GEOGRAPHICAL VARIATION IN THE NUMBER OF GENERATION GLANDS IN CORDYLID LIZARDS

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Declaration:

I, the undersigned, hereby declare that the work contained in this thesis is my own original

work and that I have not previously in its entirety or in part submitted it at any university

for a degree.

ABSTRACT

Geographic variation in the presence/absence of generation glands in females of the *cordylus-niger-oelofseni* complex was described and correlated with climatic variables. Preserved and live specimens from 96 localities in the area south of 32º30' S and west of 19º45' E in the Western Cape, South Africa, were examined for the presence or absence of generation glands. Females from western coastal localities and from the Cape Fold Mountains generally lack generation glands, while females from inland lowland localities generally possess glands. A GIS analysis was performed to determine mean annual minimum temperature, mean annual maximum temperature, potential evaporation, mean annual precipitation, mean annual fog and mean annual cloud cover measured at 14:00 for each locality. Discriminant function and canonical analyses showed a highly significant correlation between the presence and absence of generation glands and five of the six climatic variables. It is suggested that the high incidence of fog and lower temperatures experienced along the west coast as a result of the cold Benguela Current, and the high incidence of orographic fog and cloud cover in the Cape Fold Mountains may affect energy allocation in females. In order to maintain their reproductive output, females may be forced to invest less energy in secondary sexual characters such as generation glands.

Interspecific variation in the location, occurrence, number, and age of gland differentiation was investigated for cordylid lizards. For several species, data were obtained from the literature and for others data were obtained from material housed in the Ellerman Collection of the University of Stellenbosch. *Cordylus cordylus* and *C. cataphractus* were used for an evaluation of inter- and intrapopulation variation. Data for *C. cordylus* were obtained from five populations on the coastal lowlands of the Western Cape, while data on *C. cataphractus* come from the literature. The sex, snout-vent length,

and number of generation glands were recorded for a representative sample of lizards at each locality. While in all species generation glands were located in the femoral region, *Cordylus giganteus* and *C. microlepidotus* had additional generation glands elsewhere on the body. It is suggested that additional generation glands in these species may be related to an increased need for chemical communication in a low-visibility environment in the case of *C. giganteus*, and in low population densities in the case of *C. microlepidotus*. Generation glands are present in all cordylid males, but females of some species may lack these glands. All cordylids display sexual dimorphism with regards to the presence and number of generation glands, with males having significantly more glands than females, indicating that these glands may play a more important role in males than in females. Low sexual dimorphism in at least two species suggests that generation glands may be equally important in both sexes of these species. In females, the presence of generation glands is closely regulated by climate. There is no indication of differences in generation gland profile between ground-dwelling and rock-dwelling species, except that in at least three ground-dwelling species, generation glands are already present at birth. In other species these glands differentiate only later in life. In the males of most species, generation glands start to differentiate prior to or at sexual maturity with a drastic increase in number when asymptotic body size is reached. The considerable inter- and intrapopulation variation in gland number in females, but not in males, underscores the hypothesis that the presence of generation glands in females is energy-linked and closely regulated by climate. An opposite geographic pattern of generation gland number in *C. cataphractus*, suggests that other factors than climate must be responsible for a difference in gland number and a difference in population density is suggested as explanation.

To test for differences in aggression levels, females with and those without generation glands were staged in two experiments. In one experiment, females from different

localities were staged against each other, while females from the same locality were paired in another experiment. No significant difference in aggression levels between females with and those without generation glands were observed in both experiments. It is suggested that generation glands do not exclusively function in territorial marking, but rather as a scent enhancer for femoral gland secretions.

In order to elucidate the functional significance of generation glands, male response to female gland stimuli was investigated for 12 male *C. cordylus*. Males were presented with different female gland secretions (femoral, generation, and femoral and generation gland secretion combined) and water was used as a control. Total number of tongue-flicks and number of tongue-flicks directed at the secretion were counted. No significant difference was observed in the number of tongue-flicks between the different treatment groups. Males did, however, display leg-waving and head-rubbing behaviour. At present, the behaviour displayed by male *C. cordylus* in the presence of female gland stimuli, is unclear. The general absence of tongue-flicking of sandpaper labelled by female gland stimuli may be because of a flaw in the experimental design or because the experiment was conducted outside the breeding season. The display of leg-waving and head-rubbing behaviour, however, suggests that cordylids do not exclusively rely on tongue-flicking for detection of conspecifics. The real function of generation glands thus still remains unresolved and further studies are needed to clarify this.

UITTREKSEL

Geografiese variasie in die aan-/afwesigheid van generasiekliere in wyfies van die *cordylus-niger-oelofseni* kompleks is beskryf en met klimaatsveranderlikes gekorreleer. Gepreserveerde en lewende materiaal, afkomstig van 96 lokaliteite in die area suid van 32°30′ S en wes van 19°45′O in die Wes-Kaap, Suid-Afrika, is vir die aan- of afwesigheid van generasiekliere ondersoek. Generasiekliere is oor die algemeen afwesig in wyfies van westelike kuslokaliteite en die Kaapse Plooiberge, terwyl dit aanwesig is in wyfies van die binnelandse laagland lokaliteite. 'n GIS-analise is gedoen om die gemiddelde jaarlikse minimum temperatuur, gemiddelde jaarlikse maksimum temperatuur, potensiële verdamping, gemiddelde jaarlikse neerslag, gemiddelde jaarlikse mis en gemiddelde jaarlikse wolkbedekking, gemeet teen 14:00, vir elke lokaliteit te bepaal. Diskriminante-funksie en kanonieke analises toon 'n hoogs beduidende korrelasie tussen die aan- en afwesigheid van kliere en vyf van die ses klimaatsveranderlikes. Daar word voorgestel dat die hoë voorkoms van mis en lae temperature wat langs die weskus ervaar word as gevolg van die koue Benguela Seestroom, en die hoë voorkoms van orografiese mis en wolkbedekking in die Kaapse Plooiberge, die energie-toekenning in wyfies beïnvloed. Om hulle voortplantingsuitset te handhaaf, word wyfies moontlik gedwing om minder energie in sekondêre geslagskenmerke, soos generasiekliere, te belê.

Interspesifieke variasie in die posisie, voorkoms, aantal en tyd van klierontwikkeling is vir verskeie gordelakkedisspesies ondersoek. Data vir verskeie spesies is vanuit die literatuur verkry, terwyl data vir ander spesies vanaf material in die Ellerman-versameling van die Universiteit van Stellenbosch verkry is. *Cordylus cordylus* en *C. cataphractus* is vir 'n evaluering van inter- en intrapopulasie variasie gebruik. Data vir *C. cordylus* is vanaf vyf populasie wat op die kuslaaglande van die Wes-Kaap voorkom, ingesamel,

terwyl data oor *C. cataphractus* vanuit die literatuur verkry is. Geslag, snoet-kloaak-lengte en die aantal generasiekliere is aangeteken vir 'n verteenwoordigende aantal individue van elke populasie. Terwyl generasiekliere in die femorale wyk van alle spesies aangetref word, het *Cordylus giganteus* en *C. microlepidotus* ook addisionele generasiekliere in ander dele van die liggaam. Daar is tot die gevolgtrekking gekom dat addisionele generasiekliere in hierdie spesies moontlik verwant is aan 'n groter behoefte aan chemiese kommunikasie in 'n lae sigbaarheidsomgewing in die geval van *C. giganteus*, en in 'n lae populasiedigtheid in die geval van *C. microlepidotus*. Generasiekliere word in alle gordelakkedis mannetjies aangetref, maar kan afwesig wees in wyfies van sommige spesies. Alle gordelakkedisse toon geslagsdimorfisme met betrekking tot die aanwesigheid en aantal generasiekliere, en mannetjies het beduidend meer kliere as wyfies, wat daarop dui dat hierdie kliere moontlik 'n meer belangrike rol by mannetjies as by wyfies speel. Lae geslagsdimorfisme in ten minste twee spesies stel voor dat generasiekliere moontlik ewe belangrik in beide geslagte van hierdie spesies kan wees. In wyfies word die aanwesigheid van generasiekliere sterk deur klimaat gereguleer. Daar is geen aanduiding van verskille in generasieklierprofiel tussen grondbewonende en rotsbewonende spesies nie, behalwe dat generasiekliere reeds by geboorte teenwoordig is in ten minste drie grondbewonende spesies. In ander spesies ontwikkel hierdie kliere eers later. In mannetjies van die meeste spesies, begin generasiekliere voor of tydens geslagsrypheid ontwikkel, met 'n drastiese toename in aantal kliere wanneer asimptotiese liggaamsgrootte bereik word. Die aansienlike inter- en intrapopulasie variasie in aantal kliere in wyfies, maar nie in mannetjies nie, ondersteun die hipotese dat die teenwoordigheid van generasiekliere in wyfies aan energie gekoppel is en deur klimaat gereguleer word. 'n Teenoorgestelde geografiese patroon in die aantal generasiekliere in *C. cataphractus*, stel

voor dat ander faktore as klimaat vir die verskil in aantal kliere verantwoordelik is en 'n verskil in populasiedigtheid word as verklaring voorgestel.

Om vir verskille in aggressievlakke te toets, is wyfies met en wyfies sonder generasiekliere is teenoor mekaar getoets in twee eksperimente. In een eksperiment is wyfies van verskillende lokaliteite teenoor mekaar getoets, en in 'n ander eksperiment is wyfies van dieselfde lokaliteit teenoor mekaar getoets. In beide eksperimente is geen beduidende verskille is in die aggressievlakke van wyfies nie waargeneem nie. Daar word voorgestel dat generasiekliere nie uitsluitlik in gebiedsafbakening funksioneer nie, maar dat dit eerder as reukversterker vir die femorale kliere dien.

Om die funksie van generasiekliere vas te stel, is die reaksie van 12 mannetjie *C. cordylus* op klierstimuli van wyfies getoets. Verskillende kliersekresies (femoraal, generasie, en femoraal en generasie gekombineerd) van wyfies is mannetjies voorgelê, en water is as 'n kontrole gebruik. Totale aantal tongskiete en aantal tongskiete wat na die sekresie gerig is, is getel. Geen beduidende verskil in die aantal tongskiete is tussen die verskillende groepe waargeneem nie. Mannetjies het egter beenwaai- en kopvryfgedrag getoon. Die rede vir die gedrag wat deur *C. cordylus* mannetjies in die teenwoordigheid van klierstimuli van wyfies vertoon word, is egter tans onduidelik. Die algemene afwesigheid van tongskiete na skuurpapier wat met klierstimuli van wyfies gemerk is, mag moontlik wees as gevolg van 'n fout in die eksperimentele ontwerp of omdat die eksperiment buite die paarseisoen uitgevoer is. Die vertoning van beenwaai- en kopvryfgedrag stel egter voor dat gordelakkedisse nie uitsluitlik op tongskiete staatmaak om lede van dieselfde spesie waar te neem nie. Die ware funksie van generasiekliere is dus steeds onopgelos en verdere studies word benodig om dit duidelikheid hieroor te kry.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

The Cordyliformes is a clade of scincomorph lizards partitioned into two families, the Gerrhosauridae and the Cordylidae (Lang 1991). The former occurs on Africa and Madagascar, while the latter is restricted to Africa, mainly southern Africa. The Cordylidae is presently subdivided into two genera, *Platysaurus* and *Cordylus* (Frost *et al.* 2001). The Cordylidae and Gerrhosauridae differ in several major ways:

1. Gerrhosaurids are all active foragers, while cordylids are sit-and-wait foragers (Cooper *et al.* 1997; Mouton & Van Wyk 1997).

2. Gerrhosaurids have a well-developed prey-chemical discrimination ability, but cordylids lack this ability (Cooper *et al.* 1995; Mouton *et al.* 2000b).

3. Gerrhosaurids are mostly ground-dwelling, while the vast majority of cordylids are rock-dwelling (Mouton & Van Wyk 1997; Branch 1998).

4. Gerrhosaurids are oviparous, while cordylids, excluding *Platysaurus*, are viviparous (Mouton & Van Wyk 1997; Branch 1998).

5. Gerrhosaurids generally lack generation glands, while all cordylids have well-developed generation glands (Van Wyk & Mouton 1992).

6. All gerrhosaurids are solitary species with simple social structures, while cordylids show a wide range of structures, some species occurring in very dense colonies and some even displaying permanent grouping behaviour (Mouton & Van Wyk 1993; Branch 1998; Mouton *et al.* 1999; Whiting 1999).

7. Gerrhosaurids have a summer gonadal cycle, while all cordylids, including *Platysaurus* species, have a winter cycle (Van Wyk & Flemming 1992; Van Wyk & Mouton 1995).

8. Gerrhosaurids are suspected to all have prenuptial reproductive cycles, while some cordylids have postnuptial cycles.

9. All gerrhosaurids have thick osteoderms, while cordylids display extensive variation, from thick osteoderms to the complete absence of osteoderms (unpublished data).

10. There are no melanistic gerrhosaurids, while there are several melanistic cordylids (Mouton 1986a; Mouton & Van Wyk 1995).

By mapping the characters on a phylogenetic tree and by using outgroup comparison and parsimony methods, Mouton & Van Wyk (1997) showed that, within Cordyliformes, sit-and-wait foraging, lack of prey chemical discrimination, and a rupicolous lifestyle co-evolved in the immediate ancestor of the Cordylidae, while viviparity evolved in the immediate ancestor of the *Cordylus* clade. The highland distribution and viviparous reproductive mode of the latter clade suggest that the ancestor became adapted to cool climates, in other words that the clade had a cold origin. Mouton & Van Wyk (1997) provide further evidence that the transitions to a sit-and-wait foraging mode, lack of prey chemical discrimination ability, and a rock-dwelling lifestyle in the ancestor of the Cordylidae may also have been adaptations to a cold environment, or at least may have served as preadaptations, facilitating the invasion of highland areas by the *Cordylus* clade. The distribution of extant species suggests that the Cordylidae/Gerrhosauridae divergence occurred in southern Africa, the former diversifying in the extreme southern regions of the subcontinent and the latter in the south-central regions. The rupicolous lifestyle and

resultant low vagility of early cordylids probably prevented the family from reaching Madagascar before its separation from Africa.

It is possible that the possession of generation glands and the wide range of social structures found in the Cordylidae are direct consequences of the transitions to a sit-and-wait foraging strategy and a rock-dwelling lifestyle in the immediate ancestor of the Cordylidae. Among lizards, sit-and-wait foragers that are also rock-dwellers, are normally highly visible to conspecifics while perching on rocks for basking and feeding purposes, and tend to be highly territorial and in many cases also have a polygynous mating system (Stamps 1983; Mouton & Van Wyk 1993). The function of epidermal glands in territorial marking has also been demonstrated in a number of species (Alberts 1992; Cooper *et al*. 1996; Martin & López 2000). What is, however, unclear at present is what the determining factors of the extreme variation in epidermal gland profile in the Cordylidae are and to what extent this variation reflects variation in social structure among species. To understand the evolutionary history of the family Cordylidae and to identify those evolutionary forces that were involved in the diversification of the family, we, however, first need to fully understand the ecological significance of variation in characters that differ among extant species.

1.2 EPIDERMAL GLANDS IN LIZARDS

Lizards possess a variety of epidermal glands, including precloacal, cloacal, femoral and generation glands (Simon 1983; Van Wyk & Mouton 1992). The femoral pores of lizards were first described and used as a taxonomic character by Linnaeus in 1758 (Cole 1966a). Since then, several authors used the presence of femoral pores, the number of pores, the position of the pores in the scales, and the nature of the scales bearing femoral pores as a taxonomic character (Duméril & Bibron 1834; Cole 1966a). In 1786, Duvernoy was the

first to note that these pores are associated with glandlike structures (Rastogi 1975). Schaefer (1902) noted that besides femoral pores, anal and preanal pores are also present in lizards. In general, the glandlike epidermal organs of lizards are classified into four categories based upon their topographical positions: abdominal, femoral, inguinal, and preanal (Cole 1966b; Maderson 1970).

Two basic categories of these holocrine, epidermal glands may be recognised in lizards, namely femoral glands and generation glands (Cole 1966b; Maderson 1970; Van Wyk & Mouton 1992; Mouton et al. 1998). Femoral glands are exocrine glands that are tubulo-follicular in appearance (Van Wyk & Mouton 1992). Generation glands are usually present as patches of scales that show concavities in the centre of the outer scale surface, housing glandular material (Maderson 1970; Van Wyk & Mouton 1992). In lizards, two types of generation glands have been described: "escutcheon scales" (Taylor & Leonard 1956; Maderson 1967) and "β-glands" (Maderson 1968).

In the family Cordylidae, femoral glands are arranged in a single row on the ventral aspect of the thigh (FitzSimons 1943; Loveridge 1944; Van Wyk & Mouton 1992; Mouton et al. 1998). They are typically tubulo-follicular in structure and secrete protruding plugs of waxy material (Van Wyk & Mouton 1992; Mouton et al. 1998).

A structure closely related to the femoral glands is what FitzSimons (1943) calls "swollen modified glandular scales". Maderson (1972) described these glandular scales in sphaerodactyline and eublepharine gekkonid lizards as generation glands. In 1980, Moody used the term "callous glands" for the same pre-cloacal structures in agamids. Lang (1991) referred to the patches of glandular scales in cordylid lizards also as callous glands. He points out, though, that he does not thereby implicate homology with similar epidermal structures in other squamates. In a comparison of the histology of the glandular scales or callous glands of cordylid lizards with other epidermal glandular structures described in

squamates, Van Wyk & Mouton (1992) found that these glands superficially resemble what Maderson & Chui (1970) termed "generation glands".

Three types of generation glands have been identified in cordylid lizards (Van Wyk & Mouton 1992). The protruding glands show some resemblance to the β-glands of gekkonids (Van Wyk & Mouton 1992). The pit glands are characterised by a concavity filled with secretion material (Maderson 1967, Van Wyk & Mouton 1992). These glands were first described as showing resemblance to the escutcheon scales, but in a recent study it was found that they are actually closer to the β-glands of gekkonids (Searby 2002). In *Platysaurus* sp. and *Cordylus capensis*, generation glands in the femoral region, differ in overall appearance from both protruding and pit generation glands. Histologically these glands appear to represent an intermediate generation gland-type (Van Wyk & Mouton 1992) and it is also the form that most resembles the gekkonid-glands described by Maderson (1967, 1970). Generation glands normally occur as a patch of glandular scales adjacent and anterior to the femoral glands (Van Wyk & Mouton 1992; Mouton *et al*.1998).

1.3 TAXONOMIC VARIATION

The lizard families differ considerably with regard to epidermal glands (Branch 1998). Members of the Scincidae and Chamaeleonidae totally lack epidermal glands. In the Agamidae, preanal pores are present in males, while in the Gekkonidae, preanal and femoral pores are usually present in males. In most species of the Lacertidae, femoral pores are present in both sexes. In the Gerrhosauridae, femoral pores are present, although it could be absent in females. All members of the Cordylidae possess both femoral and generation glands. While these glands are always present in males, they may be present or absent in females.

According to Broadley (1971), epidermal glands could prove to be a useful taxonomic character and several authors have used the presence or absence of femoral glands, the number of femoral glands and the presence or absence of generation glands in females as taxonomic characters in their studies on the Cordylidae (Broadley 1971; Mouton 1986b; Mouton & Van Wyk 1990; Branch 1998).

1.4 POSSIBLE FUNCTION

The morphology of epidermal glands of particular lizard species has been described fairly extensively (Cole 1966b; Maderson 1968; Maderson 1970; Maderson & Chui 1970; Van Wyk & Mouton 1992; Mouton *et al.* 1998), but few studies have been done on the function of these glands. In many lizard families, epidermal glands producing secretions are found on ventral surfaces (Cole 1966b), where they are perfectly situated for marking substrates, especially during locomotion (Maderson 1970; Cooper *et al.* 1996). Maderson (1970) suggests that the exact function of these secretions may vary in different taxa. In many species of lizards and amphisbaenians exhibiting epidermal glands in the femoral or cloacal region, the pores of these glands are plugged with holocrine solid secretions that serve as semiochemical sources. Many authors assume that these glands are mainly associated with reproduction and demarcation of territory (Jared *et al.* 1999).

In his review of femoral glands, Cole (1966b) discussed some of the hypotheses proposed by several authors on the possible function of femoral glands. He stated that proposing additional hypothetical functions of these glands would be a futile attempt until there are more data available on the chemistry of the secretion, and behavioural and experimental studies are more conclusive. In recent studies, femoral glands have been implicated in chemical communication (Cooper *et al.* 1996; Martín & López 2000; Ruddock 2000).

Chemical signals as means of communication are widespread among animals (Stoddart 1980) and many species rely on these signals for attracting mates as odours may convey a great deal of information about the condition of the individual (Moore *et al.* 1997; Penn & Potts 1998). It is thus no surprise that chemical cues also play an important role in the intraspecific communication of lizards (Halpern 1992; Mason 1992) and several studies have shown pheromonal detection in different species, based on epidermal gland secretions (e.g. Cooper & Vitt 1984; Alberts 1989; López *et al.* 1998). Intraspecific communication also plays an important role in spatial use and mating systems.

Lizards have a relatively complex system of social communication. This includes a wide variety of visual displays (Carpenter & Ferguson 1977), as well as various glands associated with chemical communication (Mason 1992). Field and laboratory studies on iguanid lizards suggest that femoral gland secretions function in conspecific recognition and range marking (Alberts 1993). In a study by Cooper *et al.* (1996), male *Cordylus cordylus* showed a greater rate of tongue-flicking of male- rather than female-labelled tiles. The authors suggested that this may be an indication that femoral and generation gland secretions are used for territorial marking.

The variation in the development and use of chemical senses in lizards appears to be related to taxonomic group (Halpern 1992). The ascalabotans (Chamaeleonidae, Agamidae, Iguanidae, Gekkonidae, and Xantusiidae) depend heavily on visual signals, whereas the autarchoglossans (Lacertidae, Scincidae, Teiidae, Cordylidae, Gerrhosauridae, Helodermatidae, Varanidae) depend heavily on chemical as well as visual signals (Halpern 1992; Mason 1992). The general emerging picture for cordylid lizards coincides with this in that these lizards make use of a wide range of visual clues, but also rely heavily on pheromonal cues (Cooper *et al.* 1996; Ruddock 2000).

At present, very little data are available on the function of generation glands, and to date, no behavioural studies have been done on lizard responses to generation gland secretions (Ruddock 2000).

1.5 SIGNIFICANCE OF THE STUDY AND KEY ASPECTS

The lizard family Cordylidae is endemic to Africa and includes 57 species of which 41 occur in South Africa. Cordylids are considered to be the most typical lizards of South Africa (Branch 1998) and because of the high visibility of most forms, they are important wildlife components at many ecotourism destinations (Loubser *et al.* 2001). All *Cordylus* species are CITES-listed; many of the rock-dwelling species have extremely limited distribution ranges and many are threatened by habitat alteration, indiscriminate burning regimes, alien vegetation and global warming (Baard *et al.* 1999). Baseline biological information on cordylids lizards is urgently needed to secure their survival. To date, herpetological studies in South Africa have mainly been concerned with taxonomic issues and extremely little biological information on the local lizard fauna is available.

Being endemic, the family Cordylidae offers many unique opportunities to study phenomena of international interest. These include, amongst others, the functional significance of epidermal glands in social structures. The relatively small range of the Cordylidae and the fact that a large number of species, including representatives of all lineages, occur in South Africa, make it the ideal family to study many of these issues of international interest in a phylogenetic context. The considerable variation in epidermal gland profile in the Cordylidae is unique among lizards and provides an ideal opportunity to investigate the functional significance of these glands from an ecological perspective.

The main aims of my study were to describe the geographic patterns in the presence and number of generation glands, and to obtain baseline data on the role of epidermal

glands in the functioning of social structures of the Cordylidae. For this, I investigated the following key aspects:

1. Geographic patterns in the presence/absence of generation glands in cordylid lizards and possible correlations with climatic parameters.

2. Inter- and intraspecific variation in generation gland number, sexual dimorphism, and age at which glands differentiate in cordylids.

3. Possible correlation between aggression levels and the presence/absence of generation glands in females of a cordylid species.

4. Differential male response to epidermal gland secretions of females with femoral and generation glands in a cordylid species.

This study is a product of the ongoing research done by the Cordylidae Research Team, which forms part of the Vertebrate Functional Biology Group, based at the University of Stellenbosch. It forms part of a long-term project of which the goal is to conduct a phylogenetic analysis of epidermal glands and social structures within the Cordylidae family.

CHAPTER 2

ENVIRONMENTAL FACTORS DETERMINING THE PRESENCE/ABSENCE OF GENERATION GLANDS IN GIRDLED LIZARDS: A CASE STUDY OF THE *CORDYLUS-NIGER-OELOFSENI* COMPLEX

2.1 INTRODUCTION

Several studies reported geographic variation in the presence/absence of generation glands in female cordylid lizards. Herselman *et al.* (1992) found that female *Cordylus capensis* lack generation glands in the northern, central and southwestern parts of the species' range. In the southeastern parts of the range, these glands are, however, present in females. Cordes *et al.* (1995) reported that female *C. cordylus* from Saldanha along the west coast of South Africa lack generation glands, but that females from Gansbaai along the south coast have these glands. These authors suggested that the observed pattern is climate-related, females from cool areas lacking these glands. At Saldanha there is a zone of upwelling of cold water in the Atlantic Ocean (Taunton-Clark 1982), while the Gans Bay locality, on the other hand, is associated with the warm Agulhas current in the Indian Ocean. Cordes *et al.* (1995) based their argument on the fact that females of montane cordylid species generally lack generation glands. Retief (2000), however, established that in *C. cataphractus*, females from montane areas have considerably more generation glands than females from lowland areas. Clearly, a more detailed analysis of geographic variation in the presence of generation glands in female cordylids is needed. Knowledge of the factors responsible for geographic variation in the presence of these glands in females may also help understand the function of these glands in cordylids.

The aims of this part of my study were, firstly, to describe patterns of geographic variation in the presence/absence of generation glands in females for selected cordylid

species and, secondly, to search for environmental correlates. If a correlation can be found between geographic variation in the presence/absence of glands and climatic parameters, it will provide a new angle to study these glands from, and with the aid of carefully designed laboratory and field experiments, I may be able to elucidate the functional significance of these glands.

The *cordylus-niger-oelofseni* complex is ideal for an analysis of geographic variation in the presence/absence of generation glands. These three species are so closely related that there is still some doubt whether they should be seen as one or three separate taxa (Branch 1988; Mouton & Van Wyk 1990; Brody *et al*. 1993; Daniels *et al*. 2004). All three are rock-dwelling species. The Cape girdled lizard, *Cordylus cordylus*, has an extensive distribution in the southwestern and southern coastal areas of the Western Cape, and through the Cape Fold Mountains (Branch 1998). It is considered a lowland form (Mouton & Oelofsen 1988). *Cordylus oelofseni* is a melanistic species occurring as several isolated populations in the western Cape Fold Mountains (Mouton 1987; Mouton & Van Wyk 1990), while *C. niger*, another melanistic species, is restricted to the Cape Peninsula and the Saldanha Bay region (Mouton 1987; Mouton & Van Wyk 1990).

2.2 MATERIALS AND METHODS

2.2.1 Study area

The area south of 32°30′S and west of 19°45′E in South Africa was selected as study area (Fig. 2.1), because of the availability of distributional data for the three selected species, as well as climatic data. The distributional data were obtained from a three-year survey during which the lizard fauna of the study area was sampled extensively (Mouton 1986a). The study area falls within the winter rainfall region of the Western Cape, and includes a wide range of topographical features. The coastal platform in the west forms an extensive
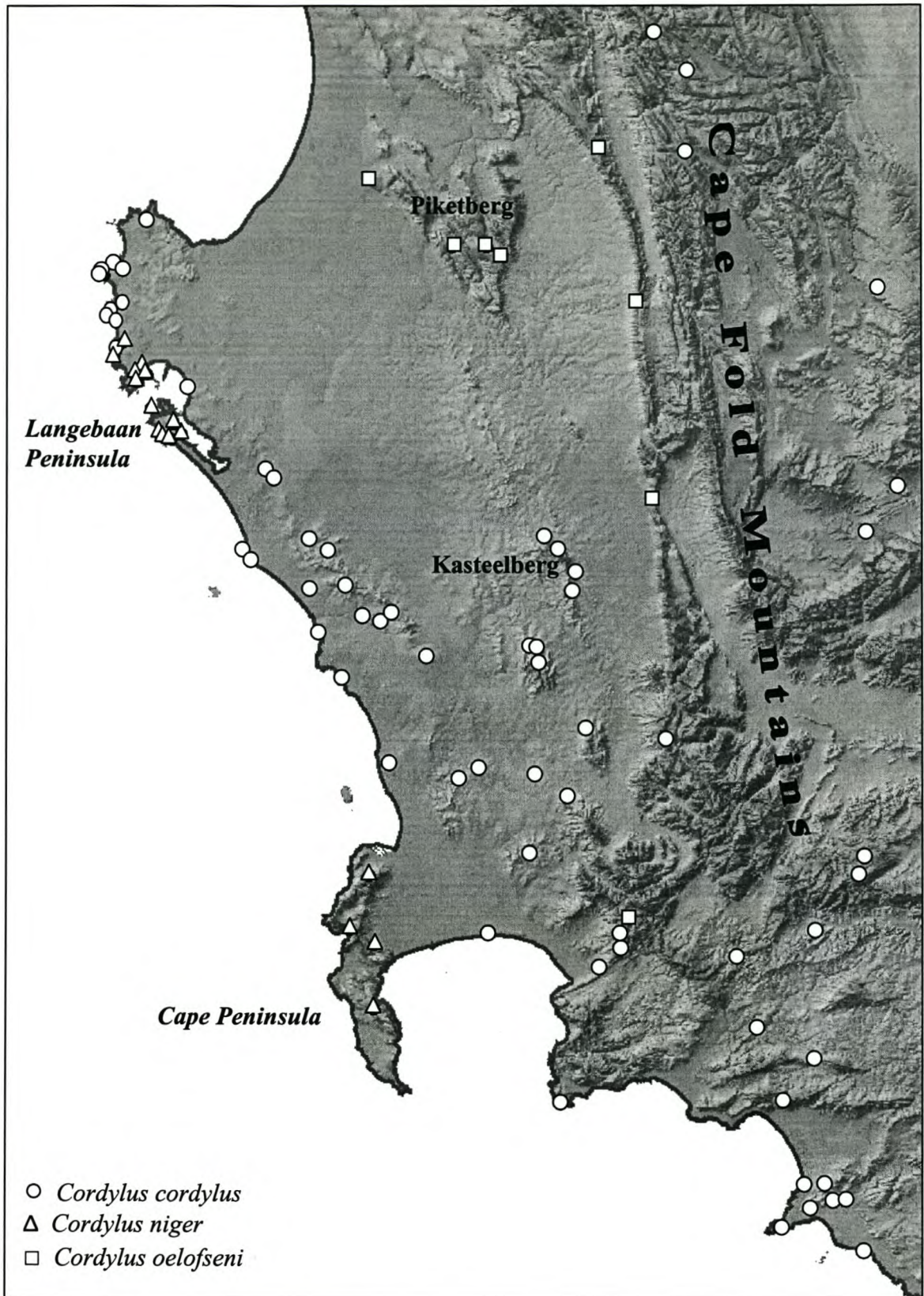
Figure 2.1. Topographical map showing localities where females of the *cordylus-niger-oelofseni* complex were collected.

coastal lowland area lacking orographic features of high altitude, with Kasteelberg and Piketberg Mountains being exceptions (Fig. 2.1). These lowlands stretch inland to the Cape Fold Mountains, of which many parts are in the proximity of 1 000 m above sea level. Several offshore islands and two peninsulas, the Cape Peninsula and the Langebaan Peninsula, are also found in the western section of the study area (Fig. 2.1). Along the west coast, the north-flowing cold Benguela Current has a strong influence on the climate of this region (Brown & Jarman 1978), while the warm Agulhas Current along the south and east coast has a less dramatic effect on the adjacent coastal regions.

2.2.2 Specimens and morphological data

Preserved specimens of *Cordylus cordylus* (N = 334), *C. niger* (N = 119) and *C. oelofseni* (N = 69), housed in the Ellerman Collection at the University of Stellenbosch and collected from 96 localities in the study area (Fig. 2.1), were examined for the presence of generation glands. In most cordylid species, generation glands only start to develop with the onset of sexual maturity (Mouton *et al.* 1998), and thus only adult lizards were used in this study. In preserved specimens, adult females were identified by internal inspection as those individuals containing advanced vitellogenic follicles or oviductal eggs.

Localities not represented by large enough sample sizes in the Ellerman Collection, were revisited to obtain additional material, and to ensure that local variation was accounted for. At these localities, adult lizards were caught by hand or with a short noose and released after measurements were taken. Females were distinguished from males by smaller heads and the absence of hemipenal bulges.

Digital callipers were used to measure snout-vent-length (SVL) to the nearest 0.01 mm. Although Cordes *et al.* (1995) reported the size at sexual maturity for *C. cordylus* and *C. niger* at a SVL of 55 mm, we used a SVL of greater than 60 mm for *C. cordylus* and *C.*

niger, and greater than 55 mm for *C. oelofseni* to identify adult lizards in the field. This was done because of the difficulty to accurately sex smaller individuals without internal inspection. Presence/absence of generation glands in preserved specimens was observed with the aid of a stereomicroscope and in live specimens, with the aid of a magnifying glass (10X).

2.2.3 Data analysis

GIS analysis -- A GIS analysis was conducted to establish whether any correlation exists between the absence or presence of generation glands in females and specific climatic indices. Each locality was analyzed spatially with the available data on climate. Two climatic data sets were used for the GIS analysis. The first was obtained from Department of Agriculture (Elsenburg), but was originally developed for the South African Atlas of Agrohydrology and Climatology by Shulze (1997). These data were already in GIS format (ESRI Shape Files) in the form of regularly spaced points (1.6 x 1.6 km intervals) representing mean annual minimum temperature (TMIN), mean annual maximum temperature (TMAX), potential evaporation (APAN), and mean annual precipitation (MAP). The second set comprises long-term weather data (WB42 database) that were obtained from the South African Weather Bureau. Since these data were not yet in GIS format, the location of each weather station had to be mapped and its corresponding weather data captured. These included mean annual fog (FOG_YR) and mean annual cloud cover measured at 14:00 (CLOUD14_YR). The climatic information for each of the localities was interpolated from both these data sets by means of an inverse distance weighting (IDW) interpolation. Aspect (direction of slope) is directly proportional to solar radiation, and thus regarded as an important factor in lizard thermoregulation. Because of

the scale we were working on, it was, however, not possible to include the aspect variable in our analysis.

Statistical analysis -- Data obtained from the GIS analysis were analysed statistically using the Statistica 6.0 software package. Probability values (*P*) smaller than 0.05 were considered significant for all statistical analyses performed. Based on the presence/absence of generation glands, the localities were separated into three zones, namely a coastal, a lowland and a montane zone,. Two different multivariate statistical procedures, discriminant functional analysis and canonical analysis, were used to compare the climatic variables - TMIN, TMAX, APAN, MAP, FOG_YR and CLOUD14_YR - of the three zones.

2.3 RESULTS

2.3.1 Geographic patterns

The study area can be divided into three zones in terms of the presence/absence of generation glands in females of the *cordylus-niger-oeloefseni* complex: a western coastal zone where generation glands are absent in all females; a coastal lowland zone where generation glands are present in all or most females; and an eastern montane zone where generation glands are absent in all or most females (Fig. 2.2).

Generation glands are absent in all *C. niger* and *C. cordylus* females occurring on rocky outcrops along the west coast (Fig. 2.2). In the Cape Fold Mountains, all females of *C. oelofseni*, as well as most of *C. cordylus*, also lacked generation glands (Fig. 2.2). Female *C. cordylus* occurring inland on the coastal lowlands all have generation glands, except for specimens from Kanonkop, Hangklip and the top of Kasteelberg, where generation glands were absent in females (Fig. 2.2). At some of the localities where females possess generation glands, intralocality variation was observed in the presence/absence of these
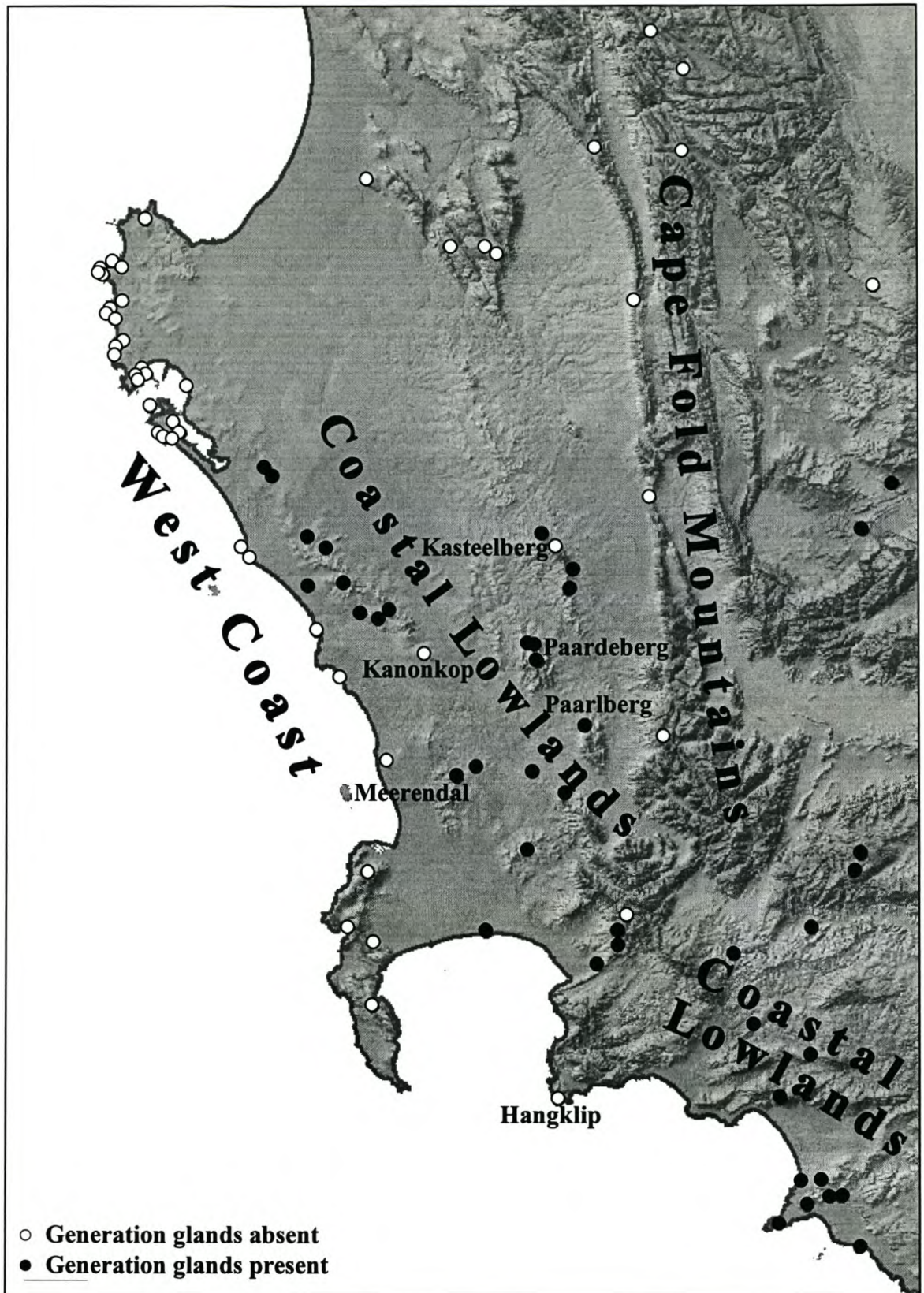
Figure 2.2. Map showing the three zones in terms of the presence/absence of generation

glands in females of the *cordylus-niger-oelofseni* complex.

glands. At Meerendal, Paarlberg, Paardeberg and the lower slopes of Kasteelberg (Fig. 2.2), generation glands were present in some females, but completely absent in others.

2.3.2 Ecological correlates

Discriminant analyses on the climatic data of the three zones in the study area indicated that a high percentage of localities could be correctly classified into their specific zones (Table 2.1). In the three-zone discriminant function analysis it was shown that function 2 accounted for 57% of the variance (Table 2.2). Although TMIN showed the greatest and CLOUD14_YR the least influence on the presence/absence of generation glands in females (Table 2.3), there was no significant difference between the influences of the six climatic variables tested ($P > 0.05$). The variables gave a nearly complete separation of the three groups (Tables 2.1-2.3, Fig. 2.3), and a significant correlation was found between the presence or absence of generation glands and five of the six climatic variables ($P < 0.05$). Only CLOUD14_YR was insignificant ($P = 0.34$). Figures 2.4-2.7 give a graphic representation of the mean annual climatic conditions encountered in the study area.

2.4 DISCUSSION

The results of my study show that the presence/absence of generation glands in females in the *cordylus-niger-oelofseni* complex strongly correlates with climatic parameters.

   The climate of the west coast of South Africa is strongly influenced by the cold Benguela Current in the Atlantic Ocean. A direct effect of this current is a cooler zone along the coast (Schulze 1965; Meigs 1966; Brown & Jarman 1978), with three major, semi-permanent upwelling zones that occur along the coastline (Taunton-Clark 1982). Associated with the upwelling zones, is a high incidence of fog caused by winds blowing from the warmer part of the ocean across the cool belt near the shore (Meigs 1966; Shulze

Table 2.1. Percentages of localities in the study area classified in a discriminant analysis of six climatic variables.

	Predicted zone membership		
Actual zone membership	Coastal	Lowland	Montane
Coastal	86.1	13.9	0
Lowland	8.3	86.1	5.6
Montane	0	18.2	81.8

Table 2.2. Performance parameters for the discriminant functions in an analysis of six climatic variables in the three zones (coastal, lowland, montane) occurring in the study area.

Discriminant function	Eigenvalue	% Variance	Canonical correlation
1	3.916	11.6	0.893
2	0.751	57.1	0.655

Table 2.3. Standardized canonical discriminant function coefficients for function 2, and values of Wilk's lambda, for a discriminant analysis of six climatic variables in the three zones (coastal, lowland, montane) occurring in the study area. Climatic variables: mean annual minimum temperature (TMIN), mean annual maximum temperature (TMAX), potential evaporation (APAN), mean annual precipitation (MAP), mean annual fog (FOG_YR) and mean annual cloud cover measured at 14:00 (CLOUD14_YR).

Variables	Standardized coefficient	Wilk's lambda
TMIN	1.069	0.209
TMAX	-1.155	0.147
APAN	-0.088	0.129
MAP	-0.701	0.142
FOG_YR	0.355	0.127
CLOUD14_YR	-0.171	0.119

Figure 2.3. Plot of the scores of the localities from the study area on the first two functions of a discriminant function analysis based on six climatic variables.
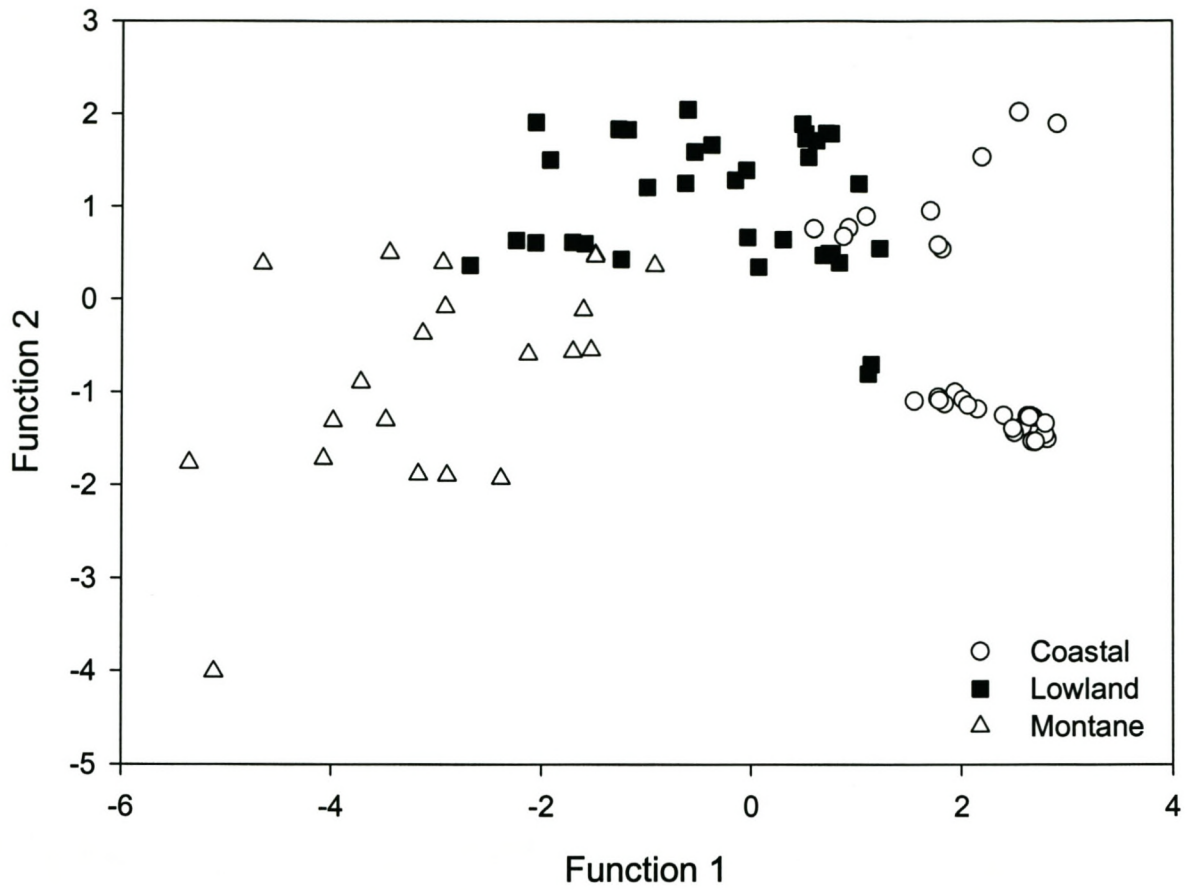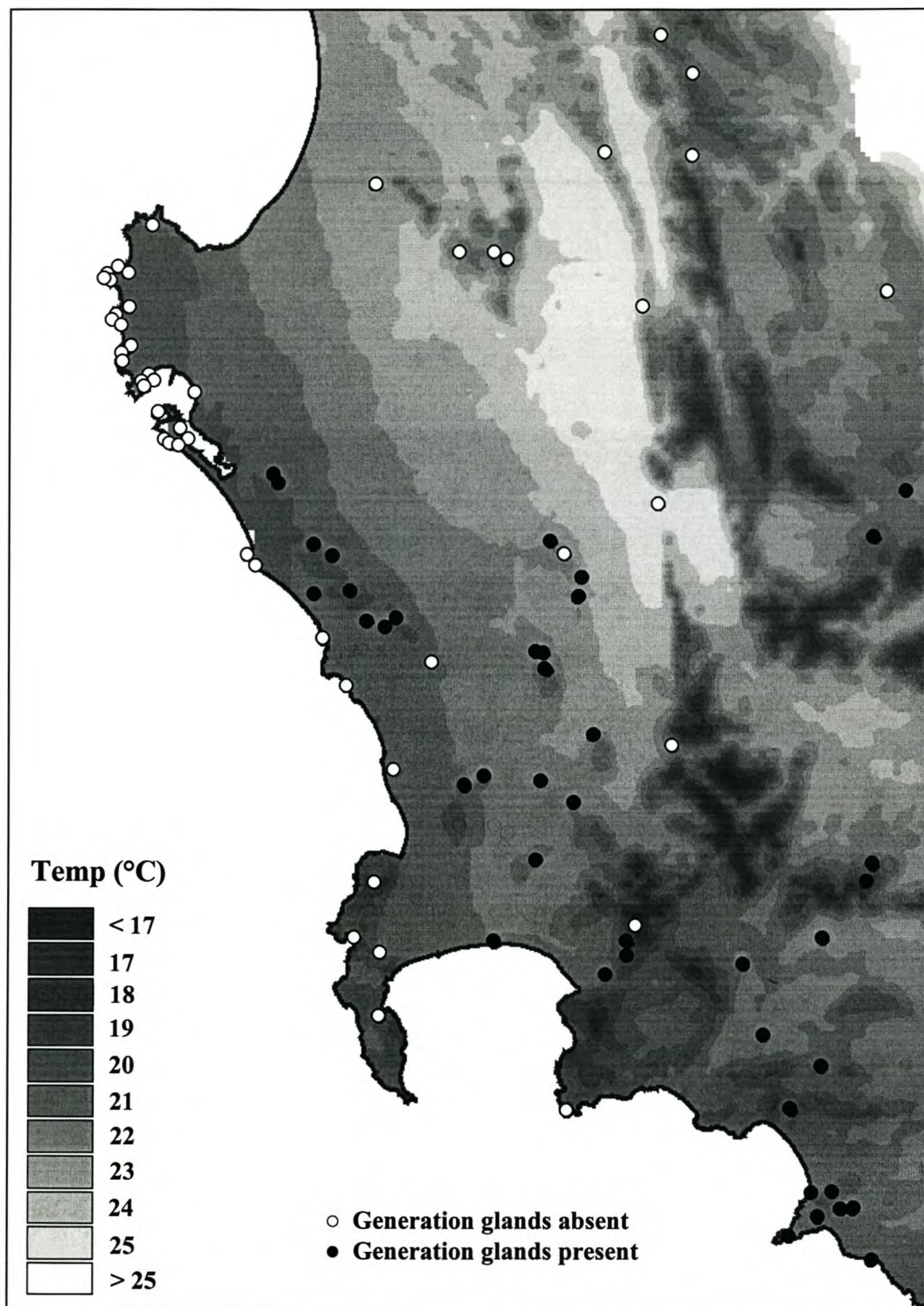
Figure 2.4. Mean annual maximum temperature for the study area.
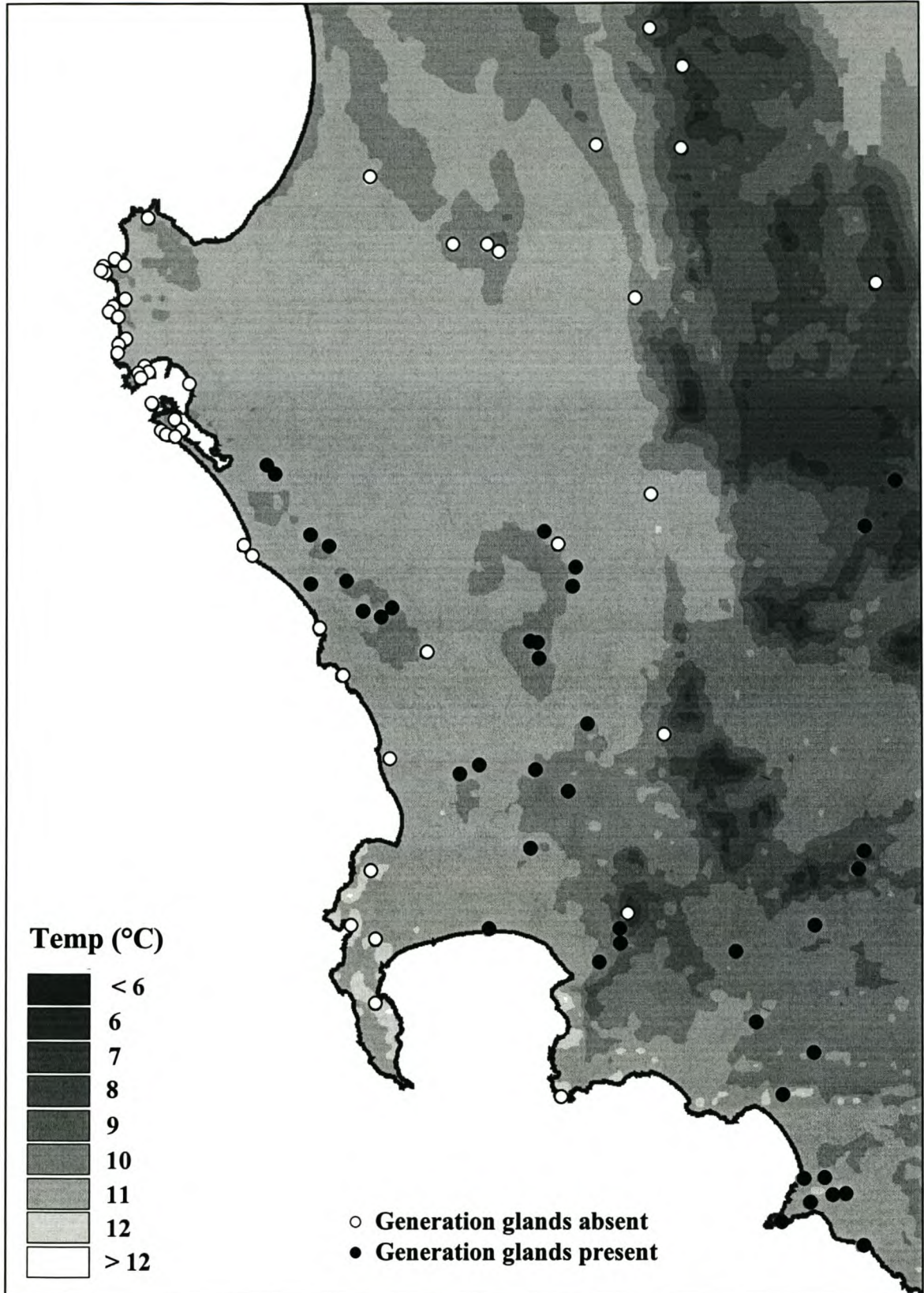
Figure 2.5. Mean annual minimum temperature for the study area.
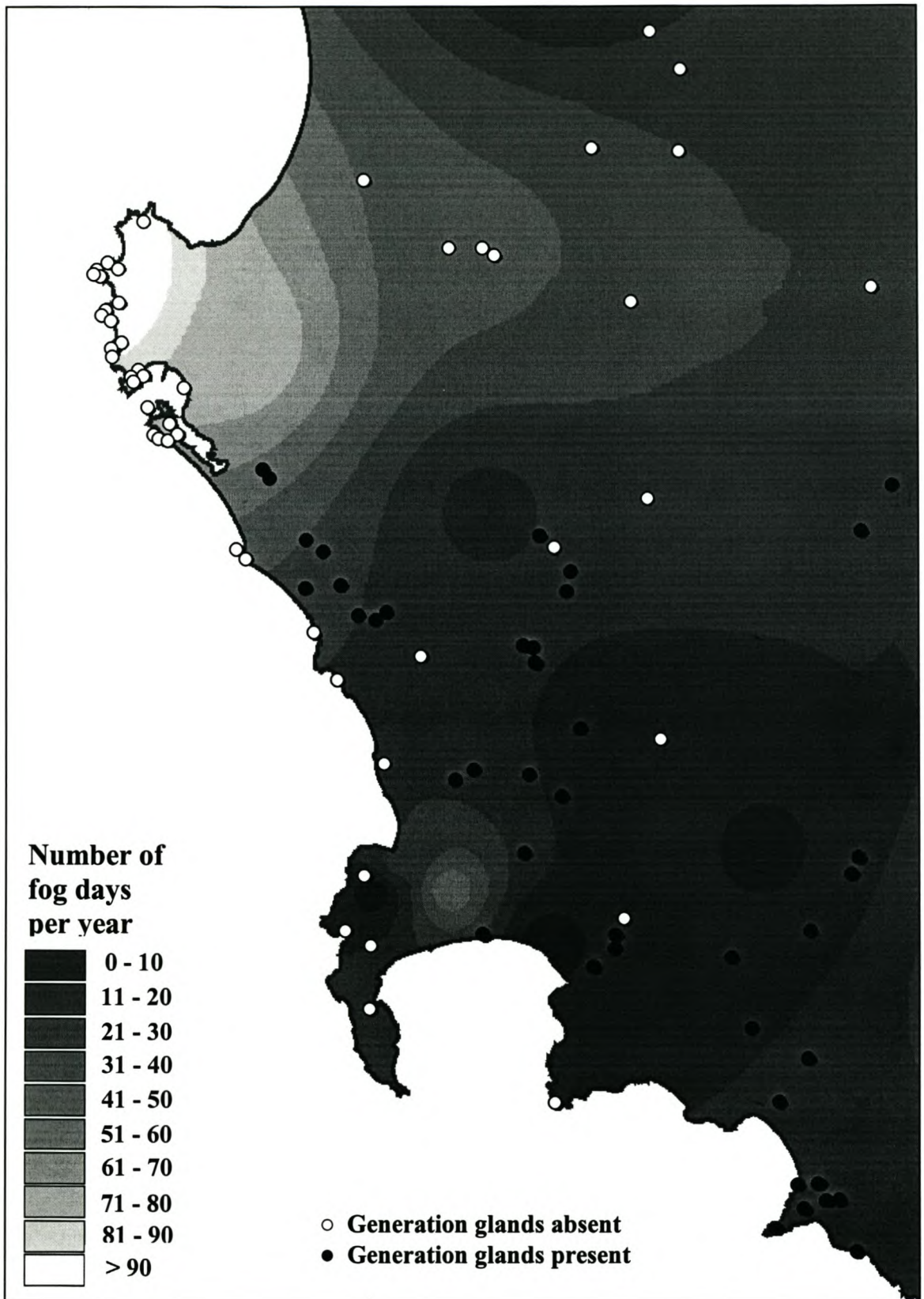
Figure 2.6. Mean annual number of days with fog for the study area.

**Cloud cover
(in eighths)**

	0 – 2.25
	2.26 – 2.40
	2.41 – 2.55
	2.56 – 2.60
	2.61 – 2.75
	2.76 – 2.90
	2.91 – 3.05
	3.06 – 3.20
	3.21 – 3.35
	> 3.35

○ **Generation glands absent**
● **Generation glands present**

Figure 2.7. Mean annual cloud cover for the study area.

& McGee 1978). The large amount of cloud cover and fog encountered causes a marked decrease in solar radiation (Schulze & McGee 1978) and these areas therefore receive less sunshine (6-8h per day) than the adjacent areas on the coastal lowlands (8-9.5h per day) (Badenhorst 1990). The absence of generation glands in female *Cordylus niger* and *C. cordylus* occurring along the west coast (Fig. 2.2), correlates well with the cool climate of the region. *C. niger*, being restricted to insular and peninsular situations, may experience even more extreme climatic conditions than *C. cordylus*.

The coastal lowland areas experience warmer temperatures than the west coast and montane areas. This is because advective sea fog seldom reaches inland areas far from the coast due to the general absence of land breezes (Badenhorst 1990). The warm Agulhas Current along the southern and eastern coast of South Africa does not have such a direct influence on the climate of the adjacent coastal areas as the cold Benguela Current has on the western coast, and warmer sea temperatures are experienced along this coastline (Isaac 1949; Swart 1956). Because of the high temperatures of the surrounding water, pronounced formation of fog is not induced (Badenhorst 1990). Apart from females from Kanonkop, Hangklip and the top of Kasteelberg, all female *C. cordylus* from the coastal lowlands possess generation glands (Fig. 2.2). The presence of generation glands in females from these regions correlate with the warmer climates experienced in the coastal lowlands in contrast to the cool climate of the west coast.
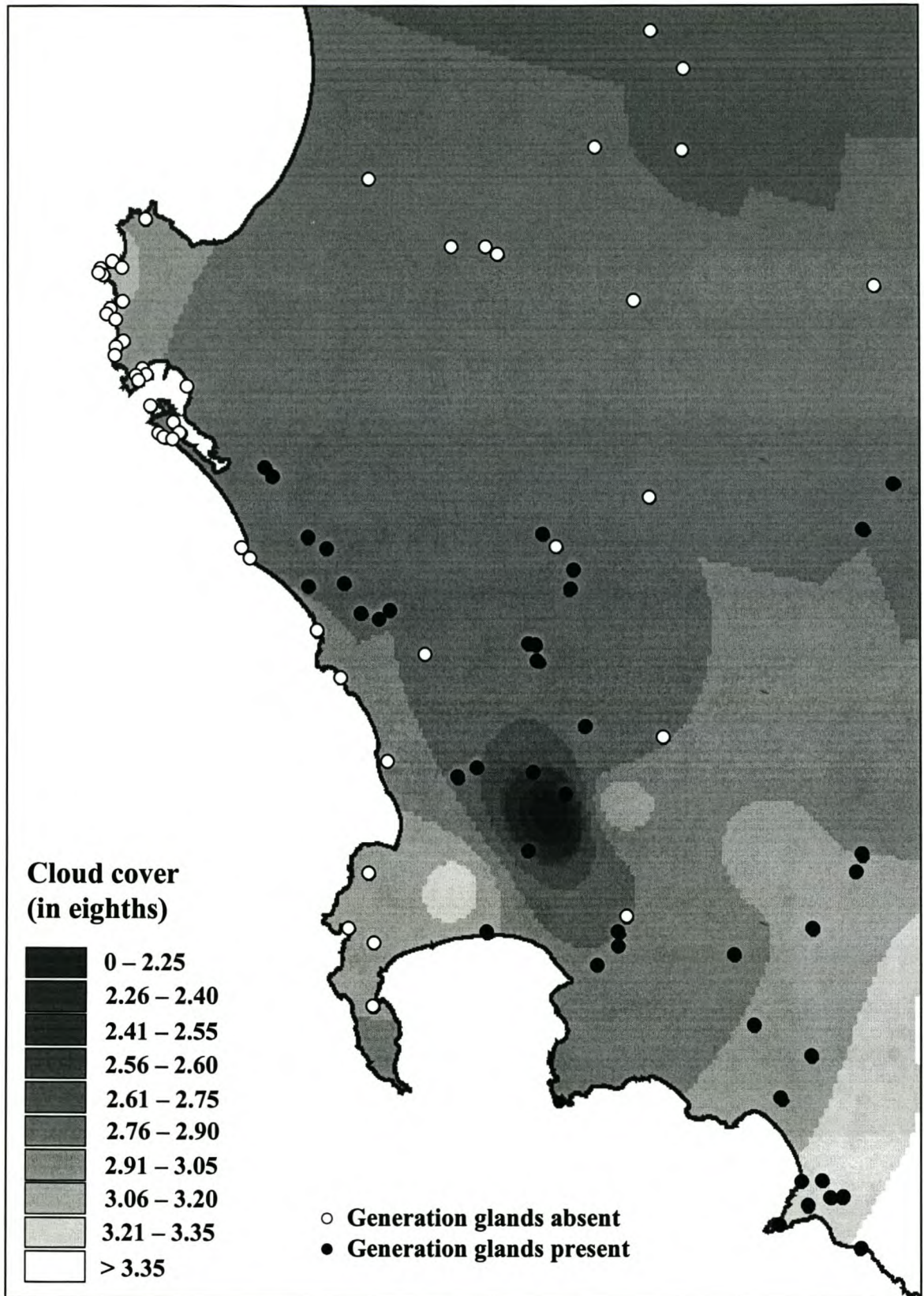
Altitude may have a decisive influence on mountain climates (Fuggle 1981) and in summer and winter, the Cape Fold Mountains experience lower temperatures than the lowlands (Swart 1956). Cloudy, cold and wet conditions are characteristic of mountainous areas (Barry 1981; Barry & Chorley 1982), while orographic fog is another important ecological factor in mountains (Badenhorst 1990). Areas along the extreme western flanks of the Cape Fold and Piketberg Mountains receive lower mean annual hours of sunshine

(7-8h per day) than the coastal lowlands due to cloud cover and fog (Badenhorst 1990). All female *Cordylus oelofseni* and most female *C. cordylus* occurring at higher altitudes in the Cape Fold Mountains lack generation glands, and this correlates with the cool climate experienced in mountainous areas. Although mountains experience cooler temperatures than the lowlands, higher temperatures are possible in the valleys (Swart 1956). The presence of generation glands in female *C. cordylus* occurring in the Cape Fold Mountains (Fig. 2.2) may thus be explained by the fact that these localities are situated in areas of lower elevation between areas of higher elevation.

All members of the genus *Cordylus* are heliothermic, and thus utilize direct solar radiation to obtain preferred body temperatures during times of activity. Thick and complete cloud cover, as well as fog, can form a significant barrier to solar radiation, especially in the infrared part of the spectrum (Barry & Chorley 1982). Reptiles, being ectothermic, have a high body temperature requirement and rely on solar radiation for energy (Bogert 1949; Huey 1982); regions with prevailing overcast and cloudy conditions are thus unsuitable habitats for these animals (Bogert 1949). The heat budget of members of the *cordylus-niger-oelofseni* complex occurring along the west coast or at high altitudes in the Cape Fold Mountains may thus be seriously affected by this constraint (Badenhorst 1990). While the results of this study showed a significant correlation between fog and the absence of generation glands, cloud cover had an insignificant correlation with the presence/absence of generation glands. A possible explanation is that the occurrence of fog is mainly restricted to the coastal region along the west coast and mountainous areas (orographic fog) (Fig. 2.6). Clouds on the other hand, can affect a far greater area than fog, covering even the whole study area when a cold front is moving over. Cloud cover, being not restricted to certain areas, will therefore have a more random effect in any specific area

(Fig. 2.7). It is also possible that a lack of weather stations in certain parts of the study area may have influenced the results obtained.

Adult male cordylids, in contrast to females, always possess generation glands, even in cool areas. It is possible that the difference in the presence of generation glands in males and females may be the result of differential energy allocation. Several authors have discussed differential energy allocation in females as an adaptation to different climatic regimes. Mouton & Van Wyk (1993) found geographic variation in the presence or absence of sexual dichromatism in females of the Drakensberg crag lizard, *Cordylus melanotus*. Females from the warmer, lower altitude localities were, like the males, brightly coloured, while females from cooler, higher altitude localities lacked bright coloration. Ruby & Baird (1994) observed intraspecific variation in the behaviour of the lizard *Sceloporus jarrovi* at different altitudes. Females from high elevations showed low levels of territoriality while females from low elevations showed much higher levels of aggression. In the cooler coastal and montane areas, members of the *cordylus-niger-oelofseni* complex experience fewer hours of sunshine or lower maximum temperatures, and females from these areas probably have less energy available to invest in secondary sexual characters than females from warmer areas. In order to maintain their reproductive output they probably invest available energy in their offspring rather than in generation glands. Generation glands have been implicated in territorial marking (Cooper *et al*. 1996; Mouton *et al*. 1998) and females investing more energy in reproduction, will probably also have less energy available to invest in territoriality. Because territoriality is usually accompanied by displaying, fighting and chasing (Carpenter 1967), in other words, aggressive behaviour towards intruders, females with glands are likely to show more aggression towards intruders than females that lack glands. These statements are,

however, still speculative and carefully designed field and laboratory experiments will be conducted to test this and to help put the function of these glands into perspective.

It is interesting to note that while Herselman *et al.* (1992) found no intrapopulation variation in *Cordylus capensis* (glands were either present or absent in all individuals), *C. cordylus* displayed intrapopulation variation regarding the presence/absence of generation glands at a number of localities. Extensive sampling of the area is necessary though, before more conclusive statements can be made.

A good correlation between the presence/absence of generation glands in females and climatic parameters does not necessarily establish a causal relationship. Hypotheses about selective forces are difficult to test, and we can only attempt to infer process from pattern (Harvey & Pagel 1991). However, the actual selective forces involved remain speculative even if two variables are tightly linked (Shine 2002) (i.e., generation glands consistently absent in cool climates). By manipulation of environmental factors (e.g. temperature) under controlled laboratory conditions we can potentially clarify whether or not the habitat does indeed impose the selective force.

Mouton & Oelofsen (1988) proposed a model for the distribution of melanistic cordylid species in the Western Cape, South Africa, according to which the distribution of these species is correlated with cooler environments. Their model proposed that melanism evolved during a glacial period and the rapid amelioration of the climate following this led to the contraction of these cold-adapted forms. This opened the way for the expansion of the warm-adapted, non-melanistic, inland form from the southern coastal lowlands into the coastal lowlands of the west coast (Mouton & Oelofsen 1988). An electrophoretic analysis (Brody *et al.* 1993) confirmed that melanistic and non-melanistic forms represent different gene pools. A recent genetic study (Daniels *et al.* 2004) reinvestigated the status of the *cordylus-niger-oelofseni* complex and found that *C. cordylus* populations along the west

coast in which females lack generation glands and populations from the coastal lowlands where females have generation glands, does in fact not represent different gene pools. This suggests that the presence/absence of generation glands is truly under climatic control.

CHAPTER 3

VARIATION IN GENERATION GLAND PROFILE AMONG CORDYLID LIZARDS: A SEARCH FOR PATTERNS

3.1 INTRODUCTION

The superfamily Cordyliformes is partitioned into two families, the Gerrhosauridae and the

Cordylidae (Lang 1991). Both families are restricted to sub-Saharan Africa, with the

Gerrhosauridae also present on Madagascar. While the Gerrhosauridae has a fairly even

spread of species over the southern subcontinent, the Cordylidae shows a distinct

clustering of species in the extreme south (Mouton & Van Wyk 1997). The

Gerrhosauridae-Cordylidae divergence was accompanied by a shift from active foraging to

a sit-and-wait foraging, and from a ground-dwelling lifestyle to a rock-dwelling one.

Mouton & Van Wyk (1997) are of the opinion that these shifts indicate that the Cordylidae

had a cold origin. The shifts in foraging mode and lifestyle and the subsequent evolution

of viviparity in the ancestor of the *Cordylus* clade, allowed the invasion of highland areas,

to the effect that cordylids today occur over a wide range of habitats, from sea level to

mountain top (Mouton & Van Wyk 1997).

The shifts in lifestyle and foraging mode in the immediate ancestor of the Cordylidae

were also accompanied by the evolution of well-differentiated generation glands. These

glands are present in all *Platysaurus* species, the basal genus in the family (Frost *et al*.

2001), while gerrhosaurids apparently lack these glands, although there are indications that

some species may have some glandular activity in the scales immediately anterior to the

femoral pores (Mouton, pers. comm.). As pointed out in Chapter 1, the possession of well-

differentiated generation glands by cordylids may be related to their rock-dwelling lifestyle

and sit-and-wait foraging mode. Although the majority of extant cordylids are rock-

dwelling, at least some species are ground-dwelling. The phylogenetic tree for the Cordylidae (Frost *et al*. 2001), however, shows that the ground-dwelling forms are all derived forms, in other words, that their ground-dwelling lifestyles represent reversals. If the possession of well-differentiated generation glands are indeed related to a rock-dwelling lifestyle, a reversal from a rock-dwelling lifestyle to a ground-welling one would probably also encompass changes in the generation gland profile of the species under question. Several independent reversals occurred in the family, offering the opportunity to investigate the relationship between lifestyle and generation gland profile.

If the diversity in lifestyle, social structure, and geographical distribution in the Cordylidae is also expressed in the generation gland profile of species, it may be worthwhile conducting a comparative analysis of generation gland profile in the family and to search for common patterns. The identification of such patterns may help to elucidate the functional significance of generation glands. Large data sets on generation glands are already available for a number of species and it should thus be possible to conduct such an analysis.

The aims of this study were firstly to investigate interspecific variation in the presence, location, and number of generation glands, sexual dimorphism in generation gland profile, and the age at which generation glands first start to differentiate in different species, and secondly, to investigate inter- and intrapopulation variation in generation gland profile for a number of species. In order to do so, I made use of existing data as well as data collected during this study.

3.2 MATERIALS AND METHODS

3.2.1 Interspecific variation

Generation gland data for the following species were used in this analysis: *C. cataphractus*

(Mouton *et al.* 1999; Retief 2000), *C. macropholis* (Mouton *et al.* 1998; Mouton *et al.* 2000a), *C. melanotus* (Mouton & Van Wyk 1993), *C. microlepidotus* (Gagiano 2003), *C. niger* (Cordes *et al.* 1995), *C. oelofseni* (Mouton & Van Wyk 1990; Chapter 2), *C. nebulosus* (Costandius, unpublished data), and *C. peersi* (Mouton, unpublished data). Data for *C. cordylus* were collected in the field, while small data sets were obtained for a number of species of which preserved material is housed in the Ellerman Collection of the University of Stellenbosch. The species included were: *Platysaurus broadleyi, P. capensis, P. intermedius wilhelmi, C. aridus, C. campbelli, C. capensis, C. cloetei, C. coeruleopunctatus, C. giganteus, C. imkeae, C. jordani, C. lawrenci, C. mclachlani, C. minor, C. namaquensis, C. polyzonus, C. pustulatus, C. rhodesianus, C. spinosus, C. tasmani, C. tropidosternum jonesi, C. vittifer* and *C. warreni*. Data on *C. langi* were obtained from the National Museum in Bloemfontein. Information on femoral glands was obtained from Branch (1998).

3.2.2 Inter- and intrapopulation variation

Cordylus cordylus and *C. cataphractus* were used for the analysis of inter- and intrapopulation variation in generation gland profile. The area south of 33°00′S and west of 19°00′E in the southwestern corner of South Africa was selected as the study area for *Cordylus cordylus* (Fig. 3.1). Five localities were selected to represent a range of climatic regimes on the lowlands. Specimens of *C. cordylus* were collected at the following localities: Swartberg, Joostenberg, Rondeberg, Klipberg, and the lower slopes of Paardeberg (Fig. 3.1). Table 3.1 gives a summary of the environmental variables for each locality.

All *C. cordylus* encountered at each locality were captured by hand or with a short noose. Digital callipers were used to measure snout-vent length (SVL) to the nearest 0.01
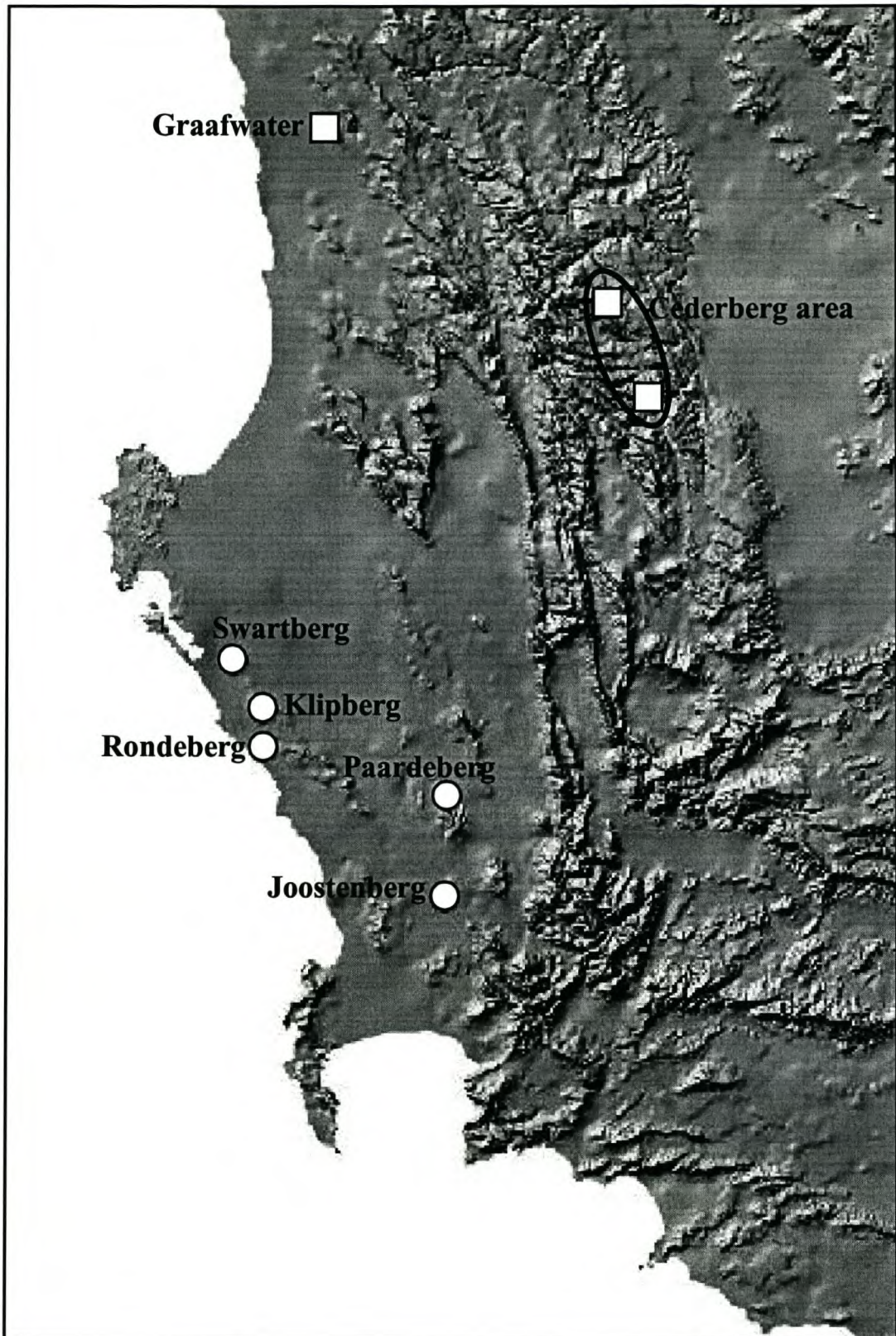
Figure 3.1. Localities in the study area where *Cordylus cordylus* (○) and *C.*

cataphractus (□) were sampled.

Table 3.1. Elevational and climatic data for the five localities where *Cordylus cordylus* was sampled. (TMIN = mean annual minimum temperature; TMAX = mean annual maximum temperature; FOG = mean number of days with fog; and CLOUD = mean annual cloud cover measured at 14:00)

Locality	Altitude (m)	TMIN (°C)	TMAX (°C)	FOG	CLOUD
Joostenberg	280	11.1	22.29	20.75	2.49
Klipberg	260	11.03	20.84	39.05	2.89
Paardeberg	380	10.61	22.05	21.99	2.83
Rondeberg	180	11.47	21.03	34.84	2.9
Swartberg	280	11.06	20.93	55.18	2.87

mm. Males were distinguished from females by their larger heads and the presence of hemipenal bulges. Small individuals (SVL < 60 mm) were difficult to sex accurately and were classified as indeterminates. A few small individuals from each locality were sacrificed for sex determination. By using a magnifying glass (10X), generation glands were counted on both thighs and the total number recorded for each individual. Lizards were released after being subjected to measurement. Preserved material from the Ellerman Collection (University of Stellenbosch) were also included in the analysis.

Data for *C. cataphractus* were obtained from two previous studies on a coastal lowland population and on an inland population (Fig. 3.1). The coastal lowland locality is near Graafwater, 20 km from the coast along the west coast of South Africa, and the inland locality is in the Cederberg Mountains, northeast of Citrusdal near Kromrivier and Sanddrif (Fig 3.1). The Graafwater population was thoroughly sampled and described by Mouton *et al.* (1999), while the Cederberg population was sampled by Retief (2000). The same procedures were followed for obtaining data on generation glands as for *C. cordylus*.

3.2.3 Data analysis

For the species for which representative data sets were available, Kruskal-Wallis tests were performed to test for possible differences in the number of generation glands among males, as well as females. Pearson correlation tests were used to test for correlations between SVL and number of generation glands for the different sexes of each species. Mann-Whitney U-tests were used to test for differences between males and females. Graphs of snout-vent-length against number of generation glands were drawn for males and females of each species to illustrate variation in number and time of generation gland differentiation.

Data sets were tested for normality and homogeneity of variances using Kolmogorov-Smirnov's and Levene's tests, respectively. For between-population comparisons of the number of generation glands in males and females, a one-way analysis of covariance (ANCOVA), with SVL as a covariate, was performed, followed by Sheffé's ad hoc test. Mann-Whitney *U*-tests were used for two-sample comparisons where the data were not normally distributed. Pearson correlation tests were used to test for correlations between SVL and number of generation glands for each sex at each locality.

3.3 RESULTS

3.3.1 Interspecific variation

3.3.1.1 Location, occurrence and number of generation glands

All species investigated possess generation glands. In all cases, the generation glands occur on the ventral aspect of the thighs as a patch of glandular scales adjacent and anterior to the femoral glands. In two species, generation glands occur, in addition to the femoral region, also elsewhere on the body. In *C. microlepidotus*, these glands are also found in the inguinal region, as well as on the dorsal body on both sides of the vertebral line. In *C. giganteus*, generation glands are also found in the anterior antebrachial (forearm) region.

While males of all species investigated possess generation glands, these glands are absent in females of several species. In all species where females do not have femoral glands, generation glands are also absent. This group includes all *Platysaurus* species, *C. polyzonus*, *C. jordani*, *C. langi*, *C. maclachlani*, *C. namaquensis*, *C. peersi*, *C. pustulatus*, *C. lawrenci*, *C. campbelli*, and the *C. warreni* species group. In a number of species where femoral glands are present in females, generation glands are absent, however. These include *C. niger*, *C. oelofseni*, *C. nebulosus*, *C. giganteus*, *C. rhodesianus*, *C. melanotus*, and *C. tropidosternum*. In *C. cataphractus*, *C. coeruleopunctatus*, *C. cordylus*, *C.*

macropholis, *C. minor*, *C. cloetei*, *C. imkeae*, *C. aridus*, *C. tasmani*, *C. vittifer*, *C. spinosus*, *C. capensis*, and *C. microlepidotus*, females have active femoral glands as well as generation glands. In at least three of these species, *C. capensis*, *C. coeruleopunctatus* and *C. cordylus*, females may lack generation glands in some geographical areas and have these glands in other areas.

Using only data for males of the *Cordylus* species for which representative data sets are available, *C. capensis* has the most generation glands ($P < 0.01$ for all cases), followed by *C. cataphractus* from the Cederberg (P < 0.001 for all cases, except *C. coeruleopunctatus* and *C. polyzonus*). These two species have more than double the number of glands recorded for most other species, with the exception of *C. coeruleopunctatus*, *C. polyzonus* and *C. melanotus* (Table 3.2). Even female *C. cataphractus* from the Cederberg and female *C. capensis* have more glands than the males of other species (Table 3.2). *Cordylus aridus*, *C. cloetei*, *C. minor*, and *C. macropholis* have the lowest number of generation glands for males across the species examined (Table 3.2), but a representative data set was only available for *C. macropholis*. When counting all generation glands and not only those in the femoral region, males of *C. microlepidotus* have by far the most glands.

In all species where females also have generation glands, males have significantly more glands than females (Table 3.2, 3.3; Fig. 3.2-3.8). The difference in number of glands between males and females was the smallest in *C. cataphractus* from the Cederberg and in *C. macropholis* (Table 3.3; Fig. 3.4a, 3.8).

Dorsal generation glands were present in both male and female *C. microlepidotus*, with adult females (mean ± SD: $14 ± 25.65$) having significantly less glands than adult males (mean ± SD: $135 ± 59.96$; Mann-Whitney *U*-test, $T = 4815.0$, $P < 0.001$). Inguinal generation glands were only present in males of this species.

Table 3.2. Interspecific variation in generation gland number in the Cordylidae family.

Species	N	Range of number of generation glands	Maximum SVL
Platysaurus broadleyi			
Males	7	91 - 146	78.75
Females	6	0	77.13
Platysaurus capensis			
Males	2	102	78.78
Females	2	0	44.83
Platysaurus intermedius wilhelmi			
Males	1	36	73.21
Females	1	0	70.32
C. aridus			
Males	2	4 - 6	63.37
Females	5	4 - 5	64.4
C. capensis			
Males	18	14 - 148	99.48
Females	36	0 - 92	107.23
C. cataphractus (Cederberg)			
Males	21	39 - 109	114.53
Females	27	2 - 75	111.02
C. cataphractus (Graafwater)			
Males	44	1 - 56	122.95
Females	37	0 - 16	115.41

C. cloetei

| Males | 2 | 13 | 63.41 |
| Females | 4 | 4 - 5 | 68.38 |

C. coeruleopunctatus

| Males | 6 | 36 - 84 | 74.68 |
| Females | 19 | 0 - 30 | 76.34 |

C. cordylus

| Males | 38 | 7 - 28 | 86.73 |
| Females | 88 | 0 - 17 | 92.17 |

C. giganteus

| Males | 10 | 40 - 58 | 190.0 |
| Females | 2 | 0 | 177.97 |

C. imkeae

| Males | 1 | 22 | 52.8 |
| Females | 3 | 8 - 12 | 62.87 |

C. lawrenci

| Males | 3 | 17 – 22 | 64.73 |
| Females | 1 | 0 | 47.95 |

C. macropholis

| Males | 45 | 7 - 15 | 72.17 |
| Females | 103 | 0 - 18 | 76.87 |

C. mclachlani

| Males | 9 | 15 - 38 | 62.7 |
| Females | 21 | 0 | 72.25 |

C. melanotus

Males	140	0 - 77	127
Females	140	0	108

C. microlepidotus

Males	23	19 – 50	160.62
Females	28	0 - 14	149.13

C. minor

Males	4	10 – 16	63.7
Females	5	0 - 5	71.95

C. namaquensis

Males	1	21	67.32
Females	4	0	78.85

C. nebulosus

Males	21	6 - 37	76.6
Females	44	0	78.92

C. niger

Males	71	0 - 32	86.24
Females	118	0	91.49

C. oelofseni

Males	25	16 – 46	62.93
Females	36	0	68.32

C. peersi

Males	20	17 - 32	85.73
Females	20	0	87.82

C. polyzonus

Males	7	13 - 82	100.37
Females	34	0	115.95

C. pustulatus

Males	-	-	-
Females	1	0	70.71

C. spinosus

Males	3	32 – 46	84.58
Females	1	12	79.36

C. tasmani

Males	-	-	-
Females	4	0 - 4	65.17

C. tropidosternum jonesi

Males	3	14 – 28	64.11
Females	2	0	75.8

C. vittifer

Males	1	28	73.4
Females	4	0	74.17

C. warreni

Males	3	36	117.54
Females	-	-	-

Table 3.3. Sexual dimorphism in number of generation glands for species for which representative data sets where available. Question marks indicate a lack of data. $P < 0.001$ in all between species comparisons.

Species	N	Mean no. of generation glands ± SD	Size at sexual maturity (mm)	Size at gland differentiation (mm)
C. capensis				
Males	18	95.0 ± 31.08	80	90
Females	36	19.06 ± 31.21	80	93
C. cataphractus				
Graafwater				
Males	21	27.82 ± 15.37	95	80
Females	27	1.59 ± 3.62	95	105
Cederberg				
Males	44	61.75 ± 5.56	90	70
Females	37	54.0 ± 16.75	81	83
C. coeruleopunctatus				
Males	6	51.33 ± 19.05	?	50?
Females	19	8.73 ± 11.44	?	50
C. cordylus				
Males	38	18.28 ± 5.32	55	45
Females	88	6.36 ± 2.93	55	55
C. macropholis				
Males	45	10.61 ± 2.12	56/51	Present in
Females	103	8.35 ± 1.89	60	neonates

C. mclachlani

Males	9	28.56 ± 8.44	?	45
Females	21	0	?	-

C. melanotus

Males	140	31.96 ± 19.12	80	80
Females	140	0	80	-

C. microlepidotus

Males	23	24.00 ± 8.98	110	70
Females	28	3 ± 4.78	110	105

C. nebulosus

Males	21	22.95 ± 9.13	?	60
Females	44	0	?	-

C. niger

Males	71	18.83 ± 8.62	55	42
Females	118	0	55	-

C. oelofseni

Males	25	30.0 ± 8.0	?	45
Females	26	0	?	-

C. peersi

Males	20	26.50 ± 3.51	?	70
Females	20	0	?	-

C. polyzonus

Males	7	57.86 ± 23.22	83	70
Females	34	0	87	-

Figure 3.2. Scatterplot of number of generation glands versus body size in male and female *Cordylus cordylus*.
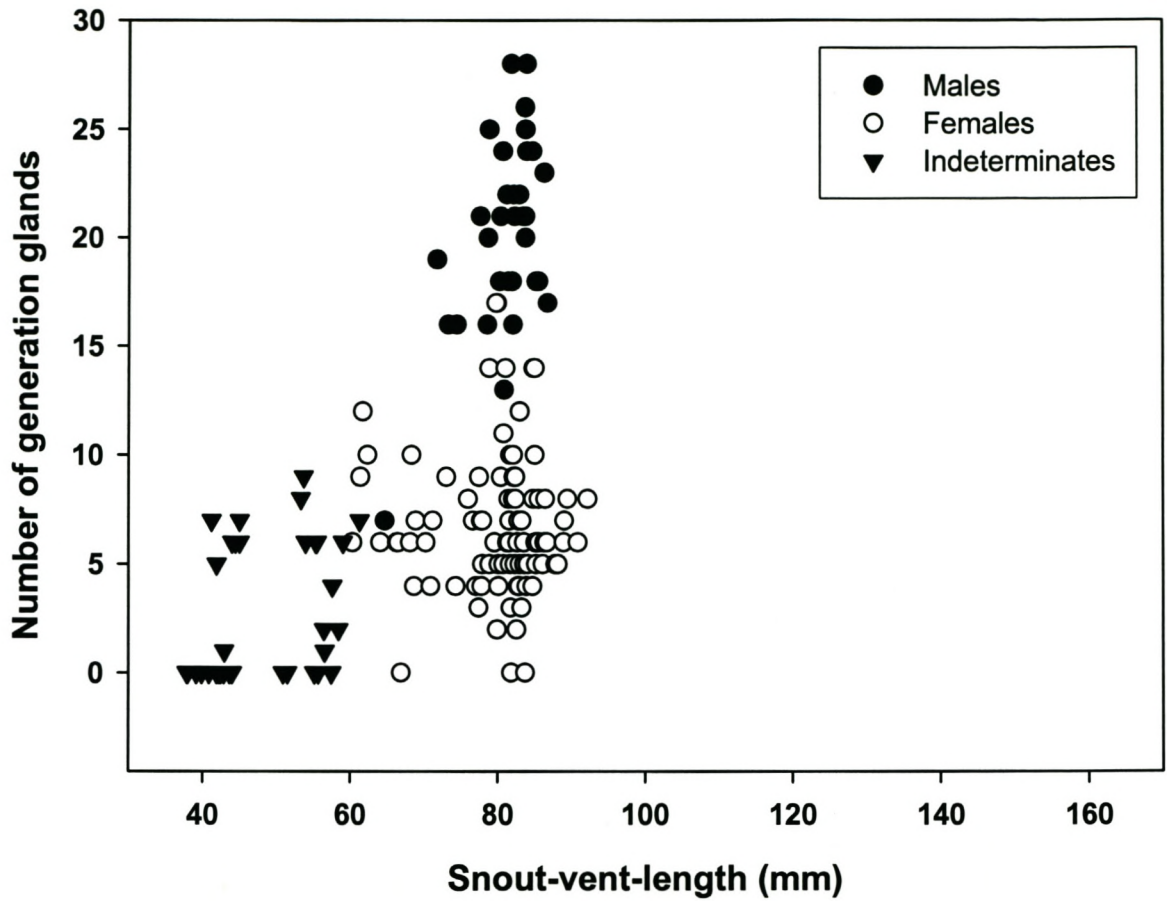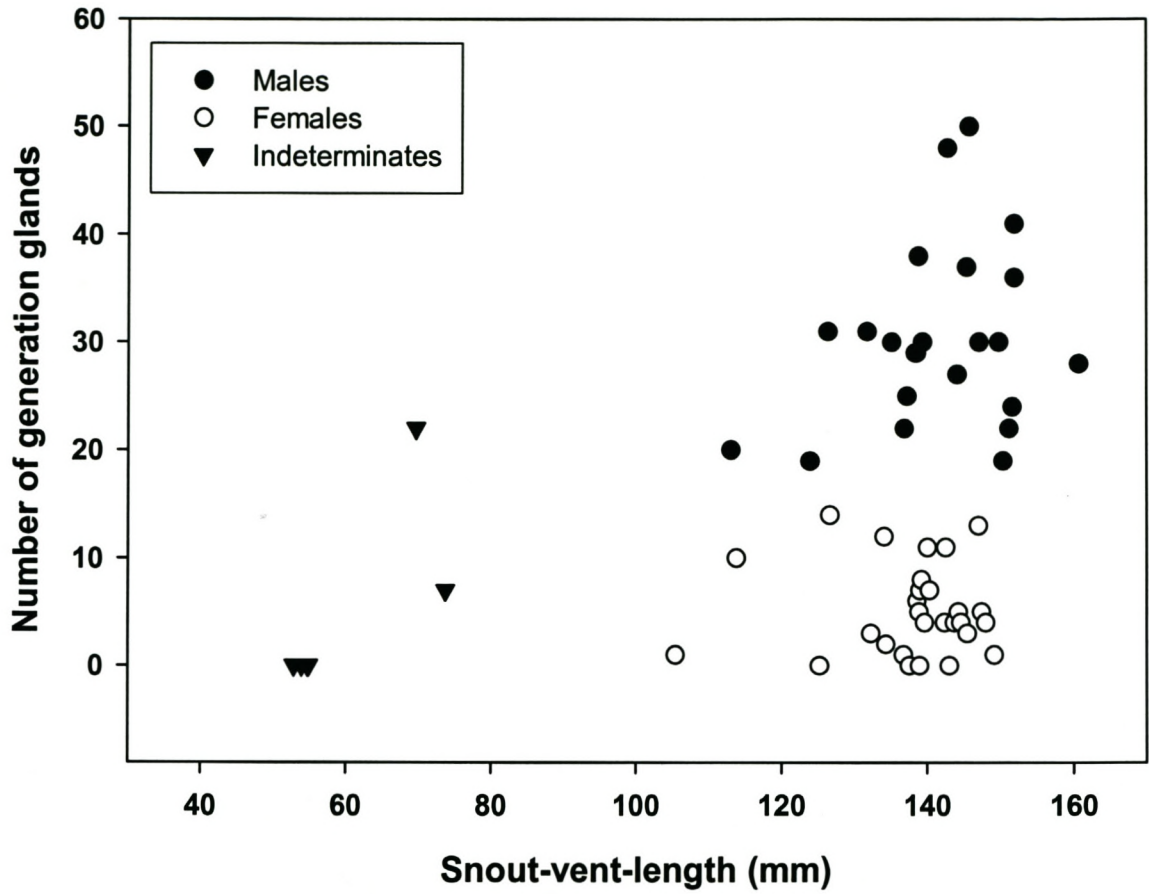
Figure 3.3. Scatterplot of number of generation glands versus body size in male and female *Cordylus microlepidotus*.
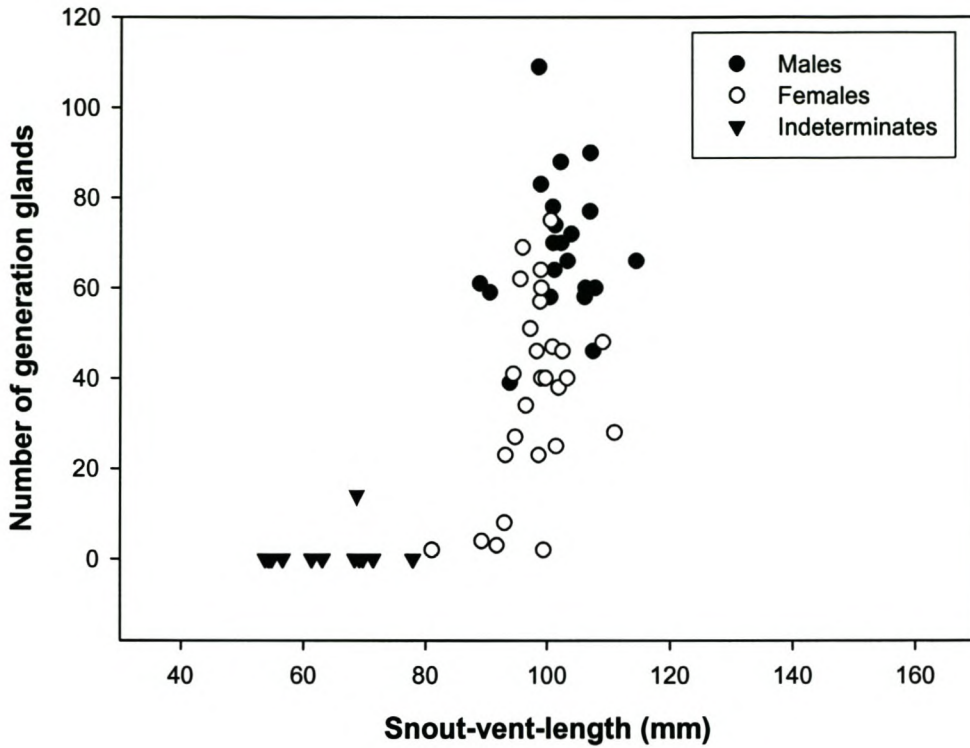
Figure 3.4a. Scatterplot of number of generation glands versus body size in Cederberg male and female *Cordylus cataphractus*.
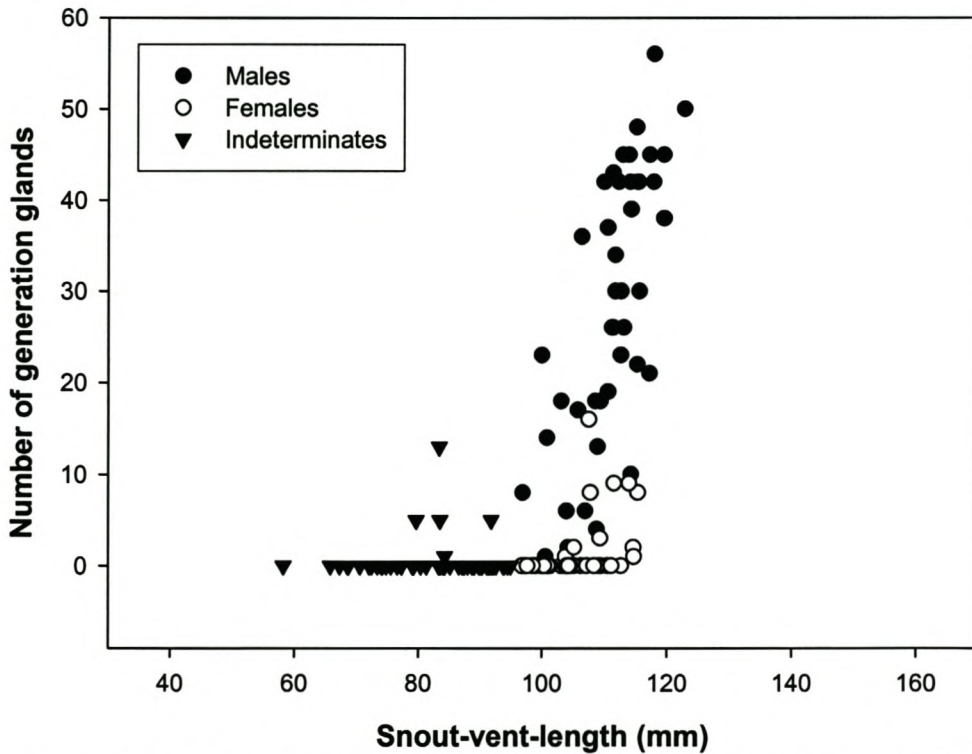

Figure 3.4b. Scatterplot of number of generation glands versus body size in Graafwater male and female *Cordylus cataphractus*.

In females, *C. capensis* and Cederberg *C. cataphractus* have significantly more generation glands than females of all the other species where females have these glands ($P < 0.005$ in all cases) (Table 3.2).

3.3.1.2 Age (size) at generation gland differentiation

In females of *C. cordylus*, *C. microlepidotus*, and Cederberg *C. cataphractus*, generation glands start to develop with or just after the onset of sexual maturity (Table 3.3, Fig. 3.2-3.4a). In *C. capensis* and in the Graafwater population of *C. cataphractus*, only the largest females develop generation glands (Fig. 3.4b, 3.5). In males, generation glands may already start to develop before sexual maturity is reached (Table 3.3, Fig. 3.2-3.8). *C. macropholis* (Fig. 3.8) and *C. tasmani* were the only species where generation glands were already present in neonates of both sexes. The presence of generation glands in neonates of *C. tropidosternum* has also been documented by Mouton *et al.* (1998).

Females of *C. coeruleopunctatus*, *C. cordylus* and *C. microlepidotus* all showed insignificant correlations between number of generation glands and SVL ($P > 0.05$ for all three species). This correlation was, however, significant in female *C. capensis* (Pearson correlation coefficient = 0.479, $P < 0.005$), *C. macropholis* (Pearson correlation coefficient = 0.325, $P < 0.05$) and *C. cataphractus* from the Cederberg (Pearson correlation coefficient = 0.427, $P < 0.05$) and Graafwater (Pearson correlation coefficient = 0.429, $P < 0.05$).

In males, the number of glands is more closely correlated with SVL, with most species showing a gradual increase in generation gland number with increasing SVL (*C. capensis*: Pearson correlation coefficient = 0.480, $P < 0.05$; *C. cataphractus* (Graafwater): Pearson correlation coefficient = 0.721, $P < 0.001$; *C. macropholis*: Pearson correlation coefficient = 0.241, $P < 0.05$; *C. melanotus*: Pearson correlation coefficient = 0.592, $P < 0.0001$; *C. microlepidotus*: Pearson correlation coefficient = 0.368, $P < 0.001$; *C. nebulosus*:

Figure 3.5. Scatterplot of number of generation glands versus body size in male and female *Cordylus capensis*.

Figure 3.6. Scatterplot of number of generation glands versus body size in male and female *Cordylus melanotus*.
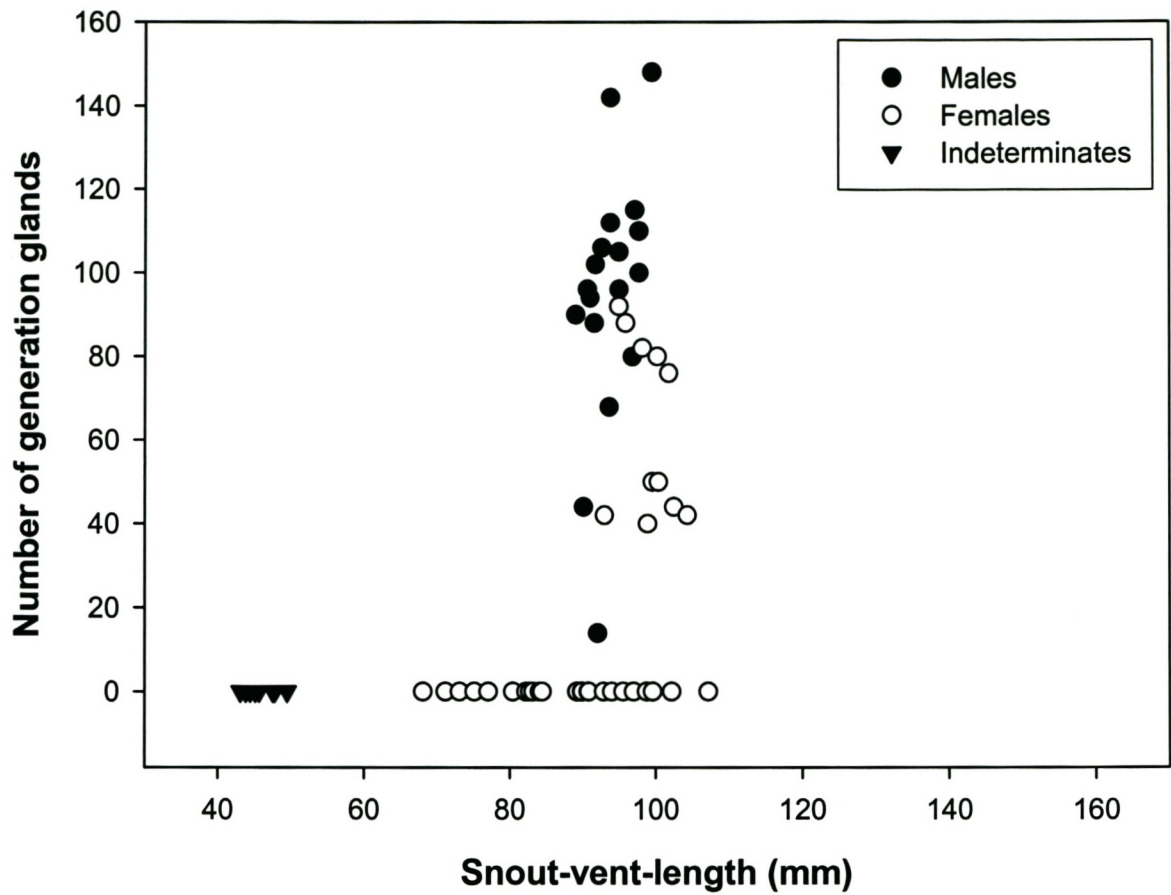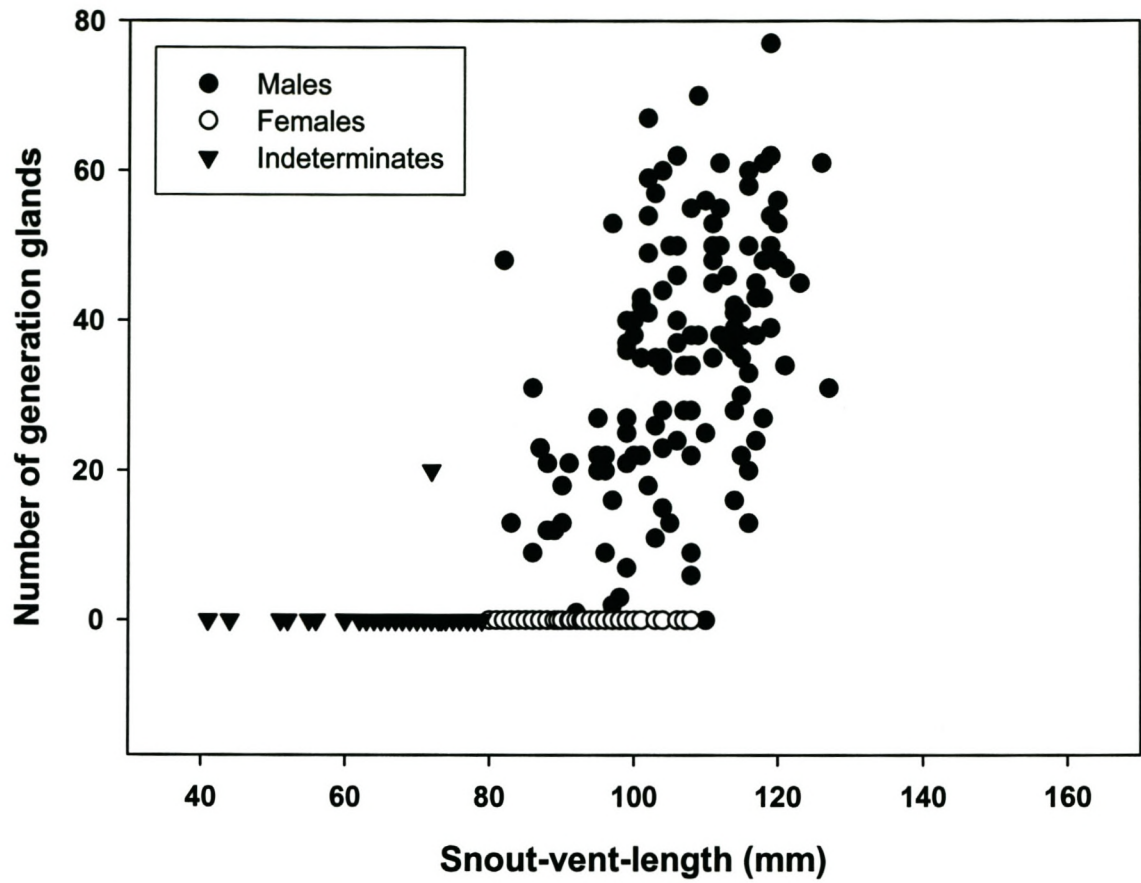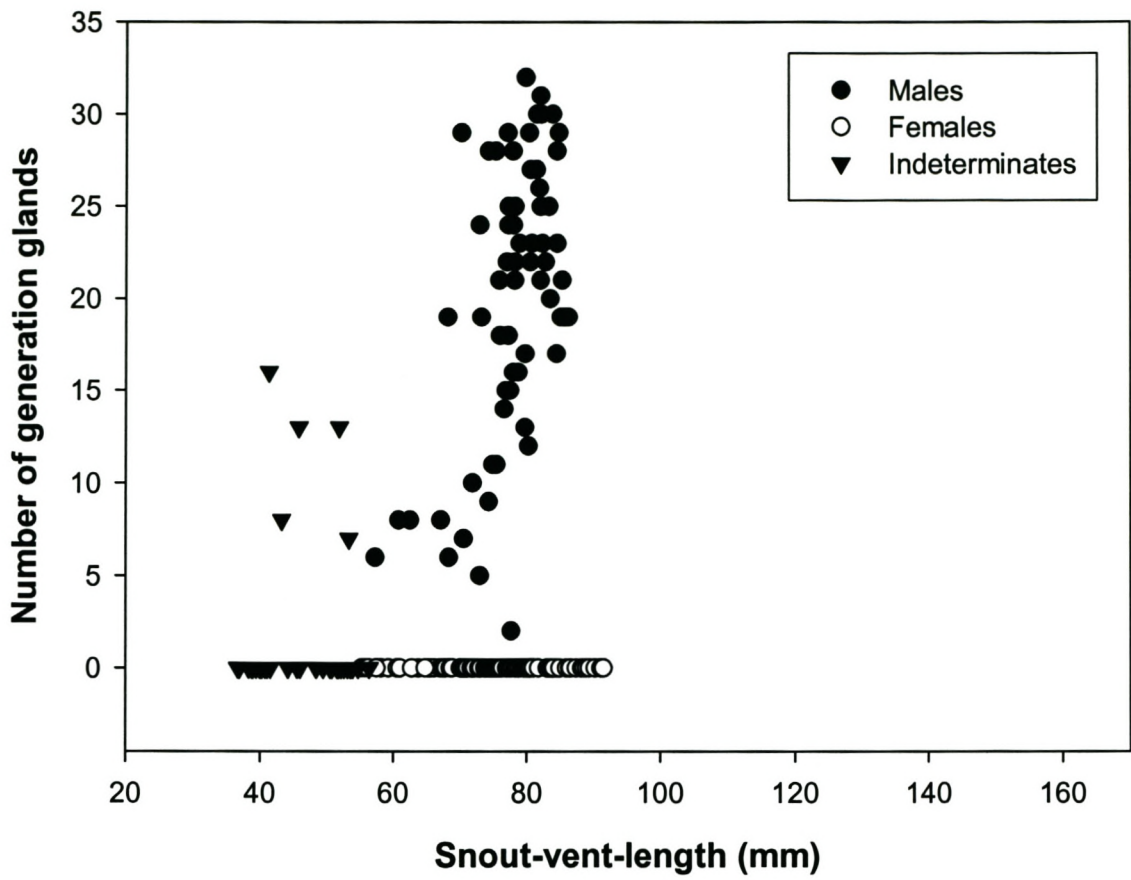
Figure 3.7. Scatterplot of number of generation glands versus body size in male and female *Cordylus niger*.
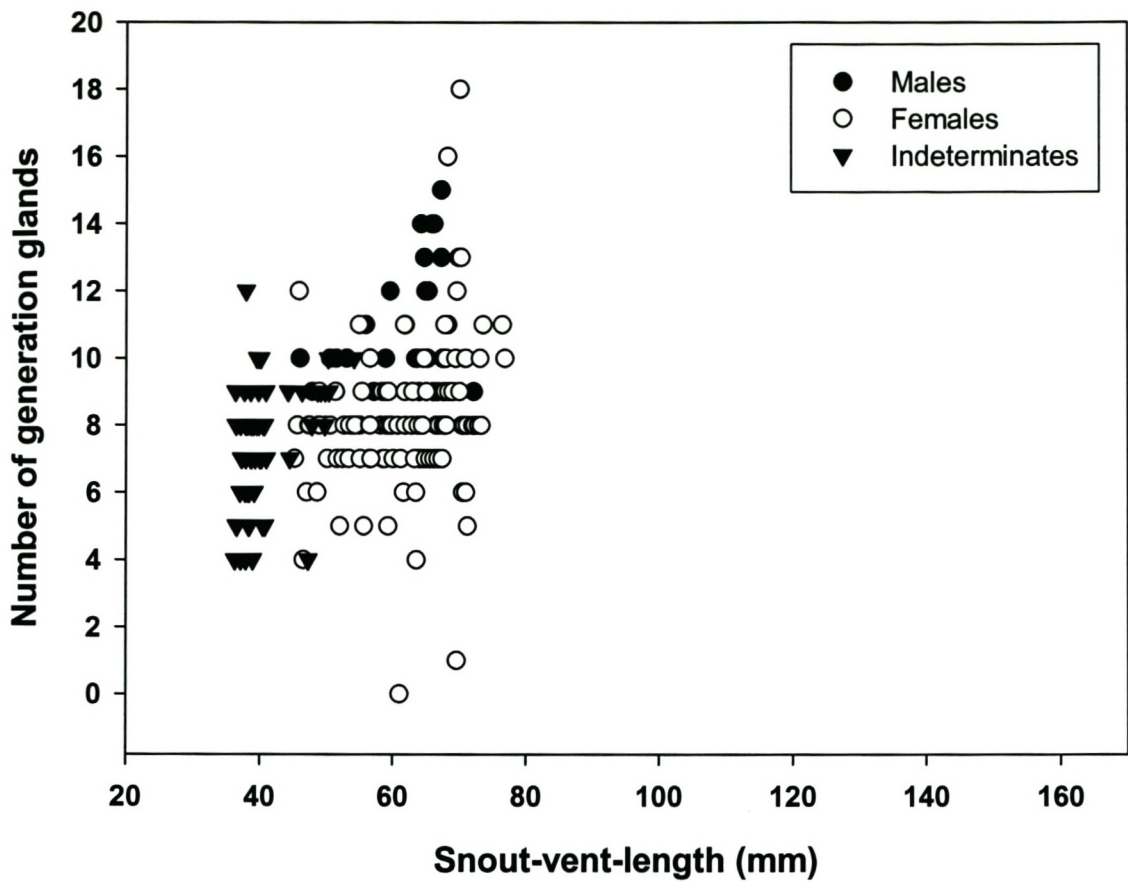
Figure 3.8. Scatterplot of number of generation glands versus body size in male and female *Cordylus macropholis*.

Pearson correlation coefficient = 0.705, *P* < 0.001; *C. peersi*: Pearson correlation coefficient = 0.568, *P* < 0.01; *C. polyzonus*: Pearson correlation coefficient = 0.784, *P* < 0.05). Number of generation glands is not correlated with SVL in *C. coeruleopunctatus*, *C. mclachlani*, *C. niger* and *C. cataphractus* from the Cederberg, while males of *C. cordylus*, only showed a significant correlation between gland number and SVL at Rondeberg (Pearsons correlation coefficient = 0.911, *P* < 0.005) and Klipberg (Pearsons correlation coefficient = 0.77, *P* < 0.005). Cordes *et al.* (1995) also found no significant correlation between gland number and SVL in their studies on *C. cordylus* and *C. niger*.

The pattern of gland differentiation differs between males and females. After differentiation at sexual maturity, generation glands in females show only a slight increase or remain more or less constant in number with increasing SVL. Female *C. cataphractus* from the Cederberg and female *C. capensis*, however, showed quite a dramatic increase in generation gland number, with some females even having the same number of glands than similar-sized males (Fig. 3.4a, 3.5). Males of most species, on the other hand, show a slight increase in gland number with increasing SVL after initial differentiation, but a sudden increase in number when asymptotic body size is reached (Fig. 3.2-3.8).

3.3.2 Interpopulation variation

I found interpopulation variation in the number of generation glands among the five populations of *Cordylus cordylus* investigated in this study. Females showed a significant difference in gland number among the five localities (ANCOVA: *F* = 6.04, *P* < 0.001) (Table 3.4a, Fig. 3.9). Number of generation glands in females from Rondeberg did not differ significantly from number of glands in females from any of the other localities. Females from Paardeberg had significantly fewer glands than females from Joostenberg and Klipberg, while the Klipberg females also had significantly more glands than females

Table 3.4. Summary of the post hoc comparison (Sheffé), showing significant differences among localities for females (a), but not for males (b). Localities that differ significantly from others are shaded and marked in bold.

(a)	Locality	1	2	3	4	5
1	Swartberg					
2	Joostenberg	0.060170				
3	Rondeberg	0.636295	0.979742			
4	Paardeberg	0.666130	**0.009563**	0.172192		
5	Klipberg	**0.005227**	0.941474	0.774442	**0.001079**	

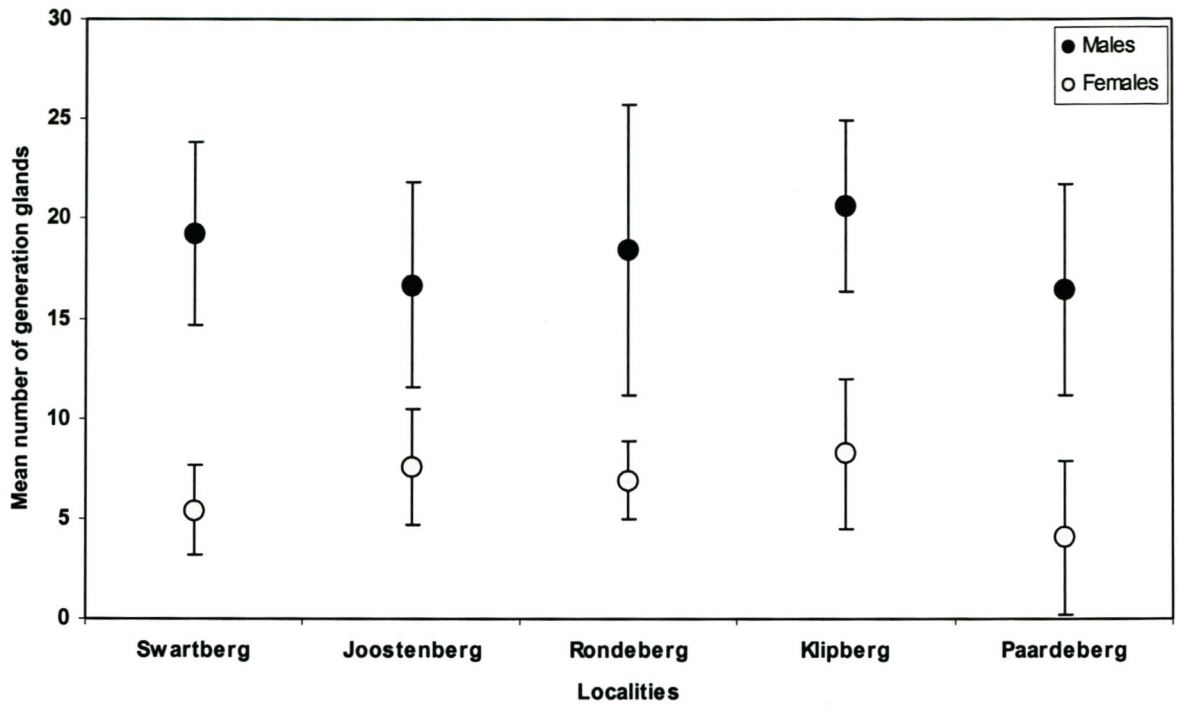| (b)	Locality	1	2	3	4	5
1	Swartberg					
2	Joostenberg	0.523136				
3	Rondeberg	0.996314	0.938451			
4	Paardeberg	0.648432	0.999971	0.939434		
5	Klipberg	0.958134	0.296430	0.911139	0.396755	

Figure 3.9. Interpopulation variation in mean generation gland number for male and female *Cordylus cordylus* from different lowland localities. Errors bars indicate standard deviation from the mean.

from Swartberg. In males, the number of generation glands did not differ significantly among the five localities (ANCOVA: $F = 2.395$, $P = 0.060$) (Table 3.4b, Fig. 3.9).

Cordylus cataphractus also displayed interpopulation variation in the number of generation glands between lowland and highland populations. Both males and females from the Cederberg population had significantly more generation glands than individuals from the Graafwater population (t-test: $P < 0.001$ for both sexes). Cederberg males had almost double and females more than four times the number of generation glands than males and females from Graafwater, respectively (Fig. 3.4a,b).

3.3.3 Intrapopulation variation

In all the *C. cordylus* populations occurring on the lowlands, considerable intrapopulation variation in the number of generation glands was recorded for both sexes (see Table 3.5 for details, Fig. 3.10). In males, generation gland number ranged from low to high, while in females, numbers ranged from zero to low. At Swartberg, Joostenberg and Klipberg, only one or two females from the whole sample lacked generation glands. At Paardeberg, one third of the adult females ($N = 15$) lacked generation glands, in comparison to the other localities, where less than 5% of adult females in the population had no generation glands (Table 3.5). The two females lacking generation glands from Joostenberg and Klipberg had SVL's of 69.63 mm and 66.91 mm, respectively, while all the other females without glands from the other localities all had a SVL greater than 80 mm.

Cordylus cataphractus also displayed extreme intrapopulation variation, with generation glands ranging from none to 16 in females from Graafwater, and none to 75 in females from the Cederberg population. At Graafwater, generation glands were only present in the largest females. In males, the number of generation glands ranged from

Table 3.5. Summary of the intralocality variation and sexual differences in *Cordylus cordylus* populations occurring on the coastal lowlands.

| Locality	*n*	Mean ± SD	Min.	Max	No. of females without glands	Z	P
Swartberg							
Males	21	19.238 ± 4.625	7	28		6.354	< 0.001
Females	44	5.0 ± 2.214	0	12	2		
Joostenberg							
Males	18	16.667 ± 5.134	8	27		5.154	< 0.001
Females	30	7.567 ± 2.873	0	16	1		
Rondeberg							
Males	5	18.429 ± 7.254	7	26		3.051	< 0.005
Females	6	6.923 ± 1.977	4	10	0		
Klipberg							
Males	10	20.6 ± 4.299	16	28		4.525	< 0.001
Females	28	8.25 ± 3.758	0	17	1		
Paardeberg							
Males	9	16.44 ± 5.294	10	24		3.846	< 0.001
Females	15	4.067 ± 3.845	0	11	5		
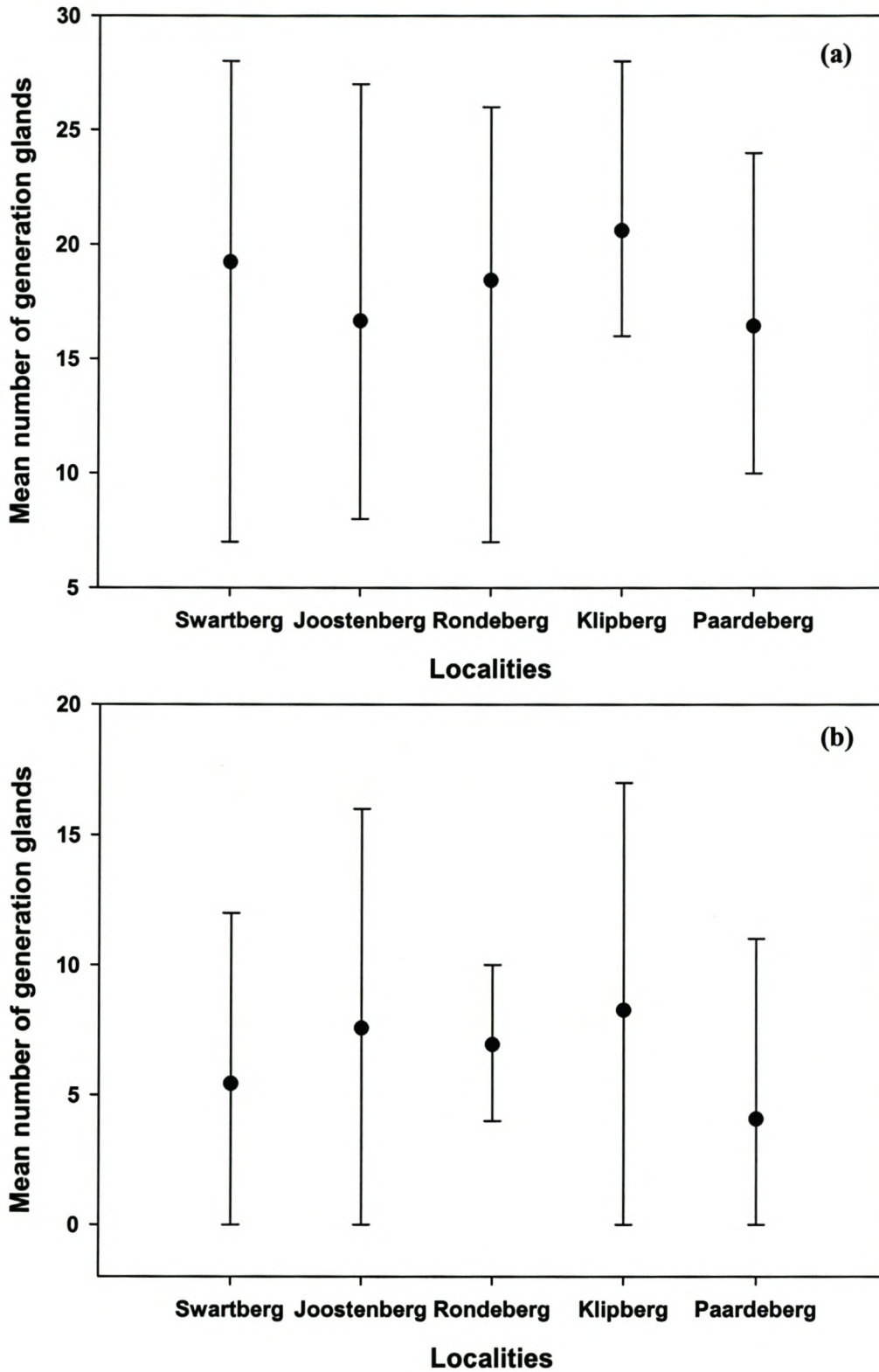
Figure 3.10. Intralocality variation in gland number for (a) male and (b) female *Cordylus cordylus*. Error bars indicate minimum and maximum number (i.e. range) of generation glands for each locality.

none in small individuals to 56 in larger males for the Graafwater population, and none to

109 at the Cederberg population.

3.4 DISCUSSION

3.4.1 Demography of generation glands

The Cape crag lizard, *Cordylus microlepidotus*, and the sungazer, *C. giganteus*, are unique

among cordylids in that generation glands are not restricted to the femoral region. As it is,

there are also unique aspects about the general biology of the two species. *Cordylus*

giganteus is one of few ground-dwelling species in the family and is the only species that

lives in burrows (Van Wyk 1992; Branch 1998). In all other cordylids, the location of

generation glands on the body is such that the marking of substrates is possible. The

femoral, precloacal, and inguinal regions are almost in continuous contact with the

substrate in most species. I have personally observed that lizards drag the precloacal and

femoral regions over the substrate by lifting the hind feet. This behaviour has also been

observed by Wirminghaus (1990) for *C. cordylus*, Griffith (unpublished data) for *C.*

melanotus, and Effenberger (pers. comm.) for *C. cataphractus*. The dorsal generation

glands in *C. microlepidotus* are also located where they can be used for marking the insides

of crevices. In contrast, the forearm region of *C. giganteus* does not seem to be the ideal

position for generation glands for marking substrates. Cordylids are all heliothermic

baskers and during basking the front part of the body is usually raised, either towards the

sun or away from the sun. Cordylids are also sit-and-wait foragers and for better vision, the

front part of the body also needs to be raised when not basking. Why then do we find

generation glands on the front legs of *C. giganteus*? Could it be that these generation

glands are not used for marking substrates, but rather as a direct chemical signal on the

body itself? In such a case, the generation gland secretions would function solely for

intraspecific communication and not for navigational purposes by the bearer. One can reason that soil is an unsuitable substrate for depositing generation gland secretions on and that this is the reason why these glands are now also located on the front legs, but male *C. giganteus* still has well-developed femoral glands, as well as generation glands in the femoral region. How do these glands function then? *Cordylus giganteus* is a sit-and-wait forager and living in flat grassland habitats where visibility to conspecifics is poor, chemical communication may be extremely important in locating mates and this may be the reason for the evolution of additional generation glands in an exposed area of the body.

Unlike most other cordylids, *C. microlepidotus* occurs in very low densities (3 lizards/ha and lower) (Gagiano 2003; Cunningham, pers. comm.; Mouton, pers. comm.). Gagiano (2003) concluded that, in their search for mates in the low density populations in which they occur, both males and females of this species are probably forced to scout large areas. This does not appear to be done from a permanent home site, but the lizards rather seem to adopt a nomadic lifestyle during this period. One can speculate that the increased numbers of glands in this species is somehow related to locating mates in low density populations. The high number of glands may be essential for amplifying the chemical signal which will allow individuals to locate mates in low density populations or for males that already secured a mate, to deter other suitors at the female's home site. More detailed information on long-term movement patterns is, however, required to come to firmer conclusions.

3.4.2 Occurrence and number of generation glands

It was previously thought that males of *Cordylus langi* and the three grass lizard species, *C. anguinus*, *C. macrolepis* and *C. aeneus*, lack generation glands, but we now know that these glands are present in males of *C. langi* (Bates, pers. comm.) and in at least two of the

grass lizard species (Mouton, pers. comm.). A proper investigation of *C. macrolepis* will probably show that males of this species also possess generation glands. While generation glands are probably present in all male cordylids, females display considerable variation in the presence of these glands. Three broad epidermal gland profiles can be identified for cordylids:

i) femoral glands and generation glands present in both males and females (M+ +/F+ +).

ii) Femoral and generation glands present in males, but only femoral glands present in females (M+ +/F+ -).

iii) Femoral and generation glands present in males, but absent in females (M+ +/F- -).

Within each category, further subdivisions are possible, based on the demography of generation glands, intraspecific variation in the presence and number of generation glands, and time of differentiation of these glands. With the exception of the *warreni* species group, all species in the last category, i.e. where females lack both femoral and generation glands, occur in the western arid regions of southern Africa. Although the Namibian species were not included, the phylogenetic tree of Frost *et al.* (2001) indicates that at least some of the species in this category are not closely related (e.g., *C. polyzonus, C. peersi, C. mclachlani*, and *C. lawrenci*) and that this condition evolved more than once in the family.

In Chapter 2, it was shown that the presence of generation glands in female *C. cordylus* is closely linked to climate. This also seems to be the case for other cordylid species. Melanistic cordylid species usually occur in cool areas, i.e. at high elevations in mountainous areas (*C. nebulosus, C. oelofseni*) or along the west coast of South Africa (*C. niger, C. peersi*), where the climate is strongly influenced by the cold Benguela Current (Brown & Jarman 1978). It is thus no surprise that females of these melanistic species generally lack generation glands (Mouton & Van Wyk 1990; Cordes *et al.* 1995; Costandius, unpubl. data; Mouton, unpubl. data). In other species occurring in

mountainous areas, generation glands are also absent in females (*C. melanotus*), or they have significantly fewer glands than females of other species where these glands are present (*C. microlepidotus*). Energy allocated to reproduction depends on the daily and annual energy budget (Congdon *et al.* 1982; Anderson & Karasov 1988; Dunham *et al.* 1989; Porter 1989; Grant & Porter 1992). Reproduction in females may be more energy demanding than reproduction in males, and this may even be more costly in extreme climates. It is, however, interesting to note that males of melanistic species and species occurring in mountainous areas (*C. melanotus*, *C. nebulosus*, *C. niger*, *C. peersi*, *C. capensis*, *C. cataphractus* from the Cederberg, *C. microlepidotus*) had, in general, more generation glands than males occurring elsewhere (Table 3.2). *Cordylus langi*, which is closely related to *C. melanotus* and *C. microlepidotus* (Frost *et al.* 2001), and only occurs at exceptionally high altitudes (2000 – 3000 m.a.s.l.), also have a high generation gland count (maximum number of generation glands recorded is 50; Bates, pers. comm.).

Direct comparison of the number of generation glands present in individual species is complicated by the fact that the maximum number of glands that could be present is in a way related to scale size. All other things being equal, the maximum number of glands that a species with large scales (and thus less scales) in the femoral region can have, will be less than the number that a species with small scales can have. It is probably really the total glandular area that should be compared among species and not the number of glandular scales per se. *Cordylus polyzonus* and *C. capensis*, two of five species that show high generation gland counts, have small body scales when compared to most other *Cordylus* species (FitzSimons 1943, Loveridge 1944) and the high count must therefore be interpreted with care. The other species with a high count, *C. cataphractus*, has normal-sized scales. This species is unique among cordylids in that it is a group-living species.

Obviously, one cannot simply link the high count to group-living, but there may be a connection as will be explained later on.

Cordylus macropholis and *C. tasmani*, two of the ground-dwelling species for which data are available, have low generation gland counts in both males and females. Could the low number of generation glands in these species perhaps be a result of the terrestrial habitats they occur in? Because of extreme limb reduction in grass lizards, males can at most only possess one or two generation glands. The low number of generation glands in grass lizards has therefore nothing do to with their non-rock-dwelling lifestyle. Males of two other ground-dwelling species, *C. giganteus* and *C. tropidosternum*, have gland counts in the same range as for other cordylid males. This, and the fact that a number of rock-dwelling species, such as the *C. minor* species group (Mouton & Van Wyk 1994), also have very low gland counts (even lower than *C. macropholis*), indicate that a low number of generation glands is not restricted to species occurring in terrestrial habitats.

The fact that all cordylid species are sexually dimorphic as far as generation gland number is concerned, is a clear indication that the possession of generation glands is a secondary sexual character and more important to males than to females. In two species, *C. cataphractus* and *C. macropholis*, the degree of dimorphism is much smaller than in other cordylids. As pointed out earlier, *C. cataphractus* is a group-living species and *C. macropholis* a ground-dwelling one. The low dimorphism may be an indication that generation glands assumed a function in these species that is equally important to females than to males and which is probably not territorial marking. *Cordylus macropholis* forms aggregations of up to 14 individuals in the succulent plant, *Euphorbia caput-medusae*, and although these aggregations normally include only one adult male, they may include several adult females (Mouton *et al*. 2000; Nieuwoudt *et al*. 2003). *Cordylus cataphractus*

likewise occurs in groups, normally consisting of one adult male and one or more adult females. Large groups may, however, include several adult males (Mouton *et al.* 1999).

3.4.3 Age (size) of generation gland differentiation

Results of this study show that in cordylids, generation glands typically start to differentiate at or after the onset of sexual maturity in females and before or at the onset of sexual maturity in males. In *C. macropholis, C. tasmani, and C. tropidosternum*, generation glands are, however, already present in neonates (Mouton *et al.* 1998). All three are ground-dwelling species (Branch 1998) and it would appear that the presence of generation glands in neonates is in some way related to the ground-dwelling lifestyle of these lizards.

Differences between males and females in the time of differentiation of generation glands suggest that the differentiation of these glands revolves around a difference in energy allocation between the sexes. After initial differentiation, the number of generation glands in females of all species appears to remain more or less constant with increasing body size. This suggests that females first invest energy in growth, until they reach sexual maturity when they also invest some energy in generation glands. After this, they invest most of their energy in reproduction. Males, not having the burden of giving birth, can already afford to invest energy in generation glands at an earlier age than females. The slow increase in generation gland number with increasing body size after initial differentiation suggests that males may invest most of their energy in growth during this period. When they reach their asymptotic body size they again invest energy in generation glands, resulting in a sudden increase in gland number.

Generation glands have been suggested to function in chemical communication, specifically territorial marking (Cooper *et al.* 1996), but it is possible that generation

glands only function as a scent enhancer for the femoral glands. The absence of or a low number of generation glands in younger cordylid males may mean that they produce a weaker scent as opposed to a larger male with a greater number of glands. This may prove to be advantageous for smaller individuals trying to avoid conflict with larger lizards and who would not be able to compete successfully against adult individuals.

3.4.4 Inter- and intrapopulation variation

Cordylus cordylus does not only display geographic variation in the presence/absence of generation glands (Chapter 2), but also in the number of generation glands among different populations where these glands are present in females. The considerable interpopulation variation in gland number in females within such a small geographic area as surveyed in this study, indicates that generation glands are a very plastic character in females and underscores the hypothesis that the number of glands in females is closely regulated by climate.

Both adult male and female *C. cordylus* display intrapopulation variation in the number of generation glands. It is virtually impossible to evaluate intrapopulation variation in gland number in males. The issue at stake here is variation in the final number of glands that individual males will develop. Because of the sudden increase in gland number when asymptotic body size is reached, variation in the largest size class will always be considerable. This size class will include males in various stages of gland differentiation. It will only be possible to evaluate intrapopulation variation in gland number in males of species where the maximum number of generation glands develops before asymptotic body size is reached. In females, generation gland number is not correlated with SVL and this sex display high intrapopulation variation. The absence of generation glands in some females may be explained by their still relatively small body sizes, but these glands can

also be absent in even very large females. The significance of high intrapopulation variation in large, adult females is unclear at this stage, but may relate to differences in microclimate experienced by individuals. This ties in with the earlier suggestion that generation glands in females are closely regulated by climate, perhaps even on a microscale. The absence of generation glands in some females, suggest that these glands do not play such an important role in this females as in males.

In many other *Cordylus* species, females have less generation glands at high altitudes than at low altitudes (Chapter 2). Geographic variation in gland number in *C. cataphractus* does not seem to follow the same pattern, as both sexes from the Cederberg Mountains have more generation glands than individuals from the lowland population at Graafwater. This may be an indication that factors other than climate may have an influence on gland number in *C. cataphractus*.

According to Retief (2000), the Cederberg locality is less densely populated than the Graafwater locality and, although only speculative at this stage, it is possible that population density may influence generation gland number in *C. cataphractus*. Generation glands in this species may perhaps function as a method to help individuals find their way back to their home crevices when going on foraging excursions. Like in the case of *C. microlepidotus*, it would make sense in low-density populations for *C. cataphractus* to invest in more generation glands, and thus a well-developed chemical communication system. In a high density population visual communication would be a more appropriate option and also energetically much cheaper. Whether there is any correlation between population density and number of generation glands is an aspect that certainly needs further investigation.

3.5 CONCLUSION

The fact that generation glands are present in all male cordylids and that all species display sexual dimorphism in the presence/absence of these glands or in gland number, indicates that the possession of generation glands is a secondary sexual character and that these glands have a more important function in males than in females. Low sexual dimorphism in the number of generation glands in at least two species, suggests that these glands may have an equally important function in males and females in at least some species. In males of most species, generation glands dramatically increase in number when asymptotic body size is reached and it is hypothesized that it may be disadvantageous for small males to already have many glands, as they will not be able to defend their territories against larger males. There are indications that gland number may be related to population density, but this requires further investigation. There is no indication that reversals from a rock-dwelling lifestyle to a ground-dwelling one resulted in a change in general gland profile as was predicted. The presence and number of generation glands in females is strongly correlated with climate, and this sex displays considerable variation in epidermal gland profile. In at least one species, however, other factors such as population density seem to override the climatic effect.

CHAPTER 4

AGGRESSION IN *CORDYLUS CORDYLUS* FEMALES WITH AND WITHOUT GENERATION GLANDS

4.1 INTRODUCTION

Lizards are normally considered to be visually orientated animals (Simon 1983) and territorial defence is generally associated with aggressive behaviours towards intruders, which may include displaying, fighting and chasing (Carpenter 1967). Lizards, however, also possess a wide array of epidermal glandular structures that seem to be involved in inter- and intraspecific communication (Quay 1972; Simon 1983). Although very little is actually known about the functional significance of these glands (Alberts 1992, 1993), many authors assume that they are mainly associated with communicating reproductive status and demarcation of territory (Jared *et al*. 1999).

The Cape girdled lizard, *Cordylus cordylus*, is a rockdwelling species with a wide distribution in the Western and Eastern Cape of South Africa (Branch 1998). Territoriality in this species is associated with site defence (Ruddock 2000), and both sexes are reported to be highly territorial and aggressive (Burrage 1974; Wirminghaus 1990; Ruddock 2000). Territories are maintained even outside the breeding season (Ruddock 2000), with each sex being more aggressive towards consexuals than to the opposite sex (Burrage 1974; Cooper *et al*. 1996).

Both sexes of *C. cordylus* have femoral glands and generation glands. Being located on the ventral surface of the thigh, generation glands might play an important role in chemical communication, specifically territorial marking. While adult males always possess generation glands, the presence/absence of these glands in females is strongly correlated with climate; generation glands are absent in females from cool areas and present in

females from warmer areas (Chapter 2). Burrage (1974), Wirminghaus (1990) and Ruddock (2000), in their studies of aggression in *C. cordylus*, did not take this variation in the presence/absence of generation glands in females into account and their observations were only made on lizards from populations where all females possessed generation glands.

The variation in the presence of generation glands in females has been ascribed to energy constraints (Chapter 2). In general, the amount of activity time a lizard has available each day, may seriously affect its energy budget (Niewiarowski 2001), which in turn will have an effect on the amount of energy that can be allocated towards reproduction (Congdon *et al.* 1982; Anderson & Karasov 1988; Dunham *et al.* 1989; Porter 1989; Grant & Porter 1992). Aggression and active generation glands are both considered secondary sexual characters (Maderson & Chui 1981; Cooper & Guillette 1991), and by investing available energy in reproduction, female *C. cordylus* may not be able to afford to invest too much energy in these characters as well.

The aim of this study was to test for differences in the level of aggressiveness in female *C. cordylus* with and those without generation glands. I predicted that, because of energy constraints, females without generation glands would show lower levels of aggression than females with generation glands.

4.2 MATERIALS AND METHODS

4.2.1 Study animals

Adult female lizards were collected from two localities along the west coast of South Africa. Females without generation glands were from the farm Kliprug, 2 km north of Saldanha, and females with generation glands from Swartberg on the farm Klipvlei, inland from Langebaan. Lizards were caught by hand or with a short noose and transported to the

University of Stellenbosch. At the completion of trials, all lizards were returned to the site of capture.

4.2.2 Experiment 1

Housing - Ten females with (Swartberg) and 10 without generation glands (Kliprug) were housed in glass terraria (400 x 600 x 300 mm) divided in half, using a removable divider. Each terrarium therefore housed two female lizards: one with, and one without, generation glands. The divider visually isolated the lizards from each other. The sides of the terraria were blackened to islolate them from other lizards. Each compartment had a shelter consisting of two pieces of hardboard and two wooden spacers. Water and food were available *ad libitum*. Room temperature was kept at 32°C under a 10L:14D photoperiod. Lizards were allowed to settle in their new environment for a period of 10-14 days before trials commenced.

Testing - Trials were conducted during June 2003, thus outside the mating season. Ruddock (2000) found *C. cordylus* to remain territorial, even outside the mating season, while Searby (2002) reported that the generation glands remain active throughout the year. To test for possible differences in the aggressive behaviour of females with and females without generation glands, I staged encounters between pairs of females (glands present vs glands absent). Prior to testing, the food and water bowls, as well as the partitioning were removed. The behaviour of both lizards at each trial was then observed for a period of 30 minutes. To observe behaviours unobtrusively, interactions were observed on a monitor in an adjacent room and recorded on video tape for reanalysis.

4.2.3 Experiment 2

Housing - Twenty females with and 20 without generation glands (from the same two localities as above) were kept under exactly the same conditions as described for Experiment 1.

Testing - Trials were again conducted outside the mating season, between July and August 2003. In this experiment, I staged encounters between pairs of females from the same locality. This resulted in 10 pairs of females with generation glands from Swartberg and 10 pairs of females without generation glands from Kliprug. The procedures followed, were exactly the same as in Experiment 1.

4.2.4 Data analysis

Stereotyped behaviours are already known for *C. cordylus* (Wirminghaus 1990; Ruddock 2000) and aggression scores were allocated for each lizard according to behaviours displayed during the staged encounter (Table 4.1). All behavioural act data were converted to frequency of behavioural acts per unit time. The degree of aggressiveness of each was assessed by using the behavioural frequencies to calculate an index of aggressiveness, that takes into account both the level of aggressiveness reached by a lizard, as well as the intensity of each behavioural act (Ruddock 2000). An aggression index was calculated for each lizard:

$$IA = \sum f (AS)$$

where   IA = Index of aggressiveness

f = Frequency of behavioural act

AS = Aggression score for behavioural act

To test for differences in aggression between females with and without generation glands, I compared the aggression indices of females with generation glands to those of

Table 4.1 List of agonistic behaviours displayed by *Cordylus cordylus* during staged encounters (compiled and adapted from Wirminghaus 1990 and Ruddock 2000).

Behaviour	Description	Score
Tongue-flicking	Investigative behaviour where tongue is extruded, touches the object of interest and is then retracted	1
Passive threat	Lizard humps lower back or inflates body and raises head.	1
Head-rubbing	Inflates body and rubs side of head against substrate. Follows contorted display or separately.	1
Lateral tail-wag	Lateral wagging of the tip of the tail	1
Push-ups	Bobbing movement of the head and body from fully or semi-elevated position.	2
Contorted threat	Body is fully puffed and swings tail and head as far as possible to one side. Performed after another adult was seen.	2
Shuffle-walk	Lizard walks in jerky, shuffling manner with inflated body held flat along substratum.	3
Arched body-tilt	Lizard arches tail and tilts entire back and top of head towards other individual.	3
Face-off	Two lizards observe each other before and during an aggressive encounter.	3
Circling	Two lizards circle each other with small, jerky movements, dragging their bellies. Both individuals tilt their bodies and heads towards each other.	4
Biting	One or both lizards bite its opponent. End-result of a number of the above displays.	4
Chasing	Lizard runs towards intruder and gives chase. Usually after aggressive display or biting when one lizard attempts to run away.	4

females without generation glands from Experiment 1. I did the same for the females from Experiment 2.

To test if females display different levels of aggression towards females from the same locality and females from a different locality, I compared the data of the following groups: 1) females with generation glands (Exp. 1) and females with generation glands (Exp. 2) (different localities); 2) females with generation glands (Exp. 2) and females with generation glands (Exp. 2) (same locality); 3) females without generation glands (Exp. 1) and females without generation glands (Exp. 2) (different localities); and 4) females without generation glands (Exp. 2) and females without generation glands (Exp. 2) (same locality).

Data were tested for normality and homogeneity using Kolmogorov-Smirnov and Levene's tests, respectively. Where data sets were not normally distributed, Mann-Whitney U-tests were used for two-sample comparisons. Probability values (P) smaller than 0.05 were considered significant for all statistical analyses performed.

4.3 RESULTS

In both experiment 1 and 2, females with, as well as those without generation glands, displayed high levels of aggression, with no significant difference between the two experimental groups (Table 4.2).

I also found no significant difference in the aggression levels of females when comparing the data from the first experiment with the data from the second experiment. There was no significant difference in level of aggressiveness between females with glands from different localities and females with glands from the same locality (Table 4.2). Likewise, there was no significant difference in aggression level between females without

Table 4.2 Test statistics for the different experimental groups.

Experimental Groups	Mean aggression levels± SD	Z	P
Females with glands (Exp. 1) vs	51.818 ± 70.173		
		0.121	0.904
females without glands (Exp. 1)	45.200 ± 67.315		
Females with glands (Exp. 2) vs	63.393 ± 80.220		
		0.925	0.355
females without glands (Exp. 2)	54.544 ± 91.135		
Same localities			
Females with glands (Exp. 2) vs	57.367 ± 79.680		
		0.117	0.907
females with glands (Exp. 2)	69.419 ± 84.592		
Females without glands (Exp. 2) vs	46.784 ± 86.606		
		-0.128	0.898
females without glands (Exp. 2)	63.304 ± 99.496		
Different localities			
Females with glands (Exp. 1) vs	51.818 ± 70.173		
		-0.413	0.680
females with glands (Exp. 2)	63.393 ± 80.220		
Females without glands (Exp. 1) vs	45.200 ± 67.315		
		0.292	0.770
females without glands (Exp. 2)	54.544 ± 91.135		

glands from different localities and females without glands from the same locality (Table 4.2).

4.4 DISCUSSION

In this study, I have demonstrated that there is no difference in aggression levels displayed by females with and those without generation glands. In fact, both groups displayed high levels of aggression. It thus seems that the presence/absence of generation glands is not directly correlated with aggression (territoriality) in *C. cordylus* females.

In their study on *Sceloporus jarrovi*, Ruby & Baird (1994) observed intraspecific variation in aggression in females occurring at different altitudes, with females from high elevations being less territorial than females from low elevations. They suggested that investing too much time and energy in higher levels of aggression might not be advantageous for the survival and reproductive success of females of *Sceloporus jarrovi* occurring at higher altitudes. Perhaps, by not investing any energy in generation glands, female *C. cordylus* from cool areas may be able to maintain high levels of territoriality.

In other lizard species, femoral glands have been implicated in territorial marking (Alberts 1989, 1992; López *et al.* 1998). Cooper *et al.* (1996) found that *C. cordylus* males showed a greater rate of tongue-flicking towards tiles labelled by males than by females, and the authors suggested that the reason for this may be that femoral glands function in territorial marking. They did not, however, discriminate between femoral and generation glands in their experiments, and tiles were marked by secretions of both these glands. The absence of variation in aggression in *C. cordylus* females suggests that femoral glands may indeed be the main glands involved in territorial marking in this species. Generation glands may then perhaps only serve as a method to enhance the scent of the femoral glands and may play a secondary role in the marking of territories.

Cordylus cordylus can discriminate between the sexes (Cooper *et al*. 1996), as well as self-produced pheromones and pheromones produced by consexuals (Cooper *et al*. 1999). All female *C. cordylus* have femoral glands (Cordes *et al*. 1995) and, while females from cool areas may not have generation glands (Chapter 2), they may still mark their territories by means of their femoral glands. Demarcation of territories by glandular secretions is not necessarily linked to aggressive defence of territories, and females without generation glands may thus still display aggression towards intruders.

While it is possible that generation glands might affect the persistence of femoral glands, the function of generation glands remains unknown. Further behavioural studies on the response of lizards to gland secretions are needed to clarify the specific function of femoral and generation glands. Aggression studies on cordylid species where femoral glands are absent in females, such as *Cordylus polyzonus*, will be useful to determine if there is perhaps any correlation between territoriality and the presence of femoral glands.

CHAPTER 5

MALE RESPONSE TO FEMALE GENERATION GLAND SECRETIONS IN THE CAPE GIRDLED LIZARD, *CORDYLUS CORDYLUS*

5.1 INTRODUCTION

Chemical communication plays an important role in the daily interactions of lizards (Simon 1983; Halpern 1992; Mason 1992; Cooper 1994). By tongue-flicking, they obtain chemical information from the environment (Cooper 1994) for locating prey, detecting predators, identifying conspecifics, discriminating sex, identifying eggs, aggregating, marking territories and related spacing behaviour, and for exploratory behaviour (see review by Simon 1983). A range of epidermal/cloacal glandular structures seem to aid in this inter- and intraspecific communication in lizards (Quay 1972; Simon 1983).

In the family Cordylidae, epidermal glands of the femoral region are present in males of all species, but females show variation in the presence/absence of these glands (FitzSimons 1943; Loveridge 1944; Van Wyk & Mouton 1992; Mouton *et al.* 1998). Laboratory and field studies indicated that cordylid lizards detect gland secretions and use it for sex discrimination and discrimination between self-produced and conspecific chemical cues (Cooper *et al.* 1996; Cooper *et al.* 1999; Ruddock 2000). Studies on the function of epidermal glands in cordylids had focused mainly on femoral glands or glands in general, i.e. they did not discriminate between the various glands associated with chemical communication. While femoral glands have been suggested to play a role in intraspecific communication (Ruddock 2000), no behavioural studies have been attempted to determine the function of generation gland secretions, although it has been suggested that they may function in association with the femoral glands in territorial marking (Cooper *et al.* 1996; Mouton *et al.* 1998).

The Cape girdled lizard, *Cordylus cordylus*, has a wide distribution in the Western Cape (Branch 1998). It is a highly territorial species, with both sexes displaying territorial behaviour (Burrage 1974; Wirminghaus 1990; Ruddock 2000). Femoral glands are found in both sexes, and while generation glands are present in all males, females show geographic variation in the presence and number of these glands (Chapter 2, 3). Because they are situated in the femoral area of the body, generation glands are well placed to function in territorial marking. In order to shed some light on the possible function of generation glands, I tested male response to female gland secretions by presenting male *C. cordylus* with different female gland stimuli.

5.2 MATERIALS AND METHODS

5.2.1 Subjects and Maintenance

Twelve adult male and 12 adult female *C. cordylus* were collected from Swartberg on the farm Kliprug, southeast of Langebaan, South Africa. Lizards were caught by hand or with a short noose and transported to the University of Stellenbosch. Males were distinguished from the females by their larger heads and the presence of hemipenal bulges. Prior to experiments, male lizards were maintained in four groups of three each in glass terraria (400mm x 600mm x 300 mm). Each compartment had three shelters consisting of two ceramic tiles and wooden spacers. Room temperature was kept at 32°C under a 12L:12D photoperiod. Food and water were available *ad libitum*.

5.2.2 Testing

Trials were conducted at the end of November 2003. Previous studies have shown that *C. cordylus* can differentiate between the sexes, as well as between self-produced pheromones

and those of another conspecific, based on chemical stimuli (Cooper *et al.* 1996; Cooper *et al.* 1999). In this experiment, I tested the ability of male *C. cordylus* to detect and respond differentially to female gland secretions from glands occurring in the femoral region. Each male was presented with four conditions: female femoral gland stimuli (FGS), female generation gland stimuli (GGS), female femoral and generation gland stimuli combined (FGGS), and water as a control (H_2O). These conditions were tested in a randomised block design with each male only tested once for each condition.

A glass terrarium was divided in half and used as a test arena. This allowed me to observe two lizards at the same time. Each test arena contained a newspaper substrate and a ceramic tile placed in the middle of the arena. Female chemical stimuli were obtained by gently rubbing fine sandpaper (grade: P800) over the glands of a female immediately prior to testing. Each female was used as a donor twice (each set of glands were rubbed only once). The piece of sandpaper, marked with female gland secretion, was then placed in the centre of the tile.

Trials were conducted between 1000 hours and 1600 hours; this allowed the lizards to reach their preferred body temperature before trials commenced. To start a trial, the experimenter slowly placed a lizard in each test arena and covered the lizard completely with her hand, gently pressing it down against the substrate. This kept the lizards from immediately fleeing to the far end of the arena after being released (Cooper *et al.* 1999). Lizards were observed from an adjacent room for a period of 30 minutes. I counted all tongue-flicks, as well as tongue-flicks directed or touching the piece of sandpaper.

5.2.3 Data analysis

Data were examined for homogeneity of variance using Hartley's test. Analysis of variance

for a single-factor experiment for a randomised block (repeated measures) design was performed. Individual comparisons were done by means of Newman-Keuls tests.

5.3 RESULTS

In nearly all of the trials, lizards started exploring the test arena after about five minutes, and in all but one trial, lizards tongue-flicked their surroundings. No significant difference was, however, observed in the total number of tongue-flicks between the four experimental groups ($\chi^2 = 1.297$, $P = 0.730$). Although lizards explored the test arena, few tongue-flicks were actually directed toward the piece of sandpaper labelled with female secretions (Table 5.1), and no significant difference was found in the number of tongue-flicks directed at female gland stimuli between the four experimental groups ($\chi^2 = 2.314$, $P = 0.510$) (Table 5.2). Most of the males, however, walked over the female-labelled piece of sand paper at least once during a trial.

In 11 trials, I observed males to perform a behaviour described by Nieuwoudt *et al.* (2003) as a leg wave (Table 5.1). This behaviour has not been reported for *C. cordylus* before. In 12 trials, I also observed males to perform a head-rub (Table 5.1). In the H_2O-trials, leg-waving and head-rubbing was done as a behaviour on its own. In all three of the other trials, these two behaviours were either performed as a behaviour on its own, or both behaviours were displayed during the same trial.

5.4 DISCUSSION

Ruddock (2000) has demonstrated that both sexes of *Cordylus cordylus* maintain their territories even outside the breeding season, with each sex being more aggressive towards

Table 5.1. Summary of male response to different female gland secretions: total number of tongue-flicks, number of tongue-flicks directed at female gland stimuli, total number of leg-waves and head-rubs displayed trials. (H_2O = water; FGS = femoral gland secretions; GGS = generation gland secretions; FGGS = femoral and generation gland secretions combined)

Experimental group	Mean number of tongue-flicks during trials $\pm$ SD	Mean number of tongue-flicks directed at female gland stimuli $\pm$ SD	Number of leg-waves displayed	Number of head-rubs displayed
H_2O	15.17 ± 9.77	0.08 ± 0.29	2	1
FGS	16.25 ± 9.30	0.25 ± 0.62	2	4
GGS	27.17 ± 34.97	0.33 ± 0.65	5	4
FGGS	19.25 ± 17.82	0.08 ± 0.29	2	3

Table 5.2. Summary of the Newman-Keuls tests, indicating no significant difference in number of tongue-flicks directed at female gland stimuli between the four experimental groups.

Experimental group	1	2	3	4
1 H$_2$O				
2 Femoral gland secretion	0.689171			
3 Generation gland secretion	0.605992	0.681772		
4 Femoral + generation gland secretion	1.000000	0.413447	0.437106	

members of the same sex than to the opposite sex (Cooper *et al.* 1996). Searby (2002), on the other hand, has found generation glands to remain active all year round and it is therefore very likely that they are involved in chemical communication, and specifically territorial marking.

The general absence of tongue-flicking by males of sandpaper labelled by female gland secretions, may perhaps be the result of several reasons: 1) there really are no effects; 2) the experiment was conducted outside the mating season; 3) the presence of two males may have caused interference with responses; 4) the texture of the sandpaper might have inhibited responses; 5) males might have spent too much time near the wall while exploring the test arena to pay much attention to paper near the middle of the arena; or 6) a flaw in the experimental design. On the other hand, the results of this study give support to the findings of Cooper *et al.* (1996), that individuals showed a lower rate of tongue-flicking to conspecifics of the opposite sex. A repeat of this experiment with male *C. cordylus* as donors, would perhaps give a clearer indication of the possible function of generation glands in this species.

The head-rubbing and leg-waving behaviour displayed by *C. cordylus* males in the presence of female gland stimuli, is still unclear at present. Nieuwoudt *et al.* (2003) described leg-waving in *Cordylus macropholis*, as a submissive behaviour displayed by juveniles in response to aggression from an adult. Also in other species, this response is thought to stop aggressive behaviour of the more dominant lizard (Carpenter & Ferguson 1977). Head-rubbing, on the other hand, is an agonistic behaviour (Wirminghaus 1990; Ruddock 2000). The display of leg-waving behaviour in association with an agonistic behaviour, suggest that in *C. cordylus*, leg-waving may be an aggressive display.

The general absence of tongue-flicking of sandpaper labelled by female gland stimuli, but the display of agonistic and submissive(?) behaviour in the presence of female gland stimuli,

may be an indication that cordylids do not exclusively rely on tongue-flicking for detection of conspecifics. The function of generation glands thus still remains unresolved and further studies are needed to clarify the real function of these glands.

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