2001; Stadelmann *et al.*, 2004). Bats in this genus have thus become locally adapted to the habitats in which they forage to the extent that they cannot be taxonomically grouped solely on the basis of morphology. Morphology within the *Myotis* is highly convergent and not necessarily a reflection of phylogenetic relationships (Ruedi and Mayer, 2001; Fenton and Bogdanowicz, 2002). Similarly, *Chaerephon pumilus* varies in morphology, echolocation and diet between habitats in different geographic regions (Jacobs *et al.*, 2004). In Daubenton's bat (*Myotis daubentoni*), body size increases with temperature, which corresponds with increasing latitude, and certain cranial characters vary more with geography while dental characters vary with climate (Bogdanowicz, 1990). In *Lasiurus cinereus semotus*, echolocation calls vary with habitat (Barclay *et al.*, 1999).

The horseshoe bats in the family Rhinolophidae belong to a single genus, *Rhinolophus*, which may have originated in Southeast Asia (Bogdanowicz and Owen, 1992; Maree and Grant, 1997) or possibly Europe (Csorba *et al.*, 2003). However, a more recent genetic study of the evolution of echolocation in this family by Eick *et al.*, (2005), suggests the family originated in Africa. Rhinolophids occur in all regions of the world

except for the Americas and the polar regions (Skinner and Smithers, 1990; Taylor, 2000).

There are ten species of rhinolophids in South Africa: *Rhinolophus blasii*, *R. capensis*, *R. clivosus*, *R. darlingi*, *R. denti*, *R. fumigatus*, *R. hildebrandtii*, *R. landeri*, *R. simulator*, and *R. swinnyi*. This diversity is likely the result of changes in rainfall and temperature that transformed the landscape and isolated bat populations in the Plio-Pleistocene. Climatic cycles created diverse patches of temporary habitat and it was probably the availability of hospitable day roosts within these patches that allowed rhinolophid bats to spread through southern Africa (Maree and Grant, 1997).

*Rhinolophus clivosus* and *R. capensis*, a species endemic to the Cape Floristic Region (CFR) in South Africa, provide an opportunity to study local adaptation. *Rhinolophus clivosus* is a medium-sized rhinolophid with a wide geographic range including diverse habitats such as savannah woodland, forest edge and desert. Skinner and Smithers (1990) suggest the absence of *R. clivosus* in the semi-desert region of Botswana may be due to the lack of hospitable day roosts. This species occurs in tropical and temperate areas of the Old World from southern Africa to eastern and northern regions of the continent (Simmons, 2005) and the Middle East (Csorba *et al.*, 2003; Simmons, 2005). The South African subspecies, *Rhinolophus clivosus zuluensis*, is just one of six recognized subspecies (Csorba *et al.*, 2003). Geographic variation within a species as a consequence of local adaptation to a variety of habitats may result from such a wide distribution (Aspetsberger *et al.*, 2003).

In the Cape Floristic Region of the south-western cape of South Africa, *R. clivosus* is sympatric with *R. capensis*, a smaller endemic species (Herselman and

Norton, 1985). *Rhinolophus capensis* belongs to the *capensis* subgroup which includes *R. denti*, *R. swinnyi*, *R. simulator* and *R. adami* (Bogdanowicz, 1992). This subgroup is the result of diversification within Africa after one or several colonization events extending from North Africa (Maree and Grant, 1997).

### RESEARCH AIMS

If *Rhinolophus clivosus* has become locally adapted to the Cape Floristic Region, its phenotype should converge on that of the endemic *R. capensis* and diverge from that of *R. clivosus* populations elsewhere in South Africa. This assumes that the endemic *R. capensis* represents a phenotype which is locally adapted to the Cape Floristic Region. However, more than one niche may be available to rhinolophid bats in the CFR, such that the two species may occupy separate niches (Van Valen, 1965) with no need for morphological convergence. Their niches may be partitioned temporally, with differences in microhabitats (Skinner and Smithers, 1990), reproductive timing or peak foraging times, or spatially, with differences in terms of roosting location within the caves and microhabitat use. Differences in diet may also reflect spatial and temporal niche partitioning. There is however some evidence that their niches are the same or overlap. *Rhinolophus clivosus* (FA 51-57 mm; Skinner and Smithers, 1990) and *R. capensis* (FA 46-51.8 mm; Skinner and Smithers, 1990) overlap slightly in body size as well as parameters of flight morphology (McDonald *et al.*, 1990). *Rhinolophus clivosus* flies at low altitudes, foraging between shrubs below the canopy level (Herselman and Norton, 1985), where it feeds primarily on moths and small beetles (Rautenbach, 1982). The preferred diet of *R. capensis* is unknown, but Herselman and Norton (1985) observed

these bats flying at low altitudes to catch low-flying insects. Both bats fly over the milkwood forest (*Sideroxylon inerme*) at the De Hoop Guano Cave (McDonald *et al.*, 1990), but whether they glean and perch-hunt in the CFR remains to be observed.

I investigated whether *R. clivosus* has become locally adapted to the Cape Floristic Region by comparing the morphology and ecology of *R. clivosus* and *R. capensis* at De Hoop Nature Reserve to determine, first, if their niches were similar, and second if their phenotypes converged. I then compared the phenotype of *R. clivosus* at De Hoop with that of a general phenotype for *R. clivosus* taken from several different localities elsewhere in South Africa to determine if *R. clivosus* at De Hoop has diverged from the general South African phenotype. I also compared the phenotype of *R. clivosus* at De Hoop with that of *R. clivosus* at Sudwala, a subtropical grassland site, to determine whether *R. clivosus* has diverged from a local population inhabiting a different environment.

## CHAPTER 2

### METHODS

#### Study site

This study was done in the De Hoop Nature Reserve (34°26'S/20°25'E; McDonald *et al.*, 1990) near Bredasdorp, in the Western Cape Province of South Africa from January to November 2005. The De Hoop Nature Reserve lies on South Africa's south-western shore and encompasses 18,762 ha of coastal and inland habitats (McDonald *et al.* 1990). De Hoop lies within the winter rainfall region of southern Africa, where at least 50% of the yearly precipitation occurs between April and October (Proches *et al.*, 2005). De Hoop receives most of its rainfall between May and September and enjoys hot, dry summers. The average daily minimum temperature in the region is 15° Celsius in January and drops to 6° Celsius in July. Average daily maximum temperatures are 28° Celsius in midsummer and 17° Celsius in winter (McDonald *et al.*, 1990).

The reserve is situated in the fynbos biome of the Cape Floral Kingdom, which is one of six floral kingdoms of the world. The Cape Floristic Region (CFR) encompasses just 90,000 square kilometres, less than 4% of the land area of southern Africa, and yet this "mosaic" of local habitats boasts 42% of the estimated total plant species in southern Africa, of which 65% are endemic (Goldblatt, 1997). The fynbos biome within the CFR is unique in that it supports such a high level of plant endemism and species diversity, yet it is depauperate in mammalian species diversity and endemism (Bigalke, 1979). Primary producer biomass is low relative to comparable biomes on other continents and a natural

burn cycle of between four and forty years constrains the types of plants able to survive in the biome (Cowling *et al.*, 1997).

The De Hoop Guano Cave is situated in limestone cliffs overlooking milkwood (*Sideroxylon inerme*) forest on the northern perimeter of the De Hoop Vlei (34°26'S/20°25'E: McDonald *et al.*, 1990) and is used as a day roost by both rhinolophid species. Approximately 24,000 rhinolophids overwinter in the cave but only about 1,200 of them roost there in summer (McDonald *et al.*, 1990). Three other insectivorous bat species — *Miniopterus natalensis*, *Myotis tricolor* and *Nycteris thebaica* — roost in the cave during summer, bringing the total roosting population estimate to between 200,000 and 300,000 bats. This makes the Guano Cave a rare, and therefore important, roosting site (McDonald *et al.*, 1990).

### **Species, Age and Reproductive Condition**

Bats were captured in a 6-m mist-net positioned across a small side entrance to the Guano Cave and in a harp trap positioned outside the same entrance or located in a clearing in the forest 5 m below the cave entrance. The species, age class, and sex of each bat were recorded. Bats were identified to species by echolocation call peak frequency and dental morphology using the taxonomic keys in Skinner and Smithers. (1990) and Taylor (2000). The frequency of echolocation calls used to identify species were obtained from hand-held bats and determined with either a heterodyne detector (Pettersson Elektronik AB, Tallbacksvagen 51, S-756-45 Uppsala, Sweden) or from recordings of echolocation calls using either the Anabat detection system with AnaLook software (v. 4.8b, Chris Corben, P.O. Box 2323 Rohnert Park, CA 94927), or with a Pettersson bat detection

system (Pettersson Elektronik AB, Tallbacksvagen 51, S-756-45 Uppsala, Sweden) and BatSound Pro software (v. 3.31; Pettersson Elektronik, Uppsala, Sweden).

Juvenile or sub-adult bats were identified by the presence of cartilaginous epiphyseal plates at the metacarpal-phalangeal joints (Anthony, 1988). To exclude pregnant bats from the study, I used abdominal palpation to detect the presence of a foetus in later stages of development. (Racey 1988)

## **Morphology**

Right forearm length was measured to the nearest 0.1 mm with dial callipers. The mass of each bat, following the evacuation of faeces, was measured to the nearest 0.001 g using a digital scale (Ohaus Corporation, 19A Chapin Rd. P.O. Box 2033 Pine Brook, New Jersey 07058).

A digital photograph of the extended right wing and tail (extended as in Saunders and Barclay, 1992) was taken with a Nikon CoolPix 5600 (Nikon Corporation, 2-3 Marunouchi 3-chome, Chiyoda-ku Tokyo 100-8331, Japan) digital camera and imported into Sigma Scan Pro software (v. 5.0; Systat Software GmbH, Schimmelbuschstr 25 D-40699 Erkrath, Germany). The area of the hand-wing, arm-wing and interfemoral membrane was measured to the nearest 0.1 mm<sup>2</sup> and wingspan to the nearest 0.01 mm (after Norberg and Rayner, 1987). These measurements were used to calculate wing area, wing loading, aspect ratio, and wingtip shape index ( $WL = Mg/S$  where M is body mass, g is acceleration due to gravity and S is total wing area;  $AR = B^2/S$  where B is wingspan;  $I = T_s / (T_1 - T_s)$  where  $T_s$  is the ratio of hand-wing to arm-wing area and  $T_1$  is the ratio of hand-wing to arm-wing length; Norberg and Rayner, 1987).

## **Echolocation**

Echolocation calls of hand-held bats were recorded directly into a Compaq nx7010 notebook computer connected to a Pettersson bat detector (Pettersson Elektronik AB, Tallbacksvagen 51, S-756-45 Uppsala, Sweden) through a high speed sound card (National Instruments Corporation, 11500 N Mopac Expwy, Austin, Texas 78759-3504). Peak frequency (kHz), call duration (ms) and pulse interval (ms) were measured from the second harmonic using BatSound Pro (v. 3.31; Pettersson Elektronik, Uppsala, Sweden). Calls were analyzed at a sampling rate of 44, 100 Hz (16 bits, mono) and a threshold of 15 (Schoeman and Jacobs, 2003). Only one call was analyzed for each individual bat to avoid pseudo-replication. Calls with a high signal-to-noise ratio were selected for measurement to ensure that the call, and not background noise, was being measured. Peak frequency was measured from the FFT power spectrum (Schoeman and Jacobs, 2003). Call duration and pulse interval were measured in a Hanning window. Call duration was measured from the initiation of the CF (constant frequency) component of the call to the terminus of the downward sweeping FM (frequency modulated) component of the call. Pulse interval was measured from the end of an individual call to the initiation of the next individual call. Duty cycle was calculated as the sum of signal durations divided by the sum of pulse intervals during a 500 ms time window (von der Emde and Schnitzler, 1986).

## **Dietary Analysis**

### **Faecal pellet collection**

Bats were placed in cotton bags immediately after capture (Whitaker, 1988) and held until the following afternoon, when faecal pellets were collected and stored in labelled and sealed plastic bags for analysis. To avoid researcher bias during faecal analysis, each bat was given a number to be used as a sample label, so that the researcher was unaware of the species from which the faeces were taken.

### **Faecal pellet analysis**

Ten faecal pellets from each bat were chosen. Pellets were immersed in a petri dish of 96% ethanol and water and teased apart under a dissecting microscope (magnification 10.5 - 45x). Insect fragments including legs, wings and antennae were identified to order using taxonomic keys (Scholtz and Holm, 1985; Whitaker, 1988) and a reference slide collection of insect parts compiled by Corrie Schoeman, for insects collected in the Cape Floristic Region. A visual estimate of the relative percent volume of each order in a pellet (to the nearest 5-10%) was made and the relative percent volume of each order in a sample was calculated after Whitaker (1988). Insect fragments were mounted on slides for future reference.

Body length, the distance between the tip of the head and the posterior margin of the terminal body segment, was used as a measure of the size of insects consumed by the bats. Although Jones (1990) used wingspan as a measure of body size, total body length was used here because wings were not always intact. Body length of the prey consumed was estimated as follows. The total body length of whole insects from four orders comprising 25 families (N=170) caught in the light trap was measured using a dissecting

microscope and dial callipers, or a compound microscope, depending on the size of the insect. A total of 75 complete tarsal segments from four orders (Lepidoptera  $n = 10$ ; Coleoptera  $n = 29$ ; Hemiptera  $n = 12$ ; Diptera  $n = 24$ ) were extracted from faecal pellets and measured under a compound microscope (to the nearest  $\mu\text{m}$ ; magnification 40x), from the margin of the tibial joint to the tip of the claw. The equation generated from the regression of total body length on tarsal length in whole insects was used with measurements of complete tarsal segments extracted from faecal pellets to determine the body length of the insects the bats ate (to the nearest 0.01 mm).

### **Insect collection**

Insects were sampled with a 22-watt battery-powered black-light trap (BioQuip Products, 2321 Gladwick St., Rancho Dominguez, CA 90220) each night bats were caught. The trap stood approximately 2 m off the ground on top of a vehicle on the vlei floor (when dry) below the Guano Cave or suspended 3 m off the ground in the forest below the cave. Insects were dehydrated at  $60^\circ$  for 48 hours and weighed by order to calculate the relative dry biomass of each order and to determine the relative availability of each order. As light traps give a biased sample of the insect fauna available to bats in a given area — for example, Lepidoptera may be over-represented in light traps because they are more attracted to light than other insects — only limited inferences were made based on the data (Kunz, 1988). Light trap data have however been used in previous studies to investigate opportunism in bats (Jones, 1990; Brack and LaVal, 2006).

## Flight Behaviour

Flight patterns of *R. capensis* and *R. clivosus* were observed at De Hoop by following light-tagged individuals. Chemiluminescent fishing lures weighing 0.2 g (Pokee Fishing Tackle Company Ltd., Tainan, Taiwan) were glued to the mid-dorsal region of 6 *R. capensis* and 7 *R. clivosus* with Skinbond surgical adhesive (Smith and Nephew, Key Largo, FL) to observe flight behaviour (McDonald *et al.*, 1990) on November 25<sup>th</sup> and 26<sup>th</sup> 2005. Two observers were positioned in each of three locations: on rocks below the forest at the edge of the full vlei, in the forest below the cliffs, and on the cliffs above the forest. Light-tagged bats were released at ten-minute intervals from the edge of the vlei below the forest. Observers with check sheets used digital watches to record the amount of time (s) they saw the bats in each habitat zone. The habitat was partitioned into narrow zones to investigate small differences in habitat use between the two species. The zones were as follows: aerial along the cliff face, aerial above the forest, 1 m above the canopy, less than 1 m above the canopy, within the forest canopy, below the canopy, greater than 1 m above the forest understory, less than 1 m above the forest understory, perching in the forest, aerial over the reeds, greater than 3 m above the reeds, greater than 1 m above the reeds, less than 1 m above the reeds, 1 m above the reeds, at reed level, aerial over the water, greater than 1 m above the water and less than 1 m above the water. Observers recorded the bat's altitude or distance from vegetation, the bat's flight path (e.g. vertically or horizontally erratic or straight) and estimated the bat's relative flight speed (fast or slow). One bat at a time was released and observers recorded behaviour until the bat was lost from view. Observations were recorded only for bats that were in sight all of the time

to avoid the possibility of a previously released bat returning and being mistaken for the bat just released. Observers were unaware which species was released.

### **Statistical Analyses**

Phenotypic convergence between *R. capensis* and *R. clivosus* at De Hoop was assessed using discriminant function analysis of morphological data. Phenotypic divergence between *R. clivosus* at De Hoop and elsewhere in South Africa was investigated in a similar manner, as was phenotypic divergence between *R. clivosus* at De Hoop and Sudwala. Squared mahalanobis distances were used to investigate the magnitude of morphological convergence or divergence.

Multivariate statistical methods such as discriminant function analysis provide biologically relevant results when they are used to explore and describe ecological systems, which are complex and involve many variables that interact with each other to produce a given pattern. Not only do multivariate techniques consider the contributions of a large number of interacting variables to a particular ecological pattern, but they are also fairly robust to autocorrelation and violations of normality and homoscedacity (McGarigal *et al.*, 2000). Bats, like small insectivorous mammals and birds, forage and interact in three-dimensional space and therefore multivariate statistical methods that describe these interactions using multiple morphological variables are appropriate (Kingston *et al.*, 2000). Furthermore, morphological variables used to describe bats are often correlated. For example, wing loading is correlated with mass and wing area; aspect ratio is correlated with wing area and wing span. Discriminant function analysis has been used in previous studies to reveal morphological variables that best discriminate between different bat species (e.g. Fenton and Bogdanowicz, 2002). Not only does discriminant

function analysis uncover the variables distinguishing most between species, but it is also useful to determine how different, overall, each species or group is from each of the other species or groups in the analysis by calculating squared mahalanobis distances.

However, because discriminant function analysis assumes independence between variables, principal components analysis was done on the November data only, which included mass, wingspan, wing area, wing loading, aspect ratio and tip shape index. November data were used in this and subsequent analyses because only the data for this month had a sufficiently large number of males and females of each species. Data were imported into Statistica (v. 7.0; StatSoft Inc., 2300 East 14<sup>th</sup> St. Tulsa, OK 74104) and standardized for homoscedacity using the formula  $X' = (X - \mu)/\sigma$  where  $X$  is a given value,  $\mu$  is the mean of  $X$ , and  $\sigma$  is the standard deviation of  $X$  (Zar, 1984). Data were tested for homoscedacity with Levene's test and for normality with the Komolgorov-Smirnov test. Sexual dimorphism was examined using individual t-tests for *Rhinolophus clivosus* ( $n = 14$ ) and individual Mann-Whitney U-tests for *Rhinolophus capensis* ( $n = 22$ ), as the data for *R. capensis* did not meet the parametric assumption of homoscedacity. Sexual dimorphism was accommodated by using equal proportions of males and females in the morphological analyses. Data for *R. clivosus* elsewhere in South Africa consisted of bats from each of four locations (Knysna,  $n = 4$ ; Kokstad mines,  $n = 4$ ; Koggelbeen,  $n = 2$ ; Sudwala,  $n = 10$ ) and were collected by Corrie Schoeman, David Jacobs and Samantha Stoffberg. Knysna is a temperate forest site located in the Western Cape Province; Kokstad is a subtropical grassland site in KwaZulu-Natal; Koggelbeen is a semi-arid site in the Northern Province Nama-Karoo; and Sudwala is a subtropical lowveld grassland site located in Mpumalanga. As *R. clivosus* populations from several

different localities were included, the mean values of the morphological variables used in the analysis reflect a general South African phenotype with which to compare *R. clivosus* at De Hoop. However, this method may mask local phenotypic differences, so comparing the De Hoop population to that of a single different locality was necessary to confirm the divergence of *R. clivosus* at De Hoop. As sufficient data were available for Sudwala, the principal components and discriminant function analysis were repeated using data for this population.

The principal components analysis created composite variables in the form of independent principal components. As the number of principal components was not known *a priori*, the number of principal components was chosen by cross-validation, which uses the input data to generate an appropriate number of principal components to describe the data. Previous studies comparing morphological variables between sympatric species have used principal components analysis to determine which morphological variables account for the three-dimensional spacing, or lack thereof, between species (e.g. Kingston *et al.*, 2000). Principal components analysis determines which morphological variables load highest on each component so that the meaning of the components is not lost during further analysis. The significant principal components were then subjected to discriminant function analysis.

Least squares regressions of wingspan, wing area, wing loading, aspect ratio and echolocation call peak frequency controlled for phylogenetic effects and body size were used to determine phenotypic convergence between *R. capensis* and *R. clivosus* at De Hoop in terms of the degree to which each species deviated from the allometry for the South African Rhinolophidae.

Log-transformed variables were regressed against body mass under the phylogenetic least squares (PGLS) model to control for phylogenetic effects and to ensure the independence of data points (Martins and Hansen, 1997). PGLS regressions were done using the software programme Compare (v. 4.6, Department of Biology, University of Oregon, Eugene, <http://work.uoregon.edu/~COMPARE>). The PGLS model allows an investigation of the degree to which the interspecific variation in morphological traits can be explained by phylogenetic history or adaptation (Martins and Hansen, 1997). The PGLS model also allows robust interspecific comparisons to be made when only a small clade is available (Housworth *et al.*, 2004). Previous studies have also incorporated methods of controlling for phylogeny to account for statistically non-independent groups (e.g. Kingston *et al.*, 2000). A molecular phylogeny for ten South African rhinolophid species including *R. capensis* and *R. clivosus* generated by Samantha Stoffberg (unpublished data) was used in the PGLS analysis. Morphological data collected by David Jacobs, Corrie Schoeman and Samantha Stoffberg from nine of the South African rhinolophid species (*R. blasii* n = 2, *R. darlingi* n = 10, *R. denti* n = 10, *R. fumigatus* n = 2, *R. hildebrandtii* n = 11, *R. landeri* n = 2, *R. simulator* n = 9, *R. swinnyi* n = 10) and from *R. clivosus* (n = 20) elsewhere in South Africa were included in the least squares regressions. The means of the morphological traits for *R. clivosus* and *R. capensis* at De Hoop were then plotted separately on each regression for the South African Rhinolophidae.

The niches of *R. capensis* and *R. clivosus* at De Hoop were compared along several dimensions including morphology, diet and flight behaviour. The previously described discriminant function analysis was used to determine whether *R. capensis* and

*R. clivosus* occupy the same morphological niche. Several dietary aspects such as taxonomic composition, prey size and diversity were compared to examine whether the two species occupy the same dietary niche.

Multivariate statistical methods were used to investigate whether diets of *R. capensis* and *R. clivosus* at De Hoop are similar in taxonomic composition. The advantage of taking a multivariate approach is that the bats may be compared in terms of their whole diets, which consist of multiple taxa in various proportions. The following statistical analyses were performed using Primer software (version 6.0; Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, United Kingdom, 2005). Although the program is most commonly used by marine ecologists to study species distribution patterns in relation to environmental variables (e.g. Field *et al.*, 1982), Bowie *et al.* (1999) have used Primer to analyze the similarity between the diets of two bat species. In the case of bat dietary analysis, Primer may be used to analyze the similarities in species distribution and relative volume of prey items across all possible pairs of individual bats in the analysis. The non-parametric methods Primer uses make few assumptions about the data, and are therefore advantageous for analyzing ecological data (Clarke, 1993). For more discussion on the uses, advantages and disadvantages of Primer see Field *et al.* (1982), Clarke (1993) and Clarke and Warwick (1994). Differences in dietary composition between *R. capensis* and *R. clivosus* were compared within each month and over the year using data from months for which data were available for both species (March, May, July and November). Dietary composition was compared between the two species in months during which the vlei was dry (January and March) and when

the vlei was full of water (May, July and November) to determine whether dietary relationships between *R. capensis* and *R. clivosus* changed between the two periods.

Percentage volume data from faecal analysis for *R. capensis* and *R. clivosus* at De Hoop were arcsine-transformed for homoscedacity (Zar, 1984). The transformed data was arranged into a matrix containing the five types of prey found in faecal pellets, in columns, and the individual bats for which dietary data was collected, in rows. A triangular similarity matrix was generated using the Bray-Curtis measure of similarity ( $\delta_{jk} = \sum |Y_{ij} - Y_{ik}| / \sum (Y_{ij} + Y_{ik})$ , where  $Y_{ij}$  is the percentage volume for the  $i^{\text{th}}$  prey type for the diet of the  $j^{\text{th}}$  bat;  $Y_{ik}$  is the percentage volume for the  $i^{\text{th}}$  prey type for the diet of the  $k^{\text{th}}$  bat; and  $\delta_{jk}$  is the sum of the dissimilarity between the diets of the  $j^{\text{th}}$  and  $k^{\text{th}}$  bats across all prey types; Bray and Curtis, 1957), which calculates the similarity between every possible pair of individuals in the matrix. The Bray-Curtis formula is robust because joint zero counts do not affect the formula (Field *et al.*, 1982). This method is appropriate for dietary data in particular because dietary data contains a considerable number of zero values (Bowie *et al.*, 1999). As values of  $\delta_{jk}$  range from 0 (identical values) to 1 (no values in common), two bats that do not eat Diptera, for example, will be similar because both do not eat Diptera, whereas a bat eating Diptera and a bat not eating Diptera will be dissimilar.

Once the similarity matrix was created, cluster analysis using a group-average sorting method was used to place bats in groups according to similar dietary composition (Bowie *et al.*, 1999). Clusters are joined based upon the average similarity between all of the individuals in one group and the average similarity between all of the individuals in the other group. The group-average sorting method generates a dendrogram which

depicts a hierarchy of similarities in which groups of bats with more similar diets cluster together (Field *et al.*, 1982). The cut-off level that provided the most useful interpretation of the cluster pattern (Bowie *et al.*, 1999) was chosen at 16% similarity for November, 6% for the year, 31% for the dry period, and 6% for the period when the vleis were full of water. The cut-off levels are arbitrary and were selected according to their informative value (Field *et al.*, 1982).

Non-metric multidimensional scaling (MDS) was used to validate the clusters generated in the dendrogram (Bowie *et al.*, 1999) because two adjacent samples on a dendrogram may not always be the most similar (Field *et al.*, 1982); the dendrogram is useful as a simple visualization of a general group clustering pattern. MDS is a preferred ordination method because it tolerates the large number of zeros inherent in dietary data matrices (Field *et al.*, 1982; Bowie *et al.*, 1999). MDS displays the similarity between each pair of bats in the analysis, measured by the Euclidean distance between them, as the distance between pairs of points plotted in two- and three-dimensional space. Some distortion is involved in compressing these distances into fewer dimensions (Field *et al.*, 1982), so stress values indicate the accuracy with which the distances between points on the ordination plot represent the true similarities between pairs of bats. Distances between points are regressed on their corresponding Euclidean distances, or dissimilarities. Stress is then measured by the goodness of fit of the regression line. The closer the fit, the lower the stress, and the more accurate the MDS plot. Shepard diagrams display such a regression line and may be used to assess the relative suitability of compressing data into two or three dimensions (Field *et al.*, 1982; McGarigal *et al.*, 2000).

To determine whether dietary differences between *R. capensis* and *R. clivosus* at De Hoop were significant, one-way analysis of similarities (ANOSIM) was performed. Once significant dietary differences were discovered, similarity percentage analysis (SIMPER) was done using the original dietary matrix to reveal which prey type best characterized each bat species and which types of food best discriminated between species (Bowie *et al.*, 1999).

Similarities in the size of prey consumed were investigated to determine whether the bats were partitioning resources based upon prey size. A Mann-Whitney U-test was used with insect size data for January, July, August and November (*R. capensis* n = 24, *R. clivosus* n = 21). These were the only months for which sufficient data were available as prey items were well-chewed. The analysis was done in Statistica (v. 7.0). The non-parametric test comparing medians was used as means may be affected by the bats eating one very large or very small insect, which would mask any underlying pattern.

To determine the relative degree of dietary specialization between the bats, Simpson's diet diversity index (DDI) was calculated for each species for the months in which volumetric dietary data was available for both species (January, March, May, July and November). The DDI was calculated after Simpson (1949) and has been used in previous studies of dietary breadth (e.g. Barlow, 1997; Brack and LaVal 2006), and is given as  $DDI = 1/\sum P_i^2$ , where  $P_i$  is the proportion of each insect order in the species' diet (Simpson, 1949). The DDI was used as an estimate of the number of orders eaten in equal proportions.

To examine whether *R. capensis* and *R. clivosus* were taking prey items in proportion to their availability, the mean percent volume of each taxon in the diet of the

two bats for each month and the mean percent dry biomass of each taxon collected in light trap samples were subjected to a Chi-square test (Jones, 1990) in Statistica (7.0). This analysis was done to find out whether the bats were foraging opportunistically, by consuming the prey most available, or preferentially selecting their prey.

Habitat use was compared to determine whether *R. capensis* and *R. clivosus* share the same spatial niche. Habitat use was compared at two spatial levels, course-grained and fine-grained, to examine large and small differences between the bats. A habitat-use index was calculated for each individual by the formula  $HU = \sum (H \cdot t_h / t)$  (where H is the rank of the habitat zone,  $t_h$  is the time spent in a zone of rank H, and t is the total time for which the bat was observed; Aldridge and Rautenbach, 1987). A mean habitat-use index was calculated for each species. Seven habitat zones were identified according to the degree of spatial and acoustic clutter encountered, after Aldridge and Rautenbach (1987). These zones were further condensed into aerial, edge and clutter zones. Non-metric multidimensional scaling was used to determine the degree of overlap in habitat use between the two species at this course scale and at a finer scale. The percentage time each bat spent in the narrow habitat zones in which observers recorded flight behaviour was standardized by the total time each bat was observed. Data were then imported into Primer (v. 6.0) and examined in a similar manner to the dietary analysis.

## CHAPTER 3

### RESULTS

#### Morphology

Female *R. clivosus* had longer forearms than male *R. clivosus* ( $t_{7,12} = 5.0$ ,  $P < 0.001$ ; Table 1). Female *R. capensis* had larger forearms (Mann-Whitney U-test,  $U = 26.5$ ,  $P < 0.03$ ), mass (Mann-Whitney U-test,  $U = 15.0$ ,  $P < 0.01$ ) and wing loading (Mann-Whitney U-test,  $U = 27.0$ ,  $P < 0.03$ ; Table 1) than male *R. capensis*. Sexual dimorphism was accommodated by using equal proportions of males and females in the morphological analyses (*R. capensis*: males  $n = 11$ , females  $n = 11$ , total  $n = 22$ ; *R. clivosus* at De Hoop: males  $n = 7$ , females  $n = 7$ , total  $n = 14$ ; *R. clivosus* elsewhere in South Africa: males  $n = 10$ , females  $n = 10$ , total  $n = 20$ ).

Differences in mass, wing area and wingspan between *R. capensis*, *R. clivosus* at De Hoop and *R. clivosus* elsewhere in South Africa were statistically significant (ANOVA  $F_{6, 102} = 23.3$ ,  $P < 0.001$ ), as were differences in aspect ratio, wing loading and tip shape index (ANOVA  $F_{6, 102} = 3.5$ ,  $P < 0.01$ ). Tukey's multiple comparison tests revealed that both *R. clivosus* populations had a greater mass, longer wingspan and larger wing area than *R. capensis* (all  $P$ 's  $< 0.001$ ). The *R. clivosus* populations were similar in aspect ratio (Tukey's  $P = 0.49$ ) and wing loading (Tukey's  $P = 0.48$ ) though *R. clivosus* elsewhere had a slightly higher aspect ratio and wing loading than *R. clivosus* at De Hoop (Table 2). *Rhinolophus clivosus* elsewhere had a higher aspect ratio and wing loading than *R. capensis* (all  $P$ 's  $< 0.01$ ). *Rhinolophus clivosus* at De Hoop and *R. capensis* were similar in aspect ratio (Tukey's  $P = 0.17$ ) and wing loading (Tukey's  $P = 0.25$ ) although

**Table 1.** Means and standard deviations of morphological variables for male (n = 11) and female (n = 11) *R. capensis*, male (n = 7) and female (n = 7) *R. clivosus* at De Hoop, and male (n = 10) and female (n = 10) *R. clivosus* elsewhere in South Africa. The De Hoop data from November 2005 is reported.

Species	Sex	Right forearm (cm)	Mass (g)	Wingspan (cm)	Wing area (cm <sup>2</sup> )	Aspect ratio	Wing loading (Nm <sup>-2</sup> )	Tip shape index
<i>R. capensis</i>	F	5.0±0.1	13.7±2.0	29.5±2.5	164±26.8	5.3±0.2	8.3±1.6	1.6±0.5
<i>R. capensis</i>	M	4.9±0.1	11.2±0.6	29.4±1.4	164±18.4	5.3±0.2	6.8±0.7	1.4±0.2
<i>R. clivosus</i> De Hoop	F	5.7±0.1	17.6±0.6	34.6±1.4	215±18.8	5.6±0.3	8.1±0.8	1.5±0.2
<i>R. clivosus</i> De Hoop	M	5.4±0.1	17.1±1.0	33±1.5	204±22.3	5.4±0.3	8.4±1.4	1.4±0.4
<i>R. clivosus</i> South Africa	F	5.5±0.2	18.8±1.9	34.4±1.4	213±12.7	5.6±0.3	8.7±1.0	1.4±0.2
<i>R. clivosus</i> South Africa	M	5.3±0.1	17.8±1.7	33.6±1.3	200±11.5	5.6±0.3	8.7±0.8	1.5±0.2

**Table 2.** Means and standard deviations of morphological variables for *R. capensis* (n = 22), *R. clivosus* at De Hoop (n = 14) and *R. clivosus* elsewhere in South Africa (n = 20). Data for *R. clivosus* elsewhere are courtesy of David Jacobs, Corrie Schoeman and Samantha Stoffberg.

Species	Right forearm (cm)	Mass (g)	Wingspan (cm)	Wing area (cm <sup>2</sup> )	Aspect ratio	Wing loading (Nm <sup>-2</sup> )	Tip shape index
<i>R. capensis</i>	5.0±0.1	12.5±1.9	29.5±2.0	164±22.4	5.3±0.2	7.6±1.4	1.52±0.4
<i>R. clivosus</i> De Hoop	5.5±0.2	17.3±0.9	33.8±1.6	209±20.6	5.5±0.3	8.2±1.1	1.45±0.3
<i>R. clivosus</i> SA	5.4±0.2	18.3±1.8	34±1.4	207±13.4	5.6±0.3	8.7±0.9	1.46±0.2

*R. clivosus* at De Hoop had a slightly higher wing loading and aspect ratio than *R. capensis* (Table 2). All three groups were similar in tip shape index (all  $P$ 's  $> 0.8$ ), although both *R. clivosus* populations had a slightly lower tip shape index than *R. capensis* (Table 2).

Principal components analysis on the morphology of *R. capensis*, *R. clivosus* at De Hoop and *R. clivosus* elsewhere identified three significant principal components. Mass, wingspan and wing area loaded highest on PC1, wing loading loaded highest on PC2 and aspect ratio and tip shape loaded highest on PC3. Thus PC1 was labelled "size," PC2 was labelled "wing loading" and PC3 was labelled "wing shape." PC1 accounted for 46.5%, PC2 accounted for 23.1%, and PC3 accounted for 14.3% of the total variance between groups.

Discriminant function analysis performed on the component scores of the three principal components showed PC1 best distinguished between the groups (F-to-remove<sub>2, 53</sub> = 124.8, Wilks' Lambda = 0.17,  $P < 0.001$ ), followed by PC2 (F-to-remove<sub>2, 52</sub> = 4.4, Wilks' Lambda = 0.15,  $P < 0.0001$ ). Principal component 3 was not included in the model (F-to-enter<sub>2, 51</sub> = 0.22, Wilks' Lambda = 0.15,  $P = 0.80$ ). Two discriminant functions were identified, and standardized coefficient values of the canonical scores showed PC1 loading highest on the first discriminant function and PC2 loading highest on the second discriminant function (Table 3). The first discriminant function was therefore labelled "size," and the second discriminant function was labelled "manoeuvrability." The first discriminant function accounted for 99.5% of the total variance but the second discriminant function only explained 0.5% of the total variance.

Only the first discriminant function was significant ( $P < 0.001$ ), again showing that body size best differentiated the three groups of bats.

The distance between the group centroids of *R. clivosus* at De Hoop and *R. clivosus* elsewhere was close to being significantly different from zero ( $P = 0.06$ ) and the smallest squared Mahalanobis distance ( $D^2 = 0.72$ ) occurred between these two groups. This is expected as these bats belong to the same species. Distances between the group centroids of *R. capensis* and *R. clivosus* at De Hoop and of *R. capensis* and *R. clivosus* elsewhere in South Africa were significantly different from zero ( $P < 0.001$ ). Squared Mahalanobis distances were smaller between *R. capensis* and *R. clivosus* at De Hoop ( $D^2 = 17.8$ ) than between *R. capensis* and *R. clivosus* elsewhere ( $D^2 = 24.4$ ), suggesting *R. clivosus* has converged morphologically upon *R. capensis* (Figure 1).

A principal components analysis comparing the morphology of *R. capensis*, *R. clivosus* at De Hoop and *R. clivosus* at Sudwala again identified three significant principal components. Mass, wingspan and wing area loaded highest on PC1; wing loading and tip shape loaded highest on PC2; and aspect ratio and tip shape loaded highest on PC3. PC1 was labelled "size," PC2 was labelled "wing loading," and PC3 was labelled "wing shape." PC1 explained 46.7% of the total variance between groups, PC2 accounted for 20% of the total variance, and PC3 explained 13.5% of the total variance.

The discriminant function analysis showed that PC1, or body size, best distinguished between the groups ( $F\text{-to-remove}_{2,41} = 234.2$ , Wilks' Lambda = 0.06,  $P < 0.001$ ) followed by PC3 ( $F\text{-to-remove}_{2,41} = 172.5$ , Wilks' Lambda = 0.05,  $P < 0.001$ ) and PC2 ( $F\text{-to-remove}_{2,41} = 136.2$ , Wilks' Lambda = 0.04,  $P < 0.001$ ). Two discriminant functions were identified. Both discriminant functions were significant. Standardized

**Table 3.** Standardized and factor structure coefficients for principal components chosen in forward stepwise discriminant function analysis comparing the morphology of *R. capensis*, *R. clivosus* at De Hoop and *R. clivosus* elsewhere in South Africa.

	Discriminant Function 1		Discriminant Function 2	
Variable	Standardized coefficient	Factor structure coefficient	Standardized coefficient	Factor structure coefficient
Principal component 1	-1.05	-0.92	0.07	0.38
Principal component 2	-0.40	-0.06	-0.98	-1.0
Variance explained (%)		99.5		0.5

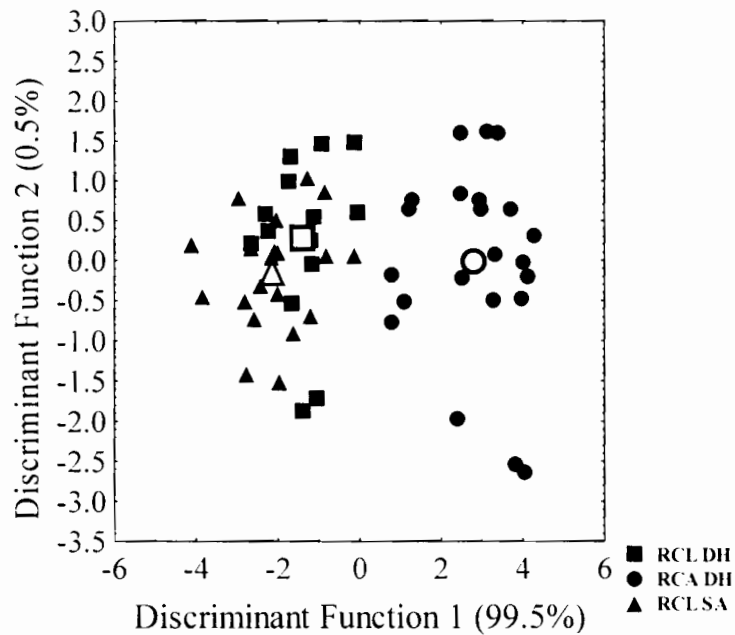
**Table 4.** Standardized and factor structure coefficients for principal components chosen in forward stepwise discriminant function analysis comparing the morphology of *R. capensis*, *R. clivosus* at De Hoop and *R. clivosus* at Sudwala.

	Discriminant Function 1		Discriminant Function 2	
Variable	Standardized coefficient	Factor structure coefficient	Standardized coefficient	Factor structure coefficient
Principal component 1	-0.20	-0.14	-1.01	-0.95
Principal component 2	2.04	0.27	-0.43	-0.07
Principal component 3	-2.02	-0.21	0.15	0.02
Variance explained (%)		57.2		42.8

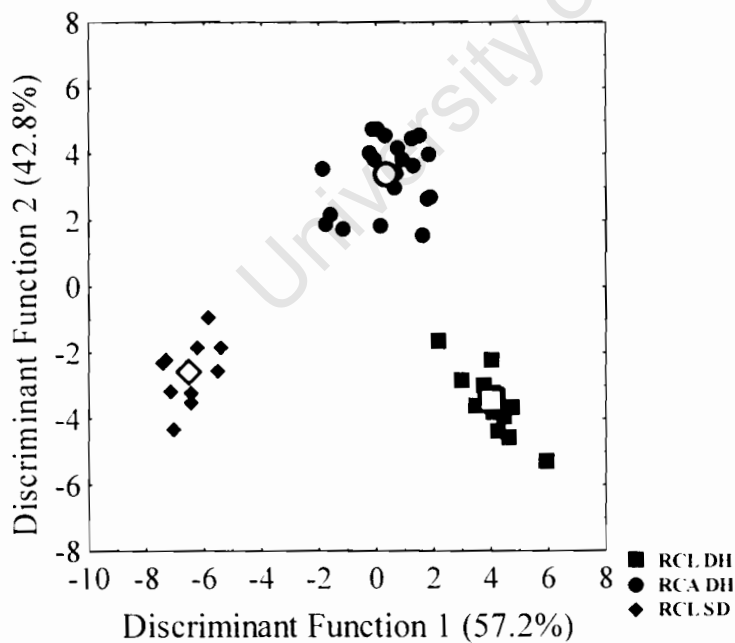
coefficient values of the canonical scores showed discriminant function 1 mainly dealt with PC2 and PC3, while discriminant function 2 dealt mainly with PC1 (Table 4). Therefore discriminant function 1 was labelled “manoeuvrability and agility” and discriminant function 2 was labelled “size.” Discriminant function 1 explained 57.2% of the total variance between groups while discriminant function 2 accounted for 42.8% of the total variance. *R. clivosus* at De Hoop and Sudwala were separated from *R. capensis* along discriminant function 2, body size. *R. capensis* and *R. clivosus* at De Hoop were separated from *R. clivosus* at Sudwala along discriminant function 1, suggesting *R. clivosus* at De Hoop has converged in wing loading upon *R. capensis*.

Distances between group centroids were significantly different from zero for all the groups ( $P < 0.001$ ). The squared Mahalanobis distance between *R. capensis* and *R. clivosus* at De Hoop ( $D^2 = 60.5$ ) was smaller than that between *R. capensis* and *R. clivosus* at Sudwala ( $D^2 = 83.0$ ) and more importantly between *R. clivosus* at Sudwala and *R. clivosus* at De Hoop ( $D^2 = 111.7$ ). This suggests that *R. clivosus* at De Hoop has diverged slightly from *R. clivosus* at Sudwala and converged upon *R. capensis* (Figure 2).

Regressions of mass on wingspan, wing area, wing loading and aspect ratio for the South African Rhinolophidae (Figure 3 a-d) revealed a strong positive correlation between mass and wingspan ( $r = 0.95$ ,  $p < 0.001$ ; Figure 3a), mass and wing area ( $r = 0.96$ ,  $p < 0.001$ ; Figure 3b), and mass and wing loading ( $r = 0.94$ ,  $p < 0.001$ ; Figure 3c), but there was no significant correlation between mass and aspect ratio ( $r = -0.07$ ,  $p = 0.85$ ). *Rhinolophus capensis*, *R. clivosus* at De Hoop and *R. clivosus* elsewhere fell within the 95% confidence limits for all of the morphological parameters. Thus none of the bats

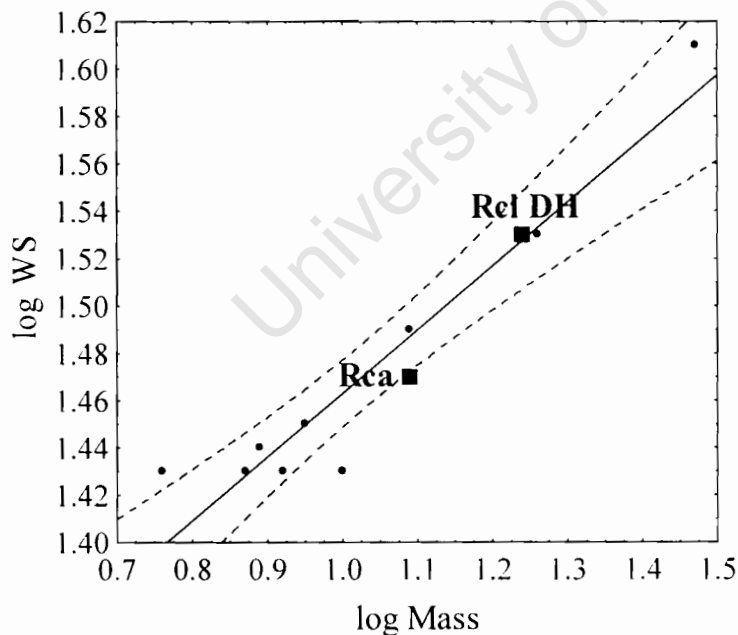


**Figure 1.** Plot of discriminant function 1 vs. discriminant function 2 illustrating the position of *R. capensis* (Rca), *R. clivosus* at De Hoop (Rcl DH) and *R. clivosus* elsewhere (Rcl SA) in multivariate morphological space. Open boxes are group centroids.

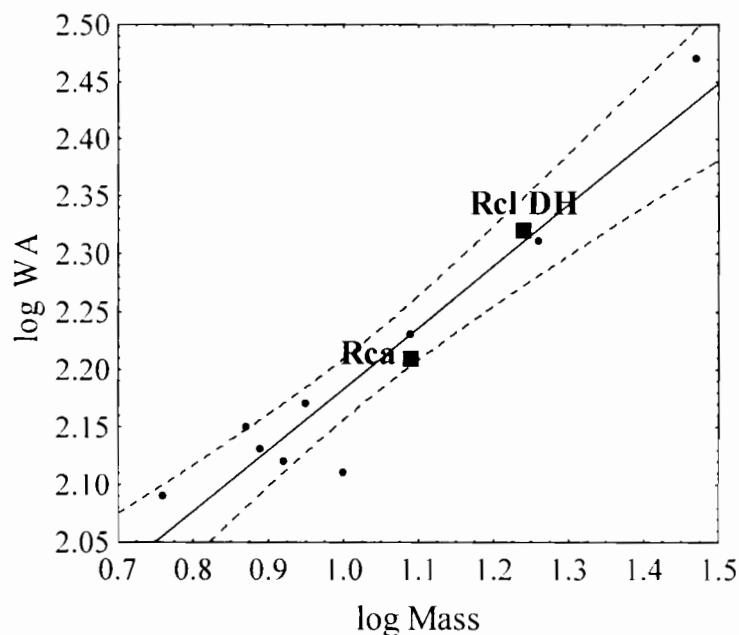


**Figure 2.** Plot of discriminant function 1 vs. discriminant function 2 illustrating the position of *R. capensis* (Rca), *R. clivosus* at De Hoop (Rcl DH) and *R. clivosus* at Sudwala (Rcl SD) in multivariate morphological space. Open boxes are group centroids.

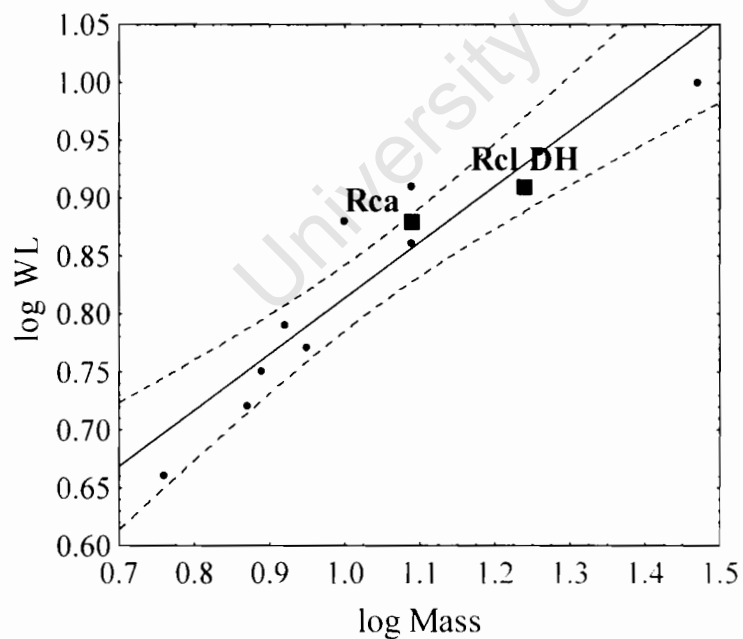
deviated from the allometry for the family, consistent with the absence of strong selection on any of the morphological characters. *Rhinolophus clivosus* at De Hoop was smaller than *R. clivosus* elsewhere in South Africa in terms of mass and wingspan, although not significantly so (*Tukey's P* = 0.23 and 0.95, respectively). The *R. clivosus* populations had a similar wing area (*Tukey's P* = 0.94). Thus as a consequence of having a smaller body size (see DFA above), *R. clivosus* at De Hoop had a lower wing loading than *R. clivosus* elsewhere. However, this difference in wing loading was not significant (*Tukey's P* = 0.48). Wing loading was not significantly different between *R. capensis* and *R. clivosus* at De Hoop, either (*Tukey's P* = 0.25). *Rhinolophus clivosus* at De Hoop has undergone a reduction in body size with a correlated reduction in wing loading, aspect ratio and tip shape index.



**Figure 3a.** Least squares regression of wingspan on mass showing the allometric relationship for the South African Rhinolophidae. Neither *R. capensis* (Rca) nor *R. clivosus* (Rcl DH) deviates from the relationship for the family.



**Figure 3b.** Regression of wing area on mass showing the allometric relationship for the South African Rhinolophidae. Neither *R. capensis* (Rca) nor *R. clivosus* (Rcl DH) deviates from the allometric relationship for the family.



**Figure 3c.** Regression of wing loading on mass showing the allometric relationship for the South African Rhinolophidae. Neither *R. capensis* (Rca) nor *R. clivosus* (Rcl DH) deviates from the allometric relationship for the family.

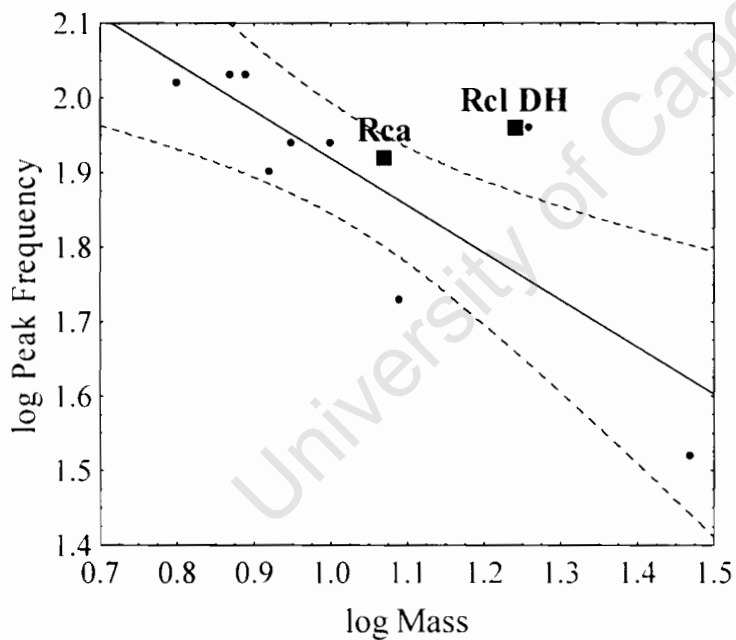
In summary, the major difference between the three populations is in size as indicated by the DFA in which the convergence of *R. clivosus* at De Hoop on *R. capensis* is mainly along axes associated with size. Furthermore, in conjunction with the regression results, *R. clivosus* at De Hoop has retained the wing shape typical of the South African Rhinolophidae while undergoing a reduction in body size such that the De Hoop population is just a scaled down version of the other two *R. clivosus* populations.

### **Echolocation**

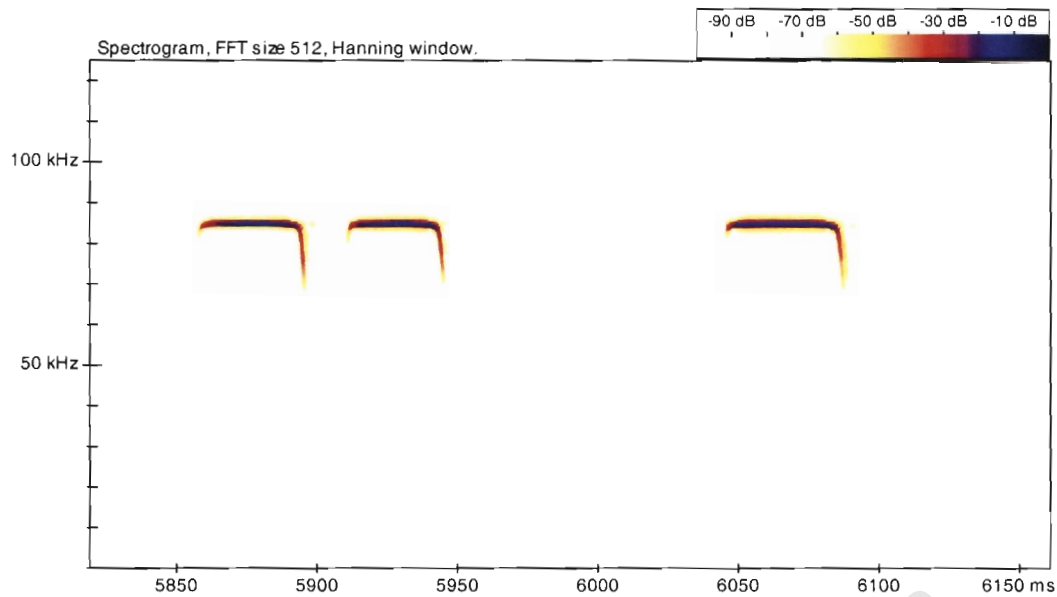
The echolocation calls of *R. clivosus* at De Hoop and *R. capensis* were separated in peak frequency by eight kilohertz (Mann-Whitney U-test,  $U = 0.0$ ,  $P < 0.001$ ; Table 5). The results of least squares regression performed on the November data (*R. capensis*  $n = 19$ , *R. clivosus*  $n = 18$ ) revealed *R. clivosus* at De Hoop had a higher frequency call relative to its body size because it fell outside the 95% confidence limits for the regression (Figure 4). Both bats used a short FM component at the start of the call (Figure 5). Pulse intervals were shorter for *R. capensis* than for *R. clivosus* (Mann-Whitney U-test,  $U = 67$ ,  $P < 0.01$ ). Both bats used a down-sweeping FM component of similar bandwidth at the end of each call (Mann-Whitney U-test,  $U = 111$ ,  $P = 0.07$ ). Both species had long duration (Mann-Whitney U-test,  $U = 170$ ,  $P = 0.98$ ) high frequency CF calls characteristic of bats foraging for fluttering insects in cluttered environments (Table 5). They both emitted high duty-cycle calls, but *R. capensis* emitted slightly higher duty-cycle calls than *R. clivosus* (Mann-Whitney U-test,  $U = 84$ ,  $P < 0.01$ ).

**Table 5.** Ranges, means and standard deviations of echolocation call parameters for *R. capensis* (n = 19) and *R. clivosus* at De Hoop (n = 18).

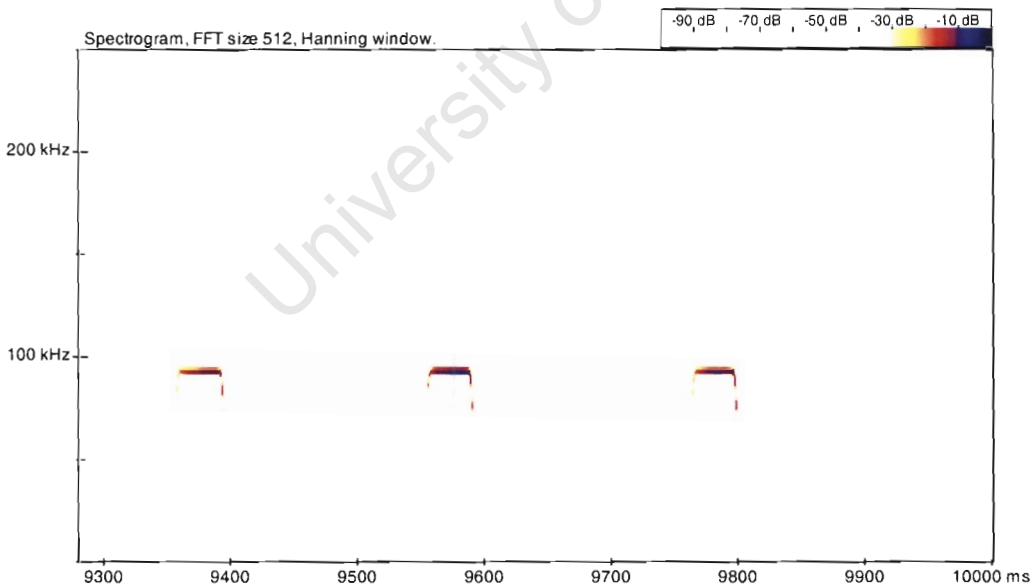
Species	Peak frequency (kHz)	Range peak frequency (kHz)	Duration (ms)	Duty cycle
<i>R. capensis</i>	84.0±1.2	82.2-86.0	38.2±6.4	0.95±0.25
<i>R. clivosus</i> De Hoop	92.0±0.92	90.3-93.5	37.5±7.0	0.73±0.19



**Figure 4.** Regression of mass on echolocation peak frequency for the South African Rhinolophidae. *R. clivosus* at De Hoop deviates from the allometry for the family and calls at a higher peak frequency than expected for its mass.



**Figure 5a.** The calls of *R. capensis* are composed of a short upward FM sweep (which may or may not be present) at the start of the call, a longer duration constant CF component, and a short downward FM sweep.



**Figure 5b.** The calls of *R. clivosus* are similar in structure to those of *R. capensis*, but the downward FM sweep is of longer duration.

## Diet

When volumetric data were analysed over the months for which sufficient dietary data for both species were obtained – January, March, May, July, and November – the diets of the two bats (*R. capensis*  $n = 56$ , *R. clivosus*  $n = 38$ ) were significantly different (ANOSIM R-statistic = 0.177,  $p = 0.001$ ). The significant difference in diet is most likely due to individual variation in dietary composition, because overall the proportions of Lepidoptera and Coleoptera consumed by *R. capensis* and *R. clivosus* were very similar (Table 6 and 7) and the results of a two-sided test for differences in proportions showed the bats ate similar proportions of Lepidoptera and Coleoptera ( $P = 0.65$  and  $P = 0.61$ , respectively). When dietary composition was examined within each month, the two species consumed similar proportions of Lepidoptera and Coleoptera (all z-scores  $P > 0.1$ ). Similarity percentage analysis showed that the diets of *R. capensis* and *R. clivosus* overlapped (Table 6; Figure 6 and 7). The two species shared a dietary similarity of 40.01%. Within-species dietary similarity was not that much higher, with 51.32% for *R. capensis* and 47.92% for *R. clivosus*. Lepidoptera contributed the most to the within-species similarity for *R. capensis*, at 45.2%. Coleoptera contributed the most to the within-species similarity for *R. clivosus*, at 29.6%. The food categories contributing most to the dissimilarity between the diets of the two bat species were Lepidoptera, which accounted for 24.46% of the dissimilarity between the diets of the two species, and Coleoptera, which accounted for 22.85% of the dissimilarity between the diets of the two species.

**Table 6.** SIMPER analysis results for 2005 (January, March, May, July and November).

	<i>R. capensis</i> n = 56 (560)	<i>R. clivosus</i> n = 38 (380)	<i>R. capensis-R. clivosus</i>
Prey type	Average per cent similarity within species group 51.32	Average per cent similarity within species group 47.92	Average per cent dissimilarity between species groups 60.99
Coleoptera	4.21	29.6	22.85
Lepidoptera	45.2	17.13	24.46
Neuroptera	0	0.01	8.94
Hemiptera	1.49	1.13	1.26
Diptera	0.42	0.05	3.47

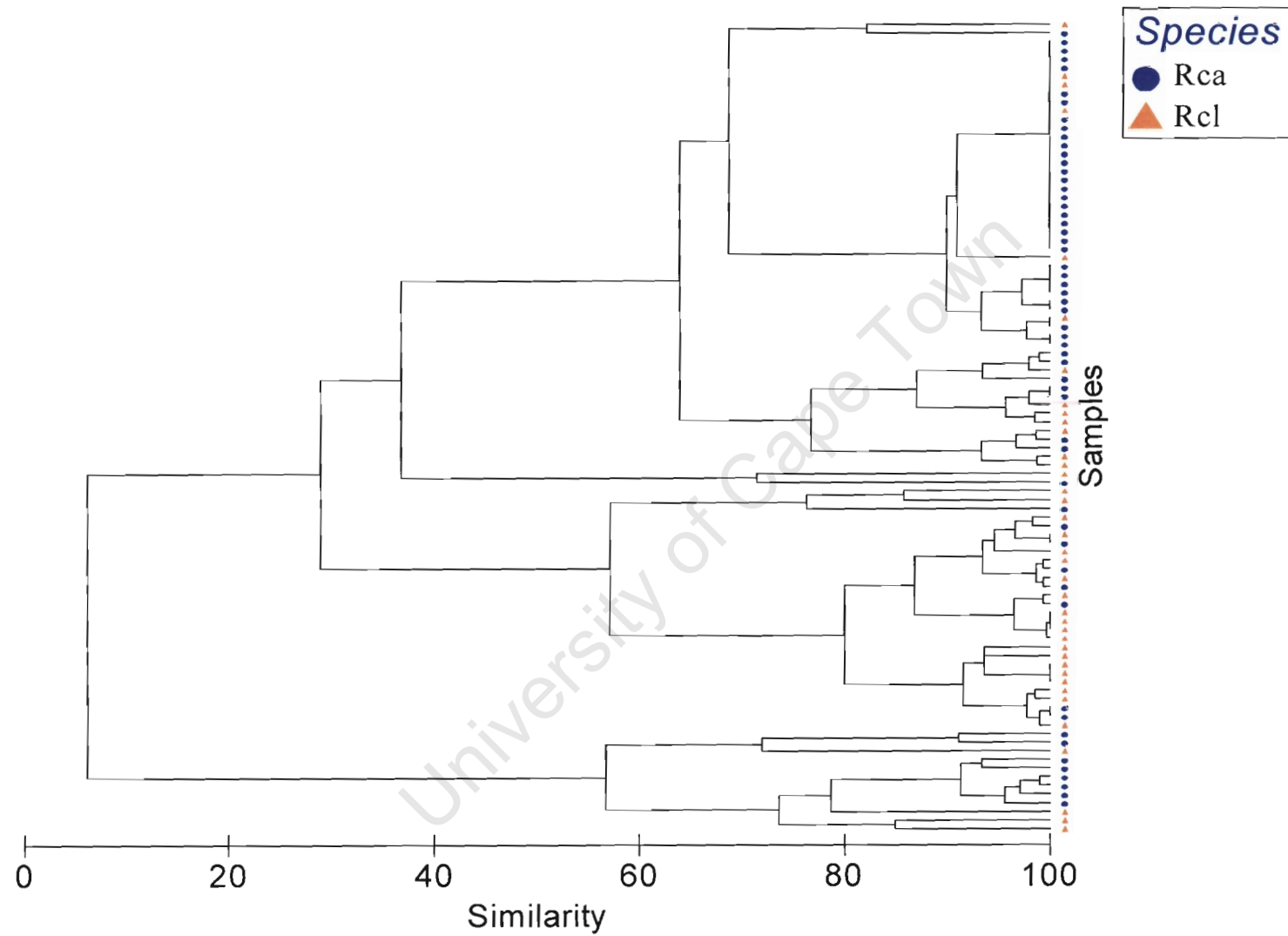
**Table 7.** Average relative percent volume of prey types found in diets of *R. capensis* and *R. clivosus* for pooled 2005 data (January, March, May, July and November). Numbers in parentheses denote number of faecal pellets analyzed.

Prey type	<i>R. capensis</i> n = 56 (560)	<i>R. clivosus</i> n = 38 (380)
Coleoptera	0.30	0.34
Lepidoptera	0.542	0.48
Neuroptera	0.014	0.02
Hemiptera	0.11	0.12
Diptera	0.034	0.04

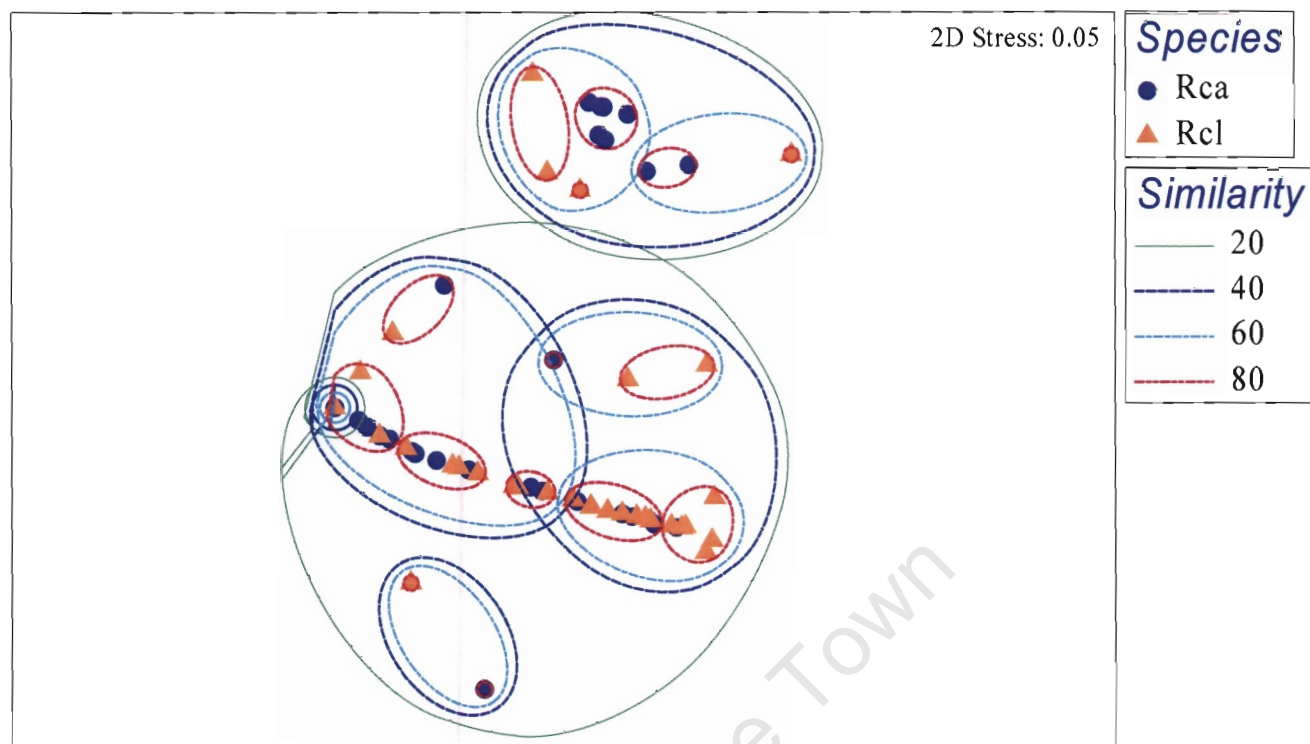
The dendrogram (Figure 6) illustrates two dietary groupings: one cluster consisting largely of *R. capensis* with some *R. clivosus* and a second cluster containing mainly *R. clivosus* but also *R. capensis*. Bats cluster further into four subgroups. Dietary overlap is indicated by the clusters not being exclusive to either species.

The MDS plot (Figure 7) illustrates dietary overlap between the two species. The low stress level of the MDS plot (0.05) indicates the compression of the dietary matrix into two-dimensional space has accurately preserved the similarities and differences between individual bats. The plot is consistent with the dendrogram, dividing bats into

two major groups with *R. capensis* mainly forming the group at the top of the plot and *R. clivosus* mainly forming the group at the bottom of the plot. The bats share a 20% dietary similarity within each of these groups and are further divided into four groups in which individuals share a 40% dietary similarity. Group A at the top of the plot consists of *R. capensis* and *R. clivosus* with Hemiptera in their diets. Group B on the left contains *R. capensis* and *R. clivosus* eating mainly Lepidoptera or eating mainly Lepidoptera with Coleoptera, in similar proportions. Most of the bats in Group B actually share a 60% dietary similarity, and Group B partially overlaps with Group C. Group C on the right of the plot is formed by *R. capensis* and *R. clivosus* consuming mainly Coleoptera with similar proportions of Lepidoptera and Hemiptera. The *R. capensis* and *R. clivosus* in the region of overlap between Group B and C are bats consuming both Lepidoptera and Coleoptera, with proportions of one taxa more similar to Group B and proportions of the other taxa more similar to Group C. Group D at the bottom of the plot contains *R. capensis* and *R. clivosus* with Neuroptera in addition to Lepidoptera and Coleoptera in their diets. As ten faecal pellets were analysed for each of the two individuals in Group D, these two bats were not considered outliers. The small amounts of Neuroptera consumed by both species (Table 7) could be the result of single opportunistic capture events.



**Figure 6.** Dendrogram showing relationships in dietary composition between *R. capensis* and *R. clivosus* at De Hoop in 2005.



**Figure 7.** Non-metric multidimensional scaling plot of similarities in dietary composition between *R. capensis* and *R. clivosus* at De Hoop in 2005. The bats form two main groups each sharing a 20% similarity in diet, and divide further into four subgroups sharing a 40% similarity in diet.

The dietary overlap between the two species may have been due to the fact that the diets of *R. capensis* and *R. clivosus* at De Hoop varied markedly in composition from month to month. Differences in the relative proportions of all taxa in the diet of *R. capensis* across the months of January, March, May, July and November 2005 were statistically significant, except for Neuroptera (*Kruskal-Wallis*  $H_{4, 56} = 5.2$ ,  $P = 0.26$ ). *Rhinolophus capensis* ate very small proportions of Neuroptera, in January. Multiple comparisons showed *R. capensis* consumed proportionately more Coleoptera in January than in July or November, and more in November than in May (*Kruskal-Wallis*  $H_{4, 56} = 33.16$   $P \ll 0.001$ ; all z-scores less than 4.06,  $P < 0.001$ ; Table 8). Proportions of

Lepidoptera were larger in November than in January or July, but smaller than May; *R. capensis* ate a larger proportion of Lepidoptera in March than in July (*Kruskal-Wallis*  $H_{4, 56} = 43.67$ ,  $P < 0.001$ ; all z-scores less than 5.78,  $P < 0.001$ ; Table 8). Proportions of Hemiptera in the bat's diet were significantly larger in July than in any other month of the year sampled (*Kruskal-Wallis*  $H_{4, 56} = 50.36$ ,  $P < 0.001$ ; all z-scores less than 4.38,  $P < 0.001$ ). *R. capensis* consumed Diptera in July only (*Kruskal-Wallis*  $H_{4, 56} = 47.50$ ,  $P < 0.001$ ; all z-scores less than 3.83,  $P < 0.001$ ). The diet of *R. clivosus* showed a significant difference only in the proportions of Lepidoptera (*Kruskal-Wallis*  $H_{4, 38} = 9.70$ ,  $P < 0.05$ ) eaten over the course of the months sampled. *R. clivosus* ate a considerably larger proportion of Lepidoptera in May than in July ( $z = 2.9$ ,  $P = 0.01$ ; Table 8).

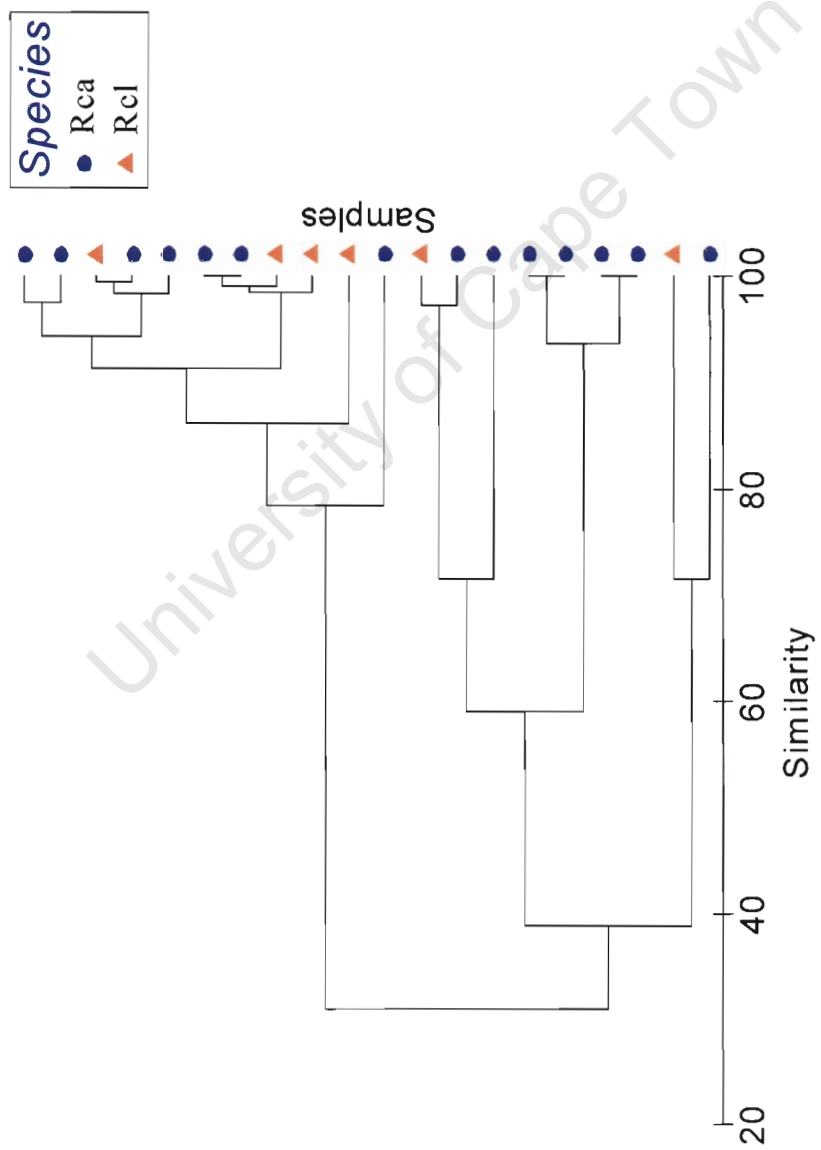
In April 2005, a flash flood left the De Hoop vlei submerged in more than 1 m of water for the rest of the winter and much of the following summer. Different relationships occurred between the diets of *R. capensis* and *R. clivosus* during the period when the De Hoop vlei was dry and when the vlei was full of water. ANOSIM results for the period when the vlei was dry (January and March; *R. capensis*  $n = 14$ , *R. clivosus*  $n = 6$ ), show the diets of the two species were similar to each other (R-statistic = -0.058,  $p = 0.691$ ). Results of SIMPER analysis were consistent with this finding, showing the bats were 45.15% dissimilar in diet. Within-group similarity was slightly higher for *R. clivosus* (58.89%) than for *R. capensis* (51.14%). Both bats ate Coleoptera and Lepidoptera, but *R. capensis* ate more Coleoptera than *R. clivosus* did, with Coleoptera composing 53% percent of the diet of *R. capensis* and 50% percent of the diet of *R. clivosus*. *R. clivosus* ate more Lepidoptera than *R. capensis* did, with Lepidoptera making up 46% percent of the diet of *R. clivosus* and 37% of the diet of *R. capensis*. The taxa

most responsible for differences between the groups were Coleoptera, with an average contribution of 43.62%, Lepidoptera, with a contribution of 40.16%, and Neuroptera to a lesser degree, with a contribution of 13.46% to the average dissimilarity between groups. Individuals of both species ate Neuroptera, but *R. clivosus* consumed a greater proportion of Neuroptera than *R. capensis*. However, neither species preyed upon Neuroptera to a great degree: only one *R. capensis* and two *R. clivosus* consumed Neuroptera. The dendrogram (Figure 8a) illustrates three main groups of bats each consisting of both bat species. The two-dimensional MDS plot (Figure 8c) which has a low stress value (0.03), also depicts three main groups of bats, with 40% similarity within each group. It further divides the bats into four main groups with 60% similarity within each group. Group A, in which most of the bats cluster, contains bats of both species which ate mainly Coleoptera and to a lesser extent Lepidoptera; bats of both species in Group B ate mainly Lepidoptera and to a lesser extent Coleoptera, with one *R. capensis* additionally consuming Hemiptera. Group C is formed by *R. capensis* eating Coleoptera and Lepidoptera in more or less equal proportions; and Group D contains one *R. capensis* and one *R. clivosus* which ate large proportions of Neuroptera.

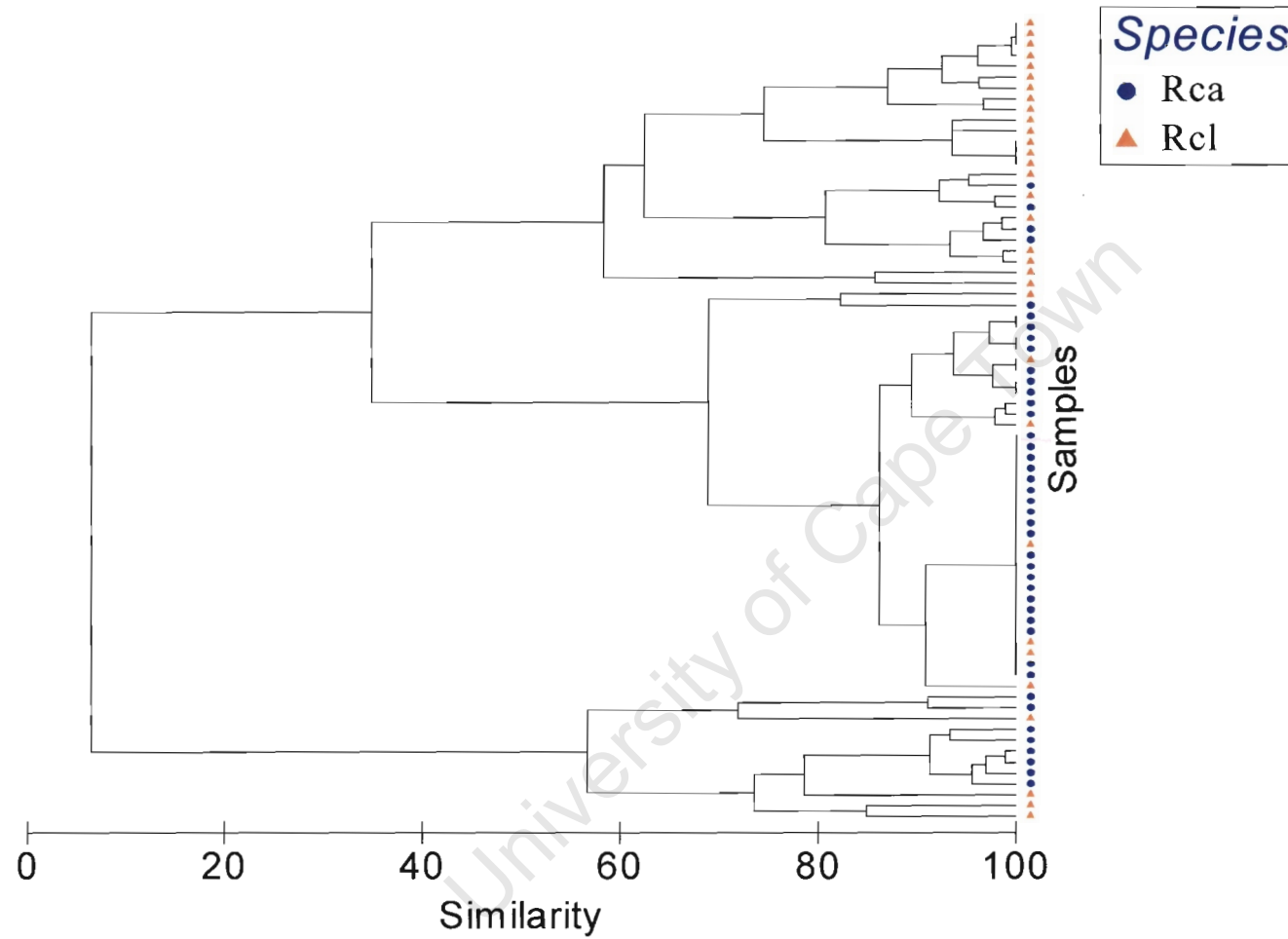
The diets of *R. capensis* and *R. clivosus* diverged after the vlei filled with water (Figure 8b and d). Results of ANOSIM analysis (May, July and November; *R. capensis* n = 42, *R. clivosus* n = 32) show a significant difference in dietary composition between the two species (R-statistic = 0.26,  $P = 0.001$ ) with a between-group dissimilarity of 63.15%. The diets of the two bats were distinguished from each other by the proportion of Lepidoptera, which accounted for 40.86% of the average dissimilarity between species, and to a lesser extent, by the proportion of Coleoptera, which accounted for 35.42% of

**Table 8.** Seasonal dietary composition for *R. capensis* and *R. clivosus* in 2005, by average relative percent volume of prey types found in faecal samples. The dietary diversity index (DDI) values reported in the table suggests that the diet of *R. capensis* is more specialized than that of *R. clivosus* during all of the months for which volumetric data were available for both species, except for January, when the diet of *R. clivosus* was slightly more specialized than that of *R. capensis*. \*\* indicates prey was eaten in extremely small amounts.

	January		March		May		July		November	
Prey type	<i>R. capensis</i> n = 9 (90)	<i>R. clivosus</i> n = 3 (30)	<i>R. capensis</i> n = 5 (50)	<i>R. clivosus</i> n = 3 (30)	<i>R. capensis</i> n = 12 (120)	<i>R. clivosus</i> n = 5 (50)	<i>R. capensis</i> n = 9 (90)	<i>R. clivosus</i> n = 5 (50)	<i>R. capensis</i> n = 22 (220)	<i>R. clivosus</i> n = 14 (140)
Coleoptera	0.64	0.66	0.26	0.742	0.12	0.31	**	0.40	0.22	0.28
Lepidoptera	0.23	0.19	0.74	0.255	0.88	0.69	0.08	0.02	0.74	0.68
Neuroptera	0.09	0.15	0	0.003	0	0	0	0	0	0
Hemiptera	0.04	0	0	0	0	0	0.70	0.35	0	0.04
Diptera	0	0	0	0	0	0	0.22	0.24	0	0
DDI	2.10	2.01	0.62	1.63	1.26	1.75	1.82	2.99	1.06	2.22

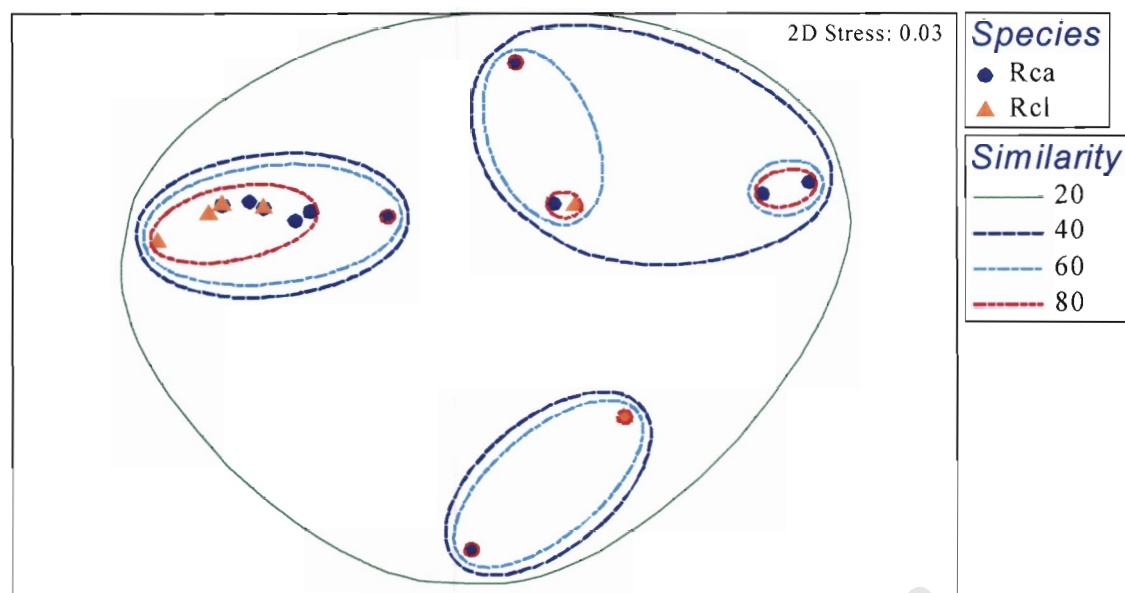


a.

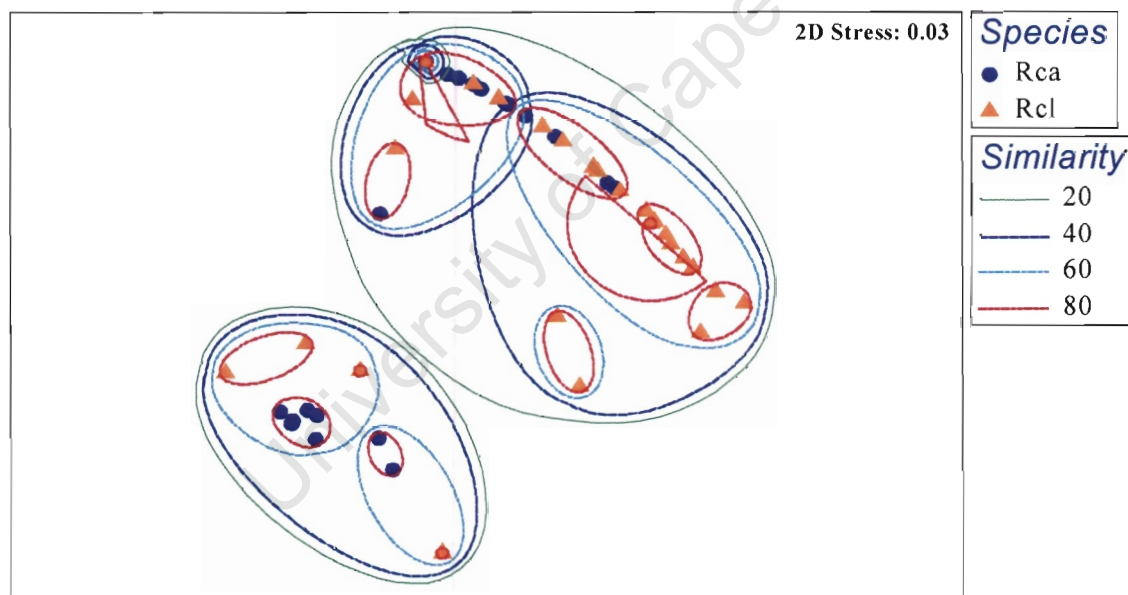


**b.**

**Figure 8 (a-b).** Dendrograms showing dietary relationships between *R. capensis* and *R. clivosus* when the vleei was dry (a) and when the vleei was full of water (b).



c.



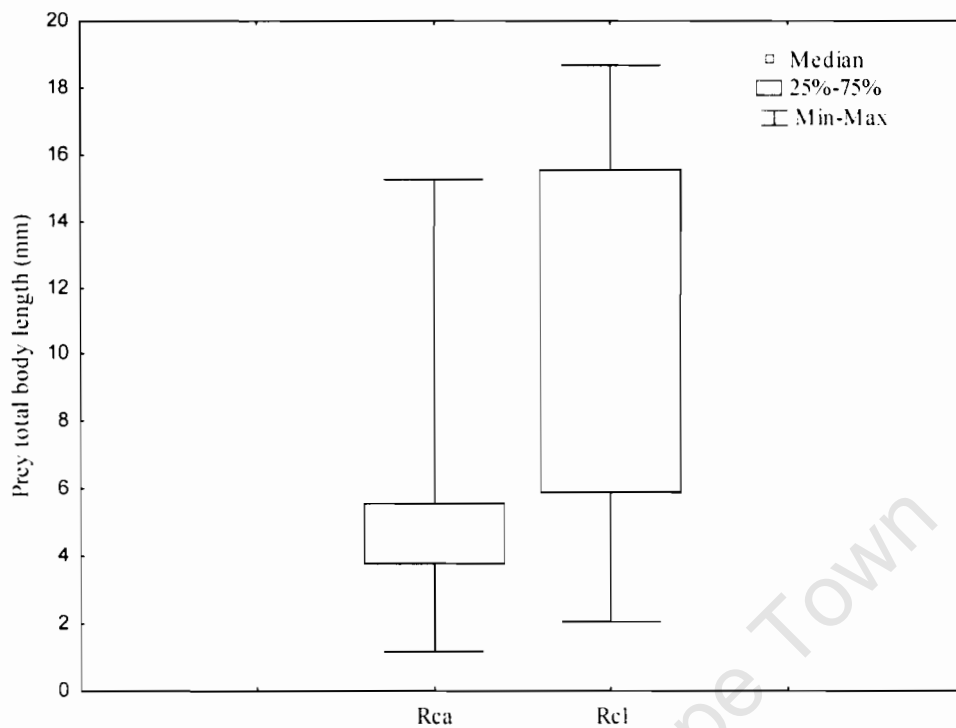
d.

**Figure 8 (continued).** Multidimensional scaling plots showing greater dietary overlap between the two species when the vlei was dry (**c**) than when the vlei was full (**d**). In (**c**) all bats shared a 20% dietary similarity as indicated by the green line around all of them. In (**d**) the bats are divided into two subgroups within which individuals shared a 20% dietary similarity.

the average dissimilarity between species. Hemiptera accounted for 16.81% of the average dissimilarity between species. *Rhinolophus capensis* consumed greater proportions of Lepidoptera than *R. clivosus* did (68% for *R. capensis* and 52% for *R. clivosus*), with Lepidoptera contributing 92.68% to the average similarity within the *R. capensis* group but only 39.88% to the average within-group similarity for *R. clivosus*. *R. clivosus* ate larger proportions of Coleoptera than *R. capensis* did (11% for *R. capensis* and 27% for *R. clivosus*), with Coleoptera accounting for 56.53% of the average within-group similarity for *R. clivosus*. Average percent similarity within the *R. capensis* group was greater when the vlei was full than it was when the vlei was dry (58.55% vs. 51.14%). The opposite was true for *R. clivosus* (46.55% vs. 58.89%).

The diet of *R. clivosus* did not change significantly after the vlei filled with water. *Rhinolophus clivosus* ate similar proportions of Coleoptera when the vlei was dry as when the vlei was full (*Kruskal-Wallis*  $H_{1,38} = 1.88$ ,  $P = 0.17$ ). The proportion of Lepidoptera in the bat's diet did not change after the vlei flooded either (*Kruskal-Wallis*  $H_{1,38} = 0.55$ ,  $P = 0.46$ ). When the vlei was dry, *R. capensis* ate considerably larger proportions of Coleoptera than it did when the vlei was full (*Kruskal-Wallis*  $H_{1,56} = 17.44$ ,  $P < 0.001$ ).

The median size of prey consumed by *R. capensis* (4.7 mm) was significantly smaller than that consumed by *R. clivosus* (13.9 mm; Mann-Whitney U-test,  $U = 101$ ,  $P < 0.001$ ). However, there was some overlap in the range of prey sizes eaten by the two species. The prey *R. capensis* ate ranged in size from 1.2-15.2 mm and that of *R. clivosus* ranged in size from 2.1-18.7 mm (Figure 9).



**Figure 9.** Box and whisker plot showing the overlap in the range of prey sizes consumed by *R. capensis* and *R. clivosus* at De Hoop. The median prey size eaten by *R. clivosus* is larger than that eaten by *R. capensis*.

The diet of *Rhinolophus capensis* (DDI 0.62-2.01) seemed only slightly more specialised than that of *R. clivosus* (DDI 1.63-2.22) during all of the months sampled except for January. The diets of *R. capensis* and *R. clivosus* show a similar trend over the course of the year. The bats' diets decreased in diversity between January and March, increased between March, May and July, and decreased again in November (Table 9). Dietary diversity for both bats increased during the winter months when the abundance of flying insects would be expected to be the lowest. The reason for this increase in dietary breadth could be that two main components of the bat community, *Miniopterus natalensis* and *Myotis tricolor*, are absent from De Hoop during the winter; or that the abundance of preferred insects during these months decreases; or alternatively that the

water in the vleis may support a greater diversity of insects than normally expected during winter, with the bats preying opportunistically upon these insects.

Both *R. capensis* and *R. clivosus* appeared to prey opportunistically upon Coleoptera, Lepidoptera, Diptera and Hemiptera when their diets were examined over the course of the entire year ( $\chi^2 = 0.95$ ,  $df = 3$ ,  $P = 0.81$  and  $\chi^2 = 0.33$ ,  $df = 3$ ,  $P = 0.95$ , respectively). When the bats' diets were examined within each month, however, the two species seemed to be foraging selectively on certain orders during particular months.

*Rhinolophus clivosus* appeared to feed selectively on Coleoptera in May, July and August, consuming significantly more Coleoptera than expected from the abundance of Coleoptera in light trap samples for these months ( $\chi^2 = 15.1$ ,  $df = 4$ ,  $P < 0.01$ ).

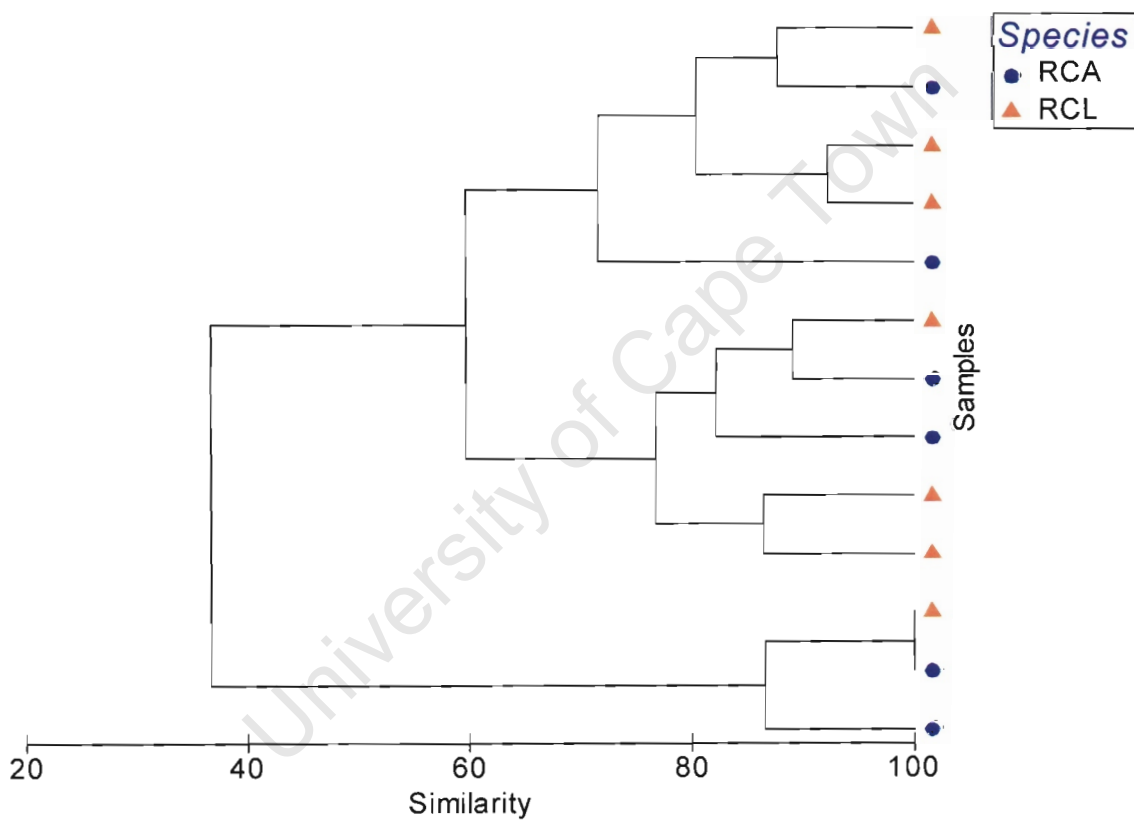
*Rhinolophus capensis* appeared to prey selectively upon Lepidoptera in January, March, May and November, eating more Lepidoptera than expected from the abundance of Lepidoptera in light trap samples ( $\chi^2 = 76.4$ ,  $df = 4$ ,  $P < 0.001$ ). *Rhinolophus clivosus* also appeared to forage selectively on Lepidoptera, consuming more Lepidoptera than expected in January, March, and May ( $\chi^2 = 29.3$ ,  $df = 4$ ,  $P < 0.001$ ). *Rhinolophus capensis* appeared to prey selectively upon Diptera in January, eating significantly more Diptera than expected from the abundance of Diptera in light trap samples ( $\chi^2 = 102.4$ ,  $df = 4$ ,  $P < 0.001$ ) as did *R. clivosus* ( $\chi^2 = 123.1$ ,  $df = 4$ ,  $P < 0.001$ ). The apparent opportunism and selectivity in diet should be considered with caution as the inherent bias in light-trapping samples might account for the significant differences between the abundance of certain orders and the proportions of the various orders consumed.

## Flight Behaviour

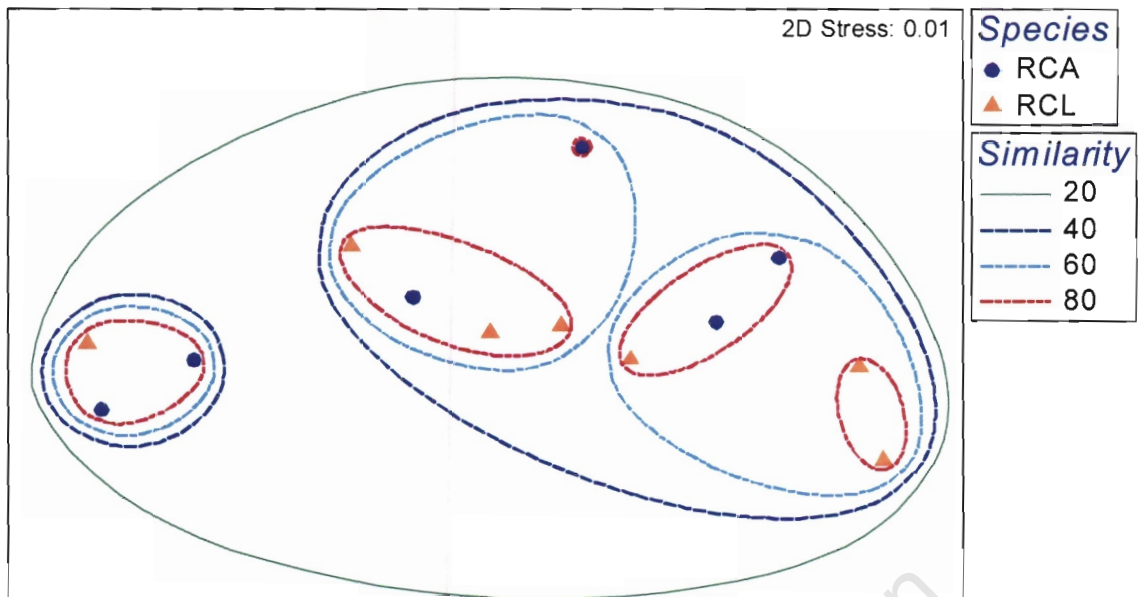
When examined at a broad scale, differences in flight behaviour between *R. capensis* and *R. clivosus* were small. The habitat-use indices of the two species were not largely different. *R. clivosus* (H-U index 3.2) used a slightly greater diversity of habitats than *R. capensis* (H-U index 2.3). The degree of clutter *R. capensis* and *R. clivosus* encountered did not differ significantly (ANOSIM r-statistic = -0.1,  $p = 0.82$ ). The dendrogram (Figure 10), which shows *R. capensis* and *R. clivosus* do not form two distinct groups, supports this result. The multidimensional scaling plot (Figure 11) shows the bats are divided into three groups, with Group A at the far left consisting of bats spending most of the time observed in clutter, Group B in the centre consisting of bats spending similar proportions of time in both cluttered and aerial environments, and Group C at the far right consisting of bats spending most of the time observed in open spaces. Each group is made up of both *R. capensis* and *R. clivosus* individuals.

Bats released from the vleij below the Guano Cave forest flew either over the water or directly in the direction of the forest and then disappeared into the cave or out of view. *R. clivosus* spent the greatest proportion of time flying over the water or perching whereas *R. capensis* spent the most time flying along the cliff face and low over the reeds, at reed-level (Figure 12 and 13). *R. capensis* was not observed perching. Both species flew over the water, and at both  $< 1\text{m}$  and  $> 1\text{m}$  in altitude, but *R. clivosus* spent more time in this zone as well as more time flying at both altitudes than *R. capensis* did. When examined at a fine scale, the differences in the time each species spent in each zone were significant (ANOSIM r-statistic = 0.2,  $p < 0.04$ ). The zones that best distinguished the two bats from each other were  $> 1\text{m}$  over water with an average

contribution of 14.4% to the differences between groups, flight at reed level, with a contribution of 12.6%, and < 1m above the reeds, with a contribution of 11.7%. *R. clivosus* spent more time flying > 1m over the water than *R. capensis* did, and also spent more time flying over the water in general than *R. capensis* did (26% versus 18%). *R. capensis* spent more time flying at reed level and < 1m above the reeds than *R. clivosus* did.

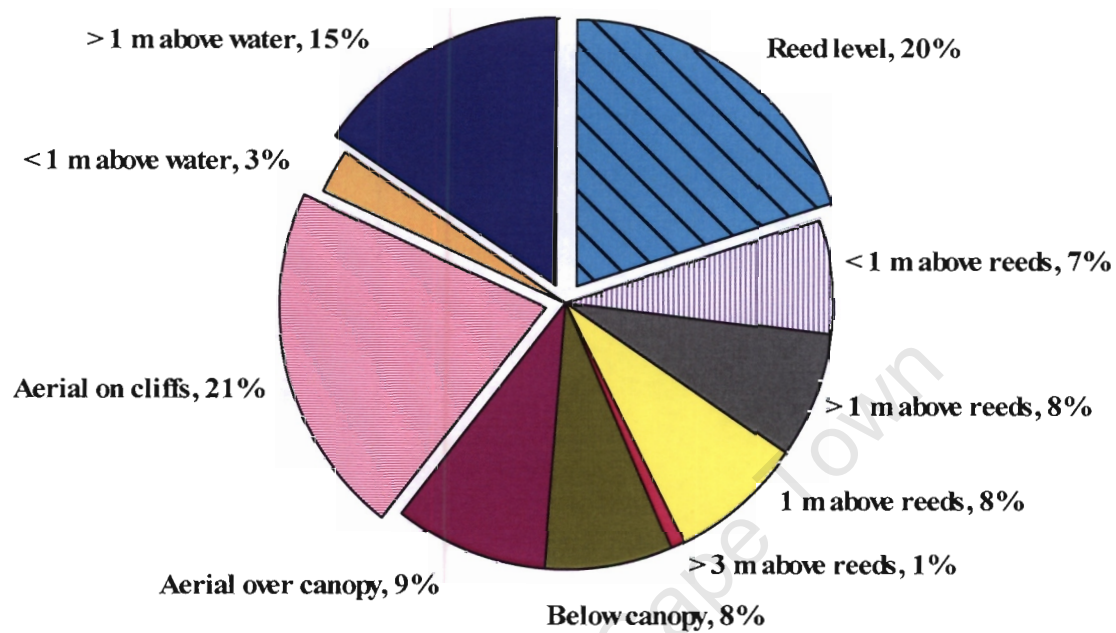


**Figure 10.** Dendrogram showing the clustering pattern based upon the degree of clutter bats encountered during flight. The bats do not form distinct clusters, showing that *R. capensis* did not spend significantly more time in spatially and acoustically cluttered environments than *R. clivosus* did.



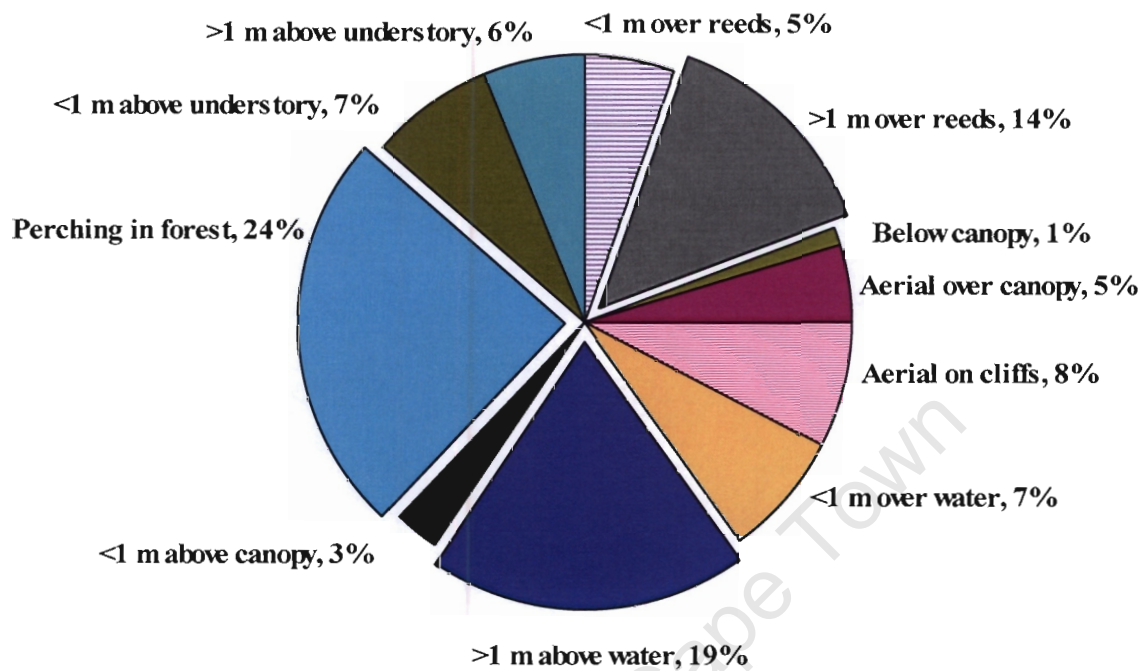
**Figure 11.** Non-metric multidimensional scaling plot illustrating the similarities in the flight space used by *R. capensis* and *R. clivosus* at De Hoop. The bats form one group sharing 20% similarity in the degree of clutter encountered, but are split into two subgroups sharing a 40% similarity and are split further into three groups sharing a 60% similarity. Group A comprises bats that spent most of the time observed flying in cluttered spaces, Group B is made up of bats that spent similar proportions of time in cluttered and open spaces and Group C consists of bats that spent most of the time observed in aerial environments.

*Rhinolophus capensis* (n = 6) 25-26 November 2005  
TOTAL TIME OBSERVED: 4 MIN 38 SEC



**Figure 12.** Pie chart showing the average percent time *R. capensis* spent in each foraging zone on the nights of 25 and 26 November 2005.

*Rhinolophus clivosus* (n = 7) 25-26 November 2005  
TOTAL TIME OBSERVED: 12 MIN 1 SEC



**Figure 13.** Pie chart showing the average percent time *R. clivosus* spent in each foraging zone on the nights of 25 and 26 November 2005.

## CHAPTER 4

### DISCUSSION

The prediction that the phenotype of *R. clivosus* should converge upon that of *R. capensis* while diverging from *R. clivosus* populations elsewhere in South Africa was supported by the results of discriminant function analysis. The main change in *R. clivosus* at De Hoop was in body size with allometric responses in wing morphology. The discriminant function analysis suggested convergence between *R. capensis* and *R. clivosus* at De Hoop with respect to body size (Figure 1 and 2). The smaller squared mahalanobis distance between *R. capensis* and *R. clivosus* at De Hoop than between *R. capensis* and *R. clivosus* elsewhere or between *R. capensis* and *R. clivosus* at Sudwala supported this convergence. Although the two species at De Hoop had similar wing loading, aspect ratio and wing tip shape, the morphological convergence between *R. clivosus* and *R. capensis* at De Hoop was slight (Table 2).

Concomitantly, and also as predicted, discriminant function analysis showed divergence between *R. clivosus* at De Hoop and both *R. clivosus* elsewhere (in wing loading; Figure 1) and *R. clivosus* at Sudwala (in wing loading, aspect ratio and wing tip shape; Figure 2). Differences between the group centroids of *R. clivosus* at De Hoop and *R. clivosus* elsewhere were barely non-significant, but when *R. clivosus* at De Hoop was compared to *R. clivosus* at Sudwala, the differences were in fact significant, although the squared Mahalanobis distance was not large. The small squared Mahalanobis distances between *R. clivosus* populations were expected and simply reflect that the three populations of *R. clivosus* belong to the same species. *Rhinolophus clivosus* at De Hoop

has thus diverged slightly from both the general South African phenotype and more so from the phenotype of a local population in a different environment (Table 2). Divergence from the latter was greater because it involved a comparison with a population which, in all likelihood, has itself become locally adapted albeit to a different habitat.

The least squares regressions support the DFA results in showing a slight convergence in wing loading between *R. capensis* and *R. clivosus* at De Hoop, but they also indicate that neither population diverged significantly from the allometry for the family. This suggests that the overall wing shape of *R. clivosus* at De Hoop has not changed from that typical of the Rhinolophidae, but that a reduction in body size with correlated changes in wing loading, aspect ratio and tip shape reduces the morphological convergence between the two species at De Hoop (Figure 3). *Rhinolophus clivosus* is simply a “scaled up” version of *R. capensis* and a scaled down version of *R. clivosus* at Sudwala or elsewhere in South Africa. The latter is similar to the finding for *Lasiurus cinereus* by Jacobs (1996), in which island populations of this species were found to have undergone a reduction in body size with a consequent reduction in wing loading, thus diverging from the mainland population. Similarly, Solick and Barclay (2006) found that the wing loading of *Myotis evotis* was lower in mountain habitats than in prairies, conferring greater manoeuvrability for bats inhabiting the densely vegetated mountains. The reduction in body size of *R. clivosus* at De Hoop with a concomitant reduction in wing loading may represent local adaptation to the environment at De Hoop. This scaling down of *R. clivosus* at De Hoop may confer greater maneuverability for bats using the fynbos vegetation.

Although the wing morphology of *R. capensis* and *R. clivosus* at De Hoop converged slightly, the echolocation calls of the two species were quite different and there was no evidence of convergence. The long duration, high frequency CF calls of rhinolophids are well-suited to navigation and prey detection in cluttered habitats (Schnitzler and Kalko, 1998; Kalko and Schnitzler, 1998), particularly in the sort of dense forest environment in which the family is suspected to have first evolved (Bogdanowicz, 1992). Constant frequency calls allow bats to detect fluttering insects such as moths against background clutter (Schnitzler and Kalko, 1998). Both *R. capensis* and *R. clivosus* at De Hoop use high frequency calls of long duration but the calls are separated in frequency by 8 kilohertz. A separation in call frequency between the two species has also been reported by McDonald *et al.* (1990) and Jacobs *et al.* (submitted). Due to the short operating range of echolocation and the need for bats to fly maneuverably (Barclay and Brigham, 1991), body size and call frequency tend to have an inverse relationship (Jones 1999). Results of the least squares regression (Figure 4) supported the negative correlation between body size and call frequency in the Rhinolophidae (Bogdanowicz, 1992) and in bats in general (Jones 1999, Kingston *et al.*, 2000). However, *R. clivosus* had a higher frequency call than predicted by its body size and deviates markedly from the allometry for the family.

Several explanations could be advanced for the increase in call frequency in *R. clivosus*. Firstly, resource competition may have maintained this separation in frequency between the two species at De Hoop. If this hypothesis were correct, however, *R. clivosus* would have consumed significantly smaller prey than *R. capensis*. The minimum size prey a bat can detect is proportional to the wavelength of its echolocation call (Vaughan,

1972), thus the bat with the higher frequency, shorter wavelength call could perhaps detect and capture smaller prey and might consume a wider range of prey sizes.

*Rhinolophus clivosus* did not consume a wider range of prey sizes. The size ranges of prey consumed by the two species overlapped considerably (Figure 9). However, the bats overlapped only slightly in the 25-75% quartiles for prey size with *R. clivosus* consuming on average, larger prey than *R. capensis* which is opposite to what would be predicted based on the wavelengths of their calls.

An alternative explanation is the Allotonic Frequency Hypothesis, which suggests bats echolocating outside the range of moth "hearing" should include a larger proportion of tympanate moths in their diet than bats echolocating within the range of moth hearing (20-60 kilohertz: Fullard, 1982). Both *R. capensis* and *R. clivosus* use frequencies inaudible to moths, so the bats should capture similar proportions of moths. As predicted by the Allotonic Frequency Hypothesis, *R. capensis* and *R. clivosus* consumed similar proportions of moths, so the Allotonic Frequency Hypothesis does not explain the higher than expected frequency of *R. clivosus*.

The difference in call frequency could also be due to habitat selection. Based upon predictions of habitat use from echolocation call structure (Aldridge and Rautenbach, 1987; Neuweiler, 1989) the higher call frequency of *R. clivosus* would lead to the prediction that *R. clivosus* would forage in more dense clutter than *R. capensis*. This was not the case, however, because the bats exhibited similar flight behaviour: *R. clivosus* and *R. capensis* encountered similar levels of clutter (Figure 10 and 11). Thus the most likely explanation for the departure in call frequency of *R. clivosus* from the allometry for the family is social communication (Jacobs *et al.*, submitted). Partitioning

of sonar frequencies may allow bats to recognise the calls of conspecifics (Kingston *et al.*, 2000). In fact, some bats “eavesdrop” on the echolocation calls of other bats to locate food patches and roosting sites (Barclay, 1982). Partitioning of sonar frequencies may also help bats avoid being conspicuous to eavesdroppers, which, while it may not allow them to monopolise patchily distributed resources, it may certainly restrict access to them (Barclay, 1982).

Heller and von Helverson (1989) collected echolocation data on 12 species of Rhinolophoidea in a Malaysian rainforest and discovered call frequencies were more evenly distributed than predicted by chance, which suggested species in the guild partitioned frequency bands. The authors suggested this partitioning of sonar frequency bands could be influenced by the need for social communication. Yet when Kingston *et al.* (2000) repeated the study including additional species, call frequencies were actually less evenly distributed than expected by chance, which would indicate bats in the guild did not partition frequency bands. As Jacobs *et al.* (submitted) noted, the hypothesis that a non-random frequency distribution is indicative of acoustic partitioning assumes the forces shaping the community are in equilibrium and that the community is stable. Furthermore, Heller and von Helverson (1989) and Kingston *et al.* (2000) incorporated rhinolophids and hipposiderids in the same guild although the call structures of the two genera differ (Jacobs *et al.*, submitted). It is possible that the increase in call frequency has allowed *R. clivosus* to use a unique frequency with respect to other rhinolophids in the numerous communities in which it has established itself, thereby maintaining such a wide geographic distribution (Jacobs *et al.*, submitted).

The morphological convergence of *R. clivosus* on *R. capensis*, without correlated convergence of echolocation, suggests firstly that the evolution of echolocation can be uncoupled from that of morphology. Morphology and echolocation in bats supposedly forms an “adaptive complex” (Arita and Fenton 1997) but the results suggest that this is not always the case. Secondly, it suggests that, morphologically, *R. clivosus* has converged upon *R. capensis* to become locally adapted to the habitat at De Hoop. However, this can only be so, if both species have similar niches at De Hoop. Indeed, the morphology, habitat use and diet of the two species at De Hoop did overlap. Despite differences in body size, discriminant function analysis showed the two species overlap in wing loading, aspect ratio and wing tip shape (Figure 1, Table 2). This overlap is due to a reduction in the body size of *R. clivosus* at De Hoop with correlated changes in wing loading, aspect ratio and wing tip shape.

Perhaps as a consequence of morphological convergence between the two species, the niches of *R. capensis* and *R. clivosus* at De Hoop also overlapped with respect to diet and habitat use. The diets of *R. capensis* and *R. clivosus* overlapped in prey type (Figure 6 and 7) and prey size (Figure 9). Both bats foraged opportunistically, which may account for the overlap in prey type. Although *R. clivosus* is larger than *R. capensis*, *R. clivosus* was more of a dietary generalist both in terms of the amounts of each prey type eaten and the variety of prey consumed. This contradicts the predictions of Barclay and Brigham (1991) that larger bats should consume a lower diversity of prey, but supported the findings of Jacobs *et al.* (submitted) which suggest that the dietary niches of the two species overlap. The slightly more generalist diet of *R. clivosus* might facilitate coexistence with *R. capensis* and possibly other bat species at De Hoop. Its generalist diet

may be what has allowed it to become established in the De Hoop bat community as well as in other bat communities in diverse habitats throughout its extensive geographic range.

The median prey size consumed by *R. clivosus* was larger than that consumed by *R. capensis* despite the fact that *R. clivosus* had a higher call frequency. Although its higher call frequency may have allowed *R. clivosus* to detect slightly smaller fluttering prey, a difference of 8 kilohertz between the call frequencies of *R. capensis* and *R. clivosus* may not translate into a functional difference, as differences in wavelength between calls of high frequency are actually smaller than they are between calls of lower frequency. The actual difference between the wavelengths of the pulses the two bats emit is a mere 0.4 mm, which is smaller than any prey item eaten by either bat. Contrast this difference in wavelength with that between the calls of bats at 30 and 38 kHz, which is 2.5 mm. The difference in the size of insects *R. capensis* and *R. clivosus* are able to detect is therefore marginal (Jacobs *et al.*, submitted). *Rhinolophus clivosus* may simply consume slightly larger prey than *R. capensis* because it is energetically efficient for a larger bat to do so (Barclay and Brigham, 1991). The size of prey consumed by the two species overlapped, however, and this in addition to the overlap in prey type and diversity of prey eaten by the bats is evidence for dietary niche overlap between *R. capensis* and *R. clivosus* at De Hoop.

Dietary niche overlap between *R. capensis* and *R. clivosus* at De Hoop was further supported by similarities in habitat use. Rhinolophids tend to have low aspect ratio wings with rounded tips and low wing loading for slow, maneuverable flight close to vegetation (Norberg and Rayner, 1987). *Rhinolophus capensis* and *R. clivosus* are no exception. Despite differences in body size, the bats overlapped in wing loading, aspect ratio and tip

shape index, such that *R. clivosus* is simply a scaled up version of *R. capensis*. Therefore, aerodynamically speaking, the bats' flight performance should be similar. On a broad scale, the flight behaviour of *R. capensis* and *R. clivosus* was indeed similar. The low habitat diversity indices for the bats showed both *R. capensis* and *R. clivosus* flew in clutter. There was no difference in the level of clutter in which the bats flew (Figure 11 and 12). A fine-scale examination revealed small differences in flight behaviour which did not translate into a difference in the degree of clutter the bats encountered (Figure 9 and 10). However, future studies should directly test the maneuverability of *R. capensis* and *R. clivosus* to determine whether small morphological differences in wing loading and aspect ratio lead to functional differences in flight capability (e.g. Jones *et al.*, 1993). The overlapping niches of *R. capensis* and *R. clivosus* at De Hoop supports the view that the morphological convergence of *R. clivosus* on *R. capensis* is an indication that *R. clivosus* has become locally adapted to the habitat at De Hoop. However, the convergence is small and this may be due to factors that mitigate against local adaptation.

Niche overlap between *R. capensis* and *R. clivosus* at De Hoop could bring the two species into competition for limited resources. Competition may have prevented the phenotype of *R. clivosus* at De Hoop from converging to a greater extent upon that of *R. capensis*. Partitioning of insect prey with respect to size may have prevented the body size of the two species from converging any further. However, differences in median prey size may be explained by optimal foraging rather than resource partitioning. No direct evidence of resource partitioning at De Hoop was found. It is nevertheless possible that the two species may partition the resources along dimensions not considered in this study. For example they may do so by foraging at different times or using different foraging

sites. Future studies should focus on the details of the foraging behaviour of these two species. Should such studies find evidence for competition it may help elucidate the factors mitigating against local adaptation.

Gene flow between the *R. clivosus* population at De Hoop and *R. clivosus* populations elsewhere may be another factor contributing to the small phenotypic divergence between *R. clivosus* at De Hoop and *R. clivosus* at Sudwala and elsewhere in the country. Gene flow would also result in less phenotypic convergence between *R. clivosus* and *R. capensis* at De Hoop. Gene flow between *R. clivosus* populations elsewhere in South Africa and *R. clivosus* at De Hoop would introduce a greater degree of genetic variability into the De Hoop population, thus preventing the local genotype, and consequently the local phenotype, from becoming fixed. Whether the *R. clivosus* population remains stable throughout the year in the Guano Cave is unknown. Determining the degree of gene flow between *R. clivosus* populations requires a genetic study, which is beyond the scope of this research.

Phenotypic plasticity may be an alternative explanation for the phenotypic convergence between *R. clivosus* and *R. capensis* at De Hoop, with consequent divergence from *R. clivosus* elsewhere in South Africa and at Sudwala in particular. The correspondence between the phenotype of *R. clivosus* and the environment at De Hoop could be due to a developmental interaction between genes and the environment rather than local adaptation. In the former case, the same genotype could give rise to different phenotypes along an environmental gradient. Solick and Barclay (2006) suggested the divergence in extremity size between mountain and prairie populations of *Myotis evotis* with no corresponding divergence in body size could indicate plasticity in wing and ear

size. However, to distinguish between genetic adaptation and phenotypic plasticity requires knowledge of the genetic basis of phenotypic traits or reciprocal transplantation experiments (Merckx and Van Dyck, 2006). Knowledge of the former is currently unavailable although recent evolutionary development studies (e.g. Sears *et al.*, 2006) show promise in this regard. Reciprocal transplantation experiments are not possible with flying vertebrates. Teasing apart phenotypic plasticity and local adaptation was beyond the scope of this study, and provides an avenue for further exploration into the mechanisms by which speciation occurs.

In keeping with the hypothesis that the focal species should not only converge on the local endemic species but also diverge from populations of the focal species elsewhere, *R. clivosus* at De Hoop has diverged slightly from *R. clivosus* populations elsewhere in South Africa as well as from a subtropical lowveld grassland site, Sudwala, in particular. Without any obvious barriers to gene flow between the *R. clivosus* population at De Hoop and populations of the species elsewhere, it is likely that gene flow between localities is responsible for the low level of morphological divergence between *R. clivosus* populations. Future studies should examine the genetic profile of *R. clivosus* populations from different localities across South Africa to evaluate the degree of gene flow between localities. Possible barriers to gene flow may be behavioural or geographical, and these should be investigated as well. Behavioural differences between populations of the focal species may limit the mating success of immigrant populations, and such a phenomenon has been shown in birds, for example (Bensch *et al.*, 1998). In terms of geographic barriers, *R. clivosus* is capable of short-distance migration of about

10 kilometres (Taylor, 2000), however just how far this species is able to disperse to establish itself in a new locality is unknown.

Local adaptation does not only involve morphology and ecology, but also includes life history and physiology, which may differ between populations across a climatic gradient. A study comparing the life history of *R. clivosus* in the CFR and elsewhere in South Africa might provide a further test for the hypothesis of local adaptation. Bernard (1983, 1985) suggested an overlap in the timing of parturition for *R. capensis* and *R. clivosus* in Natal. However, it would be informative to investigate potential divergence in timing of reproduction between populations of *R. clivosus* at De Hoop and populations elsewhere in South Africa with concomitant convergence of timing of reproduction between *R. capensis* and *R. clivosus* at De Hoop. McDonald *et al.* (1990) proposed such convergence at De Hoop may be advantageous to both species with regards to timing reproduction to coincide with seasonal peaks in rainfall and insect abundance. A more detailed study similar to those of Bernard (1983, 1985) and carried out at De Hoop would provide further support for local adaptation of *R. clivosus* by considering climate as a selection pressure driving or limiting it.

While my approach to examining local adaptation in *R. clivosus* is a novel one, representing a more feasible means of studying local adaptation in vertebrates than the more commonly used reciprocal transplantation method, the approach does lead to a problem of scale. When attempting to determine whether *R. clivosus* is locally adapted to the Cape Floristic Region, the fact that the CFR is in and of itself a "mosaic" (Goldblatt, 1979) of local habitats with steep ecological gradients should be kept in mind. For the purposes of this study, De Hoop was assumed to represent the habitat and climate of the

CFR to make the study more feasible. Sampling several different localities within the CFR would have revealed whether *R. clivosus* has become locally adapted to a specific habitat within localities both in the CFR and elsewhere in South Africa rather than to the CFR in general. Therefore, the most that can be concluded from this study is that *R. clivosus* may have become locally adapted to De Hoop rather than to the entire CFR.

The advantage to comparing the phenotype of *R. clivosus* with that of the locally occurring endemic *R. capensis* is that the study could be carried out in the field. The methods took advantage of the fact that the locally occurring focal and endemic species are sympatric. Presumably, the focal species has had sufficient evolutionary time to become locally adapted, provided natural selection is the driving force behind shaping its phenotype. My approach also raises problems of opportunity. It requires localities where sympatric and closely related endemic and focal species co-occur. Such opportunities may be rare. However studies of local adaptation in higher vertebrates, using reciprocal transplantation, may be possible using an approach similar to that of Merckx and Van Dyck (2006). For example, reciprocal transplantation of bird eggs into different localities would allow phenotypic changes during the course of the individuals' development to be recorded. Cross-fostering in birds should be easy as eggs could be transplanted into nests in different localities. The birds would need to be banded and recaptured to collect phenotypic data during juvenile and adult stages. Survivorship and appropriate indices of fitness would be compared for individuals in their locality of origin versus the locality into which they were transplanted, as in Ayres and Scriber (1994) to detect local adaptation. The phenotype of adults in their locality of origin would be compared with the phenotype of adults in the locality into which they were transplanted. If natural

selection were responsible for local adaptation in phenotype, the phenotype of adults in a new locality would be similar to that of adults in the locality of origin. However, if phenotypic plasticity were the mechanism behind local adaptation, the phenotype of individuals in a new locality would be correlated with the new locality and different from that of individuals in the locality of origin.

The novel approach used in this study provides a feasible way to examine local adaptation of vertebrates in the field. Although this study provides evidence for local adaptation in *R. clivosus* at De Hoop, whether the cause of this local adaptation is genetic adaptation (Merck and Van Dyck, 2006) or phenotypic plasticity awaits future advances in genomics and evolutionary biology.

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## APPENDIX

Abbreviations used in the text:

ANOSIM – analysis of similarities

AR – aspect ratio

B – wingspan

CF – constant frequency

DDI – diet diversity index

DFA – discriminant function analysis

FFT – fast Fourier transform

FM – frequency modulated

g – acceleration due to gravity

HU – habitat use

I – wingtip shape index

M – body mass

MDS – multi-dimensional scaling

PC – principal component

PCA – principal components analysis

PGLS – phylogenetic least squares

S – total wing area

SIMPER – similarity percentage analysis

$T_1$  – the ratio of hand-wing length to arm-wing length

$T_s$  – the ratio of hand-wing area to arm-wing area

WL – wing loading