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CONSERVATION OF *CIRCELLIUM BACCHUS FABRICIUS* (COLEOPTERA: SCARABAEIDAE): GENETIC POPULATION STRUCTURE AND ECOLOGICAL PREFERENCES

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Conservation of *Circellium bacchus* Fabricius (Coleoptera: Scarabaeidae): genetic population structure and ecological preferences.

by

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ABSTRACT

Mitochondrial DNA (mtDNA) analyses was used to determine population structure of a rare dung beetle, *Circellium bacchus* Fabricius (Coleoptera: Scarabaeidae). Polymerase Chain Reaction (PCR) amplified fragments were analysed for Restriction Fragment Length Polymorphisms (RFLPs). A specific gene section, cytochrome oxidase subunit I and II (COI\COII), was targeted for the analyses. Sixty-two beetles were analysed from nine different localities throughout their present distribution in the south and eastern Cape Province. The RFLP analyses revealed 28 haplotypes. The majority of the haplotypes were unique, private and closely related; two haplotypes from adjacent populations were shared. The most significant finding of this study is the separation of *C. bacchus* maternal lineages into two distinct clades (separated by 4.74 % sequence divergence). The eastern clade consists of a single population at Addo Elephant National Park and the second, larger western clade is formed by the remaining populations in the western area of this species' distribution. The most striking barrier to gene flow between the two clades is the Afromontane forest at Knysna presumed to have separated the clades during the late Pleistocene with the expansion of the

westerly air currents over the southern tip of Africa. Ensuing expansion and retraction of the forest habitat during cyclical glacial and interglacial periods is proposed to have lead to fynbos fragmentation and the subsequent shallower phylogenetic structure within the western clade as no conspicuous zoogeographic barriers are currently present between the different populations.

In conjunction with the molecular analyses ecological factors were also considered mainly to establish *C. bacchus'* habitat specificity. It was found that *C. bacchus* density was highest in natural, undisturbed vegetation in both Addo Elephant National Park and Buffalo Valley Game Farm. Furthermore, dung preference for feeding and breeding purposes was determined by providing dung of buffalo, elephant, rhinoceros and cattle under controlled conditions. As there was no consistent pattern of preference for a specific dung type for feeding it was concluded that *C. bacchus* is a generalist in terms of feeding. However, the dung preference study for breeding clearly showed that *C. bacchus* preferred the moist and pliable dung textures of cattle and buffalo dung. A survey of the species that co-occur with *C. bacchus* was also conducted at both sampling localities (Addo Elephant National Park and Buffalo Valley Game Farm) and showed that there were no other dung beetle species that can apparently effectively compete with *C. bacchus.*

The most important consideration in the conservation of *C. bacchus* is the distinctness of the two clades. These two clades should be regarded as separate Management Units (MUs) and translocations between the areas in which they occur should not be considered an option to increase viable population sizes outside of the areas represented by the two clades. Addo Elephant National Park and the De Hoop Nature Reserve are the only official nature conservation areas in which extant populations of *C. bacchus* are presently found. It is therefore essential that the co-operation of the local farm owners be obtained in conservation actions to ensure the survival of this enigmatic dung beetle.

dedicated to Enslin, pa Chris, ma Lois, Louise, Chris jnr.

and all other creatures with more than four legs.....

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CHAPTER ONE

GENERAL INTRODUCTION

The history of human settlement seems to be a history of ecosystem fragmentation with the primary cause being the exploitation and destruction of natural habitats for urban and agricultural development. The inevitable consequence is the disruption of ecological processes and fragmentation of once continuous habitats (Tilman *et al.* 1994). These fragmented and sometimes isolated habitats often bring about a loss of species that usually occur in large patches of uninterrupted habitat (Samways & Moore 1991; Tilman *et al.* 1994) and can lead to changes in the genetic composition and demography of the remnant populations (Klein 1989). Moreover, the loss of certain species may result in the invasion of alien species giving rise to an altered habitat and low animal species richness. Tilman *et al.* (1994) went so far as to say that "fragmentation is the most serious threat to biological diversity, and it is the primary cause of the present extinction crisis".

Small, fragmented populations are affected not only by genetic factors but also environmental and demographic processes (Menges 1992). Individuals in these small populations can experience a decrease in reproduction for non-genetic reasons due to a threshold that is so low in numbers that the population cannot recover (Lande 1988). This is called the Allee effect (Andrewartha & Birch 1954) and may be caused, among others, by density dependent mating success in a species with limited dispersal capability such as the flightless dung beetle *Circellium bacchus*. Fragmentation can further bring about the deterioration of habitat quality near an ecological boundary making the edges unsuitable for the species' survival and therefore difficult, if not impossible, to cross from one fragmented habitat to the next. This inevitably leads to the isolation of populations such as is the case in the extant *C. bacchus* populations.

Irrespective of the current heterozygosity levels in these small fragmented populations, extinction can be brought about by stochastic demographic fluctuations. However, apart from these demographic risks, small populations also face genetic risks among which inbreeding depression and loss in genetic variation through drift are the main features. Rapid inbreeding in small populations increases homozygosity of deleterious mutants that are usually kept rare by selection in large, natural populations (Wright 1977), and could lead to an increased

susceptibility of organisms to infections (O'Brien 1994). The loss of genetic variation within populations may lead to a loss of evolutionary adaptability to environmental changes (Lande 1988) which can be detrimental to a species' survival in times of change.

Traditionally, conservation efforts have focused on the preservation of large vertebrates and their habitats (Hafernik 1992). In contrast, invertebrate conservation has received little attention in spite of their ecological importance in ecosystem stabilisation, energy and nutrient transfer, maintenance of trophic structures and plant pollination to name few (Levin 1983; Kellert 1993). This may partly be due to the view that invertebrates are not regarded as "important" in conservation efforts due to their diversity and shear numerical abundance (representing more than 90% of all animal species; Erwin 1983). Some people seem to support a general misconception by believing that the disappearance of any one invertebrate species would be of less ecological importance than the disappearance of one of the larger mammal species (Jones 1987). In an attempt to redress this the International Union for the Conservation of Nature and Natural Recourses (IUCN) publishes Red Data Books on invertebrates (Wells *et al.* 1983) which provided the first compilation of threats at a global level to a variety of invertebrates. Since the first of these were published more attention has been focused on invertebrate conservation due their usefulness in commercial applications as well as their ecological and economic importance and possible use as indicators of environmental quality (Bishop & Cook 1981; but see Simberloff 1998).

Taxonomy of *Circellium bacchus*

Circellium bacchus is an ideal example of an invertebrate in need of protection. It is an endemic, flightless, ball rolling (telecoprid) dung beetle. As with the majority of dung beetles, *C. bacchus* belongs to the family Scarabaeidae, subfamily Scarabaeinae. It is one of the largest dung beetles in Africa as well as globally with their size ranging in length from 22-47mm.

Circellium bacchus is a monotypic species with no obvious close relatives (Scholtz & Coles 1991). It was previously treated as falling within the subtribe Scarabaeina by Jansens (1938) and Ferreira (1968). However, Cambefort (1978) transferred *C. bacchus* to the subtribe Canthonina and it is still treated as such by contemporary taxonomists (Scholtz & Howden 1987; Scholtz & Coles 1991). Cambefort (1978) based his classification on various morphological features and this has subsequently been supported by an ecophysiological

studydone by Chown *et al.* (1995) which confirmed that *C. bacchus* is physiologically more similar to various Canthonines than to any of the Scarabaeines.

Past and present distribution of *Circellium bacchus*

Based on IUCN criteria *C. bacchus* might be considered "vulnerable" due to decreasing population numbers (see chapter three), possibly as a result of over-exploitation of their habitat due to extensive transformation and destruction as well as other environmental disturbances. Yet over the years *C. bacchus* has become an entomological as well as a conservation enigma with a body of anecdotal evidence accumulated in various scientific papers, as well as in the popular media on the species' possible range contraction, low reproductive rate as well as probable dependence on elephant and rhinoceros dung for survival. It has been suggested that *C. bacchus* is a habitat specialist with their once wide distribution presently restricted to the eastern and southern Cape. A vegetation map of the southern and eastern Cape Province with the extant *C. bacchus* populations superimposed is shown in Figure 1.1. Habitat fragmentation together with a concomitant reduction in their numbers has led to the legislative protection of *C. bacchus* (The Cape Province Nature and Environmental Conservation Ordinance no 24/1992). Their possible extensive historic distribution is reflected in major natural history museum collections held in Paris, London and South Africa which contain specimens that were collected as far afield as the Transvaal (Blyde Rivier), Namibia (Kombat, Windhoek), Zimbabwe (Victoria Falls; see Coles 1993).

Chown *et al* (1995) hypothesised that the past penetration of *C. bacchus* into savannah areas was facilitated by the species' use of constant, large supplies of black rhinoceros dung that is excreted in middens. Moreover, they hypothesised that the physiological changes which accompanied wing reduction in this species may be considered pre-adaptations to the arid conditions of the eastern Cape thereby ensuring the survival of *C. bacchus* in an environment that is otherwise unfavourable for most other large telecoprids. These authors suggest that *C. bacchus* has suffered range contraction as a consequence of habitat destruction and the disappearance of large mammals over the last century. The artificially high number of this species currently found in Addo Elephant National Park (personal observation) may be a direct trade-off due to the conservation management which focused on the Addo Elephant population. The extreme K-selected nature of *C. bacchus* due to their flightless nature and their slow reproductive turnover and longevity compared to other invertebrates as well as their

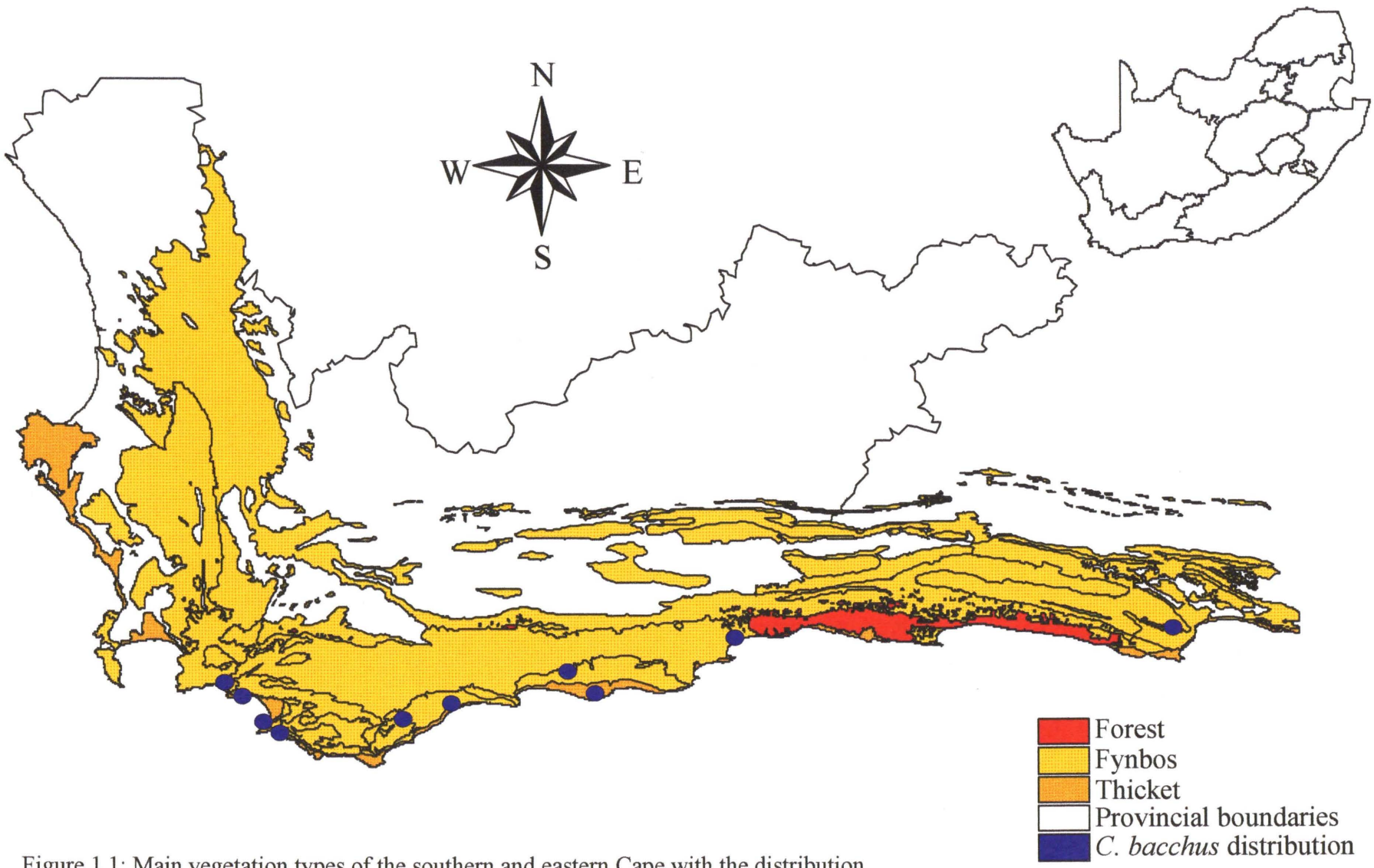


Figure 1.1: Main vegetation types of the southern and eastern Cape with the distribution of *Circellium bacchus* superimposed.

habitat specificity makes this one of the most unique endemic invertebrate species in South Africa.

Due to its uniqueness *C. bacchus* has received considerable attention from scientists and the general public alike leading to the proposal that it be treated as a flagship species. A Flagship Species has traditionally been regarded as a "charismatic, large vertebrate, used as an icon to anchor conservation campaigning due to public interest as well as sympathy" (Simberloff 1998). For the specific purpose of this project the word vertebrate inevitably needs to be replaced by invertebrate when defining a flagship species. *Circellium bacchus* can be used for the purpose of broader conservation objectives focusing on the ecosystem (function and structure) in which they occur rather then on the individual species itself (Simberloff 1998) but nonetheless using the individual species to obtain the necessary focus on the particular ecosystem.

Conservation of genetic diversity is an important component of biodiversity conservation (Wilson 1984) and with the development of molecular methods the entire biological world has been opened up for genetic scrutiny including ecological as well as evolutionary time scales of genetic differentiation. Due to genetic scrutiny of the populations under study it is possible to determine populations within populations (termed metapopulations) that have differentiated to such an extent that separate management decisions are warranted (see chapter two) and the possible implications of translocations involving these populations need to be considered. Translocations have often been regarded as tools to re-establish populations of species in their historical range and to maximise genetic variability in populations perceived to be suffering the effects of inbreeding depression (Avise 1994 but see also O'Brien & Mayr 1991). Isolated populations that are geographically distant from one another may be subjected to a diverse range of environmental conditions and may therefore be expected to adapt to their local environment. Hybridisation between specimens of such populations resulting from haphazard translocations can lead to a breakdown in the locally-adapted gene complexes as well as an irretrievable loss of the rich historical genetic records of populations (O'Brien & Mayr 1991). Translocation attempts to re-establish populations and to bolster local population sizes of *C. bacchus* on farms surrounding Addo Elephant National Park have been undertaken by SA National Parks (San Parks) employees. These practices were, however, terminated at the start

of this project with the hope that the genetic analysis may assist in the development of future policy on the conservation of *C. bacchus*.

Current concepts in conservation planning

In an attempt to help facilitate the recovery of a rare/endangered species, surveys of remaining populations are necessary in order to identify potential conservation entities (Vogler *et al* 1993). The basic entities for conservation management are populations but the question now arising is which populations should be conserved? The simple answer would seem to be "populations whose members exhibit diagnosable characters" (Vogler in press). In evaluating these diagnosable characters two important considerations need to be taken into account 1) "the uniqueness of a population"; priority is given to populations that are distinct in their DNA and 2) the "potential of a population to maintain evolutionary processes for the protection of future maximum biodiversity" (Erwin 1991).

In search of these minimal units for conservation, various operational terms have been proposed to define unique groups of organisms that should be managed separately. It has been proposed that the conservation status of populations should be based on the biological species concept (O'Brien & Mayer 1991) or on phylogenetic parameters (Avise 1992). A definition which is based on pattern rather than the extent of divergence and variation is favoured as information on ecology, behaviour, biogeography and morphology can be integrated in the delimitation of separate units for conservation purposes. The concept of Evolutionary Significant Units (ESUs) was developed as a supplemental approach to describe evolutionary distinct groups when other means of classification such as subspecies, were either inadequate or too controversial to reflect these distinctions (Ryder 1986; Legge *et al.* 1996). Evolutionary significant units focus attention on genetic (evolutionary) subdivisions within populations and can therefore provide valuable insight for conservation biology. One approach in defining ESUs is to consider the geographic distribution of haplotypes. This suggests a qualitative criterion in that ESUs should show complete monophyly of mtDNA haplotypes (Moritz 1994). However the reliable identification of ESUs is a difficult task as it ideally requires concordance between different parameters such as natural history, morphometrics, range and distribution data, protein electrophoresis, as well as analyses of mitochondrial and nuclear DNA (Ryder 1986).

In the absence of a completely concordant data set for the distinction of ESUs the use of Management Units (MUs) have been proposed (Moritz 1994). These are entities for population monitoring and demographic study, the logic being that populations that exchange so few migrants as to be genetically distinct will also be demographically independent. Therefore, current population structure, allele frequencies and short term management issues are considered when addressing MUs that are connected by such low levels of gene flow that they are functionally independent although not permitted ESU-status. It is possible that a single ESU can include several MUs, but an MU does not imply that the specific unit is sufficiently distinct to be regarded as an ESU (Avise 1994).

The determination of geographic variation in endangered species or those of special management concerns such as *C. bacchus* is important, since the existence of geographically defined types can influence management decisions and conservation planning (Robinson & Harley 1995). The understanding of population genetic structure is essential for sound species-management decisions especially since uninformed conservation decisions can harm the populations these exact actions were meant to protect.

AIMS AND OBJECTIVES

Given the controversy and doubt surrounding the past and present distribution of *C. bacchus*, the concerns about its rarity (according to IUCN categories) and the lack of knowledge regarding its ecology, this study was undertaken to address a number of issues which are central to the conservation of this seemingly endangered species. In this investigation specific emphasis was placed on the species' current distribution, its genetic population characteristics, habitat and dung preferences, and its ability to compete with other dung beetles in the utilisation of herbivorous dung. Molecular techniques were employed to determine genetic population structure in conjunction with ecological work done to investigate ecological preferences that could possibly ensure the survival of *C. bacchus.*

The aims were:

i.) to characterise mitochondrial DNA variability and partitioning among geographic populations of *C. bacchus*

ii.) to identify possible distinct populations that are to be considered separately in short and long term conservation planning and decision making involving *C. bacchus.*

iii.) to determine habitat specificity of *C. bacchus*

iv.) to determine dung preference for feeding and breeding in *C. bacchus*

v.) to survey the dung beetle communities in the two identified biomes where *C. bacchus* is presently found.

CHAPTER TWO

GENETIC POPULATION STRUCTURE

INTRODUCTION

Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) is a frequently used technique in population genetic studies (for example see Georgiades *et al.* 1994; Pope *et al.* 1996; Cronin *et al.* 1996; Lavery *et al.* 1996; Matthee & Robinson 1997). It is a "powerful and cost effective alternative to direct sequencing" especially where large numbers of specimens are screened (Dowling *et al* 1990). There are a number of unique characteristics of mtDNA that have led to its wide application in basic science as well as in more applied fields such as conservation management. The mitochondrion is a small circular molecule, present in high copy number and fairly conserved with respect to overall structure and gene order (Brown 1985; Harrison 1989). The complete mtDNA molecule encodes 13 messenger RNAs, 22 transfer RNAs, two ribosomal RNAs and a control region (Borst & Grivell 1981; Brown 1985). Mitochondrial DNA is maternally inherited (Harrison 1989) and thus not subjected to independent assortment and intermolecular recombination during transmission. In short, it provides a set of "completely linked, multiple genetic markers that record historical events that occur along one genetic lineage" (Afonso *et al* 1990).

Data generated from restriction endonuclease cleavage of mtDNA can potentially be presented in two ways. The first being distance data in which differences among molecules are measured as a single variable. The second state is known as character data in which differences are measured as a series of discrete variables, each with multiple states (Hillis & Moritz 1990). With relevance to this study the character data (site mapping) has two possible character states, e.g. the presence or absence of a specific site. In this project restriction fragment data was mapped and the presence and absence of restriction sites was used in distance and parsimony analyses to resolve evolutionary questions surrounding the conservation of *C. bacchus*.

Mitochondrial DNA analysis

The COI\COII section of the mtDNA was targeted for the RFLP analysis in an attempt to determine relationships within and between *C. bacchus* populations. This region has been frequently used in similar studies on invertebrates (Sperling & Hickey 1994; Simon *et al.*

1994) and it was regarded as an appropriate locus for resolving genetic partitioning in this species.

AIMS AND OBJECTIVES

The aims of the molecular investigation were:

i.) to characterise mitochondrial DNA variability and partitioning among geographic populations of *C. bacchus*

ii.) to identify possible distinct populations that are to be considered separately in short and long term conservation planning and decision making involving *C. bacchus*.

MATERIAL AND METHODS

Sample collection and storage

Material was collected from nine different localities covering the current distribution of *C. bacchus* (Table 2.1). Live specimens were transported to the laboratory and maintained under laboratory conditions until used for DNA extraction. Captive conditions were: relative humidity 50%, temperatures were kept constant at 28°C in climate rooms and animals were housed under 12h night/day cycles. Beetles were fed on cattle dung.

Table 2.1: Collection localities and grid references for the *C. bacchus* samples analysed in this study

Locality	Co-ordinates
Addo Elephant National Park	33° 55'S 25° 36'E
Buffalo Valley Game Farm	34° 02'S 23° 02'E
Riversdal	34° 06'S 21° 16'E
Bredasdorp	34° 32'S 20° 02'E
De Hoop (Potberg)	34° 28'S 20° 27'E
De Hoop Natuur Reservaat	34° 30'S 20° 40'E
Stilbaai (Geelkrans Natuur Reservaat)	34° 22'S 21° 24'E
Stilbaai	34° 21'S 21° 23'E
Jongensfontein	34° 26'S 21° 20'E
Grootbos	34° 05'S 21° 49'E
Hawston (Afdak)	34° 23'S 19° 08'E

Soft tissue extraction and purification

Mitochondrial DNA was extracted from soft tissues that included the fat body, trachea and thoracic muscles. All extractions were done following Rodger & Bendich (1985) with minor modifications since this method consistently resulted in the highest concentration of DNA.

Total genomic DNA was extracted by grinding material (frozen in liquid nitrogen) using a pre-cooled porcelain mortar and pestle. The powdered material was placed in a sterile 1.5 ml centrifuge tube together with 500 ml hexadecyltrimethylammonium bromade (CTAB) buffer, vortexed and subsequently incubated for 45 min at 37°C. After pelleting the samples the

supernatant was removed and placed in a new sterile centrifuge tube. This latter step was repeated until the supernatant was clear. The DNA was isolated using chloroform: isoamylalcohol (24:1) extraction and precipitated by adding chilled 70% ethanol. After the DNA was pelleted it was washed with cold 100 % ethanol, air dried and resuspended in tris-EDTA buffer (1 x TE). These samples were kept at 4°C for short-term usage and at -20°C for long-term storage (Rodger & Bendich 1985).

Polymerase Chain Reaction (PCR)

PCR was done in a laminar flow hood to minimise contamination and negative controls were included to test for contamination of the stock solutions and pipettes. The amplification of the double-stranded DNA was carried out in 50ml reaction volumes in a Hybaid thermal reactor. Each individual reaction comprised 5ml of 25mM $MgCl_2$, 5ml reaction buffer, 2mM dNTPs, 12.5-25 pmol of each primer, approximately 50 nanogram of total genomic DNA and 1 to 2 units of thermostable Taq polymerase. Double distilled water was added to the reaction to obtain a final volume of 50ml and 40ml of mineral oil was added to contain evaporation during PCR.

Cycling parameters were 94°C for 3 min to denature the double stranded DNA, 31 cycles at 93°C (60s), 53°C (60s), 72°C (105s) followed by an extension cycle at 72°C for 300s. PCR amplification was confirmed by electrophoresis in 1.5-2% agarose gels using 10ml of the total reaction volume. The remaining product was stored at 4°C for enzymatic digestions. PCR primers used to amplify the COI/COII genes were C1-J-2195: 5'-TTG-ATT-TTT-TTG-TCA-TCC-AGA-AGT-3' and C2-N-3661: 5'-CCA-CAA-ATT-TCT-GAA-CAT-TGA-CCA 3'; Simon *et al.* 1994).

a)

Figure 2.1: Graphic representation of the 1500bp COI/COII gene section of the mitochondrial DNA amplified for the RFLP analyses of *C. bacchus* populations. Primer positions indicated by squares. The tRNA(L) codes for Leucine tRNA.

Enzymatic manipulation of DNA

Twelve specimens were initially screened with a suite of 4 - 6 base pair (bp) recognition restriction enzymes. From these five were rejected (*Taq*I, *Hha*I *Sal*I, *Sca*I, *Xho*I) as they produced extremely complex fragment patterns making accurate scoring difficult. Fourteen restriction enzymes were used to digest 1500 bp of COI/COII.

Five to 10 ml of the amplified product (depending on the strength of the PCR reaction), was digested with 10-15 units of enzyme following recommendations of the manufacturers (Boerhinger Manheim, Promega and Amersham). Sterile distilled water was added to each sample to a volume of 20ml. The majority of the samples were incubated overnight at 37°C with the exception of *Bcl*I which was incubated at 60°C. Reactions were terminated by placing them on ice. Bromophenol blue loading dye (3ml) which contains 50% glycerol, 0.02 % bromophenol blue and 5% SDS was added to each individual reaction.

Agarose gel electrophoresis

In this study the fragments were separated in 1.5% agarose gels (Promega) with ethidium bromide added to the gels. Submarine gel electrophoresis was conducted using TBE buffer (tris-base, boric acid and EDTA) for 6 - 14h to obtain the appropriate separation of the different fragments. Fragments were visualised by UV trans illumination.

Internal consistency of the results

The sum of the lengths of the different restriction fragments should equal the total length of the amplified mtDNA gene fragment. As no size variation was evident in the mtDNA target used for the purpose of this study, the fragment sizes were scaled so that their sum equalled the overall size of the specific gene segments calculated, on average, to be 1500bp for COI/COII. Individual fragment sizes were determined by comparison to a calibration curve based on a 100 bp ladder (HC4706; Gibco BRL) that was run in conjunction with the other samples. Smaller fragments (< 50bp) were scored on mini PAGE gels.

Composite haplotypes

Each specimen was identified by a character profile (one character for each enzyme used) containing the alphabetical letters assigned to each unique fragment pattern. This resulted in a

composite haplotype which represents a unique maternal lineage (described in this investigation by an upper-case alphabetical letter).

DATA ANALYSES

Analysis of fragment data

The restriction fragment data was analysed using Restsite v1.1 (Nei & Miller 1990). This program allows for the calculation of the number of nucleotide substitutions per site within and between populations, where a large number of specimens are examined and numerous restriction enzymes are used. Standard errors were derived via 200 Bootstrap iterations.

The Jukes-Cantor correction method was employed to compensate for possible underestimated sequence divergence. Jukes-Cantor was chosen rather than the Kimura two-way correction as the latter method has an independent rating for transitions and transversions (Hillis & Moritz 1990). Regardless of the occurrence of a transition or a transversion, the result would inevitably be a loss (or less likely the gain) of a restriction site and the bias toward transitions need not be taken into account.

Genotypic diversity (haplotypic diversity) was calculated using $n(1-(f_i^2)/n-1$ (Nei & Tajima 1981), where f_i is the frequency of the ith mtDNA haplotype in a sample of size N. Genotypic diversity ranges from 0 -1. A value of 0 indicates that all specimens sampled exhibit the same genotype and a value of 1 indicates that all specimens screened are unique (Avise 1989). Genotypic diversity is a robust method for assessing within population diversity as the sample size of the population is taken into account as well as the frequency of each haplotype.

An account of genetic variance within and between *C. bacchus* populations was established by using a molecular analysis of variance (AMOVA, Excoffier *et al.* 1992). AMOVA is based on the frequency of restriction sites. This analysis produces estimates of variance components and F-statistic analogues which are denoted as F-statistics. These values (ranging from 0-1) reflect the correlation of haplotypic diversity at different levels in the population including within populations, between populations and among populations within groups. Each haplotype was treated as an allele at a single locus (see Excoffier *et al.* 1992). Effective female migrants per generation was calculated by substituting the F-st values in the following equation: Nfm = (1/F-st - 1)/2 (where Nfm is the effective female migrants per generation; (Takahata & Palumbi 1985). This value is an indication of the number of females that migrate between adjacent populations and thus account for gene flow between populations and within the species.

Construction of trees based on RFLP data

Distance analyses using UPGMA (Unweighted Pair-Group Method based on Arithmetic averages, Sneath & Sokal 1973) and NJ (Neighbor-Joining; Saitou & Nei 1987) of the RFLP data were used to construct trees. In the UPGMA method genetic relationships are graphically displayed by the joining of the least distant taxa (DeBry 1992). The first grouping is merged as a cluster and an average is calculated for this cluster's distance from all other taxa (Swofford & Olsen 1990). UPGMA assumes equal mutation rates among taxa and Jin & Nei (1991) suggested that the UPGMA method is specifically "robust when sequence divergence is high". Due to the assumption of equal mutation rates that is not always met the NJ method was also used to construct phenograms. In the NJ algorithm a distance matrix is constructed in which the divergence of each pair of nodes is calculated taking into account the average divergence from each other node (Saitou & Nei 1987). The NJ method allows for unequal rates of molecular change between taxa. Gene trees were constructed based on sequence divergence estimates obtained from Restsite (v1.1 of Nei & Miller 1990) and were used to illustrate the genetic relationships between different populations\localities as well as between the composite haplotypes.

Practical limitations of Restriction Analysis

In any RFLP analysis fragment sizes are determined by comparison to a curve of known sizes of a molecular marker and the distance that the individual fragments migrate. There is thus a possibility of error when estimating the size of a fragment and this error is correlated to the molecular weight of the fragment. The larger the fragment, the larger the amount of base pair variance. Independent construction of the fragment size curves and subsequent comparison of different curves can reduce the error variance in fragment size determination. Fragment sizes can also be determined by subtracting accurately scored fragments from the total length also taking into account that there is a possibility of undetected smaller fragments that are ignored. In this investigation ambiguous samples were run on mini PAGE gels allowing for the identification of fragments < 50bp.

Restriction site mapping

Fragment changes, unlike restriction site changes, are not always independent (Avise 1994) as the gain of a restriction site results in two new fragments of which the combined length adds up to the total uncut fragment length. The loss of a restriction site will result in the loss of two

fragments replaced by a single fragment of the same length as the two separate ones in the instance of an existing restriction site. In the case of insertions or deletions, fragments tend to co-vary across digestion profiles but the responsible site changes do not vary. While fragments from closely related specimens which have the same mobility tend to be homologous, the likelihood of convergence (two samples having fragments of the same size but produced by different cleavage sites) increases as sequences become more different (Dowling *et al.* 1990). As independence of characters is an assumption of parsimony, and fragment data clearly violate this assumption, it is preferable that RFLP data are coded as presence and absence of restriction sites rather than fragments. Site mapping resolves many of the "constraints" pertinent to fragment data. The process involves the construction of an accurate map of the sites where restriction endonucleases cleave DNA at specific nucleotide sequences. Site data also enables one to perform more stringent analysis (i.e. parsimony analysis).

In instances of low sequence divergence among mtDNA haplotypes it is possible to infer single restriction site differences among pairs of haplotypes through direct examination of fragment patterns (Dowling *et al.* 1990). In these instances mapping of the exact sites are not necessary. More intricate digestion profiles can be mapped by either double digestions or partial digestions (Avise 1994). Single digestions provide only the number of restriction sites and fragments of a specific enzyme but not their positions relative to other sites, whereas double and partial digestions provide an additional advantage in that the position of a site can be determined relative to the second enzyme that was used in the digestion.

Restriction mapping for this project was done at the University of Cape Town under the supervision of Professor Eric Harley. In most instances the restriction maps of single enzymes could be inferred from single digestions (Avise 1994; Dowling *et al.* 1990), but double digestions were implemented to construct accurate maps *Hinf*I, *Msp*II and *Nde*I. The site position were used in construction of a matrix based on the presence or absence of restriction sites.

A computer programme, RESOLVE (Version 2.0 1990, Harley 1990) which was specifically designed to facilitate restriction site mapping was used. In addition this programme can be used to store, manipulate and edit the final restriction maps that are produced. RESOLVE implements a three-way method of analysis that computes all possible fragment fits of the single and double digests and from these a final map is constructed on the basis of the highest

degree of correlation between the site positions relative to each other. Enzyme sites are calculated by reference to site positions of two other enzymes that are in turn used to verify deduced site positions. Independent construction of maps further helps to increase the accuracy of the sites as the resulting maps of two independent three-way analyses can be compared and evaluated accordingly.

As a complete RFLP data set was compiled for all specimens under study it was possible to identify specimens with identical fragment patterns and this reduced the number of individual reactions that had to be done for site mapping. Furthermore, all digestions were performed on PCR amplified gene sections and thus the range of size variation between largest and smallest fragments varied from 1500bp (the uncut COI/COII section) to the smallest detectable fragments on agarose gel which were 50bp. No length variation was evident from any of the digestions and it was possible to accurately determine the fragment sizes and construct accurate maps from these.

Analysis of site data

Minimum spanning network

A minimum spanning network of the populations under study was assembled (Excoffier & Smouse 1994) by using the program MINSPNET. A distance matrice is constructed by calculating the number of base substitutions between different haplotypes and these different mtDNA types are compared in a pairwise fashion. The number of changes are summed over all the enzymes used and this allows for the different lineages to be connected in a minimum spanning network reflecting the minimum number of mutational steps between linked haplotypes.

Frequency distribution of pairwise genetic distances among individuals

As allelic relationships within a population are affected by demographic history (Slatkin & Hudson 1991; Lavery *et al.* 1996) certain characteristic features are expected under specific conditions. A population that has grown exponentially, or has undergone rapid populations growth, is expected to show a starlike phylogeny and a Poisson frequency distribution of pairwise differences between haplotypes (unimodal). On the other hand, populations that have declined are expected to show strongly structured phylogenies and multimodal frequency distributions of pairwise differences (Lavery *et al.* 1996). The frequency distribution of all

pairwise differences between individuals are computed (PAUP 4*) and compared to a Poisson distribution (Slatkin & Hudson 1991). Agreement of the observed distribution with the expected distribution was evaluated by using Kolmogorov-Smirnof test (Kolmogorov 1941).

Tree construction based on site data

Both the NJ tree and the tree based on maximum parsimony (heuristic search option using default settings) was constructed from the site data. A distance approach was implemented as all variables between individual haplotypes are included (Saitou & Nei 1987) and not only the parsimony informative characters as in parsimony analysis. Parsimony analysis is based on the assumption that attributes shared among taxa (in this instance the presence or absence of a restriction site) are due to inheritance from a common ancestor (Sober 1989). A thousand bootstrap iteration were done for both distance and parsimony analyses (Hillis & Bull 1993), but due to computational constraints only the NJ bootstrapping could be completed.

RESULTS

The most significant finding of this study was undoubtedly the separation of *C. bacchus* mtDNA lineages into two distinct clades. A single populations at Addo Elephant National Park, also the furthest east population, forms the eastern clade. All other specimens collected from the south-western Cape cluster together constituting the larger, western clade (Bredasdorp, De Hoop, Buffelsbaai, Stilbaai, Riversdal, Hawston, Kleinbaai and Grootbos). These results will be reported on in the following section after the restriction digestion work, site mapping, the phylogeny reconstruction that revealed the two clades and AMOVA investigations have been reported on.

Cytochrome oxidase subunit I and II RFLPs

The amplified COI\COII gene section (1500bp) was screened with a suit of 14 restriction enzymes. An invariant pattern was observed in one of the 14 enzymes (*Ava*I, Appendix 1); all other enzymes were polymorphic. Two fragment patterns were observed with *Dra*I, *Cla*I, *Bc*lI, *EcoR*I, *EcoR*V, *Xba*I *and Sty*I and three with *Bg*lII, *Hae*III, *Nde*I and *Rsa*I. Four restriction patterns were obtained with *Hnf*I and six restriction fragment patterns were detected with *Msp*II. Several enzymes were monomorphic within populations (i.e. *Dra*I and *EcoR*I in Addo Elephant National Park). The restriction patterns of the latter enzymes (*Dra*I and *EcoR*I) were the most informative in the delimitation of the two clades.

Circellium bacchus phylogeography

Twenty eight haplotypes were detected in the 62 study specimens. Sixteen haplotypes were represented by single specimens and four haplotypes were found in more than three specimens. All haplotypes, with the exception of V and X were restricted to discrete geographic areas; although these latter haplotypes were shared, they occurred in adjacent populations. The high incidence of unique haplotypes was confirmed by an overall elevated genotypic diversity value (0.96; Table 2.3).

Table 2.2: The 28 maternal lineages detected among 62 *Circellium bacchus* specimens based on RFLP analyses of the mtDNA COI/COII segment. Numbers above the haplotypes correspond to the restriction enzymes used and the lower case letters to the digestion profile obtained. Localities are given and numbers of specimens (N) sharing the same haplotype are also presented.

Clone	Composite Haplotype														Locality	N
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
A	a	c	a	b	b	b	b	c	b	a	c	b	a	a	Addo	2
B	a	c	a	b	b	b	b	c	b	b	c	b	b	a	Addo	1
C	a	c	a	b	b	b	b	c	b	b	c	b	a	b	Addo	1
D	a	a	a	b	a	c	b	a	b	a	c	b	b	a	Addo	1
E	a	a	a	b	a	c	b	c	b	a	c	b	a	a	Addo	5
F	a	c	b	a	a	a	a	c	a	a	a	c	a	a	Bredasdorp	3
G	a	c	b	b	a	a	a	c	a	a	a	c	a	a	Bredasdorp	1
H	a	b	b	a	c	b	a	c	a	a	a	a	a	a	De Hoop	1
I	a	b	b	a	b	b	a	c	a	a	a	b	a	a	De Hoop	1
J	a	a	b	b	c	a	a	b	a	a	a	a	b	a	De Hoop	1
K	a	a	b	a	b	a	a	b	a	a	a	a	a	a	De Hoop	3
L	a	a	b	b	b	a	a	b	a	a	a	a	a	a	De Hoop	1
M	a	a	b	a	c	a	a	b	a	a	a	a	a	a	De Hoop	2
N	a	a	b	b	c	a	a	b	a	a	a		a	a	De Hoop	1
O	a	b	b	b	f	b	a	c	a	a	b	a	a	a	Buffelsbaai	2
P	a	b	b	a	f	b	a	c	a	a	b	a	a	a	Buffelsbaai	1
Q	a	b	b	a	e	a	a	c	a	a	c	a	a	a	Buffelsbaai	2
R	a	d	b	a	f	a	a	c	a	a	a	a	a	a	Buffelsbaai	3
S	a	d	b	a	f	a	a	c	a	a	c	a	a	a	Buffelsbaai	1
T	a	c	b	a	e	a	a	c	a	a	c	a	a	a	Buffelsbaai	1
U	a	b	b	b	b	b	a	c	a	a	a	a	a	a	Hawston	1
V	a	b	b	b	a	a	a	c	a	a	a	a	a	a	Kleinbaai/ Hawston	7
W	a	b	b	b	d	a	a	c	a	a	a	a	a	a	Riversdal	3
X	a	b	b	b	b	a	a	c	a	a	a	a	a	a	Kleinbaai/ Hawston/Grootbos	6
Y	a	a	b	a	b	b	a	c	a	a	b	b	a	a	Stilbaai	1
Z	a	a	b	b	c	b	a	c	a	a	a	a	a	a	Stilbaai	1
Aa	a	a	b	a	a	b	a	c	a	a	a	a	a	a	Grootbos	1
Ab	a	b	b	a	b	a	a	c	a	a	a	a	a	a	Grootbos	8

1 = *Ava*I, 2 = *Hnf*I, 3 = *Dra*I, 4 = *Cla*I, 5 = *Msp*II, 6 = *Nde*I, 7 = *Bcl*I, 8 = *Bgl*II, 9 = *EcoR*I, 10 = *EcoR*V, 11 = *Hae*III, 12 = *Rsa*I, 13 = *Sty*I, 14 = *Xba*I.

Diversity measures

The mean sequence diversity for *C. bacchus* was estimated at 1.15% (±0.53). Sequence diversity (Table 2.3) ranged from 2.20% within the De Hoop population (which had 10 specimens and seven haplotypes) to 0.28% in the Grootbos population (where ten specimens were sampled and two haplotypes detected). Genotypic diversity values were generally high and in some populations equal to one (Riversdal and Stilbaai) or very close to one (Buffelsbaai and De Hoop 0.89 and 0.91 respectively). However, low genotypic diversity values were also detected in Grootbos (0.39) and Hawston (0.47).

Divergence measures

The eastern and western clades are separated by an average sequence divergence of 4.74%. Pairwise estimates of sequence divergence between populations ranged from very low (0.01%) between populations that shared a haplotype (Kleinbaai vs Hawston) to 7.03% between De Hoop and Addo Elephant National Park (Table 2.4). The sequence divergence between haplotypes ranged from a very low 0.13% (haplotypes G and F both from Bredasdorp) to a relatively high value of 14.14% between haplotypes R and D from Addo and Buffelsbaai respectively (Appendix 2).

Table 2.3: Sequence diversity and genotypic diversity based on COI/COII of the nine populations of *Circellium bacchus*.

Population	No Specimens	No Haplotypes	% sequence diversity	SE	genotypic diversity
Addo	10	5	1.40	0.32	0.76
Bredasdorp	4	2	0.72	0.33	0.50
Buffelsbaai	10	6	1.60	0.77	0.89
De Hoop	10	7	2.20	0.63	0.91
Grootbos	10	2	0.28	0.20	0.39
Hawston	10	2	0.62	0.40	0.47
Kleinbaai	3	2	0.56	0.38	0.60
Riversdal	2	2	0.35	0.34	1
Stilbaai	3	3	2.63	1.45	1
COI\COII	62	28			0.96

Table 2.4: Percentage sequence divergence values (corrected by Jukes-Cantor) between the nine *Circellium bacchus* populations sampled based on COI/COII. Values in bold are the three populations that share haplotypes.

	Bred	Bufb	Grtb	Hawst	Klnb	Rivr	Stilb	dHoop
Addo	4.74	5.51	6.15	4.94	4.96	2.74	2.56	7.78
Bred		7.03	1.8	1.31	1.52	2.83	1.18	1.98
Bufb			1.93	2.37	2.26	1.94	2.05	2.66
Grtb				**0.55**	**0.30**	1.50	1.24	2.00
Hawst					**0.01**	0.65	0.99	1.54
Klnb						0.90	1.20	1.83
Rivr							2.27	1.37
Stilb								0.84

Addo = Addo Elephant National Park, Bred = Bredasdorp, Bufb = Buffelsbaai, Grtb = Grootbos, Hawst = Hawston, Klnb = Kleinbaai, Rivr = Riversdal, Stilb = Stilbaai, dHoop= De Hoop.

Restriction site data

High overall similarities between haplotypes permitted inferences of single site changes between haplotypes (Avise *et al.* 1990; McMillan & Bermingham 1996). However, in instances where complex digestion profiles were obtained (*Hnf*I, *Msp*II and *Nde*I) double and partial digestion were implemented to map the respective sites. Appendix 3 lists the presence and absence of restriction sites on which all subsequent analysis were based.

The minimum spanning network

The composite haplotypes (see Table 2.2) are separated by a number of nucleotide changes and this is summarised in a minimum spanning network in Figure 2.2. There were some ties among links in the connection of haplotypes in the minimum spanning network and these were resolved by using coalescent models (Crandall *et al.* 1994). For example, two equally parsimonious solutions existed between haplotyp F ↔ D, F ↔ E. However, it is more likely that F originated from E rather than from D as the latter involved a site gain which is less likely than a site loss under a neutral model of evolution (Crandall *et al.* 1994).

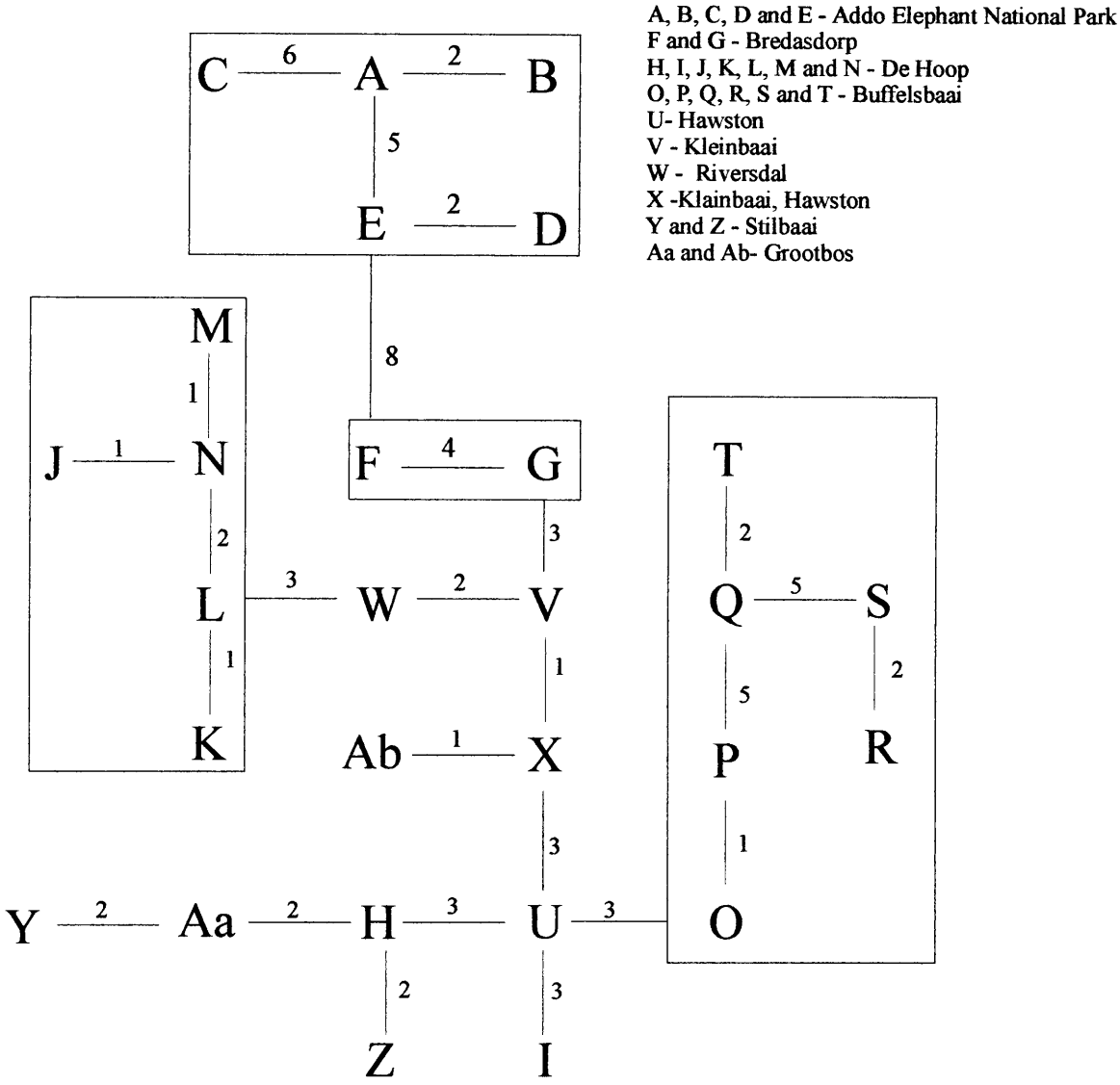


Figure 2.2: A minimum spanning network summarising the minimum number of mutational steps between the 28 mtDNA haplotypes of COI\COII. Capital letters indicate the maternal lineages and the numerals indicate the minimum number of mutational steps between haplotypes. Haplotypes that are grouped within squares are from the same populations.

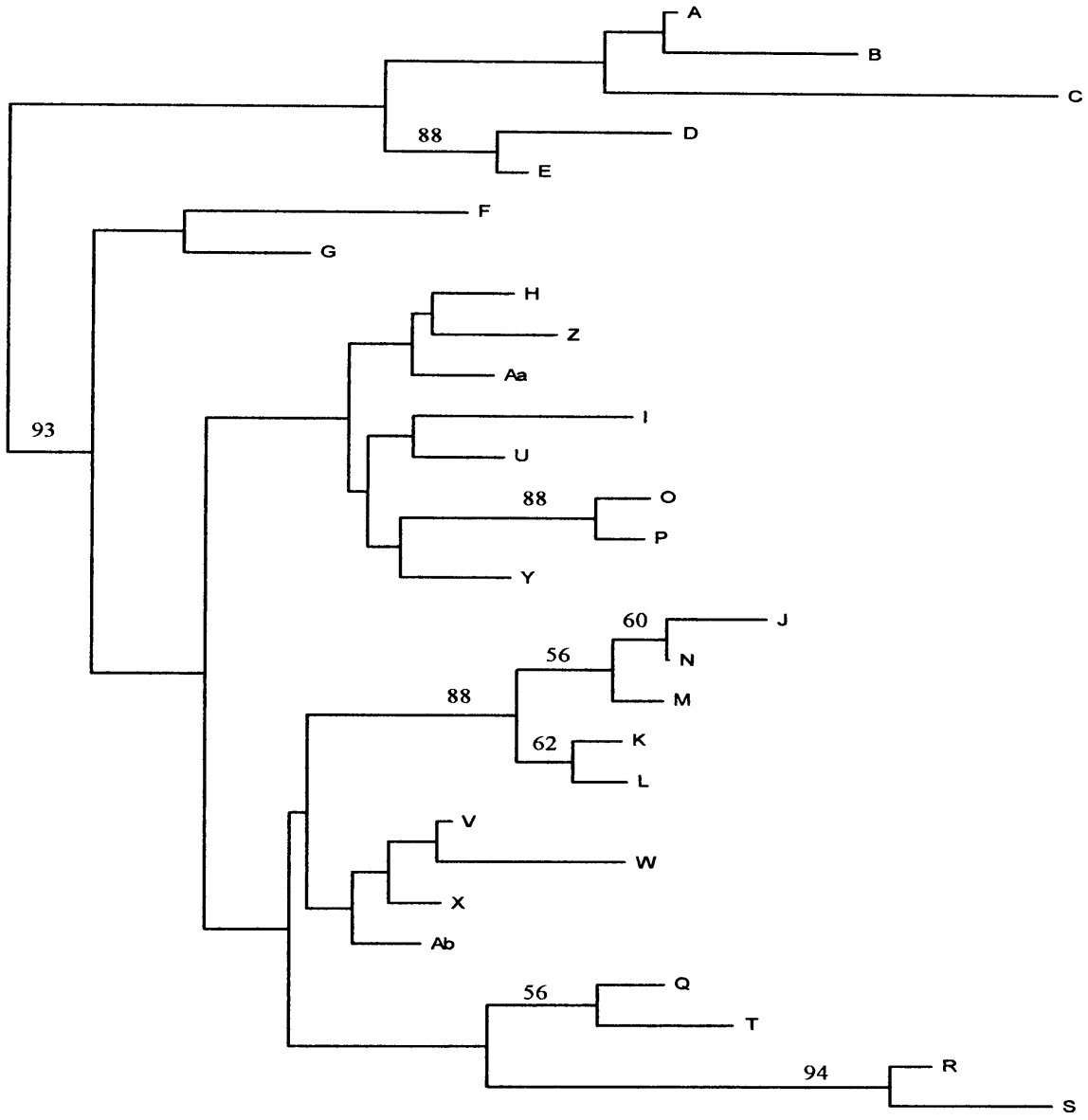


Figure 2.4: An unrooted NJ tree based on the presence and absence of restriction sites between the 28 mtDNA haplotypes detected within *C. bacchus*. Branch lengths are proportional to the number of changes. Only bootstrap support (1000 iterations) above 50% is indicated at the appropriate nodes.

Unrooted parsimony analysis based on presence and absence of restriction sites (Appendix 2), revealed 346 equally parsimonious trees (Ci = 0.38; Ri = 0.75). The 50% majority rule strict consensus tree is shown in Figure 2.5. As expected, given that the majority of the haplotypes

comprising the western clade are separated by low numbers of site changes and few parsimony informative characters (28 taxa and 19 informative characters), relationships within the western clade are poorly resolved. The only differences detected in tree topologies generated by neighbor joining and parsimony involves minor branch swapping within the western clade where the differences between haplotypes were small.

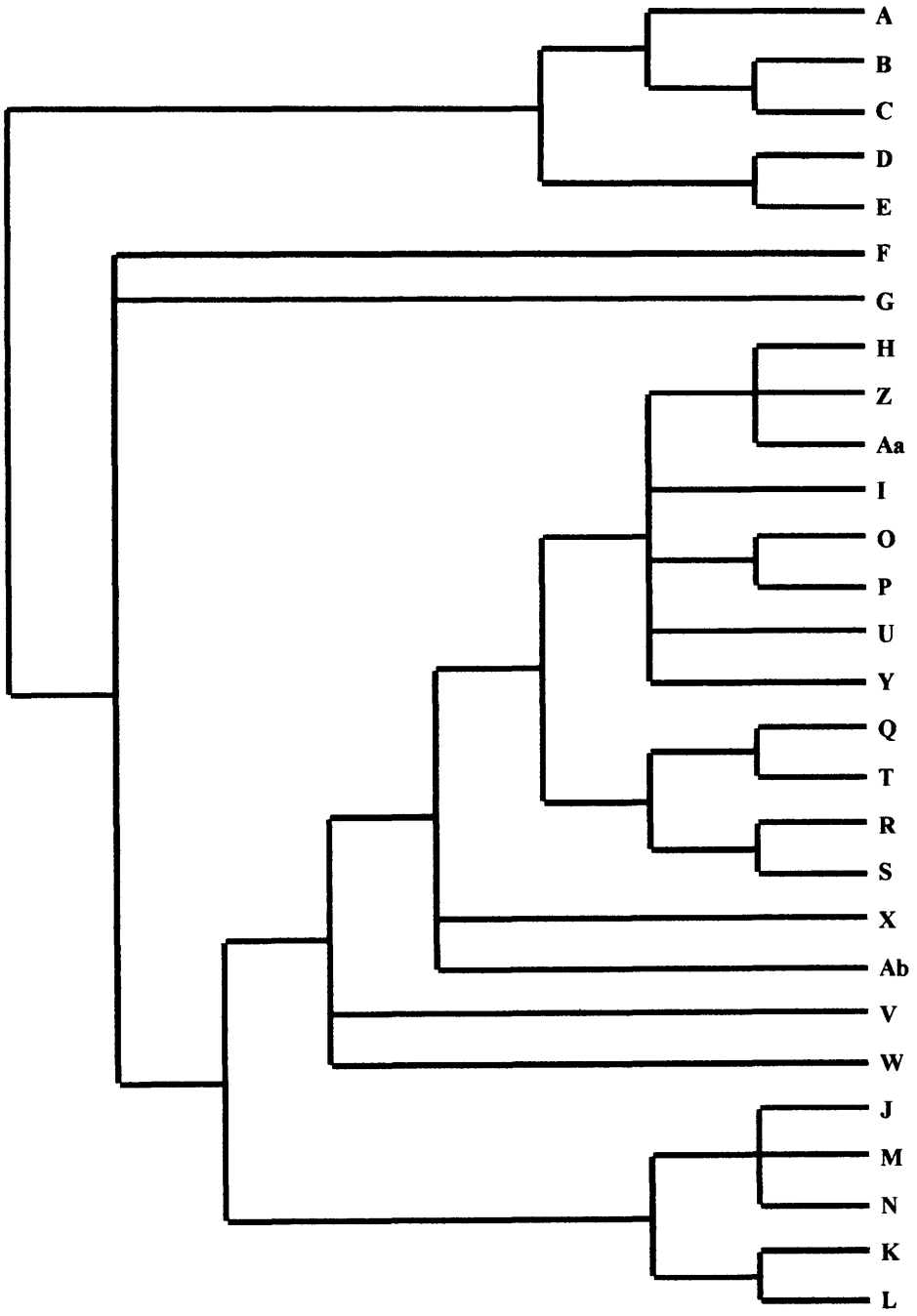


Figure 2.5: Unrooted strict consensus tree based on the presence and absence of site data.

Analysis of Molecular Variance

An Analysis of Molecular Variance confirmed the distinctness of the two clades with the highest proportion of variance calculated between them (49.81%, V_a = 3.01, $p<0.001$, F-st =0.76). However, the presence of substructures among populations in this species was also detected (63.58%, V_a = 2.53, $p<0.001$, F-st = 0.64). In order to determine wether the resulting substructure was due to the distinctness of the Addo Elephant National Park population, the eastern clade was excluded and the AMOVA repeated on data including only the western clade. Although the structure within the western clade was significant a lower F-st value (0.54) was obtained confirming that the eastern clade contributes significantly to the level of substructure detected in this species. Furthermore, the variation among populations in the western clade (54.02%, V_a = 1.60, $p<0.001$, F-st = 0.54) was only slightly higher than the variation within populations (45.98%, V_a = 1.36, $p<0.001$).

Effective female migration (N_{fm}) was the lowest between the two clades (N_{fm} = 0.16) with intermediate levels of female migration (N_{fm} = 0.43) within the western clade. A summary of these results is presented in Table 2.5 below.

Table 2.5: Results of the AMOVA for COI/COII.

	Φ-st	Φ-ct	Nfm	among group variation	among population variation	within population variation
C. bacchus	0.64	0.64	0.29	63.58 %		36.42 %
western vs eastern clade	0.76	0.50	0.16	49.81 %	26.21 %	23.98 %
western clade	0.54		0.43		54.02 %	45.98 %

DISCUSSION

The phylogenetic analysis of mtDA data revealed that *C. bacchus* populations from the south-western Cape coast and the south-eastern Cape coast comprise two genetically distinct groups. Numerous restriction site changes (8) characterise the separation of the two maternal assemblages and the minimum sequence divergence between them was calculated as 4.74%.

The overall pattern and magnitude of the mtDNA differentiation in *C. bacchus* is similar to data reported for a number of species with low dispersal capabilities in which population structure over geographic ranges were investigated (Saunders *et al.* 1986; Prinsloo & Robinson 1992; Avise 1992; Vogler *et al.* 1993). Local population structure has been shown to be partially related to the life history pattern and the dispersal capability of the species under study (Avise 1992). In this study geographic partitioning could in large part be anticipated due to *C. bacchus's* flightless nature as well as the characteristic and fragmented habitat in which they are currently found. However, *C. bacchus'* geographic structuring is particularly interesting because of the many unique, localised, yet closely related maternal lineages that characterises this species. Since there is no extensive sharing of haplotypes (apart from V and X shared between adjacent populations) sufficient time has presumably lapsed since common ancestry allowing fixtation of unique, yet closely related mtDNA lineages.

Provisional estimate for the time of separation between the two clades

Mitochondrial DNA clock calibrations that can be calculated are not absolute. Clock calibration of invertebrate mtDNA have been calculated based on several invertebrate species and for different genes for example: *Drosophila* mtDNA NADH subunit I, 2% per myr (DeSalle *et al.* 1987); Brower (1994) calculated a range of percentages based on several mitochondrial genes in a variety of Lepidopteran taxa ranging from 1.7% to 4.2% per myr and from this data he postulated a general mitochondrial substitution rate of 2.3% per myr. Furthermore, Pruser & Mossakowski (1998) worked with carabid beetles (NADH subunit I) and found substitution rates ranging between 0.98% to 2.3% per myr. These values were calibrated using the formation of land bridges that separated previously continuos species and this enabled the dating of separation events. Only two studies have used COI sequences in the calibration of a molecular clock and the rate was estimated at 1.71% per million years for invertebrates (Caccone *et al.* 1988; Knowlton *et al.* 1993). Since the majority of the genes on

which mtDNA calibration have been estimated are not COI\COII, I have used the two extremes reported in the literature (0.98 and 2.3) in estimating the temporal separation of the two major *C. bacchus* clades. The separation time of the two clades was estimated at 4.8 to 2 million years before present. This is clearly a gross estimate since separation between the two clades took place and must be viewed in that light. Nonetheless, it is helpful when speculating on possible reasons for the divergence of *C. bacchus* into two major mtDNA lineages.

Separation of the eastern and western clades

Different factors may have resulted in contemporary and historical barriers to gene flow between the two clades in *C. bacchus*. Since no shared haplotypes were detected between specimens comprising each clade, it can be assumed that these barriers have not been broached in the recent historical past. However, estimates of effective female migration between them does show that historical contact did indeed take place and this confirms the possibility of a once wider distribution for *C. bacchus*. The most likely barrier to gene flow between the two clades is the Afromontane forest at Knysna as well as numerous rivers that open into the Indian ocean (the largest being the Keurbooms Rivier, Storms River and Groot Rivier). The Afromontane forest could act as a barrier of movement from east to west as *C. bacchus* is ectothermic and thus reliant on high enough ambient temperatures to warrant activity (see Chown *et al.* 1995). As forest temperatures are usually lower, the movement of an ectothermic beetle could have been hindered. Fynbos pollen elements have been recorded at Groenvlei (bordering the extensive Knysna forest; Scott 1994), indicating that fynbos encroachment in the forest did occur, possibly opening up parts of the forest that could permit the movement of *C. bacchus*.

A gradual transition from subtropical woodland to fynbos occurred throughout the late Miocene, Pliocene and Quaternary due to a gradual shift in the prevailing climatic conditions (Scott *et al.* 1995). As forest dwelling herbivores are mostly single compared to herd dwelling grassland herbivores (Thackeray pers.com) the dung resource for *C. bacchus* became an even more ephemeral resource during time periods of retracting grasslands and expanding forests. However, the most drastic incident effecting the current climatic character of southern Africa was the expansion of the westerly air currents over the southern tip of Africa ± 3 million years before present (mybp). This lead to the displacement of former warmer systems and brought with it the current cooler climates. Throughout these transitional phases, cyclic expansion and

contraction of the woodland habitats occurred during fluctuating glacial and interglacial periods. Lawes (1990) proposed that a rise in temperature and change to summer rainfall patterns presented condition favourable for the spread of Afromontane forest and during cooler periods the forest boundary would contract (Thackeray 1987). Thus, somewhere between the warmest climatic conditions (> 3 mybp) and the inception of the cooler climates (< 3 mybp) the Afromontane forest formed an impenetrable barrier for the flightless *C. bacchus* and has remained as such throughout the Pliocene and Pleistocene epochs terminating gene flow between the two clades.

This drastic climatic incident (± 3 mybp) coincides with the rough estimate of the clade separation based on the invertebrate molecular clock. At present the Knysna forest is regarded as a relict of a much wetter climatic period, as the existence of the forest is due to evenly spread orographic mist and rain (1200mm recorded at Diepwalle) that is higher at the protected southern side of the Outeniqua Mountains than in the surrounding areas. In most other areas the temperate forests have largely disappeared. The fynbos has expanded from small patches in edaphically favourable sites to become the more dominant vegetation in the southern Cape (Acocks 1988).

Fynbos fragmentations and subsequent shallow phylogenetic structure in the western clade

In addition to the deep phylogenetic separation between the two clades, there was evidence of "shallower" structure in the western clade. Apart from the three population in close geographic proximity that share three haplotypes, and a second haplotype shared by two of these populations, all other haplotypes are private and probably reflect the fragmentation of the habitat. Most haplotypes are closely related, differing by 2 - 3 mutational steps, with some localised clustering of haplotypes shown by the minimum spanning network (for example the Buffelsbaai and De Hoop populations which also grouped together in the NJ and strict consensus trees). The shared haplotypes between adjacent populations (Grootbos, Hawston and Kleinbaai) could indicate that the geographic isolation between these populations is incomplete and recent exchange has occurred between them. In essence these three populations could thus be treated as a single panmictic population.

Since no widespread haplotypes were detected it seems likely that the once continuos *C. bacchus* population was fragmented into multiple refuges, subsequent expansion and

contraction coincides with cyclical changes in the habitat due to climatic influences. The relatively high levels of mtDNA sequence divergence between haplotypes suggest that the separation was sufficiently protracted to allow the distinctiveness of the majority of the populations. Although the overall genotypic diversity value was high (0.96, Table 2.5), two populations (Riversdal and Stilbaai) were characterised by small sample sizes and did not fit this pattern. Bredasdorp was also found to have a low genotypic diversity value, possibly for the same reason, although two haplotypes were detected in this population.

Frequency distribution

The nature of the frequency distribution of pairwise differences between haplotypes is unimodal and indicates historical exponential population growth. This initial expansion may obscure later expansions and possible retractions in population size for a very long time (Rodgers & Harpending 1992). Therefore, although *C. bacchus* populations are currently proposed to be in decline, this was not reflected in the frequency distribution of the pairwise difference between the haplotypes.

The future conservation of *C. bacchus*

In order to ensure the continued survival of *C. bacchus* it is clear that priority should be given to maintaining and safeguarding the current high levels of variation detected in *C. bacchus.* In considering various criteria for the determination of evolutionary distinct groups (Chapter 1) the application of management units is proposed rather than evolutionary significant units (ESUs) as the conclusions from this study are based on a single mtDNA gene and is not integrated with data from morphological or nuclear markers. Two management units are therefore proposed for *C. bacchus* which are dealt with in more detail in Chapter 4.

CHAPTER THREE

ECOLOGICAL PREFERENCES

INTRODUCTION

Modern agriculture and artificial grazing pastures increase the already problematical "cascading fragmentation effect" (Grant 1994) and the result of this increase is evident in the decrease of natural fauna and flora that surrounds us. Another alarming perspective is the "extinction debt" that we are facing due to extinctions that occur several generations after the initial fragmentation event took place (Tilman *et al.* 1994). They have suggested that even moderate levels of destruction and fragmentation can be the cause of time delayed extinctions. An example of the latter suggestion is the vegetation in the southern Cape which used to be dominated by shrubland in the recent historical past (Davis 1993). The arrival of Europeans several hundred years ago lead to the replacement of large amounts of this indigenous fauna and flora with foreign assemblages. Could it be that the extinction debt caused by these past events are the main determinants of species composition, fragmentation and extinction that presently occurs in this area?

Grant (1994) stated that the numbers of endangered and threatened species were growing rapidly, chiefly as a consequence of human population growth but also because of climatic change. The increasing pressure on our natural resources is forcing not only biologist but also the general public into an acute awareness as to the damage being done and damage already done to our limited natural resources. At this stage it does seem, however, that biologists world-wide are attempting to develop a theoretical framework for conservation of biotic diversity. The first step in this process would be to identify species and sub-specific taxa in need of protection together with the ecological framework essential for that specific taxon's survival. In all conservation efforts it is important to consider the feasibility of maintaining small populations in quasi-natural settings. It has been proposed that conservation of ecosystem function relies on the fact that the loss of rare species may be compensated for if these species are replaced by others that fill the same niche and function. The ideal situation would be to preserve as many undisturbed ecosystems as possible, with focus on ecological processes rather than individual populations and species (Meffe & Carroll 1994). In preserving whole ecosystems it is expected that the balance of nature will (theoretically) then conserve

biodiversity. These processes will maintain the species that play a leading roll in the maintenance of the ecosystem (including structure and function), (Franklin 1994) and would thus bring about a healthy ecosystem. Unfortunately, existing nature reserves are too small for this conservation strategy to continue unhindered, making population and often species level interventions necessary. However, the main aim of nature reserves is still to focus on the protection of areas with high incidences of biodiversity as well as endemicity.

A centre of endemism is defined as "an area delimited by the more-or less coincident distribution of taxa that occur nowhere else" (Cowling *et al.* 1992). The Cape floral kingdom, although only 89000km^2 in extent, has an extremely rich flora of 8550 species of which 73% are endemic (Hall 1987). The high incidence of floral endemism in the Cape Floristic region is paralleled by a 64.4% endemism in the dung beetle fauna (Davis 1993). These endemics are threatened not just by local landscape changes but also by global events (Samways 1994). Except for Addo Elephant National Park that falls within the south-eastern part of the Cape Floristic Region and Buffalo Valley Game Farm being on the division of the east-and-west Cape all other areas covered in this study are included in the high endimism, south-western area of the Cape Floristic Region.

Circellium bacchus, occurring within this Cape Floristic Region, is an ideal example of an endemic species threatened by habitat transformation (Coles 1993). This is possibly due to increased pressure and changes in their natural environment. This dung beetle is part of a larger community of coprophagous beetles that have a high incidence of endemicity in the southern Cape (Davies 1993). Primary reasons offering an explanation for this endemicity include winter rainfall that started during the Pliocene (3 MYbp), (Deacon 1983) and continental transgression and regression due to polar oscillation from 10-2 MYbp. These oscillations lead to calcareous sand deposits on the coasts (Deacon *et al* 1992) with a subsequent contribution to endemism due to an influence on the vegetative cover and associated fauna. These past historical changes that have caused the initial fragmentation of the habitat in which *C. bacchus* occurs are now masked by the more recent fragmentations due to human influence. This has mainly occurred through burning to encourage the growth of edible geophytes and clearance of natural vegetation for agricultural and urban purposes (Geldenhuys 1994). Indigenous shrubland is at present restricted to mountains and coastal areas and it is within the coastal "refuges" that small and isolated populations of *C. bacchus*

are presently found. Their distribution and density have changed dramatically over the last 50 - 100 years, and the few local extant populations probably owe their survival to the reserves and game farms on which they occur. These conservation areas are mainly established in areas unsuitable for agricultural or other purposes.

Palaeoecology of the habitat in which *Circellium bacchus* occurs

There are various underlying differences between the south-eastern and the south-western Cape. These differences are represented by a higher concentration of species in the south-west that decline towards the south-east (Cowling 1992); a higher species-to-genus ratio is found in the south-west and a lower incidence of endemicity is encountered in the south-eastern Cape. These differences can be explained by several contemporary ecological factors such as rainfall, altitude and climatic differences between these two areas but historical factors also need to be considered in an attempt to explain these differences. What follows is a brief overview of the climatic trends that took place in the Cainozoic Era (Cenozoic), starting at the Palaeocene to the Pleistocene and on to the Holocene epoch. Prevailing climatic conditions in southern-Africa have been proposed to have developed in the Pliocene (3MYbp, Deacon 1983). It seems that environmental deterioration started at the end of the Tertiary (65 MY), (Deacon 1983) causing widespread extinctions and differences in the glacial and interglacial periods within these two areas. Glacial periods within the south-east were drier and colder than interglacials (Deacon & Lancaster 1988) causing lower levels of precipitation in the south-east. In the south-west the glacial conditions were wetter due to frontal rains that did not affect the south-east (Cockcroft *et al* 1987).

Radiation of mammals took place in the Cainozoic Era (starting 65MY ago, also referred to as "the age of mammals", Vrba 1990) but it is also an important evolutionary time period due to modernisation of the floras paralleled by changes in the faunas (palynological evidence, Coetzee 1987) as well as significant climatic changes (Deacon *et al.* 1983; Vrba 1985). Warm and humid climates have been suggested for the latitude of the Cape in the early part of the Cainozoic (Lambrechts 1983) with temperatures changing toward cooler, drier, and more seasonal climates in the Palaeocene through to the Pleistocene. This is in sharp contrast to the uniformly mild climates of the preceding Mesozoic (Schackleton & Kennett 1975). A lowering of the sea level of 200m during a global regression in the Oligocene (30 MY) could have lead to an exposure of much of the continental shelf off the Cape and further contributed to this

environmental change (Deacon *et al.* 1983). According to Hendey (1983) the shrubland communities of the fynbos region owe their existence to the climatic changes that began in the late Tertiary (2-3 MY). This included dry seasons during which fires were a regular occurrence. These fires were terminated by heavy falls of rain that caused severe flooding. It thus seems that climate in the fynbos region changed from tropical in the Miocene to temperate in the Pliocene with rainfall initially perennial but distinctly seasonal by the early Pliocene (Hendey 1983). It is further proposed that the dry season was probably in winter and it was only later on in the Pliocene that the present dry summer and wet winter rainfall patterns were experienced in the southern Cape.

At present there are three major cells of air currents that cause the climatic conditions experienced in southern Africa (Tyson 1986). Centered over the Atlantic ocean the rain-bearing westerly currents are found, with rain-bearing easterlies over the Indian ocean and the only dry current centres over the cold Benguela in the Atlantic ocean (Davis 1997). Bimodal (autumn and spring) rainfall peaks in the eastern Cape are brought about by an expansion of the westerlies and the dry current towards the north-east. Midsummer rainfall in the east is caused by a south-westward expansion of the easterlies whilst the dry air contracts southwards bringing about the dry summers in the winter rainfall regions. The differences in rainfall peaks within the two biomes have a marked effect on the vegetation in the two biomes in which *C. bacchus* is currently found. This emphasises the need to further explore reasons for the survival of this species in two seemingly distinct biomes.

The areas under study have not escaped the changes associated and influenced by these climatic fluctuations such as vegetation cover, herbivore composition and inevitably dung beetle assemblages that are dependent on herbivores for their survival. The general fluctuation trends of the sea level in these earlier time periods have continued up until today, albeit on a smaller scale and with a decrease in the intervals between changes. During the Cainozoic period sea level changes occurred with continuing smaller time intervals between the different fluctuations (Deacon *et al.* 1983) such that the most resent fluctuation during the Quaternary has been proposed to be as short as 10 000 years (Frakes 1979). Transgressional changes in sea-level are associated with mild climate, increased rainfall and more luxuriant vegetation whereas in times of regression increased aridity that significantly modifies vegetation patterns and thus also trophic resources are brought about. It has been proposed that warmer and wetter

conditions prevailed 9000 to 6000 years ago (during the Holocene) with reports of higher wood - to - grass ratios than at present (Vrba 1990). The higher wood - to - grass ratio could have resulted in an density of herbivores paralleled by a subsequent density in dung dependent insects and both of these have slowly decreased as the weather conditions once again changed. These changes were unsuitable for large herds of herbivores and their associated dung insects, favouring the forest dwelling herbivore species which mostly occur singly. It thus seems likely that the fragmentation of the habitat in which *C. bacchus* occurs started to take place during earlier periods of sea level fluctuations. *Circellium bacchus* subsequently followed the distribution of the most suitable habitat of the herd dwelling herbivores.

Canthonine distribution

In the tribe Scarabeini there are four recognised subtribes namely Canthonina (the subtribe to which *C. bacchus* belongs), Scarabaeina, Gymnopleurina and Sisyphina (Scholtz & Howden 1987). Many African Canthonine genera are monotypic and relict remains of previous widespread populations (Scholtz & Howden 1987). Their ranges are restricted to temperate regions of southern Africa as well as the eastern highlands of central and south-central Africa (Davis 1993; Scholtz & Howden 1987). Canthonines have long been regarded as a Gondwanaland tribe with one characteristic being high levels of endemism (Davis 1993). They seem to follow the general trend that species richness declines from west to east in the southern Cape (Davis 1997). Cool southern climates, highlands as well as forest areas seem to be favoured habitats of the Canthonines (Davis 1993). It has been proposed that Canthonines are excluded from warmer regions due to competition with old world Sisyphini and Gymnopleurini (Cambefort's "exclusion hypothesis"; Cambefort 1981). Canthonines have presumably entered southern Africa via a palaeantartic route and this has been offered as a possible explanation for the present Canthonine distribution (Halffter 1974; Endrodi-Younga 1987). The current distribution of *C. bacchus* follows the same general trend as most other Canthonines in that it is restricted to the south-western and south-eastern Cape coast with a former wider distribution.

Functional groups and competition in dung beetle communities

Competition is an important determinant of community structure especially in coprophagous dung beetles that compete for an ephemeral resource (Doube *et al* 1988; Hanski & Cambefort 1991). The effect of competition in the structuring of a community will be even more pronounced a) when the utilisers overlap in both space and time and b) the position of the interacting species in the functional group hierarchy (Doube 1991). Dung beetles have different nesting behaviours and ways in which resources are utilised and this has given rise to their division into three behavioural groups. Competition between dung beetles is largely reduced due to these behavioural differences in breeding and feeding behaviour. The telecoprids are ball rollers that remove dung from the pad and provide for their larvae away from the dung source. Paracoprids tunnel beneath the dung and dwellers or endocoprids construct nests inside the dung pats. These behavioural groups have further been divided into functional groups depending on the way in which dung is utilised (Doube 1991, Table 1). Certain functional groups have competitive advantages due to adaptational differences as well as differences in dung utilisation and this has given rise to a competitive hierarchy that exists among these functional groups (Doube 1991). The competitively dominant beetles are in functional group I (FGI, large telecoprids). These dung beetles are mostly large and can rapidly remove dung from the pat. *Circellium bacchus* belongs to FGI even though it is not such an aggressive competitor as the other members of this group for various reasons (see discussion of chapter three). Due to high levels of competition between dung beetles in the same functional groups different colonisation patterns have been found among these competing species. Some prefer dung that is very fresh (1-2 days old) whilst others prefer older dung of up to 7 days or more of age (Doube 1990). The smaller telecoprids in FGII are also strong competitors as they are also able to remove dung rapidly. All Canthonines are telecoprids belonging to either FGI or FGII depending on their size (Doube 1991). Members of FGI weigh more than 400mg and those that weigh less than 400mg belong to FGII. Other strong competitors include FGIII, the fast-burying endocoprids but as their crepuscular\nocturnal activity peaks do not overlap with those of FGI and FGII, which are diurnal, competition between these groups is generally low. Subordinate to these groups are FGIV and FGV (the paracoprids). The latter two groups remain in the pad or bury dung over the course of a few days. Because of a lack of biological information on the smaller species it is difficult to distinguish FGVI (the kleptocoprids or kleptoparasites) from FGV and FGVII. Kleptocoprids use dung buried by other species for

breeding and are thus not a part of the hierarchy that exists between the different functional groups (Doube 1990). For the purpose of this project focus will be placed on the clearly distinguishable functional groups (FGI-V and FGVII) specifically FGI and FGII that contain those species of particular relevance to competing with *C. bacchus*.

There is a general lack of information on dung beetle competition between functional groups although the different ways in which resources are utilised suggests these differences have developed to avoid such competition. A competitive hierarchy has been proposed but that does not exclude the possibility of the less competitive groups having a greater competitive influence due to their sheer magnitude in numbers. The smaller not so aggressively competitive individuals from higher functional groups can thus have a more dramatic competitive influence than the dominant competitors if the smaller individuals occur in high enough numbers and thus remove dung at higher rates than the larger telecoprids. These smaller dung beetles could thus have an equal or far greater impact on the competition experienced by other dung beetles than lesser numbers of the larger competitive telecoprids given that dung is a limited resource and that there is an overlap in activity periods of the respective groups.

Table 3.1: Characteristics of the seven functional groups of dung beetles (Doube 1990).

FG	DESCRIPTION	DIEL ACTIVITY
I	Large telecoprids, >400mg dry weight	Diurnal
II	Small telecoprids, <400mg dry weight	Diurnal
III	Fast burying paracoprids	Crepuscular/nocturnal
IV	Larger slow burying paracoprids, >10mg up to 100mg dry weight	Diurnal or, Crepuscular/nocturnal
V	Smaller, slow burying paracoprids, <10mg dry weight	Diurnal or, Crepuscular/nocturnal
VI	Kleptopcoprids	Unknown
VII	Endocoprids	Diurnal

Habitat preference of *Circellium bacchus*

No published information on *C. bacchus* habitat preference in fynbos is available. On the other hand, work done by Coles (1993) in Addo Elephant National Park suggests that *C. bacchus* is more common in certain vegetation types within Addo Elephant National Park than in others. A preference is shown for the Moist or Dry Spekboomveld and large open and/or highly disturbed areas are avoided. Furthermore, Coles found that dung utilisation was higher in the more natural vegetated areas, compared to disturbed areas, due to a higher incidence of dung beetles in the former. Unfortunately he did not analyse vegetation structure nor plant density to quantify the differences in the preferred habitats. Since these beetles are flightless and their only means of locomotion is by walking, the basal plant composition and structure at ground level are clearly critical for successful dispersal. Consequently it is thus essential to determine the basal plant structure as an indication of the terrain to which these beetles are exposed.

Dung preference

Adult dung beetles feed on the liquid component of dung as it is only the fluid that can pass through their mandibular channels (Halffter & Edmonds 1982; Edwards 1991; Al-Houty & Al-Musalem 1997). They are thus unable to feed on very dry dung. Dung characteristics arise from two dichotomous attributes regarding herbivore digestion and feeding in that an animal is either a grazer versus a browser and a ruminant versus a non-ruminant. These sources of variation result in dung of different texture, moisture content as well as nitrogen and fibre content (Edwards 1991). Dung preference by dung beetles is influenced by these factors inundating to dung texture (Tribe 1976), volume and characteristic odour (Halffter & Edmonds 1982) and moisture content (Halffter & Matthews 1966). Of these moisture content is probably the most important attribute of herbivore dung for coprophagous insects (Edwards 1991). Elephant and rhino dung are generally much coarser and less pliable containing twigs, bark and leaves compared to finer textured buffalo and cattle dung. Cattle and buffalo dung is very compact (Matthiessen & Hayles 1983) and desiccation takes place at a slower rate compared to elephant and rhino dung. The desiccation rate of cattle and buffalo dung is decreased even more due to the hard crust that forms on the outer exposed surface that effectively prevents the evaporation of moisture on the inside. Elephant and rhino dung that is excreted in heaps/middens, though large, has a loose structure that leads to rapid desiccation of the outer surface. The large heaps, however, tend to hold their moisture in the core for a considerable

amount of time and dung beetles are often found feeding on the inside that is moist enough for ingestion (personal observation). Studies done on the effect of seasonal and pastural variation on herbivorous dung, and the ways these different factors influence the coprophagous insects (Edwards 1991; Macqueen *et al.* 1986; Matthiessen & Hayles 1983; Riano 1966; Al-Houty & Al-Musalam 1997), have lead to the following general conclusions: dung that is easy to manipulate and roll (for both the telecoprids and paracoprids) is preferable to semi-liquid forms although relatively dry dung offered to beetles as a single nutritional resource can be utilised. Only a few studies have specifically been done on a single species and their dung preferences but as early as 1961 Landin (in Riano 1966) stated that "dung beetles feed on any dung substratum independently of the kind of dropping". A few species of coprophagous insects are wholly specialised to a particular type of dung but it does seem that in the absence of the preferred food reasonable substitutes will also be accepted.

Preliminary but non-quantitative dung preference trials conducted by Coles (1993) involving *C. bacchus* indicated that in order of preference elephant, buffalo, cattle and rhino dung respectively were preferred for feeding. However, in this experiment (Coles 1993) unequal quantities of the four dung types were used to represent a single defecation of the four respective herbivores. These unequal quantities could have resulted in a more dominant odour from the larger elephant and rhino defecations compared to that of buffalo and cattle which may have influenced the data obtained. Coles found that elephant and rhino dung were most often preferred during the earlier part of the day with high ambient relative humidity, cool temperatures and dung moisture content at its highest. Moreover, Coles (1993) observed that feeding began at temperatures as low as 14 °C and terminated as the temperatures reached 38 °C. He further recorded *C. bacchus* feeding on a variety of other dung types such as that of monkey, human, hare as well as ostrich and he concluded that *C. bacchus* is an opportunist, feeding on any suitable dung. The experiments done by Coles (1993) to determine *C. bacchus* dung preference were repeated by using the same volumes of the four dung types in order to determine whether his results could be verified (materials and methods of this chapter).

In contrast to *C. bacchus'* feeding preference for elephant dung Coles (1993) found that the finer textured, more pliable buffalo and cattle dung seemed to be selected for the construction of brood balls but once again no statistical confidence could be placed on his conclusions and therefore a repeat of this experiment was also done (material and methods of this chapter).

The conservation of *Circellium bacchus*

Habitat specificity (specialisation), geographic range (distribution) and local population size (density) are three categories used in defining levels of rarity (Ferrar 1989). Different combinations of these three criteria result in seven categories of rarity. Coles (1993) found *C. bacchus* to comply with five of these seven categories of rarity, thus emphasising the current status of *C. bacchus* and the need to consider conservation measures for this species. Unfortunately, before the project was undertaken by Coles there was a general lack of scientific information on current and past distribution as well as insufficient information on perceived rarity and conservation of *C. bacchus*. The project undertaken by Coles coincided with a public awareness campaign at Addo Elephant National Park and the eastern Cape to highlight the scarcity and uniqueness of *C. bacchus*. *Circellium bacchus* was subsequently made one of the priority species in Addo Elephant National Park and this promoted further awareness of *C. bacchus*. The public enthusiasm generated by this publicity lead to ill-considered "re-introductions" to other areas in the eastern Cape. This resulted in *C. bacchus* specimens being translocated to farms surrounding Addo Elephant National Park and the Andries Vosloo Kudu Reserve (33°S 20′E - 26S °40′E). Fortunately these translocations were done on a small, local scale, and records kept thereof rendering remaining populations sufficiently intact for further population genetic analyses (see chapter 2).

AIMS AND OBJECTIVES

This part of the project was aimed at determining habitat, feeding and breeding preferences in *C. bacchus* in order to define conservation criteria in terms of these preferences. These preferences will be important consideration if translocations are deemed necessary or desirable, not forgetting the controversy about the original distribution of this species and the conditions for successful re-introductions of species to areas of historical distribution (Oates & Warren 1990; Samways 1994). In order to gain some insight into possible dung beetle competitors a survey of the sympatric dung beetle fauna within both of the biomes where this species is currently found was conducted. The selected areas were Addo Elephant National Park and Buffalo Valley Game Farm.

The objections of this investigation were:

i) to determine habitat preferences of *C. bacchus*

ii) to determine dung preference of *C. bacchus* for both feeding and breeding

iii) to survey sympatric dung beetle fauna in both biomes in which these beetles are currently found, namely the Valley Bushveld biome of Addo Elephant National Park as well as the Fynbos biome of Buffalo Valley Game Farm.

STUDY AREAS

Field work was undertaken in the Valley Bushveld biome of the eastern Cape in Addo Elephant National Park from 19/12/1996 - 23/12/1996 and from 9/03/1998 - 15/03/1998. In the fynbos biome at Buffalo Valley Game Farm fieldwork was conducted from 21/01/1997 - 26/01/1997. There are climatic, geographic and vegetative differences between these two study sites. Buffalo Valley Game Farm is situated at sea level on the south-east coast which results in a less extreme annual range in mean monthly temperatures (January 14.3 - 28.2 °C and July 5.8 - 18.2 °C) than Addo Elephant National Park. At Addo Elephant National Park which lies at an altitude of 75m - 125 meter above sea level mean temperatures are 13.5 - 32 °C averages in July and January respectively. Average annual rainfall at Buffalo Valley Game Farm is 850 mm and at Addo Elephant National Park 436 mm (Johnston personal communication).

Addo Elephant National Park

Addo Elephant National Park is situated in the Eastern Cape Province, 72 km from Port Elizabeth (33°28'S-25°45'E). This area is characterised by bimodal rainfall with peaks during spring (September/November) and in late summer or autumn (March to May; Davis 1987). It has been an island in a sea of increasing agricultural development since the 3rd of July 1931 (Grobler & Hall-Martin 1982). This area was proclaimed a National Park due to public pressure and it has since been a sanctuary for the elephants in particular, but also for a large variety of other indigenous fauna and flora. In 1990 the park comprised approximately 12 000 ha of pristine Valley Bushveld. Since then it has increased in size with the addition of neighbouring farmlands that had been primarily used for grazing and crop farming. Further expansion of the park has recently been approved and with the incorporation of various smaller reserve and farmlands the end result of this expanded reserve will be the third largest continuous conservation area in South Africa covering 398 000ha. The proposed areas that will be acquired includes small reserves, the Woody Cape and Tootabie reserves, 57 000ha of marine reserve, the Alexandra coastal dunefield, Bird Island and StCroix Island as well as neighbouring farmlands.

The vegetation in Addo Elephant National Park was formerly referred to as Valley Bushveld, Succulent Mountain Shrub and even Spekboomveld due to an abundance of *Portulacaria afra* (spekboom) according to Acocks (1988). However, this broad habitat category was later divided into smaller units and the particular habitat type in Addo Elephant National Park is

currently referred to as Xeric Succulent Thicket (Low & Rebelo 1996). This habitat is characterised by short, dense vegetation dominated by *Portulacaria, Schotia, Sideroxylon, Cussonia* and *Cassina* species. There is a high density of leaf-and stem-succulent shrubs such as *Portulacaria afra* which in some instances represent more than 90% coverage by the latter species (Coles 1993). *Euphorbia bothae* as well as climbers such as *Azima tetracantha, Plumbago auriculata* and *Capparis sepiaria* are also found. There are a few forbs and grasses such as *Pentzia incana* (Anchorkaroo), *Chrysocoma ciliata* (Bitterbush) and *Cynodon dactylon* (Couchgrass), (Everard 1987). This veld type is characteristic of the steep sandstone, quartzite and shale mountain slopes in the eastern and southern Cape. On the steeper southern aspects, *Portulacaria* is often rare and sometimes absent and the vegetation is more or less non-succulent shrub or even shrub forest (Acocks 1988).

Characteristics of the three chosen habitat types within Addo Elephant National Park

Three different habitat types were identified within Addo Elephant National Park, representing the most divergent combinations of herbivore presence and different plant communities in which sampling was done. Transects 1-6 were placed in pristine *C. bacchus* Xeric Succulent Thicket habitat to provide data on their occurrence in natural habitat with exposure to mega-herbivores. This area was flat, north east facing with no visible degree of erosion. Transects 7-12 were placed in a 425.4 ha protected area of natural habitat generally known as the Botanical Reserve that excludes herbivores larger than Kudu (*Tragelaphus strepsiceros*, 200 kg). The major difference in these two habitat types was the composition of herbivores that had access to the area and the degree of trampling. Transects 13-18 were placed in an area named Alva. This area used to be a privately owned cattle farm almost entirely transformed from natural bush to artificial pastures for cattle grazing. This area was added to Addo Elephant National Park in 1991 and is now managed as part of the park with various large herbivores including elephant and buffalo that graze on the lush grasses growing in this area. A number of pioneer plants are starting to establish on this north eastern convex sloping ridge.

Table 3.2: Coding system of the transects in Addo Elephant National Park

Habitat type	Transect number
Exposed veld (transect 1-6)	OA(1-6) - OF(1-6)
Plant Reserve (transect 7-12)	PG(1-6) - PL(1-6)
Alva (transect 13-18)	αA(1-6) - αF(1-6)

Buffalo Valley Game Farm

This privately owned 175 ha game farm is situated 12 km from Knysna (34°05'S-22°45'E) in a coastal fynbos and dune forest area. In places it is invaded by the exotic *Acacia saligna* (Port Jackson Willows) which were introduced from Australia early in this century to stabilise sand dunes. This game farm falls within a winter rainfall area with midwinter rainfall peaks in June or July (Davis 1987). In terms of floristics and structure, this specific fynbos region has not been rigorously defined (Cowling & Richardson 1995). Communities vary considerably in species composition between the shallow limestone and the deeper neutral sands with dominant vegetation types such as *Protea obtusifola* (Limestone sugarbush), *Chrysanthemoides monilifera* (Bietou), *Leucadendron coniferum* (Dune Conebush) and *L. galpinii* (Cowling 1983). Bushbuck (*Tragelaphus scriptus)*, Grysbok (*Raphicerus sharpei*) and Grey Rhebok (*Palea capreolus*) are the only remains of the once abundant wildlife that occurred in the surrounding areas. Bontebok (*Damaliscus pygargus*), Burchell's Zebra (*Equus burchellii*) and Black Wildebeest (*Connochaetes gnou*) were re-introduced in the recent past.

Characteristics of the three habitat types at Buffalo Valley Game Farm

Field work was undertaken in January 1997 (21/01/1997 - 26/01/1997) in the three different areas that were identified according to the herbivorous plant layer in each. The first was a north-east facing concave grassveld with dense covering by *Stenotaphrum secundatum* (coastal buffalo grass) *Cynodon dactylon* (couch grass), *Setaria verticillata* (bur bristle grass) and *Sporobolus africanus* (ratstail dropseed). A fair amount of trampling by game was evident in this area. In scattered patches, secondary regrowth was starting to establishing, mostly in the form of herbaceous shrubs. Transects 1-6 were placed in this area. The second area (transects 7-12) consisted of dense fynbos on the periphery of the sandy dunes with a slight slope leading up the dune side that led to the beach. Canopy cover mainly consisted of *Acacia saligna, Rhus crenata, Hakea suaveolens* (Sweet Hakea) and *Asclepias fruticosa* (milkwood) shrubs with a thick layer of litter and decomposing detritus covering the ground. The third was an open,

clear-cut area with secondary regrowth of the natural occurring fynbos (herbaceous cover) along one of the roads. Transects 13-18 were placed in this third habitat. No erosion was apparent in any one of the identified habitat types.

Table 3.3: Coding system of the transects at Buffalo Valley Game Farm

Habitat Type	**Transect number**
Grasses (transects 1-6)	GrsA(1-6) - GrsF(1-6)
Dunes(transects 7-12)	DuneA(1-6) - DuneF(1-6)
Fynbos (transects 13-18)	FynbA(1-6) - FynbF(1-6)

MATERIALS AND METHODS

Weather data

As dung beetle activity is strongly influenced by occurrence of rainfall, optimal trapping for a standardised survey should ideally be conducted on warm, sunny days immediately after rainfall (Davis pers. com.). This ensures that sampling coincides with peaks in species richness, density and functional complexity of the communities. Sampling in this study was conducted in two short periods under ideal environmental conditions thus ensuring that data are representative of the dung beetle community. The fieldwork at both selected areas coincided with optimal weather conditions (weather data are available).

Sampling of *C. bacchus* outside Addo Elephant National Park and Buffalo Valley Game Farm

This survey took place over a 10-day period in May 1996 (6/05/1996-16/05/1996). The beetles are known to be active at this time which represents warm periods during early winter rains. The survey began near Stellenbosch, (the most westerly known record) and ended at Knysna - the eastern limit of the southern Cape distribution. Areas from which beetles were recorded were visited and presumed suitable habitat near and between them were searched for *C .bacchus.* In all habitats where specimens were found 15 minutes were spent counting the number of beetles seen. I realise that surveying in this way is subjective and biased towards finding individuals as we only searched in areas where the presence of *C. bacchus* was already determined. However, the purpose was solely to get a rough estimate of the number of beetles present in these areas and an idea of the type of habitat that *C. bacchus* occurs in.

Pitfall trapping

Pitfall trapping has many advantages in its application. It is a non-expensive trapping technique and simple to set up in the field. This trapping method provides species inventory as well as population and community data (Davis 1995). Sampling of dung beetles in the identified habitats was done over a four day period in Addo Elephant National Park (19/12/1996 - 23/12/1996) and a five day period in Buffalo Valley Game Farm (21/01/1997 - 26/01/1997).

One hundred and eight 1-litre plastic pitfall traps were placed in each sampling area of Addo Elephant National Park and Buffalo Valley Game Farm respectively. Traps were baited with 200g of fresh dung. Elephant dung collected in the park was used in Addo Elephant National Park pitfall traps, and cattle dung collected in Pretoria was deep frozen and transported to

Buffalo Valley Game Farm for use there. Thirty-six baited traps were placed in each of the three habitats at Addo Elephant National Park and at Buffalo Valley Game Farm. Each series of 36 pitfall traps were placed in areas chosen to be as homogeneous as possible to determine within and between habitat dung beetle heterogeneity. The traps were set in transects containing six 1-litre buckets each with at least 20 m intervals between consecutive transects. The traps were sunk into the ground until the lip of the container was level with the ground surface, making it accessible to both flying and walking insects. Transects within each habitat type were designated a capital alphabetical letter (Table 3.2 and 3.3) and the individual traps in each numbered from one to six. As an example αA3 refers to the third pitfall trap in the first transect at Addo Elephant National Park in the habitat Alva. Throughout the rest of this thesis this coding system in Table 3.2 and 3.3 will be used when referring to different transects.

The traps were set for consecutive days and checked hourly for all dung beetles during daylight and at first light in the mornings for nocturnal species. Record was kept of the number and sex of *C. bacchus* specimens captured in each pitfall trap. In Addo Elephant National Park the captured individuals were removed by hand, sexed and kept overnight. One hundred of these beetles were weighed to determine the mean live mass of the beetles at Addo Elephant National Park. *Circellium bacchus* individuals captured in Addo Elephant National Park were released on the following day in the same area where the dung preference trials were conducted (see below). In Buffalo Valley Game Farm captured individuals were removed from the pitfall traps after which they were sexed and released. Mean live mass of *C. bacchus* (n=24) in Buffalo Valley Game Farm was also determined.

By the third sampling day the crust of the dung in the pitfall traps had started to dry. These samples were stirred and fresh dung was added to all the pitfall traps. The dry dung was not removed until the end of the trapping period so as not to lose the other trapped species. On the last day of sampling all pitfall traps were sorted by hand and the other dung beetle species collected. Samples from each transect were pooled and placed in preservative in individually marked jars. In the laboratory these beetles were sorted and identified to species with the assistance of a Scarabaeid taxonomist - A.L.V. Davis, University of Pretoria and an Aphodiinae specialist - G. Dellacasa from Italy. Voucher specimens were deposited in the Transvaal Museum, Pretoria.

Sympatric dung beetle fauna survey at Addo Elephant National Park and Buffalo Valley Game Farm

After the species were identified they were sorted into functional groups (FGs) on the basis of knowledge of the taxa; and since species from various genera occupy similar FGs they were lumped. Species were lumped in all genera with the exception of those with representative species in FGI (the functional group in which *C. bacchus* is placed) and those in FGII which may have a competitive effect on the members of FGI. As some of the species were sampled in both Addo Elephant National Park and Buffalo Valley Game Farm but some were only sampled in one of the respective habitats the species groups from Addo Elephant National Park will not necessarily contain the same species that are included in the Buffalo Valley Game Farm species groups. Therefore, species lumped in the different species groups are defined separately for the two biomes. An example of Addo Elephant National Park FGs are as follows: the local *Neosisyphus* species, *N. barbarossa*, *N. spinipes* and *N. rube* were lumped in the *Neosysiphus* species group since they all belong to FGII. *Sisyphus alveatus* and *Euoniticellus intermedius* were the only species surveyed in Addo Elephant National Park from the *Sisyphus* species group and the *Euoniticellus* species group. *Onthophagus sugillatus* was the only species sampled from the *Onthophagus* species group 1 but *O. lugubris* and *O. bubalus* were lumped in *Onthophagus* species group 2 from FGIV. Three *Onitis* species were lumped to form the *Onitis* species group. They were *Onitis caffer*, *O. pecaurius* and *O. alexis*. With the exception of *Colobopterus maculicollis*, a diurnal aphodiine tunneler from FGV, the remaining nine *Aphodius* species surveyed at Addo Elephant National Park were lumped. It is not known to which FGs these remaining *Aphodius* species belong but habits of individuals belonging to FGV, FGVI and FGVII were recorded. Although some of these *Aphodius* species may belong to different FGs they were nevertheless lumped in the *Aphodius* species group since they are clearly of minor importance as potential competitors for *C. bacchus*. Individuals lumped in the *Pedaria* species group are possibly from one species. *Oniticellus planatus* and *O. pictus* were lumped under the *Oniticellus* species group representing FGVII. *Epirinus flagellatus* and *E. obstusus* were lumped in the *Epirinus* species group from FGII.

In Buffalo Valley Game Farm *Sisyphus costatus* (FGII) and *Euoniticellus triangulatus* (FGIV) were the only representatives of their respective species groups. Other species groups are as follows: *Onthophagus deterrens O. sugillatus* and *O. asperulus* were grouped under *Onthophagus* species group 1 representing FGV. *Onthophagus* species group 2 consisted only

of *O. giraffa* from FGIV. *Aphodius* sp1 was the only aphodiine surveyed in Buffalo Valley Game Farm. The *Copris* species group consisted of *C. fidius* and *C. antares*. Three species were lumped in the *Epirinus* species group representing FGII. They were *E. flagellatus*, *E. hilaris* and *E. rugosus*.

Dung preference trials: breeding

Trials to determine possible preferences for dung utilised in brood ball construction were conducted in the Botanical Reserve in Addo Elephant National Park on 18/12/1996 and 19/12/1996. All brood ball formation data obtained from feeding preference trials conducted on 11/03/1998, 12/03/1998 and 13/03/1998 were also incorporated. During the dung preference trial for brood ball construction 16 sites consisting of four different dung types each were placed in 2X2m square grids with at least 5m intervals between each transect. Fresh elephant, rhinoceros and buffalo dung was collected in the park and cattle dung was collected on one of the neighbouring farms. One litre of each dung type was used in each of the 16 sites. The four dung types were positioned in randomised order relative to each other. The 16 sites were checked half-hourly and it was noted whether the beetles were merely feeding on the dung or constructing a food or brood ball. (Brood balls are twice the size of food balls - Coles 1993.) Individuals were not removed but left at the dung pad to complete brood ball formation. One hundred and eighty nine *C. bacchus* specimens that were removed from the baited pitfall traps in different areas on the previous day were released in close proximity to the sites. This was done in order to increase the number of beetles in the surrounding area.

Dung preference trials: feeding

Dung preference trials to determine possible feeding preferences were also conducted in the Botanical Reserve on 11/03/1998, 12/03/1998 and 13/03/1998. The same experimental design was used as with the previous trials involving the determination of dung preference for the construction of brood balls. Elephant, rhinoceros and buffalo dung was collected in the park. Cattle dung was collected on a nearby farm. The 16 sites were checked half-hourly and all the beetles were removed after investigating whether they were feeding or constructing a food or brood ball. The females constructing brood balls were not counted but these data were incorporated in the relevant trials where possible preferences for dung in the construction of brood balls were determined. The beetles collected and counted from the dung feeding preference trials were sexed and kept in a container and released at the end of the experiment.

In both the feeding and breeding trials 250ml of dung was taken from each of the four dung types in the 16 sites at different time intervals. During the breeding preference trial, as well as on 13/03/1998 during the feeding preference trials, dung was collected at the onset of the experiment, halfway through and at the end of the experiment. On 11/03/1998 and 12/03/1998 samples were collected at the onset as well as at the end of the experimental trial. The different dung types were placed in separate jars and deep frozen. These samples were taken to determine the differences in moisture content of the respective dung types at the different time intervals of the experiment. It was anticipated that this would give an indication of the different rates of desiccation of the various dung types. Differences in moisture content were determined by weighing ±50ml of each dung type before and after a drying period of 24hours at 60 °C. The difference calculated by subtracting the mass at the end of desiccation (W_e) from the mass at the onset of the experiment (W_o) gives a measure of mass loss due to desiccation.

Assessment of plant compositional differences and density

Techniques for assessing veld condition are usually based on estimates of proportional species composition (Hurt & Bosch 1991) and a large number of such sampling techniques exist. One of the preferred sampling methods is the step-point method that can be done by one person without specialized equipment (Mentis 1981). In the step-point method the observer walks forward along a designated transect and the percentage species composition is recorded by using the nearest plant method (Mentis 1981). Only the ground directly in front of the shoe is searched for the nearest plant. These are roughly categorised into appropriate classes. This point position is examined at every second step along a 200m transect, repeated a number of times in each habitat, resulting in 100 points for each transect which can be manipulated statistically with ease.

In the analysis of the habitat types identified in both Addo Elephant National Park and Buffalo Valley Game Farm the step-point method was used with minor modifications. Instead of categorising the plants in species categories divisions were made on the basis of woody plants (including all treelike growth forms), forbs (all shrubs and smaller bushes) and grasses. Five iterations of 200 steps were done in each of the three identified habitats in Addo Elephant National Park. The same was done in Buffalo Valley Game Farm to determine the differences in plant composition within and between the different habitats. From the vegetation data

recorded basal plant coverage, percentage frequency as well as relative frequency of each of the three chosen categories (wood, forbs and grasses) were calculated.

DATA ANALYSES

A Kruskal-Wallis one-way analysis by ranks (Zar 1984) was used to analyse *C. bacchus* density in the various habitats. In cases where the null hypothesis was rejected a non-parametric Tukey type multiple comparison of ranks was implemented to determine whether significant differences occurred between samples. In this multiple comparison pairwize differences between ranks are tabulated and differences calculated starting with the difference between the largest and smallest ranks. These calculated differences are divided by a standard error (SE) calculated from the following equation: $SE = \sqrt{\ } n(kn + 1)\backslash 12$ (Miller 1966) and the tabulated Studentized range to be used is $q_{\infty,\infty,k}$. These non-parametric methods were chosen to analyse the data as it was not always possible to transform the data to fit a normal distribution due to zero values in the different samples. This same method of analysis was used in comparison of vegetational composition of the different habitats.

Dung feeding preference data were analysed by using the Friedman two-way analysis of variance test and to determine whether significant differences occurred between samples a multiple comparison for the Friedman test was implemented. In this method of analysis the null hypothesis is rejected if Z-stat is larger than the critical value ZC, where $1 - \phi(ZC) = \alpha/(k(k-1))$, ϕ is the cumulative standard normal distribution function, α is the desired overall significance level and k is the number of groups compared. With the four groups (four different dung types) used in the dung preference trials the critical Z-values are: 2.39 for overall α of 0.10 and 2.64 for overall α of 0.05.

RESULTS

Sampling of C. bacchus *outside Addo Elephant National Park and Buffalo Valley Game Farm*

Sampling at Hawston was conducted on an overcast day and although only six individuals were counted farm workers collected more specimens that were sent to the laboratory that were subsequently used in the molecular analysis (chapter two). At Stilbaai a herd of cattle congregate at a waterhole and in this vicinity 38 specimens were counted within 15 minutes of searching. Outside this area no indication of *C. bacchus* existence was evident. On a neighbouring piece of farmland next to Buffalo Valley Game Farm 17 specimens were counted in the set time limit. At Jongensfontein 11 specimens were seen and 21 in Grootbos. At Baardskeerdersbos, Humansdorp, Grabouw, Riversdal and Elim there was no sign of any dung beetle activity at the time of this survey. All these areas in which populations were found were private owned farms with small patches of natural fynbos remaining in a sea of ploughed land, irrigation systems and crop farming.

Table 3.4. Localities and co-ordinates where *C. bacchus* was surveyed outside Addo Elephant National Park and Buffalo Valley Game Farm.

Locality	Co-ordinates	No of specimens counted
Buffalo Valley Game Farm (farmland)	34° 02'S 23°	17
De Hoop Nature Reserve	34° 30'S 20°	12
Stilbaai (Geelkrans Nature Reserve)	34° 22'S 21°	38
Jongensfontein	34° 26'S 21°	11
Grootbos	34° 05'S 21°	21
Hawston (Afdak)	34° 23'S 19°	6
Elim	34° 35'S 19°	none
Baardskeerdersbos	34° 35'S 19°	none
Humansdorp	34° 10'S 24°	none
Riversdal	34° 60'S 21°	none
Grabouw	34° 90'S 19°	none

Results of pitfall trapping

Addo Elephant National Park

A total of 509 *C. bacchus* specimens was collected in Addo Elephant National Park of which 193 were males and 316 females (sex ratio 1: 1.63; Table 3.5). Mean mass was calculated at 6.23g (n=100). The largest individual collected at Addo Elephant National Park weighed 12.96g and the smallest 1.66g. The Botanical Reserve yielded 284 (112 males and 172 females), the Exposed Habitat 205 (76 males and 129 females) and Alva 20 specimens (5 were males and 15 females). Pitfall traps in Addo Elephant National Park yielded no significant variation (at level $p < 0.05$) in *C. bacchus* density between any six individual pitfalls within any of the 18 transects (Test statistic $H_{(5,6)} = 5.00$, $p = 0.42$) nor between the six transects within the three habitats (Exposed Habitat: Test statistic $H_{(5,36)} = 3.49$, $p = 0.63$; Botanical Reserve: Test statistic $H_{(5,36)} = 4.02$, $p = 0.55$ and Alva: Test statistic $H_{(5,36)} = 3.99$, $p = 0.56$).

The Kruskall-Wallis one way analysis by ranks of *C. bacchus* density among the different habitat types in Addo Elephant National Park showed a significant difference. The Test statistic was $H_{(2;108)} = 59.73$, $p = 0.00$ and the average ranks, calculated for each habitat are given in declining order: Botanical Reserve, 73.32; Exposed Area, 68.17; Alva, 22.01. The non-parametric Tukey type multiple comparisons further showed that Alva differed significantly from both the Exposed Habitat as well as the Botanical Reserve ($p < 0.01$). No significant difference in density was evident between the Exposed area and the Botanical Reserve.

Table 3.5: *Circellium bacchus* density in the different pitfalls and transects within the three different habitats in Addo Elephant National Park. Pitfall codes according to Table 3.2 in this chapter. Total = transect density; TOTAL = habitat density.

Pitfall	Density	total	Pitfall	Density	total	Pitfall	Density	total
OA1	11	29	PG1	5	28	AlvaA1	4	5
OA2	4		PG2	11		AlvaA2	0	
OA3	4		PG3	0		AlvaA3	0	
OA4	5		PG4	5		AlvaA4	0	
OA5	4		PG5	4		AlvaA5	0	
OA6	1		PG6	3		AlvaA6	1	
OB1	13	38	PH1	2	40	AlvaB1	1	5
OB2	7		PH2	4		AlvaB2	0	
OB3	3		PH3	22		AlvaB3	0	
OB4	1		PH4	5		AlvaB4	1	
OB5	5		PH5	1		AlvaB5	0	
OB6	9		PH6	6		AlvaB6	3	
OC1	2	27	PI1	8	49	AlvaC1	0	1
OC2	9		PI2	3		AlvaC2	0	
OC3	2		PI3	4		AlvaC3	0	
OC4	3		PI4	1		AlvaC4	1	
OC5	8		PI5	23		AlvaC5	0	
OC6	3		PI6	10		AlvaC6	0	
OD1	5	43	PJ1	9	43	AlvaD1	0	6
OD2	16		PJ2	3		AlvaD2	0	
OD3	0		PJ3	7		AlvaD3	2	
OD4	6		PJ4	3		AlvaD4	2	
OD5	6		PJ5	11		AlvaD5	0	
OD6	10		PJ6	10		AlvaD6	2	
OE1	6	27	PK1	10	51	AlvaE1	0	1
OE2	4		PK2	3		AlvaE2	0	
OE3	3		PK3	2		AlvaE3	1	
OE4	4		PK4	8		AlvaE4	0	
OE5	9		PK5	18		AlvaE5	0	
OE6	1		PK6	10		AlvaE6	0	
OF1	9	41	PL1	33	73	AlvaF1	0	2
OF2	6		PL2	7		AlvaF2	0	
OF3	4		PL3	11		AlvaF3	1	
OF4	4		PL4	11		AlvaF4	0	
OF5	4		PL5	3		AlvaF5	0	
OF6	14		PL6	8		AlvaF6	1	
TOTAL	205		TOTAL	284		TOTAL	20	

Buffalo Valley Game Farm

Pitfall traps in Buffalo Valley Game Farm yielded 343 *C. bacchus* specimens of which 76 were male and 267 female (sex ratio 1: 3.51; Table 3.6) with a mean mass of 4.19g (n=24). The total number of beetles collected comprised 47 beetles (8 males and 39 females) from the Grass area, 110 from the Dunes (19 males and 91 females respectively) and 188 individuals from the Fynbos area (49 males and 139 females). There was no significant difference in *C. bacchus* density among pitfalls within any of the 18 transects in the three habitat types (Test statistic $H_{(5,6)}= 5.00$; $p = 0.42$) nor was there significant difference in their density between transects within the Dune habitat. However, between transects within the Grass and Fynbos habitats significant differences in *C. bacchus* density were found (see Table 3.7 and 3.8). In the Grass habitat transect six had a significantly less amount of *C. bacchus* specimens compared to transect one but not significantly different to all the other transects and in the Fynbos habitat transect six differed significantly to transect one but none of the others (Table 3.7).

Table 3.6: *Circellium bacchus* density in the different pitfalls and transects within the three different habitats in Buffalo Valley Game Farm. Pitfall codes according to Table 3.3 in this chapter. Total = transect density; TOTAL = habitat density.

Pitfall	Density	total	Pitfall	Density	total	Pitfall	Density	total
DuneA1	5	20	GrsA1	1	19	FynbA1	1	8
DuneA2	5		GrsA2	2		FynbA2	2	
DuneA3	1		GrsA3	3		FynbA3	2	
DuneA4	0		GrsA4	4		FynbA4	2	
DuneA5	2		GrsA5	5		FynbA5	0	
DuneA6	7		GrsA6	4		FynbA6	1	
DuneB1	1	24	GrsB1	0	4	FynbB1	2	40
DuneB2	8		GrsB2	0		FynbB2	15	
DuneB3	7		GrsB3	1		FynbB3	3	
DuneB4	0		GrsB4	2		FynbB4	9	
DuneB5	8		GrsB5	0		FynbB5	1	
DuneB6	0		GrsB6	1		FynbB6	10	
DuneC1	2	22	GrsC1	0	3	FynbC1	3	14
DuneC2	2		GrsC2	1		FynbC2	3	
DuneC3	5		GrsC3	0		FynbC3	1	
DuneC4	6		GrsC4	0		FynbC4	2	
DuneC5	5		GrsC5	1		FynbC5	3	
DuneC6	2		GrsC6	1		FynbC6	2	
DuneD1	4	18	GrsD1	0	2	FynbD1	4	42
DuneD2	2		GrsD2	0		FynbD2	10	
DuneD3	5		GrsD3	0		FynbD3	4	
DuneD4	1		GrsD4	0		FynbD4	7	
DuneD5	4		GrsD5	2		FynbD5	9	
DuneD6	2		GrsD6	0		FynbD6	8	
DuneE1	1	3	GrsE1	1	5	FynbE1	6	51
DuneE2	0		GrsE2	0		FynbE2	2	
DuneE3	0		GrsE3	1		FynbE3	10	
DuneE4	1		GrsE4	1		FynbE4	11	
DuneE5	0		GrsE5	2		FynbE5	14	
DuneE6	1		GrsE6	0		FynbE6	8	
DuneF1	5	23	GrsF1	5	14	FynbF1	2	33
DuneF2	7		GrsF2	1		FynbF2	11	
DuneF3	2		GrsF3	0		FynbF3	1	
DuneF4	3		GrsF4	5		FynbF4	8	
DuneF5	2		GrsF5	3		FynbF5	0	
DuneF6	4		GrsF6	0		FynbF6	11	
TOTAL	110		TOTAL	47		TOTAL	188	

Table 3.7: Kruskall-Wallis one way analysis by ranks of *Circellium bacchus* density in the six different transects within the Grass and Fynbos habitats at Buffalo Valley Game Farm. As there was no significant variation between transects in the Dune habitat it is not included in this table. The average ranks, calculated for each habitat are sorted in decending order. Roman numerals indicate the transect ordered by ranks, used in the non-parametric Tukey type multiple comparison to determine the sources of variation given in Table 3.8.

	Grass Habitat	Average Rank
I	transect 1	29.75
II	transect 6	22.83
III	transect 2	18.5
IV	transect 5	17.5
V	transect 3	14.25
VI	transect 4	11.25
	Fynbos Habitat	Average Rank
I	transect 5	26.08
II	transect 4	24.42
III	transect 2	21.25
IV	transect 6	18.17
V	transect 3	13.42
VI	transect 1	7.67

Table 3.8: Results of a non-parametric Tukey type multiple comparison of *Circellium bacchus* density in the six different transects within each of the three habitats at Buffalo Valley Game Farm. Average rank from Table 3.6 were used in the calculations. Significant levels at p = 0.05.

Grass Habitat	I	II	III	IV	V
II	n.s.				
III	n.s.	n.s.			
IV	n.s.	n.s.	n.s.		
V	n.s.	n.s.	n.s.	n.s.	
VI	$p < 0.05$	n.s.	n.s.	n.s.	n.s.

Fynbos	I	II	III	IV	V
II	n.s.				
III	n.s.	n.s.			
IV	n.s.	n.s.	n.s.		
V	$p < 0.05$	n.s.	n.s.	n.s.	
VI	n.s.	n.s.	n.s.	n.s.	n.s.

The Kruskall-Wallis one way analysis by ranks of *C. bacchus'* density across the three habitat types showed that there was a highly significant difference ($H_{(2,108)} = 24.26$, p = 0.00). Average ranks were as follows: Fynbos, 70.97; Dunes, 57.17; Grass, 35.36. The non-parametric Tukey type multiple comparisons showed that the density of *C. bacchus* in the Fynbos and Dune areas was significantly greater then that of the Grass area ($p > 0.01$); between the Fynbos and Dune Habitat no significant difference in *C. bacchus* density were found.

Composition of dung beetle species other than *Circellium bacchus* from the different habitats within Addo Elephant National Park and Buffalo Valley Game Farm.

Table 3.9: List of dung beetle species sampled in the habitat association surveys. ** delineates species that were sampled only in Addo Elephant National Park, * species only sampled in Buffalo Valley Game Farm. Species that do not have any asterisk were sampled in both of the above mentioned areas.

Aphodius sp1
***Aphdius* sp2**
***Aphodius* sp3**
***Aphodius* sp4**
***Aphodius* sp5**
***Aphodius* sp6**
***Aphodius* sp7**
***Aphodius* sp8**
***Aphodius* sp9**
***Aphodius* sp10**
Aphodius sp11
Colobopterus maculicollis Reiche
***Scarabaeus viator* Peringuey**
Scarabaeus savignyi M'Leay
**Garreta unicolor* (Fahraeus)
**Catharsius tricornutus* De Geer
***Pedaria* sp**
***Sarophorus tuberculatus* (Castelnau)**
***Copris antares* Ferreira**
**Copris fidius* (Olivier)
***Onitis caffer* (Boheman)**
***Onitis alexis* Klug**
***Onitis pecaurius* Lansberge**
***Euoniticellus intermedius* (Reiche)**
**Euoniticellus triangulatus* (Harold)
***Oniticellus planatus* Castelnau**
***Oniticellus pictus* (Hausman)**
Liatongus militaris (Castelnau)
**Cyptochirus ambiguus* (Boheman)
***Drepanocerus kirbyi* Kirby**

Table 3.9 cont.
Odontoloma sp
Epirinus flagellatus (Fabricius)
***Epirinus obtusus* Boheman
**Epirinus hilaris* Péringuey
**Epirinus rugosus*
***Anachalcos convexus* (Boheman)
***Neosisyphus barbarossa* Wiederman
***Neosisyphus spinipes* Thunberg
***Neosisyphus rube*
***Sisyphus alveatus* Boucomont
**Sisyphus costatus* Thunberg
Onthophagus sugillatus Klug
**Onthophagus asperulus* D'Orbigny
**Onthophagus deterens* Péringuey
**Onthophagus giraffa* Hausman
***Onthophagus lugubris* Roth
***Onthophagus bubalus* Harold
Digitonthophagus gazella Balthasar
**Caccobius obtusus* Péringuey

Addo Elephant National Park habitat association survey

A total of 1020 dung beetle specimens from 49 species excluding *C. bacchus* were collected at Addo Elephant National Park during the *C. bacchus* habitat association survey. At least one species representing each of the functional groups was surveyed in all three habitats although some functional groups were represented by a single species and sometimes only a few individuals. *Anachalcos convexus* was the only other species surveyed at Addo Elephant National Park from the same functional group as *C. bacchus*. However, their numbers were very low. The highest density of *A. convexus* was in the Exposed Habitat where five specimens were sampled. One specimen each was collected in the Botanical Reserve and Alva. Two other species with a possible competitive influence on *C. bacchus* were also surveyed at Addo Elephant National Park but once again in low numbers. They were the larger FGII species *Scarabaeus viator* (12 specimens) and *S. savignyi* (3 specimens).

Exposed Habitat

Dung beetle species composition surveyed in the Exposed Habitat is listed in Table 3.10. The smaller telecoprids (FGII) and the larger, slow-burying paracoprids (FGIV) were the dominant FGs in the Exposed Habitat. These FGs were followed by three species of the smaller, slow-

burying paracoprids (FGV) and the fast-burying paracoprid *Copris antares* from FGIII. *Oniticellus planatus* was the only representative species from FGVII. The *Aphodius* species group (FGV/FGVI and FGVII) was also present.

Botanical Reserve

Functional group II species were similar in the Exposed Habitat and the Botanical Reserve (Table 3.11) although *Scarabaeus savignyi* was only present in the Botanical Reserve and not in the Exposed Habitat (Table 3.10). The fast-burying paracoprid species (FGIII) were only represented by *Copris antares* and the species composition of FGIV was similar in the two habitats except that an additional species, *Sarophorus tuberculatus,* was encountered in the Botanical Reserve. The majority of FGV specimens were *Onthophagus sugillatus.* Fewer *Aphodius* sp were present than in Alva (Table 3.12) or the Exposed Habitat (Table 3.10). A kleptocoprid species *Odontoloma* sp (FGVI) was also surveyed in this habitat and not in either of the other two.

Alva

Functional groups in Alva (Table 3.12) were again similar to those in the Exposed Habitat and the Botanical Reserve with the exception of one species in Alva which was not encountered in either of the other two habitats. This was *Pedaria* sp, the only clearly distinguishable kleptocoprid from FGVI. Functional group II (*Neosisyphus* sp) and FGIII (*Copris antares*) were both represented by a single species. Functional group IV had the same species/species groups that were present in the Exposed Habitat. The *Onthophagus* species group1 (FGV) had the highest number of specimens in Alva compared to the other habitats. The *Oniticellus* species group from FGVII was also surveyed in Alva as was the *Aphodius* species group.

Table 3.10: Dung beetles, other than *Circellium bacchus* present at Addo Elephant National Park in the Exposed habitat. The transects are numbered according to Table 3.2 in this chapter.

		transects					
	Functional group	OA	OB	OC	OD	OE	OF
Anachalcos convexus	I		1		2		2
Scarabaeus viator	II		1		1	1	1
Epirinus sp	II	1	1	1	3	7	1
Neosisyphus sp	II	3	2	2	5	2	2
Sisyphus alveatus	II			7	2	12	17
Copris antares	III	1	1			1	
Onitis sp	IV	2	1			1	1
Euoniticellus intermedius	IV	1				1	
Liatongus militaris	IV					1	1
Onthophagus sugillatus	V	11	15	1	66	44	2
Drepanocerus kirbyi	V	1	2	1	1	1	1
Colobopterus maculicollis	V					6	5
Aphodius sp	?	5	4	4	5		2
Oniticellus sp	VII	1				1	4

Table 3.11: Dung beetles, other than *Circellium bacchus* present at Addo Elephant National Park in the Botanical Reserve. The transects are numbered according to Table 3.2 in this chapter. * Indicates species unique to this habitat.

		transects					
	Functional group	PG	PH	PI	PJ	PK	PL
Anachalcos convexus	I				1		
Scarabaeus viator	II		2			5	1
* *Scarabaeus savignyi*	II	1	1				1
Epirinus sp	II	1	5	13	3	3	4
Neosisyphus sp	II	2	1	2		1	1
Sisyphus alveatus	II	25	4	19	12	14	4
Copris antares	III					1	1
Onitis sp	IV		1		1	2	1
* *Sarophorus tuberculatus*	IV			1	1		
Onthophagus sugillatus	V	4	1		13	3	77
Drepanocerus kirbyi	V		1	1	1		1
Colobopterus maculicollis	V						1
Odontoloma sp	VI				2		
Aphodius sp	?						9

Table 3.12: Dung beetles, other than *Circellium bacchus* present at Addo Elephant National Park in Alva. The transects are numbered according to Table 3.2 in this chapter. * Indicates species unique to this habitat.

		transects					
	Functional group	αA	αB	αC	αD	αE	αF
Anachalcos convexus	I				1		
Neosisyphus sp	II			1	1		
Copris antares	III		1	1	1	1	
Onitis sp	IV		11	1	5	1	1
Euoniticellus intermedius	IV				1		
Liatongus militaris	IV		1	1		1	
Onthophagus sugillatus	V	1	201	35	24	9	4
Drepanocerus kirbyi	V	1	2	2	1		1
Digitonthophagus gazella	V	1	2	1	1	2	
Colobopterus maculicollis	V	1	11		25	6	
Aphodius sp	?	9	40		38	21	
* *Pedaria* sp	VI	1					
Oniticellus planatus	VII	1	1	2	2	1	2

Buffalo Valley Game Farm habitat association survey

Six hundred and seventy one dung beetles, other than *C. bacchus,* were collected in pitfall traps set in the three habitats at Buffalo Valley Game Farm. As discussed in materials and methods, species of the same FGs were lumped in their respective genera as was done with the data from Addo Elephant National Park. No species from the same FG as *C. bacchus* was sampled at Buffalo Valley Game Farm but all the other FGs were represented in this habitat association survey. The only species that can possibly compete with *C. bacchus* is *Scarabaeus savignyi,* one of the larger telecopdrids from FGII. They were, however, sampled only in the Fynbos habitat and in low numbers.

Fynbos Habitat

The Fynbos habitat species composition is given in Table 3.13. This habitat had the highest number of species from FGII. They were *Epirinus* sp, *Garreta unicolor, Sisyphus* sp and *Scarabaeus savignyi. Scarabaeus savignyi* and *Garreta unicolor* were unique to the Fynbos Habitat. *Catharsius tricornutus* was the only species from FGIII that was surveyed in the Fynbos Habitat and FGIV had one representative species, *O. giraffa,* from the *Onthophagus* species group 2. *Colobopterus maculicollis* and *Onthophagus* species group 1, represented FGV in the Fynbos Habitat. The *Aphodius* species were also surveyed in the Fynbos Habitat.

Dune Habitat

Epirinus flagellatus was the only representative of FGII found in the Dune Habitat (Table 3.14). Members of FGIII included *Catharsius tricornutus* and *Copris* sp. FGIV was represented by *Euoniticellus triangulatis,* a single species from *Onthophagus* species group 2 (*O. giraffa*) and *Liatongus militaris.* All of the species lumped in the *Onthophagus* species group 1 were present at Buffalo Valley Game Farm in this habitat as were *Colobopterus maculicollis* and *Digitonthophagus gazella* from FGV. *Oniticellus pictus* was the one species representative of FGVII and was unique to the Dune Habitat. The *Aphodius* species group was also present in the Dune Habitat.

Grass Habitat

Sisyphus costatus and *Epirinus* sp were the only species representative of FGII in the Grass Habitat (Table 3.15). Functional group III was present as a single species, *Catharsius tricornutus. Euoniticellus* sp, *Liatongus militaris* and *Onthophagus* species group 2 were sampled in low numbers but nevertheless represented FGIV. The widespread and numerous

Onthophagus species group 1 and *Digitonthophagus gazella* represented FGV and *Odontoloma* sp (FGVI) was also surveyed. The ubiquitous *Aphodius* species group was also included in the survey. *Cyptochirus ambiguus* from FGVII was the only species from this FG and also unique to this habitat.

Table 3.13. Dung beetles, other than *Circellium bacchus,* present at Buffalo Valley Game Farm in the Fynbos Habitat. Transects are numbered according to Table 3.3 in this chapter. * Indicates species unique to the habitat.

		transects					
	Functional group	FynbA	FynbB	FynbC	FynbD	FynbE	FynbF
* *Scarabaeus savignyi*	II						1
* *Garreta unicolor*	II						1
Epirinus sp	II	3	1				2
Sisyphus costatus	II	1					1
Catharsius tricornutus	III					1	1
Onthophagus sp group2	IV	1	2	2		2	
Colobopterus	V		1				
Onthophagus sp group1	V	4	3	2	6	4	10
Aphodius sp	?	2			6	2	1

Table 3.14: Dung beetles, other than *Circellium bacchus* present at Buffalo Valley Game Farm in the Dune Habitat. Transects are numbered according to Table 3.3 in this chapter. * Indicates species unique to the habitat.

		transects					
	Functional group	DuneA	DuneB	DuneC	DuneD	DuneE	DuneF
Epirinus sp	II	2			2		1
Catharsius tricornutus	III						1
Copris sp	III	2		1			3
Euoniticellus triangulatus	IV	1					
Liatongus militaris)	IV						1
Onthophagus sp group2	IV	1	1	2	1	1	1
Onthophagus sp group1	V	55	27	62	19	2	
Colobopterus maculicollis	V						1
Digitonthophagus gazella	V			2			
Coccobius obstusus	VI	1		1	2		
Aphodius sp	?	6	10	12	45	4	6
* *Oniticellus pictus*	VII				1		

Table 3.15: Dung beetles, other than *Circellium bacchus* present at Buffalo Valley Game Farm in the Grass Habitat. Transects are number according to Table 3.3 in this chapter. * Indicates species unique to the habitat.

		transects					
	Functional group	GrsA	GrsB	GrsC	GrsD	GrsE	GrsF
Epirinus sp	II				1	1	
Sisyphus costatus	II					1	
Catharsius tricornutus	III	1					
Euoniticellus sp	IV	1		1	1	1	1
Liatongus militaris	IV			1	1		
Onthophagus sp group2	IV	1		1	1	2	
Onthophagus sp group1	V	49	61	37	1	1	1
Digitonthophagus gazella	V		3			2	
Odontoloma sp	VI	1					
Aphodius sp	?	19	96	15	5	16	6
* *Cyptochirus ambiguus*	VII					1	

Results of the dung preference trials:

Brood ball formation

During the experiment designed to determine dung preference in brood ball construction 25 brood balls were formed from the buffalo dung, 20 from the cattle dung, three and seven from the elephant and rhino dung respectively. During the determination of possible food preferences in *C. bacchus* some of the females were also noted to constructed brood balls. Results of these totals are given in Table 3.16. Of the total of 28 brood balls 15 were constructed from buffalo dung, two from elephant and 11 from the cattle dung. No brood balls were constructed from the rhinoceros dung that was used in the feeding preference trials. In total, 40 brood balls were constructed from buffalo dung, 31 from cattle, seven from rhino and five from elephant dung. Figure 3.1 shows the number of brood balls constructed on the different dung types during two separate trials.

Combined brood ball data obtained from the four dung types during the feeding preference trial and the dung breeding preference trial were analysed using the Kruskall-Wallis non-parametric multiple comparison. As the null hypothesis was rejected (the Test statistic was $H_{(3,46)} = 21.43$, $p = 0.001$) showing that there were significant differences between brood ball construction from the four dung types a non-parametric Tukey Type multiple comparison was done to determine between which of the four dung types the differences lie. Highly significant differences were found between the number of brood balls constructed from cattle dung versus the number constructed from elephant and rhino dung (Table 3.17). There was also a significant difference between the number of brood balls constructed on buffalo dung versus the number constructed from elephant and rhino dung (Table 3.17).

Table 3.16: Number of brood balls constructed by *Circellium bacchus* from different dung types during the dung preference trial for brood ball formation as well as brood balls constructed during the dung feeding preference trials.

Date	Dung type	total number of brood balls
18/12/1996-19/12/1996	Buffalo	25
	Elephant	3
	Rhino	7
	Cattle	20
11/03/1998-13/03/1998	Dung type	total number of brood balls
	Buffalo	15
	Elephant	2
	Rhino	0
	Cattle	11

Table 3.17: Non-parametric Tukey Type multiple comparison of the number of brood balls constructed by *C. bacchus* from the four different dung types. * denotes significant differences at $p < 0.05$ and ** denotes significant differences at $p < 0.01$.

dung type	buffalo	elephant	rhino	cattle
buffalo	-	3.95*	4.12*	0.56
elephant		-	0.17	4.51**
rhino			-	4.68**
cattle				-

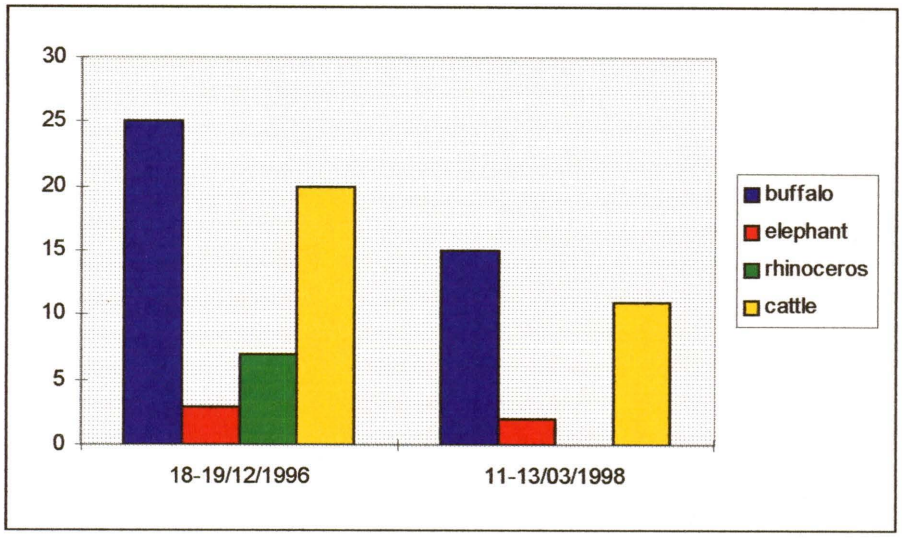


Figure 3.1: Number of brood balls constructed from for different dung types during the two respective trials.

Feeding preference

A total of 284 *C. bacchus* beetles fed on the dung placed out for the feeding preference trials. Data collected for the analyses at each one of the four dung types in the 16 sites were combined for every one of the time intervals. This resulted in a total number of beetles sampled over 16 individual dung pads at a given time for each of the dung types. On the first of the three sampling days results of the Friedman two-way analysis of variance test showed that there were significant differences between the colonisation of the four dung types (Friedman test statistic = 13.46, p = 0.0037, ZC = 2.39 for overall alpha 0.10 and ZC = 2.64 for overall alpha 0.05). Rank sums for the different dung types are given in Table 3.18. Colonisation on the elephant and rhino dung was similar but both dung types had fewer *C. bacchus* compared to the number of beetles that fed on the cattle and buffalo dung. Significant differences in dung colonisation were found between cattle and elephant dung (Z-stat = 2.59), cattle and rhino dung (Z-stat = 3.12), and rhino and buffalo dung (Z-stat = 2.46, Table 3.19). No significant difference in feeding preference was found between elephant and buffalo dung nor between elephant and rhino dung and likewise, no significant difference was found in the number of beetles that fed on buffalo and cattle dung.

The dung preference trial conducted on the second sampling day showed no significant difference between *C. bacchus* colonisation on any of the four dung types (Friedman test statistic =4.62, p = 0.2016, ZC = 2.39 for overall alpha 0.10). Friedmans rank sums are given in Table 3.18. However, there were significant differences in the colonisation of the four dung

types for the third sampling day (Friedman test statistic = 17.17, p = 0.0007, ZC = 2.64 for overall alpha 0.05; Table 3.20). The rate of colonisation of the rhino dung was significantly less than the colonisation of buffalo (Z-stat = 3.65) and elephant dung (Z-stat = 3.39). There was a highly significant difference in the number of beetles that fed on rhino versus buffalo and rhino versus elephant dung. Colonisation between the other dung types did not differ significantly.

Table 3.18: Number of *Circellium bacchus* collected from four dung types during the feeding preference trials conducted in the Botanical Reserve at Addo Elephant National Park.

	Day 1 (11\03\1998)		Day 2 (12\03\1998)		Day 3 (13\03\1998)	
Dung type	Number	Friedman rank sum	Number	Friedman rank sum	Number	Friedman rank sum
Buffalo	25	49.5	32	51.0	82	53.5
Elephant	7	35.0	10	41.0	53	51.5
Rhino	3	51.0	4	35.0	4	26.0
Cattle	23	54.5	11	43.0	30	39.0

Table 3.19: Friedman multiple comparison of dung colonisation by *C. bacchus* on day 1. ** denotes significant differences at overall alpha of 0.05 and * denotes significant differences at overall alpha of 0.10

dung type	buffalo	elephant	rhino	cattle
buffalo	-	1.93	2.46*	0.66
elephant		-	0.53	2.59*
rhino			-	3.12**
cattle				-

Table 3.20: Friedman multiple comparison of dung colonisation by *C. bacchus* on day 3. ** denotes significant differences at overall alpha of 0.05.

dung type	buffalo	elephant	rhino	cattle
buffalo	-	0.27	3.65**	1.93
elephant		-	3.39**	1.66
rhino			-	1.73
cattle				-

Since moisture content is regarded as one of the principle components effecting dung suitability, the differences in moisture content of the four dung types was determined. These values are presented in Figure 3.2. The different dung types show the same general expected pattern of moisture loss in that the moisture content declines towards the end of the day after exposure for 12 hours. The loss of moisture in all four dung types on day 2 of the experiment was slightly less than that of the first and third sampling days. There was a slight increase in the initial moisture content in buffalo and elephant dung at the onset of each of the experimental days (77.89% initially increasing to 81.43% for buffalo dung and from 69.2% - 78.49% for the elephant dung). The rhinoceros dung maintained a relatively constant moisture content (starting off at 64.9% on the first day, 64.21% on the second day and 63.38% on the third day of the experiment) and the cattle dung moisture content initially decreased from 84.4% to 78.02% and then increased to 81.96%.

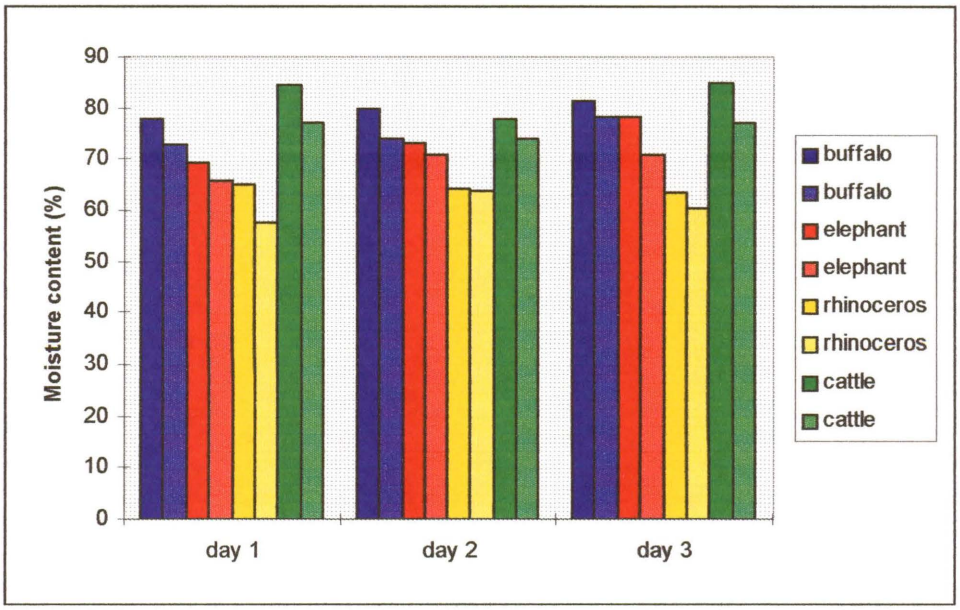


Figure 3.2: Four dung types represented in each of three experimental trials and their rates of desiccation. The darker colours indicate dung at the onset of the experiment (the first column) and the second column is dung after 12 hours of exposure. Moisture content is given as a percentage of the weight loss of the dung. The first series (8 columns) represent dung sampled on day 1; 11/3/1998, the following eight are samples collected on day 2; 12/3/1998 and the last series (8 columns) is the data obtained on the last day (13/3/1998).

Plant compositional differences

Addo Elephant National Park

From the results of a step-point method, the percentage Basal Cover was calculated for each individual plant type within each of the three habitats at both Addo Elephant National Park and Buffalo Valley Game Farm (see Tables 3.21 and 3.24). As an indication of overall vegetational cover within a habitat a value was obtained by calculating the sum of the basal cover of the specific plant types within a habitat. These values of habitat basal cover were used for comparisons within and between the different habitat types as well as within and between plant types.

Table 3.21: Results of a step-point method derived from five transects in each of three habitats targeted in Addo Elephant National Park. Vegetational cover was divided into trees, grasses and forbs. Percentage basal cover was calculated by number of direct hits in step-point analysis/total number of points X 100. Habitat percentage basal cover (bc) is the average bc between the five repetitions within a habitat.

	Exposed Habitat				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	24	7	20	51	53.8
2	14	8	31	53	
3	15	12	22	49	
4	25	5	32	62	
5	20	12	22	54	
	Botanical Reserve				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	18	20	23	56	56.2
2	28	6	25	59	
3	21	9	27	57	
4	27	6	23	56	
5	22	9	22	53	
	Alva				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	34	1	6	41	55.0
2	47	3	7	57	
3	52	0	10	62	
4	53	2	6	61	
5	51	1	2	54	

No significant difference was found in the total basal cover of the three habitat types within Addo Elephant National Park (Test statistic $H_{(2;45)} = 2.24$; $p = 0.33$). However, the basal cover of each of the individual plant types did show a significant difference between the three different habitats (Exposed habitat: Test statistic $H_{(2,45)} = 9.97$, $p = 0.007$; Botanical Reserve: Test statistic $H_{(2;45)} = 9.45$; $p = 0.089$; Alva: Test statistic $H_{(2;45)} = 9.52$, $p = 0.0086$, Table 3.22). Alva and the Exposed Area differed significantly with regard to grass basal cover as did Alva from both the Exposed Habitat and the Botanical Reserve with respect to the basal cover of trees and forbs. The Botanical Reserve and the Exposed area did not differ significantly with regards to basal cover of all three plant types.

Table 3.22: Kruskall-Wallis one way analysis by ranks and non-parametric Tukey type multiple comparisons of individual plant basal cover in the three different habitat types in Addo Elephant National Park. The average ranks, calculated for each habitat are sorted in declining order. Alphabetical letters "a" and "b" denote significant differences at level $p < 0.01$.

Grasses	Average	Comparison		
1 Alva	13.0	1 vs 3	p <0.01	
2 Botanical Reserve	6.6	1 vs 2	n.s.	
3 Exposed habitat	4.4	2 vs 3	n.s.	
Trees	Average	Comparison		
1 Botanical Reserve	10.6	1 vs 3	p <0.01	a
2 Exposed habitat	10.4	1 vs 2	n.s.	a
3 Alva	3.0	2 vs 3	p <0.01	b
Forbs	Average	Comparison		
1 Exposed habitat	10.8	1 vs 3	p <0.01	a
2 Botanical Reserve	10.2	1 vs 2	n.s.	a
3 Alva	3.0	2 vs 3	p <0.01	b

Every habitat was found to have a unique combination of the three plant types that made up the total basal cover of that specific habitat (Figure 3.3). As a result there was a significant difference in the composition of the three different plant types at each of the three habitat types (Exposed habitat: Test statistic $H_{(2,15)} = 10.15$, $p = 0.006$; Botanical Reserve: Test statistic $H_{(2;15)} = 8.9$; $p = 0.0117$; Alva: Test statistic $H_{(2;15)} = 11.86$, $p = 0.027$, Table 3.23). The Exposed area showed significant differences in the basal cover of forbs and trees with a higher

incidence of the former. Tree basal cover differed significantly from that of grass and forbs within the Botanical Reserve. Alva had significantly less trees and forbs and significantly more grasses in the total basal cover when compared to the Exposed Habitat and the Botanical Reserve.

Table 3.23: Kruskall-Wallis one way analysis by ranks and non-parametric Tukey type multiple comparison of plant basal cover of plant types in three different habitats in Addo Elephant National Park. The average ranks, calculated for each habitat are sorted in decending order. Alphabetical letters "a" and "b" denote significant differences at level $p < 0.01$ or as otherwise stated.

Plant type in Exposed habitat	Average Rank	Comparison		
forbs	11.7	1 vs 3	p<0.01	
grasses	9.3	1 vs 2	n.s.	
trees	3.0	2 vs 3	n.s.	
Plant type in Botanical Reserve	Average Rank	Comparison		
grasses	10.3	1 vs 3	p<0.05	a
forbs	10.3	1 vs 2	n.s.	a
trees	3.4	2 vs 3	p<0.05	b
Plant type in Alva	Average Rank	Comparison		
grasses	13.0	1 vs 3	p<0.01	
forbs	7.7	1 vs 2	n.s.	
trees	3.3	2 vs 3	n.s.	

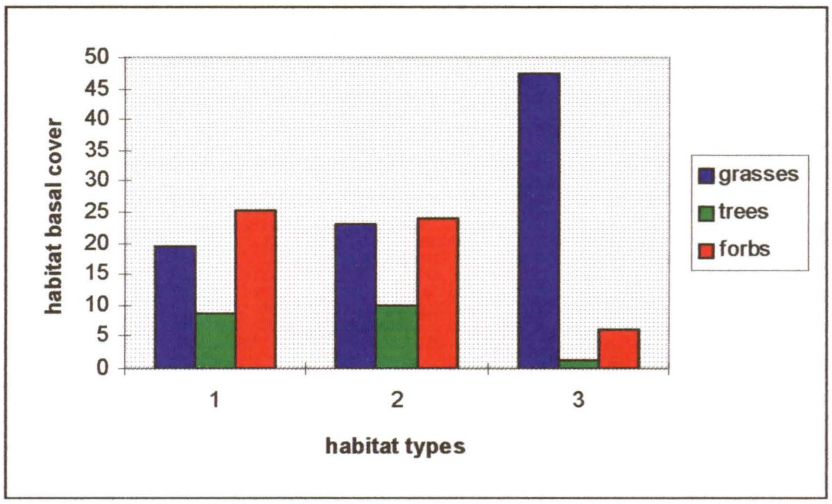


Figure 3.3: Habitat profile of Addo Elephant National Park visualising the ratio of different plant components that create the unique basal cover of each habitat. The numbers on the x-axes is as follows: 1= Exposed habitat, 2= Botanical Reserve and 3= Alva.

Buffalo Valley Game Farm

In Buffalo Valley Game Farm there were no significant differences detected in basal cover of the three selected habitats (Test statistic $H_{(2,45)} = 4.53$, $p = 0.1036$; Table 3.24) however, significant differences were noted in the basal cover of specific plant types within the Grass habitat (Test statistic $H_{(2;15)} = 12.75$, $p = 0.0017$) and the Fynbos Habitat (Test statistic $H_{(2;15)} = 12.84$, $p = 0.0016$; Table 3.25). The Dune habitat showed equal representation of grasses, trees and forbs ($H_{(2;15)} = 0.26$; $p = 0.87$; Table 3.24) and therefore no multiple comparison was done for this particular habitat. In the Grass habitat there were significantly fewer trees compared to grasses (Table 3.25). The Fynbos habitat had significantly more forbs than trees (Figure 3.4).

Table 3.24: Results of the step-point method from five transects in each of three habitats in Buffalo Valley Game Farm. Vegetational cover was divided into trees, grasses and forbs. Percentage basal cover was calculated by number of direct hits in step-point analysis/total number of points X 100. Habitat percentage basal cover (bc) is the average basal cover between the five repetitions within a habitat.

	Grass Habitat				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	60	0	7	67	70.2
2	57	0	15	72	
3	61	0	12	73	
4	58	1	11	69	
5	52	0	17	69	
	Dune Habitat				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	19	15	9	43	45.2
2	11	13	18	42	
3	13	23	14	50	
4	18	7	22	47	
5	10	17	17	44	
	Fynbos Habitat				
transect	grasses	trees	forbs	percentage bc	habitat percentage bc
1	17	0	27	44	40.0
2	16	0	24	40	
3	16	0	20	36	
4	16	0	25	41	
5	11	1	27	39	

Table 3.25: Kruskall-Wallis one way analysis by ranks and non-parametric Tukey type multiple comparisons of plant basal cover in the two variable habitat types in Buffalo Valley Game Farm. The average ranks, calculated for each habitat are sorted in decending order. Significance level $p < 0.01$.

Plant type in Grass Habitat	Average Rank	Comparison	
1 grasses	13.0	1 vs 3	$p < 0.01$
2 forbs	8.0	1 vs 2	n.s.
3 trees	3.0	2 vs 3	n.s.
Plant type in Fynbos Habitat	**Average Rank**	**Comparison**	
1 forbs	13.0	1 vs 3	$p < 0.01$
2 grasses	8.0	1 vs 2	n.s.
3 trees	3.0	2 vs 3	n.s.

Significant differences were found when the basal cover of specific plant types were compared across all three habitat types. Grasses dominated the basal cover of the Grass habitat, whereas trees were the most abundant component of the Dunes habitat. There were no significant differences in herbaceous cover between the Grass and Fynbos habitat nor between the Fynbos and Dunes habitats. However, the herbaceous cover differed significantly between the Grass and Dunes habitats (Table 3.26).

Table 3.26: Kruskall-Wallis one way analysis by ranks and non-parametric Tukey type multiple comparison of plant basal cover of each individual plant type across three different habitats in Buffalo Valley Game Farm. The average ranks, calculated for each habitat are sorted in decending order. Significance level $p < 0.01$. The letters "a" and "b" denote significant differences at $p < 0.01$.

grasses	Average	Comparison		
1 Grass	13.0	1 vs 3	p <0.01	a
2 Fynbos	5.7	1 vs 2	p <0.01	b
3 Dunes	5.3	2 vs 3	n.s.	b
trees	Average	Comparison		
1 Dunes	13.0	1 vs 3	p <0.01	a
2 Grass	5.5	1 vs 2	p <0.01	b
3 Fynbos	5.5	2 vs 3	n.s.	b
forbs	Average	Comparison		
1 Grass	13	1 vs 3	p <0.01	
2 Fynbos	7.8	1 vs 2	n.s.	
3 Dunes	3.2	2 vs 3	n.s.	

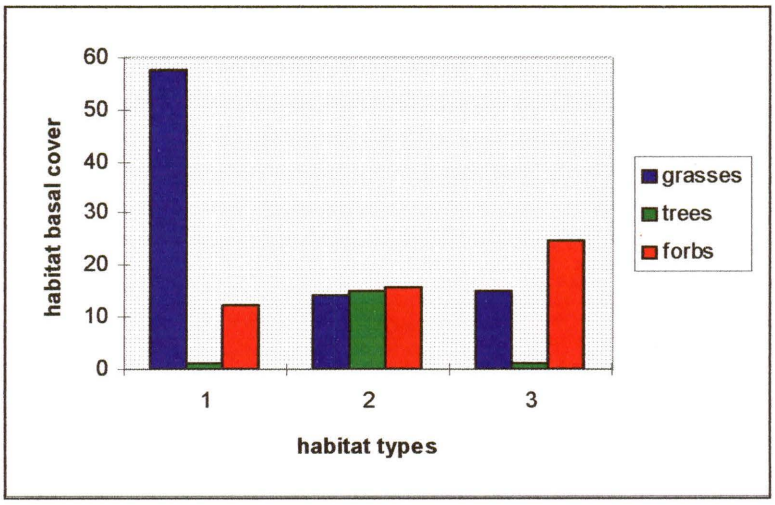


Figure 3.4: Habitat profile of Buffalo Valley Game Farm visualising the ratio of the different plant components that create the unique basal cover of each habitat. Numbers on the x-axes are: 1= Grass habitat, 2= Dune and 3= Fynbos habitat.

DISCUSSION

Various studies have shown that scarabaeine species are habitat specific (Doube 1983, Osberg & Hanrahan 1992, Davis 1996 and Ward & Seely 1996) and that a switch between species associated with different vegetation types can occur over tens of meters (Hill 1996). The principle criteria influencing distribution are environmental factors such as climate, vegetative cover and soil type (Doube 1983, Davis 1996) and biotic factors such as interactions with other fauna (Ward & Seely 1996, Lobo 1996). Soil type has a direct effect on vegetation type which, in turn, affects the distribution and density of a species (Osberg & Hanrahan 1982). Vegetation type could influence dung availability since herbivores would be unevenly distributed due to vegetational differences and dung beetle density can potentially be affected accordingly. However, this study showed that rather than dung availability, vegetative cover was paramount in influencing the density of *C. bacchus* in different areas (see also Chown & Steenkamp 1996, Hill 1996). Soil type analysis did not fall within the scope of this investigation but an analysis of the different components of habitat basal cover was done to determine the different vegetative components of the habitats in which *C. bacchus* populations occur. Significant differences in *C. bacchus* density between different habitats at both Addo Elephant National Park and Buffalo Valley Game Farm emphasise that certain habitat types are more suitable than others for the survival of this species. It would seem therefore that combinations of various factors such as soil type, vegetation structure and herbivore presence contribute to the suitability of a habitat for *C. bacchus.*

Assessments of *C. bacchus* density at the Botanical Reserve and the Exposed habitat types in Addo Elephant National Park did not differ significantly although both had significantly higher densities of *C. bacchus* than Alva. The two habitats with the same *C. bacchus* densities are covered in similar natural valley bushveld vegetation. The only difference between these sites is the exclusion of the mega-herbivores from the Botanical Reserve although antelope as large as kudu have access. The similarity in *C. bacchus* density between the Botanical Reserve habitat and the Exposed Habitat is in sharp contrast to the long held belief that mega-herbivores, specifically black rhinoceros have been central to the survival of *C. bacchus.* While elephants have long thought to have assured the survival of this species Chown *et al.* (1995) recently suggested that black rhinoceros may, in fact, have been important in assuring their survival in widely disjunct patches of dense bush. Since the black rhinoceros has disappeared

from most of its historic range, *C. bacchus* is thought to have suffered range contraction as a consequence. It does not seem to hold however, given evidence of populations surviving in areas where mega-herbivores do not occur. It is clear that *C. bacchus* is not as specialised a feeder as was previously thought. A possible explanation could be that movement of *C. bacchus* specimens in suitable habitats within the park is random. Beetles move in search of dung for feeding and breeding purposes and if suitable dung in large enough quantities is detected brood ball formation commences. The ball is then rolled away and buried in a suitable location. However, when insufficient quality or quantities of dung are found, the beetles will probably only feed and not breed. It has been estimated that these beetles may live for several years and therefore breeding probably occurs only when suitable dung is detected.

In Buffalo Valley Game Farm the Grass habitat had significantly fewer *C. bacchus* than either the Dune and Fynbos habitats, whereas the latter two habitats did not differ significantly in their densities of *C. bacchus.* Significant differences were however found in *C. bacchus* densities between transects within the Grass and Fynbos habitats and, although these differences were statistically significant, they were not biologically significant. Only one transect (the one with the least number of *C. bacchus* captured) within either of the habitats differed from one other transect (the one with the highest *C. bacchus* density). In the case of the Dune habitat at Buffalo Valley Game Farm, sampling was homogeneous as expected. As before higher densities of *C. bacchus* were found in some of the habitats at Buffalo Valley Game Farm and this underscores the observation that some habitats are more suitable than others for the survival of this species.

Several reasons may be responsible for this. First, due to locomotory constraint vegetation structure and density of a habitat is a major determinant of *C. bacchus'* environment. Whenever a tree, forb or grass is encountered during walking or ball rolling it is doubtful whether *C. bacchus* perceives it as anything other than an obstacle that needs to be passed. It is therefore possible that the structure of the vegetation types that form the basal cover of a habitat is a strong determinant for habitat suitability and therefore of *C. bacchus'* presence or absence within specific habitats. Similar results were found at Addo Elephant National Park and Buffalo Valley Game Farm with *C. bacchus* densities being highest in the untransformed, natural fynbos or renosterveld vegetation. Vegetation cover across habitat types at both these localities did not differ significantly with regard to total basal cover. In fact, the only difference detected was that the basal cover in each of the habitats comprised a unique

combination of the three different plant types. Habitats with larger proportions of dense grass cover had lower densities of *C. bacchus* as they most likely avoid these areas since their movement is impaired. *Circellium bacchus* thus clearly prefers vegetation with a high percentage forbs and trees which generally has a less dense basal layer providing more open spaces in which *C. bacchus* can move more freely and thus easily manipulate the rolling of a food or brood ball.

For some time, although a few specimens of *C. bacchus* from outside Addo Elephant National Park have been collected, this population has been regarded as the only large and seemingly healthy one of this species (Coles 1993). However, the present investigation has shown the presence of several smaller populations along the south coast between Knysna (34°02'S-23°02'E) and Hawston (34°23'S-19°08'E). Beetles were found on cattle, horse and sheep farms as well as on small, private nature reserves. As the same magnitude of sampling was not followed in all instances, comparisons on densities are clearly problematic. Where *C. bacchus* is still found on commercial farmland they are restricted to untransformed fragments of the natural habitat. It is noteworthy that the majority of the landowners where neither aware that *C. bacchus* occurs on their farms nor of their conservation status. Consequently, no overt attempts are being made towards the management of the habitat for these beetles. In other seemingly suitable areas *C. bacchus* was not found during the survey. This was not taken as a reflection that they are absent from those areas but it was assumed that the conditions were possibly not suitable for activity at the time of sampling. At Elim (34°35'S-19°45'E) and Baardskeerdersbos (34°35'S-19°34'E), local people recognised pinned *C. bacchus* specimens and commented that these beetles have been seen in larger numbers in the past although their numbers seem to have declined over the last few years.

At Grootbos, a farm managed as an ecotourism centre, beetles are regarded as being important. The management team of the farm were eager to learn more and direct efforts toward conservation of *C. bacchus*. Horse dung is available on this farm and it was mainly on the horse trails that beetles were collected. At Humansdorp (34°10'S-24°46'E), however, which appears to have suitable fynbos habitat (Acocks 1988, Low & Rebelo 1996), no *C. bacchus* specimens were noted during the survey. Farmlands in this region where previous collections have been made are currently characterised by extensive irrigation systems and intensive dairy farming. All other areas in which specimens were found were extremely isolated and localised

in small natural fynbos patches. The habitat separating these patches has been severely fragmented or completely destroyed and the possibility of contact between isolates is unlikely and therefore the future survival of the smaller populations outside the big reserves is a matter of concern. Furthermore, not a single official conservation area exists in the range occupied by *C. bacchus* between Addo Elephant National Park in the east and De Hoop Nature Reserve in the west. Moreover, with the exception of Addo Elephant National Park no awareness campaigns have ever been undertaken (Coles 1993).

Although there have been few detailed studies on dung beetle sex ratios, it seems that generally females outnumber males 3:1 (Edwards 1988) or 2:1 (Emlen & Oring 1977, Martins & Cantel 1997) during the height of their activity period. This ratio can increase towards a male bias of 10:1 (Edwards 1988) as the season progresses and more females are involved with brood care. According to Hanski (1991) sex ratios with a high female bias are indicative of a non-breeding state. The sex ration at Addo Elephant National Park was 1.6: 1 (females: males). We believe this reflects both the availability of dung in Addo Elephant National Park and the large number of females anticipated to be underground with brood balls at the time of sampling. In contrast, however, at Buffalo Valley Game Farm females outnumbered males 3.5:1 at the height of the breeding season, a ratio which possibly reflects a lack of large enough quantities of suitable dung for breeding purposes.

The size of a dung ball is correlated with the size of the beetle that constructed it (Halffter & Mathews 1966, Coles 1993) and it is generally the larger females that are able to construct larger balls since the middle and hind legs are used to gauge the brood ball. Furthermore, adult dung beetle size is largely determined by the amount of food available during larval development. Relative size and density of the dung beetle fauna in an area has been shown to be correlated with the presence, or absence, of large mammals at that locality (Cambefort 1991, Hanski & Cambefort 1991). In *Heteronitis,* which feeds exclusively on elephant dung, the larvae and hence adults were larger than specimens of the same species that feed on donkey dung as a substitute (Cambefort 1991). This could possibly explain the differences found in the mean body mass of *C. bacchus* collected from Addo Elephant National Park, which was higher than that of specimens from Buffalo Valley Game Farm. In fact, specimens from Addo Elephant National Park were generally larger than all other specimens from populations found outside of this park. These differences in overall body size and mass are

most likely explained by a lack of suitable quantities of dung outside Addo Elephant National Park.

The food preference of most dung beetles seems to be exercised when that specific dung is available in large quantities but as soon as it becomes scarce any suitable substitute will be accepted (Gordon & Cartwright 1974, Doube 1983, Hill 1996). The same holds for *C. bacchus* in that there has been speculation as to whether it is a dung specialist or generalist. However, as there are significant colonisation differences for *C. bacchus* when selecting between different dung types for feeding purposes, there is some doubt as to whether they can be regarded as complete generalists in terms of feeding. However, that they are not specialist feeders has been resolved by the presence of *C. bacchus* in areas where mega-herbivores are absent. They do exercise choice when provided with opportunity to choose between dung types but it is still uncertain if this choice is based on dung type, characteristic odour or moisture content of the dung. Various authors have suggested that dung moisture content is a stronger determinant of dung preference than dung type and it thus suggests further analysis as to the various factors that determine characteristic odour, moisture content, nitrogen content etc. of different dung types. When comparing *C. bacchus* densities on the different dung types and simultaneously taking moisture content of the dung types into account it could be concluded that it is not necessarily the dung type but also the moisture content of the dung that determines its suitability for feeding. Colonisation on buffalo and cattle dung was at its highest at the end of the experiment when the latter dung types were moist on the inside compared to the desiccated elephant and rhino dung that was almost completely deserted.

Given that a female is ready for breeding, large quantities of suitable dung are available and the environmental conditions are favourable brood ball construction is initiated (Coles 1993). Female *C. bacchus* specimens preferred the moist and pliable dung of buffalo and cattle although few attempts were made to construct brood balls on elephant and rhino dung. The attempts at brood ball formation involving rhino dung in the first trial and elephant dung during both trials, were at the onset of the experiments when the dung was fresh, sufficiently moist and probably pliable enough for brood ball construction. The desiccation profiles of the different dung types showed that the coarser elephant and rhino dung lost moisture at a greater rate than the buffalo and cattle dung. The latter dung types are thus still suitable for the construction of brood balls long after the elephant and rhino dung has become completely

desiccated. However, the large rhino middens and elephant droppings tend to stay moist on the inside of the pile and it is highly likely that beetles will attempt brood ball construction if the dung is found suitable.

Many studies have focused on the most important factors that determine distribution and density of species (Brown 1988, Chown & Steenkamp 1996, Chown *et al.* 1995, Ward & Seely 1996) and it has been shown that it is the interaction between numerous factors that ultimately define the suitability of specific areas. Specifically, suggestions have been made to explain the current distribution of *C. bacchus*. Nicolson (1987) showed that *C. bacchus'* body temperatures are elevated by solar radiation and not by endothermy, thus making them strict ectotherms (later confirmed by Chown *et al* 1995). Nicolson concluded that the ectothermic nature of *C. bacchus* can be considered a disadvantage compared to winged dung beetles and that their preference for certain dung types and reduced mobility due to flightlessness may explain their current restricted distribution. In an attempt to shed further light on distribution of this species, Chown *et al* (1995) examined and compared the distribution of a number of winged and wingless scarabaeinae from southern Africa and then compared the data to that of *C. bacchus*. In addition, an investigation of the thermal biology, water balance and respiratory metabolism was also done. They found that most other wingless canthonines follow an eastern (coastal) forests distribution throughout the subcontinent. This was confirmed by A.L.V. Davis (pers. com.). In terms of their ecophysiological adaptations *C. bacchus* is capable of metabolising lipids during dehydration as a supplement to body water and their larger size enables them to be more tolerant to desiccation compared to smaller specimens (Chown *et al.* 1995). The lack of speed (due to their exothermic physiological state) of *C. bacchus* suggests that dung, as an ephemeral resource, could be removed by other winged, endothermic competitors even before they have had time to locate and utilise it. Due to the species' tolerance of far drier conditions than most other large, competing species they are able to penetrate drier habitats.

Competitive interactions with other fauna may be regarded as one of the important determinants in the shaping of a community but it is probably the competitive interactions between members that rely on the same food source that has a greater influence on the presence or absence of a particular organism in a community (Giller & Doube 1994, Ward & Seely 1996, Lobo 1996). Although *C. bacchus* is not an active competitor, it is still placed in

FGI together with the other large telecoprids (functional groups as defined by Doube (1990). The survey of other dung beetle species that co-occur with *C. bacchus* at Addo Elephant National Park yielded a few specimens of FGI species but the numbers were low and any serious competition is unlikely. Several species in FGII were also surveyed, once again in very low numbers and the only possible competitors from this FG were the larger *S. savigny* and *S. viator*. As they were, however, sampled in such low numbers I concluded that their competitive effect on *C. bacchus* would probably also be minimal.

No species from the same FG as *C. bacchus* were collected at Buffalo Valley Game Farm. There was a total absence of large, wingless or winged, telocoprid species and therefore none that can directly compete with *C. bacchus*. The lack of competing species could be one of the reasons why *C. bacchus* is so successful in these biomes and the pre-adaptations to the arid environmental conditions further enables them to survive in an area that is unsuitable for other similar species.

CHAPTER FOUR

SUMMARY AND CONCLUSION

Management and conservation concerns often motivate the study of a particular species as was the case for *C. bacchus*. Restriction based molecular approaches are frequently used to address short term problems of direct interest in conservation management (e.g. see Georgiadis *et al* 1994; Ohland *et al* 1994; Lavery *et al* 1996). Similarly, in this study mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) data were used to address concerns on the long-term preservation of the endangered *C. bacchus*. In an earlier but ill-conceived conservation attempt *C. bacchus* specimens from Addo Elephant National Park were translocated by National Park authorities to surrounding farms in the hope of augmenting the beetle numbers in what appeared to be declining populations. No consideration had been given to the possible existence of geographic genetic partitioning within the species. The translocations were suspended to allow this investigation to determine the possibility of locally defined *C. bacchus* populations. The molecular data resulted in the separation of *C. bacchus* into two distinct mtDNA clades with the eastern clade comprising Addo Elephant National Park while the larger western clade contained the remaining populations in the western range of this species' distribution. Some evidence of shallower phylogenetic structure was found within the western clade.

Numerous characteristics of *C. bacchus* further emphasise the uniqueness and need of protection of this species i.e. it complies with various different categories and classifications that determine rarity, endangerment and extinction (see Coles 1993). Furthermore, it is endemic and South Africa's largest flightless dung beetle. The resulting separation of *C. bacchus* into two distinct clades further emphasises the importance of this species from a conservation perspective. Although mtDNA reflects the results of only the matriarchal phylogeny, and therefore the historical pattern recorded in the mtDNA remains to some extent incomplete, it is nonetheless informative. The lack of nuclear data in conjunction with mtDNA lead to the determination of Management Units (MUs). The one MU is the eastern clade that includes all other populations in the same geographic area as Addo Elephant National Park and the other MU is the western clade encompassing the remaining fragmented populations in Bredasdorp, Buffelsbaai, De Hoop, Grootbos, Hawston, Kleinbaai, Riversdal, Stilbaai, Jongensfontein and the surrounding area. The identification of MUs implies that these

populations should be treated and conserved as separate conservation entities and that translocations between them should under no instances be considered as a solution for increased viability in the extant populations or for any other reason.

Apart from the molecular work done on *C. bacchus* its ecological preferences were also determined in the two major biomes in which it is currently found. The western habitat has been defined as Fynbos or False Fynbos and the eastern habitat as Coastal Renosterveld (Acocks 1988). Low & Rebelo (1996) later redefined this area, dividing it into smaller units consisting of Limestone Fynbos, Laterite Fynbos and Dune Thicket in the western area of *C. bacchus* distribution. The eastern habitat was defined as south and south west renosterveld in the area surrounding Addo Elephant National Park (Low & Rebelo 1996) and the area separating the eastern and western distribution is known as Knysna Forest (Acocks 1988) or Afromontane Forest (Low & Rebelo 1996).

Circellium bacchus' density was highest in natural, undisturbed vegetation at both Addo Elephant National Park and Buffalo Valley Game Farm. This emphasises the importance of natural vegetation patches in ensuring healthy populations and eventual survival of *C. bacchus.* Experiments to determine dung preference for feeding supported Coles' (1993) findings that *C. bacchus* has no consistent pattern of choice in dung type and it is concluded the species is a generalist feeder. However, a definite choice was exercised with regard to the dung type utilised in brood ball formation. The finer textured and more pliable buffalo and cattle dung was preferred to the coarser elephant and rhinoceros dung types clearly indicating that the appropriate herbivores are an important consideration in ensuring successful breeding in *C. baccchus.* With regard to competition, the survey of the sympatric dung beetle fauna within Addo Elephant National Park recorded only a few species and individuals from FGI and FGII which are theoretically and ecologically similar to *C. bacchus*). Their numbers were low and any serious competition appears unlikely. At Buffalo Valley Game Farm no potential competitors from FGI nor FGII were surveyed.

Temperate regions of South Africa have been subjected to continuous fluctuations in cyclic glacial and interglacial periods over the last 3 MY (Deacon 1983). Within these fluctuation periods shorter cycles have occurred which resulted in the disruption of once continuous habitats. It is proposed that these habitat changes subsequently lead to the fragmentation of *C. bacchus* populations in the native habitat. The mountains that run along the seaboard of Africa

have probably hindered expansion of *C. bacchus'* ranges northward although it has been proposed that *C. bacchus'* distribution could once have been further inland during more favourable climatic conditions. Although there are still relict fynbos at high elevations no records of *C. bacchus* have ever been found there. This is most likely due to altitudinal elevation, decreasing temperatures and the lack of sandy soils for telecoprid beetles.

The population at Addo Elephant National Park appears to have a secure future as long as this national park is well maintained. The high numbers of mega-herbivores and antelope should ensure a large supply of feeding and breeding material for *C. bacchus.* Public awareness is maintained by numerous leaflets as well as road signs that point out the presence of *C. bacchus* in the park. At present De Hoop Nature Reserve is the only large fynbos reserve in the lowlands (Rebelo 1992). Other lowland fynbos areas are threatened and severely fragmented by agriculture, urbanisation, forestry and invasion of alien plants. Although it has been thought to be a nutrient poor resource for grazing, fynbos is often grazed by domestic livestock (Cowling 1983) which further contributes to the escalating deterioration of the fragmented habitat. The survival of *C. bacchus* on farmlands scattered throughout the western clade is thus of concern. It would thus seem that *C. bacchus* is facing extinction in these areas if nothing is done to ensure its survival. It is therefore important that farmers be made aware of its importance (and all other dung beetle fauna) and their co-operation in developing a conservation strategy for *C. bacchus* be obtained. *Circellium bacchus* could also be used as a flagship species, with specific aim it's own conservation but also to focus attention on the conservation of dung beetle communities as an integral part of ecosystem functioning.

OPSOMMING

Mitochondriale DNA (mtDNA) analises is gebruik om bevolkingsstrukture van 'n skaars miskruierspesie, *Circellium bacchus* Fabricius (Coleoptera: Scarabaeidae) te bepaal. Restriksie ensieme is gebruik om die Polimerase Ketting Reaksie (PKR) fragmente te analiseer. Die spesifieke mtDNA geenfragmente wat vir die studie gebruik is, is die sitochroomoksidase subeenhede I en II (COI\COII). Twee-en-sestig miskruiers is geanaliseer, afkomstig vanaf nege verskillende lokaliteite in die suid-en-oos Kaapprovinsie. Die meerderheid van die 28 geidentifiseerde haplotipes was uniek, privaat en naverwant; slegs twee haplotipes is gedeel tussen aangrensende bevolkings. Die mees uitstaande kenmerk van hierdie studie was die verdeling van *C. bacchus* moederlyne in twee aparte entiteite geskei deur 'n verskil in nukleotiedopeenvolgingsvolgorde van 4.74%. Die oostelike entiteit word gevorm deur 'n enkele bevolking miskruiers in Addo Olifant Nationale Park, terwyl die ander, heelwat groter, entiteit bestaan uit die oorblywende bevolkings in die westelike gedeelte van hierdie spesie se verspreiding. Die mees opvallende skeiding tussen die twee entiteite blyk die Knysnawoud te wees. Daar word aangeneem dat hierdie woud vanaf die laat Pleistoseen, as gevolg van die uitbreiding van westelike lugstrome oor die suidelike punt van Afrika, 'n ondeurdringbare skeiding tussen die ooste en weste gevorm het. Voortdurende sikliese klimaatsverandering, tydens en tussen ystydperke, het gelei tot die fragmentasie van die natuurlike fynbos habitat in die westelike deel van *C. bacchus* se verspreiding. Vandaar het die vlakker genetiese strukture in die weste moontlik ontstaan.

Tesame met die molekulêre analise is ekologiese faktore, wat moontlik 'n bydrae kon lewer m.b.t. spesifieke habitat voorkeure van *C. bacchus*, ook ondersoek. *Circellium bacchus* se digtheid was die hoogste in natuurlike, onversteurde habitatte in beide Addo Olifant Nationale Park en Buffalo Valley Game Farm. Verder is misvoorkeur vir voeding - en teeldoeleindes ondersoek deur 'n keuse van vier mistipes (buffel, olifant, renoster en bees) daar te stel. Uit die analise het dit geblyk dat daar geen konstante voorkeurpatroon van voeding was nie, en daar is tot 'n gevolgtrekking gekom dat *C. bacchus* 'n generalis is in terme van voeding. In teenstelling hiermee is gevind dat *C. bacchus* wel 'n voorkeur toon in die tipe mis wat gebruik word om broedballe mee te vorm. Vogtige, plooibare mis van buffels en beeste is verkies bo die growwer teksture van olifant en renostermis. Die resultate van 'n opname wat gedoen is in beide Addo Olifant Nationale Park en Buffalo Valley Game Farm, om te bepaal watter ander

miskruier spesies in dieselfde area as *C. bacchus* voorkom, het aangetoon dat daar geen ander miskruier spesies teenwoordig is wat direk in kompetisie met *C. bacchus* is nie.

Die belangrikste oorweging in die bewaring van *C. bacchus* is die onderskeid tussen die twee entiteite. Hierdie twee groepe behoort as aparte Bestuurseenhede gesien te word. Translokasies tussen die entiteite moet onder geen omstandighede as 'n maatstaf aangewend word om bevolkingsgetalle buite die areas te verhoog nie. Addo Olifant Nationale Park en De Hoop Natuur Reservaat is die enigste formele bewaringsareas waarbinne *C. bacchus* tans voorkom. Alle ander bevolkings, op landbougrond, se lot is dus totaal in die hande van die grondeienaars op wie se plase hulle voorkom. Dit is dus essensieël dat die samewerking van hierdie individue bekom moet word in 'n bewaringspoging vir *C. bacchus*.

REFERENCES

Acocks J.P.H. 1988. *Veld Types of South Africa.* Third edition. (eds. Leistner O.A. & Momberg B.A.) Memoirs of the Botanical Survey of South Africa No. 57.

Afonso J.M.,Volz A., Hernandez M., Ruttkay H., Gonzales M., Larruga J. M., Cabrera V. M. & Sperlich D. 1990. Mitochondrial Variation and Genetic Structure in Old World Populations of *Drosophila subobscura. Molecular Biology and Evolution* **7** (2): 123-142.

Al-Houty W. & Al-Musalem F. 1997. Dung preference of the dung beetle *Scarabaeus cristatus* (Coleoptera - Scarabaeidae) from Kuwait. *Journal of Arid Environments* **35**: 511-516.

Andrewartha H.G. & Birch L.C. 1954. *The Distribution and Abundance of Animals.* University Chicago Press, Chicago, IL.

Avise J.C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**: 1192-1208.

Avise J.C. 1992. Molcular populations structure and the biogeographic history of a regional phauna: a case history with lessons for conservation biology. *OIKOS* **63**: 62-76.

Avise J.C. 1994. *Molecular Markers, Natural History and Evolution.* Chapman Hall, New York.

Avise J. C., Lansman R.A & Shade R.O. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. Population structure and evolution in the genus Peromyscus. *Genetics* **92**: 279-295.

Avise J.C., Ankney C. D. & Nelson W.S. 1990. Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. *Evolution* **44**: 1109-1119.

Bishop J.A. & Cook L.M. 1981. Industrial melanism and the urban environment. *Advanced ecological Research* **11**: 373-404.

Borts P. & Grivell L.A. 1981. Small is beautifull - potrait of a mitochondrial genome. *Nature* **290**: 443-445.

Brown W.M. 1985. The mitochondrial genes of animals. In: *Molecular Evolutionary Genetics* R.J. MacIntyre, pp 95 - 130. New York, London: Plenum.

Brown J.S. 1988. Patch use as an indicator of habitat preference, predation risk and competition. *Behavioural Ecology and Sociobiology.* **22**: 37-47.

Brower A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA* **91**: 6491-6495.

Caccone A., Amato G.D. & Powel J.R. 1988. Rates and patterns of scnDNA and mtDNA divergence within the *Drosophila melanogaster* subgroup. *Genetics* **118**: 671-683.

Cambefort Y. 1978. Canthonina nouveaux oupeu connus d'Afrique continentale (Coleoptera:Scarabaeidae). In*: A revision of the African Canthonina (Scarabaeidae: Scarabaeinae*), by Scholtz C.H. & Howden H.F. 1987. *Journal of the Entomological Society of southern Africa.* **50** (1): 75-119.

Cambefort Y. 1991 In: *Dung beetle ecology* (eds I. Hanski & Y Camberfort) Princetown University press, Princeton.

Chown S.L., Scholtz C.H., Klok C.J., Joubert F.J. & Coles K.S. 1995. Ecophysiology, range contraction and survival of a geographically restricted African dung beetle (Coleoptera: Scarabaeidae). *Functional Ecology* **9**: 30-39.

Chown S.L. & Steenkamp H.E. 1996. Body mass and abundance in a dung beetle assemblage: optimal mass and the role of transients. *African Entomology* **4** (2): 203-212.

Cockcroft M.J., Wilkinson M.J. & Tyson P.D. 1987. The application of a present-day climatic model to the late-Quaternary in southern Africa. *Climate change* **10**: 161-181.

Coetzee J.A. 1987. Late Cainozoic palaeoenvironmentals of southern Africa. In: E.M. Van Zinderen Bakker Sr. (ed), *Antarctic glacial history and world palaeoenvironments*: 115-127. Rotterdam: Balkama.

Coles K.S. 1993. Biology and Conservation of Circellium bacchus Fabricius (Coleoptera: Scarabaeidae). MSc. thesis, University of Pretoria.

Cowling R.M. & Richardson D. 1995. *Fynbos: South Africa's unique floral kingdom.* Fernwood Press, Cape Town.

Cowling R.M. 1992. *The Ecology of Fynbos: Nutrient, Fire and Diversity.* Oxford University Press.

Cowling R.M., Holmes P.M. & Rebelo A.G. 1992 In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Edited by Richard Cowling, Cape Town. Oxford University Press.

Crandall K.A., Templeton A.R. & Sing C.F. 1994. Intraspecific phylogenetics: problems and solutions. In: Models in phylogeny reconstruction, (eds) R.W. Scotland, D.J. Siebert & D.M. Williams, Claredon Press, Oxford.

Cronin M.A., Bodkin J., Ballachey B., Estes J. & Patton J.C. 1996. Mitochondrial DNA variation among subspecies and populations of sea otters *(Enhydra lutris). Journal of mammology* **77** (2): 546-557.

Davis A.L.V. 1987. Geographic distribution of dung beetles (Coleoptera: Scarabaeidae) and their seasonal activity in south-western Cape Province. *Journal of South African Entomological Society* **50**: 275-278.

Davis A.L.V. 1993. Biogeographical groups in a southern African, winter rainfall, dung beetle assemblage (Coleoptera: Scarabaeidae) - consequences of climatic history and habitat fragmentation. *African Journal of Ecology* **31**: 306-327.

Davis A.L.V. 1995. Daily weather variation and temporal dynamics in an Afrotropical dung beetle community (Coleoptera: Scarabaeidae). *Acta Ecologica* **16**: 641-656.

Davis A.L.V. 1996. Habitat associations in a South African, summer rainfall, dung beetle community (Coleoptera: Scarabaeidae, Aphodiidae, Staphylinidae, Histeridae, Hydrophilidae). *Pedobiologia* **40**: 260-280.

Davis A.L.V. 1997. Climate and biogeographical associations of southern African dung beetles (Coleoptera: Scarabaeidae s. str.) *African Journal of Ecology* **35**: 10-38.

Deacon H.J., Hendey Q.B. & Lambrechts J.J.N. 1983. *Fynbos Paleoecology: A preliminary synthesis* (Eds H.J. Deacon, Q.B. Hendey & J.J. Lambrechts) South African National Scientific Programmes Report no 75, December 1983, CSIR, Pretoria.

Deacon H.J. 1983. An introduction to the fynbos region, time scales and palaeoenvironments. In: *Fynbos Paleaoecology: A preliminary synthesis.* (Eds H.J. Deacon, Q.B. Hendey & J.J. Lambrechts) South African National Scientific Programmes Report no 75, December 1983, CSIR, Pretoria.

Deacon J. & Lancaster N. 1988. Late Quaternary palaeoenvironments of southern Africa. Claredon Press, Oxford.

Deacon H.J., Jury M.R. & Ellis F. 1992 In: *The Ecology of Fynbos: Nutrients, Fire and Diversity.* Edited by Richard Cowling, Cape Town. Oxford University Press.

DeBry R.W. 1992. The consistency of several phylogeny-inference methods under varying evolutionary rates. *Molecular Biology and Evolution.* **9**: 537-551.

DeSalle R., Freedman T., Prager E.M. & Wilson A.C. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila. Journal of Molecular Evolution* **26**: 157-164.

Doube B.M. 1983. The habitat preference of some bovine dung beetles (Coleoptera: Scarabaeidae) in Hluhluwe Game Reserve, South Africa. *Bull. Ent. Res.* **73**: 357-371.

Doube B.M., Giller P.S. & Moola F. 1988. Dung burial strategies in some South African coprine and onitine dung beetles (Scarabaeidae: Scarabaeinae). *Ecological Entomology* **13**: 251-261.

Doube B.M. 1990. A functional classification for analysis of the structure of dung beetle assembledges. *Ecological Entomology* **15**: 371-383.

Doube B.M. 1991. Dung beetles of southern Africa: In *Dung beetle ecology* (Eds. I. Hanski and Y. Cambefort). Princeton University Press, Princeton.

Dowling T.E., Moritz C. & Palmer J.D. 1990. Nucleic acids II: Restriction site analysis. In *Molecular Systematics*, (eds.) D.M. Hillis & C. Moritz, Sinauer, Sunderland

Edwards P.B. 1988. Contribution of the female parent to the survival of laboratory reared offspring in the dung beetle *Kheper nigroaeneus* (Boheman) (Coleoptera: Scarabaeidae). *Journal of the Australian Entomological Society* **27**: 233-237.

Edwards P.B. 1991. Seasonal variation in the dung of African grazing mammals, and its consequences for coprophagous insects. *Functional Ecology* **5**: 617-628.

Emlen S.T. & Oring L.W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* **197**: 215-223.

Endrödy-Younga, S. 1987. In: *Biogeography and ecology of southern Africa*, chapter **26** (2): 797-821.

Erwin T.L. 1983. Tropical forest canopies: The last biotic frontier. *Bulletin of the Entomological Society of America* **29**:14-19.

Erwin T.L. 1991. An evolutionary basis for conservation strategies. *Science* **253**: 750-752.

Everard D.A. 1987. A classification of the subtropical transitional thicket in the eastern Cape, based on syntaxonomic and structural attributes. *South African Journal of Botany* **53**: 329-340.

Excoffier L., Smouse P.E. & Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**: 479-491.

Excoffier L. & Smouse P.E. 1994. Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: Molecular Variance Parsimony. *Genetics* **136**: 343-359.

Ferrar A.A. 1989. The role of Red Data Books in conserving biodiversity. In: *Biotic Diversity In southern Africa.* Ed. B.J. Huntley. Oxford Univesity Press, Cape Town.

Ferreira, M.C. 1968. Os escarabideos de Mozambique. I. *Revista de Entomologia de Mocambique* **10** (1&2):5-778.

Frakes 1979 in Deacon H.J., Hendey Q.B. & Lambrechts J.J.N. 1983. *Fynbos Paleoecology: A preliminary synthesis* (Eds H.J. Deacon, Q.B. Hendey & J.J. Lambrechts) South African National Scientific Programmes Report no 75, December 1983, CSIR, Pretoria.

Franklin J.F. 1994. Response. *Ecol. Apll* **4:** 208-209.

Geldenhuys C.J. 1994. Bergwind fire and the location pattern of forest patches in the southern Cape landscape, South Africa. *Journal of Biogeography* **21**: 49-62

Georgiadis N., Bischof L., Templeton A., Patton J, Karesh W. & Western D. 1994. Structure and History of African Elephant Populations: I. *Eastern and southern Africa. Journal of Heredity* **85**: 100-104.

Gordon R.D. & Cartwright O.L. 1974. Survey of food preferences of some no. American Canthonini (Coleoptera: Scarabaeidae). *Entomology News* **85**: 181-185.

Grant W.S. 1994. Multi-disiplinary Approaches for Defining Evolutionary Significant Units for Conservation. *South African Journal of Sceince* **14**: 114-123.

Grobler J.H. & Hall-Martin A. 1982. A Guide to the Addo Elephant National Park. Creda Press, Cape Town.

Halffter G. 1974. Elements anciens de l'entomofaune néotropical: ses implications biogéographiques. *Quaest. Entomol* **10**: 223-262.

Halffter G. & Edmonds W.D. 1982. *The nesting behaviour of dung beetles (Scarabaeinae): an ecological and evolutive approach.* Publication 10, Instituto de Ecologia. Mexico, D.F.

Halffter G. & Mathews E.G. 1966. The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera: Scarabaeidae). *Folia Entomologica mexicana* **12-14**: 1-312.

Hall A.V. 1987. Threatened plants in the fynbos and karoo biomes, South Africa. *Biological Conservation* **40**: 11-28.

Hanski I. 1991. In: *Dung beetle ecology* (eds I. Hanski & Y. Camberfort) Princetown University press, Princeton.

Hanski I. & Camberfort Y. 1991. Dung beetle ecology (eds I. Hanski & Y Camberfort) Princetown University press, Princeton.

Harley E.H. 1990. RESOLVE, version 3.3, University of Cape Town, South Africa.

Harrison R.G. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Tree* **4** (1): 6-11.

Hendey Q.B. 1983. Palaeontology and Palaeoecology of the fynbos region: An Introduction. In Deacon H.J., Hendey Q.B. & Lambrechts J.J.N. 1983. Fynbos Paleoecology: A preliminary synthesis. South African National Scientific Programmes Report no 75, December.

Hillis D.M. & Moritz C. 1990. *Molecular Systematics*. Sinauer, Sunderland, MA

Hillis D.M. & Bull J.J. 1993. An emperical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*. **42**: 182-192.

Hill C.J. 1996. Habitat specificity and food preference of an assemblage of tropical Australian dung beetles. *Journal of Tropical Ecology*. **12**: 449-460.

Hurt C.R. & O.J.H. Bosch 1991. A comparison of some range condition assessment techniques used in southern African grasslands. *Journal of the Grassland Society of South Africa*. **8** (4): 131-137

Jansens A. 1938. In: A revision of the African Canthonina (Coleoptera: Scarabaeidae: Scarabaeinae) by Scholtz C.H. & Howden H.F. 1987. *Journal of the Entomological Society of southern Africa*. **50** (1): 75-119.

Jin L. & Nei M. 1991. Relative efficiencies of the maximum-parsimony and distance-matrix methods of phylogeny construction for restriction data. *Molecular Biology and Evolution* **8**: 356-365.

Jones G.E. 1987. The conservation of ecosystems and species. London: Croom Helm.

Kellert S.R. 1993. Values and Perceptions of Invertebrates. *Conservation Biology* **7** (4): 845-855.

Klein B.C. 1989. Effects of forest fragmentation on dung and carrion beetle communities in central Amazonia. *Ecology* **7** (6): 1715-1725.

Knowlton N., Weight L.A., Solórzano L.A., Mills D.K. & Bermingham E. 1993. Divergence in proteins, mtDNA and reproductive capability across the *Isthamus* of Panama. *Science* **260**: 1629-1632.

KocherT.D., Thomas W.K., Meyer W.K., Edwards S.V., Paabo S., Villablanca F.X. & Wilson A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA.* **86** :6169-6200.

Kolmogorov A. 1941. Confidence limits for an unknown distribution function. *Annals of the Mathematical Statistics.* **12**: 461-463.

Lambrechts J.J.N. 1983. Soils, soil processes and soil distribution in the fynbos region: An introduction. In: Deacon H.J., Hendey Q.B. & Lambrechts J.J.N. 1983. Fynbos Paleoecology: A preliminary synthesis (Eds H.J. Deacon, Q.B. Hendey & J.J. Lambrechts).

Lande R. 1988. Genetics and the role of demography in Biological Conservation. *Science* **241**: 1455-1460.

Lavery S., Lui H. & Beckenbach T. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among ten orders of insects. *Molecular Phylogenetics and Evolution* **1**: 41-52.

Lawes M.J. 1990. The distribution of the samango monkey (*Ceropithecus mitis erythrarchus* Peters 1852 and *Ceripithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of Biogeography* **17**: 669-680.

Legge J.T., Roush R., Desalle R., Vogler A.P. & May B. 1996. Genetic criteria for establishing evolutionary significant units in Cryan's Buckmoth. *Conservation Biology* **10** (1): 85-98.

Levin M.D. 1983. Value of bee pollination to US agriculture. *Bulletin of the Entomological Society of America* **29**: 50-51.

Lobo J.M. 1996. Diversity, biogeographic considerations and spatial structure of a recently invaded dung beetle (Coleoptera: Scarabaeidae) community in the Chihuahuan desert. *Global Ecology and Biogeographic Letters* **5**: 342-352.

Low A.B. & Rebelo T.G. 1996. Vegetation of South Africa, Lesotho and Swaziland. Published by the Department of Environmental Affairs & Tourism, Pretoria.

Maqueen A., Wallis M.M.H. & Doube B.M. 1986.Seasonal changes in favourability of cattle dung in central Queensland for three species of dung-breeding insects. *Journal of the Australian Entomological Society* **25**: 23-29.

Martins E. & Cantel E.P.B. 1997. The african beetle *Onthophagus gazella* maintained in terraria at Fazenda Experimental Getulio Vargas in Uberaba (MG)-Brazil. *Revista Brasileira de Biologia.* **57** (3): 403-409.

Matthee C.A. & Robinson T.J. 1997. Molecular phylogeny of the springhare, *Pedetes capensis*, based on mitochondrial DNA sequences. *Molecular Biology and Evolution* **14** (1): 20-29.

Matthiessen J.N. & Hayles L. 1983. Seasonal changes in characteristics of cattle dung as a resource for an insect in southwestern Australia. *Australian Journal of Ecology* **8**: 9-16.

MacMillan W.O. & Bermingham E. 1996. The phylogeographic pattern of mitochondrial DNA variation in Dall's porpoise *Phocoenoides dalli. Molecular Ecology* **5**: 47-61.

Meffe G.K. & Carroll C.R. 1994. *Principles of Conservation Biology.* Sinauer, Sunderland, MA.

Menges E.S. 1992. In: *Conservation Biology: the theory and practice of nature conservation, preservation and management.* (edd. P.L. Fiedler and S.K. Jain.)

Mentis M.T. 1981. Evaluation of the wheel-point and step-point methods of veld evaluation. *Proceedings of the Grassland Society of South Africa.* **16**: 89-94

Miller R.G. Jr. 1966. *Simultaneous Statistical Inferences.* McGraw-Hill, New York. pp 272.

Moritz C. 1994. Application of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* **3**: 403-413.

Nei M. & Miller J.C. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics* **125** 873-879.

Nei M. & Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* **97**: 145-163.

Nicholson S.W. 1987. Absence of endothermy in flightless dung beetles from southern Africa. *South African Journal of Zoology*. **22** (4): 323-324.

Nigro L.M. & Grapputo A. 1993. Evolution of mitochondrial rRNA in the oriental species subgroup of Drosophila. Biochem. *Systematic Ecology* **21**: 79-83.

O'Brien S.J. & Mayr E. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* **251**: 1187-1188.

O'Brien S.J. 1994. A role for molecular genetics in biological conservation. *Proceedings of the National Academy of Sciences* **91**: 5748-5755.

Oates M.R. & Warren M.S. 1990. A Review of Butterfly Introductions in Britain and Ireland. Report for the Joint Committee for the Conservation of British Insects funded by the World Widw Fund for Nature.

Osberg D.C. & Hanrahan S.A. 1992. The spatial distribution of *Allogymnopleurus thalassinus* Klug and *A. consocius* Péringuey (Coleoptera: Scarabaeidae) in an area of mixed soil types in South Africa. *Journal of the Entomological Society of southern Africa* **55** (1): 85-92.

Prinsloo P. & Robinson T.J. 1992. Geographic mitochondrial DNA variation in the Rock Hyrax, *Procavia capensis. Molecular Biology and Evolution* **9** (3): 447-456.

Pope L.C., Sharp A. & Moritz C. 1996. Population structure of the yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1984) inferred from mitochondrial DNA sequences and microsatelite loci. *Molecular Ecology* **5**: 629-640.

Prüser F. & Mossakowski D. 1998. Low substitution rates in mitochondrial DNA in Mediterranean carabid beetles. *Insect molecular Biology* **7**: 121-128.

Riano M. 1966. Abundance and phenology of some coprophagous beetles in different kinds of dung. *Annual Zoological Fenn.* **3**: 88-98.

Robinson T.J. & Harley E.H. 1995. Absence of geographic chromosomal variation in the roan and sable antelope and the cytogenetics of a naturally occuring hybrid. *Cytogenetic Cell Genetics* **71**: 363-369.

Rodger S.O. & Bendich A.J. 1985. Extractions of DNA from milligram amounts of fresh, herbarium and mummified plant tissue. *Plant Molecular Biology* **5**: 69-76.

Rogers A.R. & Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**: 552-569.

Ryder O.A. 1986. Species conservation and systematics: the dilemma of subspecies. *Tree* **1** (1): 9-10.

Saitou N. & Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Moleclar Biology and Evolution* **4**: 406-425.

Samways M.J. & Moore S.D. 1991. Influence of exotic conifer patches on grasshopper (Orthoptera) assemblages in a grassland matrix at a recreational resort, Natal, *South Africa. Biological Conservation* **57**: 117-137.

Samwys M.J. 1994. *Insect Conservation Biology*. Chapmann & Hall. London, UK.

Saunders N.C., Kessler L.G., Avise J.C. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus* . *Genetics* **112**: 613-627.

Schackleton N.J. & Kennett J.P. 1975. Paleotemperature history of the Cenozoic and the initiation of Antarctic glaciation: oxygen and carbon isotope analyses in DSDP sites 277, 279 and 281. *Initial Reports of the Deep Sea Drilling Project* **29**: 743-755.

Scholtz C.H. & Howden H.F. 1987. A Revision of the African Canthonina(Coleoptera: Scarabaeidae: Scarabaeinae). *Journal of the Entomological Society of southern Africa.* **50** (1): 75-119.

Scholtz C.H. & Coles K.S. 1991. Descripton of the larvae of *C.bacchus* (Fabricius) Coleoptera; Scarabaeidae: Scarabaeinae*). Journal of the Entomological Society of southern Africa* **50:** 261-264.

Scott L. 1994. Palynology of late Pleistocene Hyrax middens, Southwestern Cape province, South Africa: a Preliminary report. *Historical Biology* **9**: 71-81.

Scott L., Steenkamp M. & Beaumont P.B. 1995. Palaeoenvironmental conditions in South Africa at the Pleistocene-Holocene transition. *Quaternary Science Reviews* 14: 937-947.

Simberloff D. 1998. Flagships, umbrellas and keystones: is single-species management passe in the landscape era? *Biological Conservation* **83**(3): 247-257.

Simon C., Frati F. Beckenbach A., Crespi B., Liu H. & Flook P. 1994. Evolution, Weighting and Phylogenetic Utility of Mitocondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annual Entomological Society of America* **87**(6): 651-701.

Slatkin M. & Hudson R.R. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**: 555-562.

Sneath P.MA. & Sokal R.R. 1973. Numerical taxonomy. W.H. Freeman, San Francisco, U.S.A.

Sober E. 1989, Reconstructing the past: Parsimony, Evolution and Inference. MIT Press, Cambridge, MA.

Sperling F.A.H. & Hickey D.A. 1994. Mitochondrial DNA sequence variation in the spruice budworm species complex (Choristoneura: Lepidoptera). *Molecular Biology and Evolution* **11**: 656-665.

Swofford D.L. & Olsen G.J. 1990. Phylogeny reconstruction. In: *Molecular systematics,* (eds.) D.M. Hillis & C. Moritz, Sinauer, Sunderland.

Takhata N. & Palumbi S.R. 1985. Extranuclear differentiation and gene flow in the finite island model. *Genetics* **109**: 441-457.

Thackeray J.F. 1987. Late Quaternary environmental changes inferred from small mammalian fauna, southern Africa. *Climatic Change* **10**: 285-305.

Tilman D., May R.M., Lehman C.L. & Nowak M.A. 1994. Habitat destruction and the extinction debt. *Nature* **371**: 65-66.

Tribe G.D. 1976. The ecology and ethology of ball-rollong dung beetles (Coleoptera: Scarabaeidae). M.Sc. thesis, University of Natal, Pietrmaritzburg, South Africa.

Tyson P.D. 1986. Climate change and variability in southern Africa. Oxford University Press, Cape Town.

Vogler A.P., Kinsley C.B., Glueck S.B., Hill J.M. & DeSalle. 1993. Using molecular and ecological data to diagnose endangered populations of the puritan tiger beetle *Cicindela puritana. Molecular Ecology* **2**:375-383.

Vrba E.S. 1985. Environment and evolution: alternative causes of the temporal distribution of evolutionary events. *South Africa Journal of Science* **81**: 229-236.

Vrba E.S. 1990. Mammals as a key to evolutionary theory. *Journal of Mammology* **73** (1): 1-28.

Wells S.M., Pyle R.M. & Collins N.M. 1983. The International Union for the Conservation of Nature and Natural Recourses Invertebrate Red Data Book. Cambridge, UK.: International Union for the Conservation of Nature and Natural Recourses.

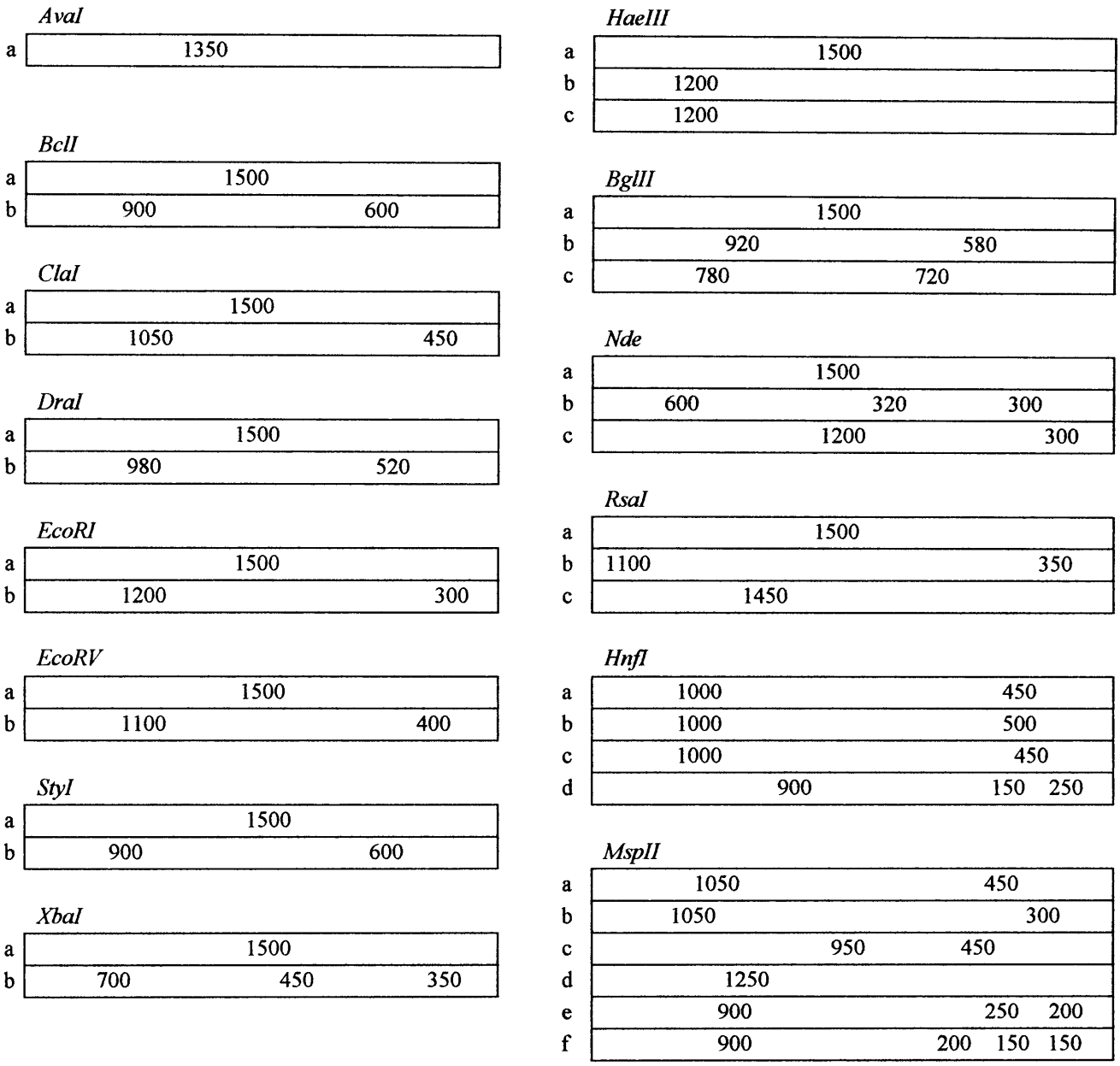
Ward D. & Seely M.K. 1996. Competition and habitat selection in Namib desert tenebrionid beetles. *Evolutionary Ecology* **10**: 341-359.

Wilson E.O. 1984. Biophilia. Harvard University Press, Cambridge, MA.

Wright S. 1977. Evolution and the genetics of populations, Experimental Results and Evolutionary Deductions. (University of Chicago Press, Chicago, vol 3).

Zar J.H. 1984. *Biostatistical Analysis*, second edition. Prentice-Hall, Inc.

Appendix 1



Schematic representation of COI/COII mtDNA restriction fragment patterns generated by a suit of 14 restriction endonucleases. Fragment sizes have been scaled so that their sum equals the estimated size of the amplified target DNA which was 1500bp.

Appendix 2

Percentage sequence divergence above the diagonal (corrected by Jukes-Cantor) and minimum number of mutational steps below the diagonal for the 28 mtDNA haplotypes in the COI/COII gene section sampled in *Circellium bacchus*. Sequence divergence values are based on the number of shared fragments between the different haplotypes. Divergence values of 0 indicate values which are smaller than 0.01.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Aa	Ab
A	*	0.66	0.82	2.28	1.19	2.73	4.21	2.03	3.00	4.16	5.64	3.73	5.56	3.68	4.11	4.74	5.20	7.82	5.88	4.43	2.88	6.10	2.70	4.20	2.10	1.23	5.03	5.33
B	2	*	0.72	2.06	2.11	4.25	5.74	3.67	4.29	5.41	10.10	6.13	9.96	6.05	5.42	6.22	7.53	10.37	8.48	6.48	4.28	7.87	4.64	6.04	3.56	2.59	6.42	7.74
C	6	6	*	3.24	2.21	5.13	6.56	3.98	4.80	8.02	0.40	7.74	0.40	7.64	5.50	6.47	8.91	11.29	9.42	8.07	4.38	8.42	5.27	6.63	3.95	2.82	6.77	9.18
D	6	6	10	*	0.81	3.20	5.67	9.46	7.14	6.75	6.07	3.87	9.58	7.34	8.30	10.02	8.66	14.14	9.59	8.25	7.70	6.56	4.66	8.19	5.95	6.92	7.11	10.50
E	4	6	8	2	*	1.91	3.72	6.91	4.77	7.13	5.41	3.35	8.95	6.83	6.48	7.54	5.63	10.75	6.27	5.39	5.83	4.44	2.53	6.10	3.74	5.28	4.78	7.33
F	10	12	16	8	8	*	0.13	2.33	1.83	3.03	3.42	3.65	2.25	2.51	7.12	6.99	4.68	5.19	5.48	4.24	2.57	0.99	3.15	1.99	2.05	2.40	1.23	1.78
G	10	12	16	10	8	4	*	3.34	2.79	3.04	3.93	3.22	3.13	2.45	1.36	1.96	1.14	5.76	2.10	0.43	2.69	1.26	2.87	2.09	3.79	2.44	2.31	2.62
H	12	14	16	14	12	8	8	*	3.28	3.53	3.33	4.03	2.18	2.90	3.46	3.13	4.54	3.84	5.50	5.01	1.70	1.97	2.54	2.79	0.45	0.87	0.98	2.24
I	8	10	12	12	10	8	8	4	*	5.92	2.79	3.46	4.82	5.44	4.28	3.98	5.37	4.44	6.10	4.54	3.60	4.43	2.74	4.77	1.91	3.35	2.69	4.30
J	16	16	20	10	12	8	6	8	12	*	2.09	1.58	0.96	0.48	5.66	6.57	6.20	5.68	7.30	5.86	3.95	2.45	3.02	3.26	2.64	1.93	3.31	3.96
K	14	16	18	12	12	6	6	8	8	4	*	0.49	1.17	1.63	4.47	3.80	3.29	3.08	4.14	3.13	4.03	2.80	1.91	3.04	1.58	3.45	2.52	2.47
L	13	15	17	11	11	7	5	9	9	3	1	*	1.63	1.11	3.58	4.47	3.82	3.60	4.62	3.63	3.12	2.08	1.21	2.33	2.28	2.57	3.25	3.04
M	16	18	20	12	12	6	6	6	10	2	2	3	*	0.48	6.02	5.40	5.14	4.15	5.89	4.90	4.25	2.44	3.04	3.29	1.56	2.36	2.19	2.73
N	15	17	19	11	11	7	5	7	11	1	3	2	1	*	5.10	6.02	5.61	4.63	6.32	5.35	3.34	1.74	2.33	2.58	2.25	1.52	2.91	3.29
O	10	12	14	14	12	12	10	4	6	10	10	9	10	9	*	0.39	1.74	2.53	2.76	2.30	1.76	3.59	2.38	2.64	2.55	3.37	3.50	3.30
P	11	13	15	15	13	11	11	3	5	11	9	10	9	10	1	*	1.21	2.03	2.28	1.80	2.21	4.28	3.04	3.30	2.22	3.84	3.16	2.79
Q	14	16	18	12	10	10	8	6	10	8	8	9	6	7	6	5	*	1.66	1.18	0.68	3.57	4.02	2.57	2.87	4.31	5.38	4.56	2.46
R	19	21	17	19	17	13	11	9	11	11	9	10	9	10	9	8	7	*	0.42	2.09	3.51	3.41	2.62	2.84	4.82	4.46	3.75	2.44
S	17	19	15	17	15	15	13	11	13	13	11	12	11	12	9	8	5	2	*	1.63	4.55	5.07	3.68	3.95	5.01	6.05	5.29	3.59
T	12	14	18	12	10	8	6	8	12	8	8	9	6	7	8	7	2	9	7	*	4.07	4.54	3.16	3.44	4.13	5.15	4.36	3.06
U	9	11	13	13	11	9	7	3	3	9	7	6	9	8	3	4	9	10	12	11	*	1.32	1.87	0.78	2.13	1.67	1.37	1.41
V	13	15	17	11	9	7	3	5	7	5	5	4	5	4	7	8	5	8	10	7	4	*	0.90	0.57	5.02	1.78	1.94	1.09
W	15	17	19	13	11	9	5	7	9	7	7	6	7	6	7	8	5	8	10	7	6	2	*	1.17	1.81	2.32	2.77	1.71
X	12	14	16	12	10	8	4	6	6	6	4	3	6	5	6	7	6	7	9	8	3	1	3	*	3.18	2.56	2.43	0.50
Y	8	10	12	12	10	8	8	4	4	10	6	7	8	9	4	3	8	11	11	8	3	7	9	6	*	0.39	3.00	2.68
Z	10	12	14	12	10	8	6	2	6	6	8	7	6	5	4	5	8	11	13	8	3	5	7	6	4	*	0.89	3.22
Aa	10	12	14	12	10	6	6	2	4	8	6	7	6	7	6	5	8	11	13	8	3	5	7	6	2	2	*	1.8i8
Ab	13	15	17	13	11	7	5	5	5	7	3	4	5	6	7	6	5	6	8	7	4	2	4	1	5	7	5	*

Appendix 3

Presence (1) versus absence (0) restriction site matrix of mtDNA COI\COII generated by 14 restriction endonucleases and used in the parsimony analysis.

Haplotype	Restrition enzymes													
number	*AvaI*	*HnfI*	*DraI*		*ClaI*	*Msp*	*Nde*		*BclI*	*BglII*	*EcoRI*	*EcoRV*	*HaeIII*	*RsaI*	*StyI*
A	1	01001	0	1	1010	111	1		10	1	0	11	11	0	0
B	1	01001	0	1	1010	111	1		10	1	1	11	11	1	0
C	1	01001	0	1	1010	111	1		10	1	1	11	11	0	1
D	1	01001	0	1	0010	001	1		00	1	0	11	11	1	0
E	1	01001	0	1	0010	001	1		10	1	0	11	11	0	0
F	1	01001	0	0	0010	001	0		00	0	0	00	10	0	0
G	1	01001	1	1	0010	000	0		10	0	0	00	10	0	0
H	1	01000	1	0	0011	111	0		10	0	0	00	00	0	0
I	1	01000	1	0	1010	111	0		10	0	0	00	11	0	0
J	1	01001	1	1	0011	000	0		01	0	0	00	00	1	0
K	1	01001	1	0	1010	000	0		01	0	0	00	00	0	0
L	1	01001	1	1	1010	000	0		01	0	0	00	00	0	0
M	1	01001	1	0	0011	000	0		01	0	0	00	00	0	0
N	1	01001	1	1	0011	000	0		01	0	0	00	00	0	0
O	1	01000	1	1	1111	111	0		10	0	0	01	00	0	0
P	1	01000	1	0	1111	111	0		10	0	0	01	00	0	0
Q	1	01000	1	0	0111	000	0		10	0	0	11	00	0	0
R	1	10110	1	0	1111	000	0		10	0	0	00	00	0	0
S	1	01000	1	0	0111	000	0		10	0	0	11	00	0	0
T	1	10110	1	0	1111	000	0		10	0	0	11	00	0	0
U	1	01001	1	0	0111	000	0		10	0	0	11	00	0	0
V	1	01000	1	1	1010	111	0		10	0	0	00	00	0	0
W	1	01000	1	1	0010	000	0		10	0	0	00	00	0	0
X	1	01000	1	1	0100	000	0		10	0	0	00	00	0	0
Y	1	01000	1	1	1010	000	0		10	0	0	00	00	0	0
Z	1	01001	1	0	1010	111	0		10	0	0	01	00	0	0
Aa	1	01001	1	1	0011	111	0		10	0	0	00	00	0	0
Ab	1	01001	1	0	0010	111	0		10	0	0	00	00	0	0
Ac	1	01000	1	0	1010	000	0		10	0	0	00	00	0	0