The Evolution of Dung Beetle Assemblages

by

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Onthophagus neostenocerus (Australia)

Abstract

Dung beetles (Scarabaeinae) are a well studied group, not least because of their clearly defined taxonomic and ecological boundaries. The majority of preceding studies have taken a limited local-level view of dung beetle systems, where local species interactions have been assumed to play the main role in structuring assemblages. Many other generally accepted hypotheses regarding scarabaeine evolution also remain untested. This includes the notion that the two main functional nesting groups, the rollers and the tunnellers, are monophyletic sister taxa. This is the first study to investigate the processes acting upon assemblage structure at both local and regional levels, using a number of different approaches, including molecular phylogenetics and analyses of functional diversity and ecomorphology.

Based on sequence information from three gene regions (COI, 16S rRNA, and 28S rRNA) and 200 taxa, the molecular phylogenetic hypothesis suggested rolling and tunnelling each originated on multiple occasions in the Scarabaeinae, and that significant parts of the higher level taxonomy of the group were misleading. Perhaps most importantly, the phylogeny was seen to be closely associated with continental biogeographic history.

Using morphometric techniques, dung beetle assemblages were characterised by the gross functional morphology of their component species, and compared within and across regions. Whilst species richness of assemblages was seen to influence species packing, the total area of ecomorphological space (niches) occupied remained consistent. Functional groups were clearly separated from each other in morphospace, and were seen to be largely constrained by phylogenetic ancestry. However, convergence in function was seen to be accompanied by convergence in morphology where strong environmental pressures or ecological opportunity prevailed.

Small-scale species interactions were seen to be of lesser importance in structuring assemblages than large-scale historical processes. Within regions, habitat was the main driver, but across regions, phylogenetic history was the key structuring force.

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Declaration

The material contained in this thesis is my own original research except for the following parts:

(1) *Chapter 2*
Some of the gene sequences used in the phylogenetic analysis were contributed by Claire Wilsher and Jason Maté. The POY analyses and Bremer support were run on the parallel computer cluster by Alfried Vogler.

(2) *Chapter 5*
All raw morphometric measurements of the African species were collected by Claire Wilsher, and all raw measurements of the Australian species were collected by Richard Davies.

Daegan Inward

Chapter 1. Introduction

"No one fails to be surprised when he firsts finds himself in the presence of the Scarab..."

So wrote the entomologist J.H. Fabre (1918) on the extraordinary behaviour by which dung beetles are most well-known, the rolling of balls of dung. It was probably this highly complex and adaptive trait that also inspired the ancient Egyptians to give the dung beetle a leading role in their mythology (discussed in depth by Cambefort 1987). However, in addition to this remarkable strategy, there is an impressive diversity of behaviour, form and other biological attributes amongst the dung beetles. As much as anything, it is this conspicuous diversity that has fuelled the wide range of research that continues on the group to this day.

Fabre was one of the earliest workers to seriously observe and consider dung beetle behavioural and nesting strategies. Some of the most significant later pieces of work contributing to our current understanding of dung beetles include that of Halffter and Matthews (1966), who explored much new ground in general Scarabaeinae biology, followed by Halffter and Edmonds (1982) on their nesting behaviour. More recently, a comprehensive review of the work and literature existing on the group was compiled by Hanski and Cambefort (1991). Dung beetles remain a focus of much taxonomic work and, because of their well-defined ecological boundaries and potential for use as a 'model' system, are a popular study group with population and community ecologists, as well as evolutionary biologists. They are of importance to farmers due to their key roles in soil health and fertility, and offer great potential as an indicator group of environmental disturbance. The hypotheses of dung beetle evolution and radiation given mainstream acceptance (e.g. Cambefort 1991a, Zunino 1985, Halffter 1964) remain entirely untested, and sometimes implausible. There is a tendency to invoke competition as the key determinant of assemblage structure and species diversity, even though good evidence of this is scant. No robust phylogeny for the group has yet been produced, and no attempt made to compare and contrast dung beetle faunas from across the world to understand how the group has evolved under different biogeographic histories. This thesis attempts firstly to incorporate existing knowledge of dung beetle ecology into a new phylogenetic and biogeographic framework. It will utilise a number of different analytical approaches to search for patterns of convergence or divergence in

independently evolved dung beetle assemblages, and identify the key processes which have influenced the evolution of the group in different habitats and continents.

1.1 The 'dung beetles'

Animal dung is a rich resource which is sought after by a range of insects. Coleoptera are a major component of the dung insect community in almost all parts of the world, and in tropical habitats such as African savanna, are the single most species rich and abundant group to exploit the resource. As many as 16,000 dung beetles have been recorded as visiting a single pile of elephant dung (Anderson and Coe 1974). Worldwide, 'dung beetles' can be considered to include some 150 species of the subfamily Geotrupinae in the family Geotrupidae, and approximately 2000 species of Aphodiinae and 5000 species of Scarabaeinae, both in the family Scarabaeidae. All three subfamilies are members of the superfamily Scarabaeoidea, a large and well-studied group of beetles. A total evidence phylogeny of the Scarabaeoidea was recently produced by Browne and Scholtz (1999), based on a range of available data from larval and adult morphology. This suggested the Geotrupidae to be relatively distantly related to the other dung beetles, being amongst one of two basal scarabaeoid lineages. The Aphodiinae and Scarabaeinae are considered to be sister groups within another lineage which also includes the 'higher' taxa usually associated with the Scarabaeidae such as the Melolonthinae, Cetoniinae and Dynastinae. Other scarabaeoid families such as Hybosoridae and Trogidae are also sometimes attracted to dung, although this is not typical for these groups. A few other Coleoptera taxa, such as the Oxytelinae (Staphylinidae), and some adult Hydrophilidae also feed on dung, but have not diversified or speciated to the same extent as their scarabaeoid counterparts.

Dung feeding in the adult Aphodiinae and Scarabaeinae is assisted by a filtering structure within their mandibles to allow only small particles and liquids to be ingested (Hata and Edmonds 1983). However, their larvae and the Geotrupidae retain the hard biting mandibles shared by the rest of the Scarabaeoidea. The majority of species of the Geotrupidae and many Aphodiinae consume other food resources such as humus, roots and fungus (Scholtz and Chown 1995, Cambefort 1991c), with dung-feeding developing as a more derived behaviour. The great majority of Scarabaeinae however are coprophagous (exceptions are discussed below), and have evolved a wide range of complex nesting and behavioural mechanisms specifically for dealing with this

resource, the likes of which are not seen in the other groups. It is primarily for this reason that the Scarabaeinae are often considered the 'true' dung beetles.

1.2 Distribution of the dung beetle groups

Dung beetles have an interesting worldwide distribution pattern, whereby the dung insect communities of the temperate regions (particularly in the northern hemisphere) are dominated by species of Aphodiinae and Geotrupinae. In Britain for instance, there are in total 59 species of Aphodiinae (Jessop 1986) compared to only 8 species of Scarabaeinae (all of which are of the same genus *Onthophagus*). Although the coprophagous Geotrupinae are primarily restricted to the temperate regions, perhaps due to a poor competitive ability compared to the Scarabaeinae (Cambefort 1991c), the Aphodiinae are more widespread in the tropics, though they too are also much more species rich at higher latitudes. North temperate dung communities tend to have a high diversity of coprophagous beetles and flies, and a large number of predatory mites, Hymenoptera, Coleoptera, and Diptera (Hanski 1987). Tropical dung communities on the other hand tend to be dominated almost totally by Scarabaeinae, which have a pan-tropical distribution. South Africa boasts some 800 species of Scarabaeinae, compared to around 60 species of Aphodiinae (Doube 1991), and a single African savanna assemblage can contain up to 150 scarabaeine species (pers. obs., see data sets within Hanski and Cambefort 1991).

1.3 The nature of dung as a resource, and the role of competition

As a resource, dung is patchy and ephemeral, and this leads to particular dynamics within the dung insect community. All dung beetles tend to exist in multi-species assemblages, which is why they are often studied at this level. The high species richness seen in dung communities, and the great number of individuals that can be attracted to a single dung pile has led to the general assumption (underlying much of the work of the last 40 years) that dung beetle evolution is driven predominantly by competition. Competition is often invoked as a selective pressure leading to resource partitioning in communities, so reducing competitive interactions between species and facilitating coexistence (Ricklefs and Miles 1994). It is however a difficult force to quantify, and competition theories in patchy environments tend to be model-based (discussed by McLaughlin and Roughgarden 1993). Very briefly, the environmental

heterogeneity (patchiness) of the dung environment is thought to create an asynchrony among species interactions in different areas (due to fluctuating densities of species at different habitat patches), so whilst a species might potentially be competitively excluded at one site, it would experience favourable conditions elsewhere (Hanski 1991b). This theoretically enables a variety of competitively superior and inferior species to coexist in an assemblage. Increased patchiness (more numerous but smaller resources) has also been empirically demonstrated to prolong coexistence of species of different competitive abilities in a carrion environment (Kneidel 1985). Competition does indeed appear to be quite severe in tropical dung beetle communities, affecting all levels of the beetles ecology and life cycle. Competition in dung beetles is thought to take various forms, from direct interference competition often seen between adult rollers where dung balls are stolen from one another (e.g. Matthews 1963, pers. obs.), to exploitative competition where a dung resource is depleted rapidly (Hanski and Cambefort 1991b and references therein), so that later arrivals are effectively excluded. The behaviour of dung beetles is apparently altered by such interactions, for instance large tunnellers will generally not remain to feed or breed at a dung pat which has already been occupied by another pair of large tunnellers (Hanski and Cambefort 1991d).

1.4 The Scarabaeinae

The particular subjects of this study are the pan-tropical, so-called 'true' dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). This group has several characteristics which make them a particularly good model system to study at a community level. Firstly, they are well-studied in terms of their taxonomy and natural history, thus there exist various useful hypotheses regarding their evolution and biogeography to consider and test, as well as various potentially useful data sets already existing in the literature. They are also an accepted monophyletic group (Balthasar 1963, Janssens 1949, Cambefort 1991c), with the Aphodiinae as the known sister group (Browne and Scholtz 1999). This makes phylogenetic analysis and consideration of evolutionary mechanisms within the group a more straight-forward affair. As dung beetles, the great majority of species within a single species pool or assemblage have well defined boundaries and resources. This enables accurate species composition data to be gathered by trapping, which can be done passively using baits and in a quantitative manner. This in turn makes the study and characterisation of an entire assemblage, or

indeed the comparison of multiple assemblages, a much simpler and more accurate proposition than the study of a more ecologically variable group of organisms such as phytophagous beetles, which would need to be primarily collected by hand from a great variety of hosts, or to rely on more observational techniques, which cannot be easily standardized. Furthermore, the dominance of Scarabaeinae in the tropical and sub-tropical dung communities, and the evolution of different functional adaptations for dealing with dung, means that they lend themselves well to a comparative global study on adaptation and evolution. Dung beetles therefore present an ideal model with which to investigate the roles of competition, local environmental conditions, and geographic and phylogenetic history in structuring community composition.

1.5 The taxonomy of the Scarabaeinae

I have chosen to adopt a classification used in many contemporary studies, for the convenience of employing the same terminology. Thus I have accepted the "Scarabaeinae" as a subfamily of the Scarabaeidae, a rank supported by the evidence presented by Browne and Scholtz (1999), and used also the system of 12 tribes described by Balthasar (1963). Many authors in the past, including those contributing to the important review work of Hanski and Cambefort (1991), have considered the Scarabaeinae to be at family level (i.e. as Scarabaeidae), so as to then be able to use a nomenclature which divides up the 12 tribes equally into two subfamilies, the "Scarabaeinae and Coprinae". These subfamilies represent the division in nesting behaviour seen in the dung beetles, namely those that make and roll dung balls and those that do not. Whilst this might seem to make phylogenetic sense (Cambefort 1991c), I did not wish to make such a priori assumptions of relationship, and thus have avoided its use. Since this study is concerned with the Scarabaeinae alone, the rank given to the groups are not strictly important at this stage, but the use of tribes reflects the current understanding of dung beetle history, and as such, will be tested within this project. Table 1.1 presents the 12 currently recognised tribes, their key nesting behaviours, and a broad indication of their global distributions.

Cambefort (1991a) has suggested that the different tribes of the Scarabaeinae can be separated by their hypothesized relative taxonomic ages and area of origin, into 3 basic age groups. These include an 'old' group with a Gondwanan origin, a 'modern' group with a holarctic origin and an 'intermediate' group, which includes endemic tribes

falling somewhere between the 2 other groups. As such, this hypothesis of relative age provides a useful model to test, but will not be adopted here because of its *a priori* assumptions.

Table 1.1. The worldwide distribution of the currently recognised Scarabaeinae tribes across the zoogeographical regions. The key nesting strategy for each tribe and the numbers of genera (and species) are given in each case. Data extracted from Cambefort (1991a).

Tribe	Key nesting strategy	Nearctic	Neotropical	Palearctic	Afrotropical	Oriental	Australian
Canthonini	Roller	4 (24)	27 (318)	1 (3)	33 (224)	8 (47)	29 (157)
Coprini	Tunneller	1 (9)	1 (17)	1 (6)	7 (242)	4 (93)	2 (17)
Dichotomiini	Tunneller	2 (5)	20 (564)		8 (126)	4 (16)	1 (14)
Eucraniini	Roller (Carrier)		4 (19)				
Eurysternini	Dweller		1 (22)				
Gymnopleurini	Roller			1 (6)	3 (64)	4 (34)	
Oniticellini	Tunneller / Dweller	1 (1)	4 (5)	3 (14)	11 (117)	5 (33)	
Onitini	Tunneller			3 (21)	17 (163)	2 (26)	
Onthophagini	Tunneller	1 (38)	1 (95)	3 (259)	30 (1114)	10 (423)	1 (280)
Phanaeini	Tunneller	1 (9)	12 (146)				
Scarabaeini	Roller			3 (24)	10 (118)	2 (8)	
Sisyphini	Roller		1 (2)	1 (1)	3 (44)	2 (11)	

1.6 The evolution of dung-feeding

The ancestral way of life for the larvae of the Scarabaeoidea has been suggested to be soil-dwelling, with a microbe-enriched diet of humus and the adults are believed to have fed upon fungi (Scholtz and Chown 1995). Cambefort (1991c) suggested that a shift from humus to dung-feeding might have been a straight-forward step, citing Fabre's (1910) observations that some saprophagous scarabs could be reared on dung and coprophagous species could be reared with humus. Dung beetle larvae were found to have a chamber in their gut devoted to cellulose-digestion through use of bacteria (Halffter and Matthews 1971), and this is thought to provide them with their primary nutrition, and is perhaps the ancestral condition in the Scarabaeoidea. However, the

most nutrient-rich component of both humus and dung, is the 'soup' of bacteria and fungi involved in the decomposition or digestion process (Cambefort 1991c). Based on gut morphology, Halffter and Matthews (1971) suggest almost all of the adult dung beetles, and some of the larvae, receive their primary nutrition from the microbial content of their food. This would seem likely to prove more accessible and nutrient-rich than the high cellulose and lignin content of humus and herbivore dung.

From the fossil record, we know that the major groups of the Scarabaeoidea were present by the beginning of the Tertiary period (Scholtz and Chown 1995), but the record for dung beetles is unfortunately poor. The earliest evidence of Scarabaeinae activity comes from fossilized dung balls from the late Oligocene period of Chile (Halffter 1959). *Scarabaeus*, *Sisyphus* and *Onthophagus* are reported from French and Swiss deposits of a similar age (Cambefort 1991a) and so this suggests considerable divergence in body form and behaviour by this time (approximately 30 million years ago). Suggestions have been made that dung beetles might have been using dinosaur faeces in the late Cretaceous period (Jeannel 1942), and in support of this, the burrows of dung beetles are believed to have been found beneath dinosaur coprolites, some 75 million years old (Chin and Gill 1996). However, of the extant groups of dung beetles, only Geotrupidae are represented as far back in the fossil record, and so we can only surmise whether it was these or ancestral Scarabaeinae that produced the burrows. Mammals are thought to have first evolved in the Late Triassic, some 210 million years ago, but most would probably have been small, insectivorous creatures at first (Cox 2000). It is generally thought that it was the radiation and diversification of the large herbivorous ungulates during the Tertiary that provided a significant source of dung which allowed the Scarabaeinae to diversify and radiate themselves (e.g. Cambefort 1991a).

1.7 Other feeding behaviours in the Scarabaeinae

It is noteworthy that some Scarabaeinae are humus-feeding such as the Australian genus *Cephalodesmius* (Monteith and Storey 1981), and the Neotropical genera *Bdelyrus* and *Bdelyropsis* (Cambefort 1991c), whilst the African genus *Coptorhina* feeds on fungus (C.H. Scholtz pers. comm.). These examples of saprophagy have generally been considered to represent parallel or simply primitive behaviour in the group, although various species of adult Scarabaeinae are attracted to decomposing fruit (pers. obs.,

Halffter and Matthews 1966), despite also being attracted to, and nesting in dung. These are probably examples of opportunistic foraging, and support the idea that dung and decomposing plant matter similarly supply the necessary nutrients for dung beetles. Examples of beetles specializing on fruit have also been recorded however (Davis and Sutton 1997).

Another resource utilized by many species of Scarabaeinae is carrion (necrophagy). The shift towards use of animal corpses has been attributed to step-wise progression initially via the more nitrogen-rich dung of omnivorous animals (Cambefort 1991c), such as that of apes and pigs. In the search for similar protein-rich resources, carrion was also used, leading to a specialization in some groups. Necrophagy is practised in almost all dung beetle assemblages, to varying extents, but has attracted most attention in the Neotropics, where it is especially evident. Halffter and Matthews (1966) were the first to catalogue the range of feeding behaviours in the Neotropical fauna, and present evidence of a wide range of resource use, from strict coprophagy through to strict necrophagy even within a single genus. They suggest that since a single species will feed on both dung and carrion, there is little difference between them as a food resource to a dung beetle, rather they believed the choice to be a result of local ecological factors. It is this which is suggested as the primary reason for the high incidence of necrophagy in the Neotropical dung beetles, where there is a paucity of large mammals and a relative scarcity of competing necrophagous insect groups such as the Silphidae (Coleoptera). This may be a habitat-driven phenomenon, since necrophagy is more widely reported in the tropical forests of South America and South East Asia than in the African savannas. Hanski and Cambefort (1991a) suggest that this may be due to fewer large herbivorous mammals in tropical forests, but a greater incidence of omnivorous mammals whose dung is more nutritionally similar to carrion. It is less well-known as to whether the necrophagous species breed in dung or carrion, but instances have been recorded of both strategies (Halffter and Matthews 1966).

1.8 The evolution of nesting behaviour

Members of the superfamily Scarabaeoidea all have a modified prothorax and large powerful coxae for burrowing (Browne and Scholtz 1999), as well as compact, well-sclerotized bodies (Scholtz and Chown 1995) important with such a lifestyle. Scarab larvae are also suited to subterranean living, with modifications such as sieve-like

spiracles which function effectively underground whilst preventing the entry of soil (Hinton 1967). All of the Scarabaeinae are capable diggers and the majority spend at least part of their lives underground.

In order to compare dung beetle assemblages in different parts of the world, where species may or may not be taxonomically related, it is useful to be able to relate to them in terms of 'functional groups'. These relate to various key strategies that define their life histories, morphology and behaviour. Three primary functional groups can be recognised in the Scarabaeinae in particular, reflecting the three main modes of nesting and feeding. These are the 'rollers', the 'tunnellers' and the 'dwellers'. These are discussed here in order of the complexity of behaviour that each entails. Various authors use the more technical terms of endocoprids for dwellers, paracoprids for tunnellers and telocoprids for rollers, but so as to avoid unnecessary confusion, they are not used here.

1.9 The dwellers

Dwellers (sensu Cambefort and Hanski 1991) can be characterized by their relatively simple behaviour at a resource. For the purposes of this project, the dwellers will be regarded as those species which nest and feed within the dung, or at the interface of the dung and soil surface. Thus dwellers consume dung where it is deposited and eggs are usually laid directly in the dung pat. The great majority of the Aphodiinae are dwellers, and very few species actually build true nests in the soil beneath (J. Maté pers. comm.). However, three genera of Scarabaeinae can also be considered to belong to this functional group. Two of them, *Oniticellus* and *Tragiscus* are members of the old world tribe Oniticellini, which otherwise contains true 'tunneller' species (Cambefort 1982). Unlike the Aphodiinae, *Oniticellus* actually constructs a nest within the dung pat, or immediately beneath it at the soil-dung interface (Davis 1989). It has been suggested that they are poor competitors and are only common in situations where more competitively superior rollers and tunnellers are uncommon (Cambefort and Hanski 1991).

The other genus which I am designating as dwellers for the purposes of this study, is *Eurysternus* (of the monogeneric Neotropical tribe Eurysternini). These are usually considered as rollers (e.g. Cambefort 1991a), a supposition supported by their roller-like hind legs (quite extreme curvature of the tibiae). Yet in a study of their feeding and

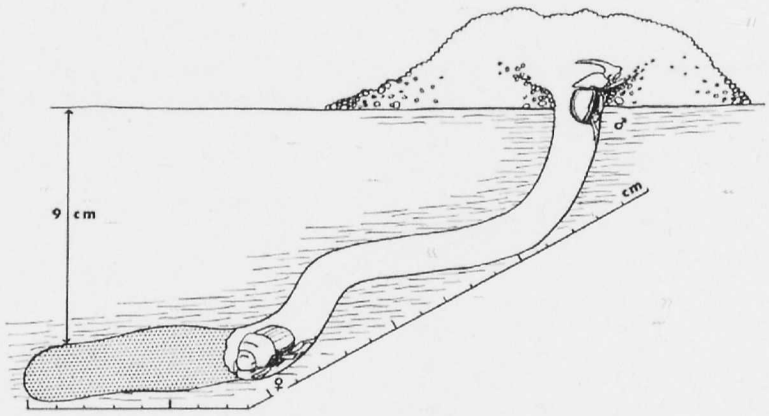
nesting behaviours, Halffter *et al* (1980) report a complete absence of ball formation for feeding purposes. Rather, eurysternines feed from the dung resource directly, often from within. Balls are in fact formed, but only by the female for reproductive purposes, and even then, these are not rolled away from the main resource, but rather formed within the dung pat at the soil interface. A shallow pit is dug beneath them and they are manoeuvred by butting with the head (Halffter *et al* 1980) - no actual rolling has ever been observed. Although the most appropriate way of classifying this behaviour remains debatable, the beetles actually remain within the main dung resource for both feeding and breeding purposes, and are thus regarded here as dwellers.

1.10 The tunnellers

Tunnellers are defined as those species which excavate tunnels directly beneath the dung resource, to the end of which dung is moved for feeding or breeding. Digging is generally performed by first loosening the soil with the head and forelegs, and pushed backwards behind the beetle. The beetle will then turn round and push the soil from the tunnel with its head, and only provision it with dung once finished (Halffter and Matthews 1966). Tunnels can be more or less complex, depending on its purpose and the species involved. Cambefort and Hanski (1991) provisionally defined seven nest types for the tunnellers based on the depth, branching structure, number of brood balls created, and the extent of male-female cooperation in its excavation. Tunnels are dug to a variety of depths by different species. In general, the smaller species construct relatively shallow nests with many brood masses. This results in greater fecundity and these species usually provide no parental care. The large tunnellers conversely tend to dig deeper tunnels and provision fewer brood masses. This generally reduces their overall fecundity, but maternal care is evident in some species (Cambefort and Hanski 1991). Plate 1.1 illustrates a typical burrow dug by a pair of tunnellers.

Tunnelling behaviour is exhibited by the Geotrupinae and a number of scarabaeine tribes, namely the Coprini, Dichotomiini, Onthophagini, Onitini, Phanaeini and the non-dwelling genera of Oniticellini. Some of these oniticellines such as *Drepanocerus* and *Cyptochirus* are 'shallow tunnellers', that is they nest in simple pits beneath the dung, or down a very short tunnel below it. This has been hypothesized to be the ancestral behaviour for Oniticellini and even the Scarabaeinae in general (Cambefort 1982).

Plate 1.1. A typical burrow dug beneath the dung resource by tunnellers, and provisioned with dung for feeding purposes. Note the sexual dimorphism in this species, *Phanaeus palliatus*, commonly seen in the tunnelling groups. Taken from Halffter and Matthews (1966).



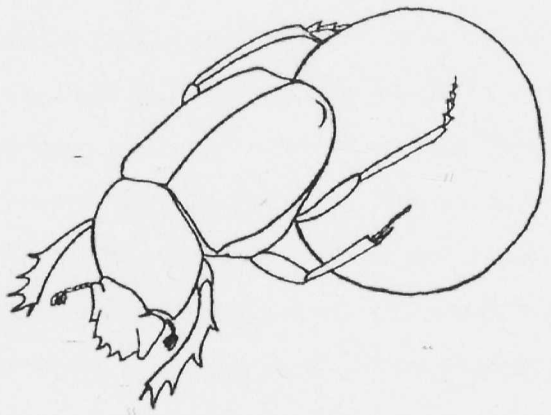
1.11 The rollers

The rollers are considered to be the most derived in terms of their behaviour, being characterised by the ability to remove and transport a part of the dung resource, by fashioning it into a ball *in situ* and then rolling it away with the hind legs. The dung is usually cut and carefully shaped to the correct size - a brood ball for nesting is generally larger than a food ball. The ball is pushed backwards, using the mid and hind legs to manipulate it (see Halffter and Matthews 1966 for a full discussion). Plate 1.2 illustrates the typical manner of this strategy. The ball can be transported some distance from the main resource before it is buried – up to 15 metres by the larger species. Rollers generally bury their balls by excavating the soil from underneath the food (Halffter and Matthews 1966, pers. obs.) and the whole process may be conducted with varying degrees of sexual cooperation, depending on the strategies of different species (Halffter and Edmonds 1982). In fact mates are often attracted by the preparation of a dung ball by one or other of the sexes (Halffter and Matthews 1966).

Tribes which exhibit rolling behaviour include the Canthonini, Gymnopleurini, Sisyphini and Scarabaeini. The Eucraniini are also often thought of as rollers (e.g. Cambefort 1991a) and are usually placed in the sub-group of rolling Scarabaeinae mentioned earlier. However, they in fact transport their food by grasping it with head

and fore-legs and carrying it, whilst running on the second and third pair of legs (Halffter and Matthews 1966 and references therein). Because of this unique behaviour, they will be designated as 'carriers' for the purposes of examining functional evolution in chapter 2. This is because the goal of overland transport might be the same as for rollers, but the method seems quite likely to be independently derived.

Plate 1.2. The typical method of rolling a dung ball, as illustrated here by a species of *Scarabaeus*. Taken from Halffter and Matthews (1966).



The transportation of dung overland has in fact also been seen in a few tunnelling species, and is perhaps noteworthy. Central American *Phanaeus* (Phanaeini) have been recorded as using the head to 'butt' pieces of dung away from the primary resource (Halffter *et al.* 1974, Gill 1991), and a variety of species of different tribes have been seen to drag dung towards a pre-formed burrow (Halffter and Matthews 1966) including members of the Onthophagini, Coprini, Dichotomiini and Phanaeini.

1.12 Dung beetles - a community, an assemblage or a guild?

An 'ecological community' is viewed in different ways by different ecologists. Strong *et al.* (1984) defined a community as a group of species that interact, or have the ability to interact with one another. In practice, studying an entire community is almost impossible unless they are very simple and ecologically isolated, and usually a simplified version or particular taxa are studied. There is however a distinctive dung community, which is composed of a variety of different functional groups. Dung feeders include numerous dipteran families such as Muscidae, Calliphoridae and

Sarcophagidae, and coleopteran groups including Staphylinidae and Hydrophilidae as well as the scarabaeoid groups. Predatory insects include nematodes, mites, Diptera as well as the beetle groups of Staphylinidae, Histeridae and Hydrophilidae which generally feed on other insects, such as fly larvae and even adult dung beetles. As well as this, mycetophagous (fungus-feeding) groups such as the cryptophagid beetles, and various parasitoid Hymenoptera contribute to the community. Thus a single group of dung beetles are best thought of as a guild or assemblage. Root (1967) defines a guild as being a group of species utilising similar resources in similar ways. Note that this does not imply any taxonomic boundaries, and that the Scarabaeinae could be further broken down into smaller guilds based on how they handle the dung (such as rollers and tunnellers). An assemblage is defined by Lawton (2000) as a taxonomically restricted group within a community. Although both terms might be applicable, in the context of my thesis, I use the term 'assemblage' rather than guild, because I am studying one particular subfamily, the Scarabaeinae, and largely excluding the Aphodiinae and Geotrupidae dung beetles from consideration. Concentrating on just the Scarabaeinae however, is justified in the sense that the other dung beetle groups play a very minor role in the tropical and sub-tropical dung communities under consideration here, whilst the Scarabaeinae form the vast majority of coprophagous organisms, in terms of diversity, abundance and biomass, to the almost total exclusion of the other groups. This also means that the strongest local species interactions occur between the scarabaeine species. Aphodiinae and Geotrupidae could not be put to one side in the same way in a northern, temperate dung community where they are more abundant and species rich.

1.13 Approaches and aims of the project

The aim of this project is to employ a number of different techniques to examine different aspects of dung beetle biology and through these, to understand how dung beetle assemblages evolve, by comparing different local and regional scarabaeine faunas. The key methodologies will include molecular phylogenetics, morphometric techniques, and multivariate ordination. A brief account of what I hope to achieve with these procedures follows here.

1.14 Molecular phylogenetics

Insect systematics has traditionally been a field in which morphological characters are the primary means of establishing taxonomic relationships, and it is really only since the advent of the polymerase chain reaction, or PCR (Mullis *et al.* 1986) that the enormous amount of untapped molecular data has become accessible on a large scale. Molecular phylogenetics interprets this information in terms of its evolutionary significance and aims to employ it in the reconstruction of genealogical relationships. Without a phylogenetic hypothesis, any theory of dung beetle evolution remains speculative and untested. Mapping a character onto a phylogeny however, enables us to differentiate between shared ancestry and the independent evolution of that character. A primary aim of this project then is to establish a phylogenetic hypothesis, so that we can investigate a number of important questions:

- When did dung feeding originate in the group? Were there multiple origins, and is there any evidence that the ancestral strategy was saprophagy?
- Is tunnelling the ancestral behaviour? Are the rollers a monophyletic group derived from the tunnellers, as expected from the apparent complexity of rolling? Where and how did the other nesting strategies such as carrying and dwelling arise?
- Are the hypothesised relationships of the tribes correct, and are they supported as being monophyletic clades?
- How have the dung beetle groups radiated in different regions of the world? Can the role of different biogeographic histories be determined? Can we infer the minimum ages of the various clades or splitting events?

1.15 Functional diversity

Because dung beetle communities are likely to be structured by a mixture of regional biogeographic processes, local ecological factors, and competitive interactions between species, a means of separating and quantifying these different influences would be highly informative. In order to compare and contrast assemblage structure between continents, and quantify the level of convergence between assemblages, we must first characterise the assemblages. One way to do this is by the functional roles played by their component species, and could include information regarding nesting strategy,

feeding behaviour or morphology. This part of the project will investigate the relative influences of the various local and regional ecological and environmental factors on assemblage structure, as well as assessing levels of convergence between independent assemblages (in different regions, but similar habitats for instance). Using multivariate statistical techniques, it is possible to analyse the variation in several related variables simultaneously. This is the true strength of the approach, since it is able to incorporate the complexity of an ecological system, rather than having to simplify it. A number of questions will be addressed with this technique:

- Within a species pool, how does the habitat type affect the structure of an assemblage?
- Within a habitat type, how does the regional history of an assemblage influence its structure?
- Under what circumstances do independently evolved assemblages converge in structure?

1.16 Morphological variation

The ecological and morphological diversity of the species within the Scarabaeinae is clearly considerable, and a means is needed to quantify the variation and to search for patterns in its distribution across different taxa, functional groups, local assemblages and regional faunas. One way to do this is to measure and compare morphology, since this reflects the key aspects of an organism's life history. Used in conjunction with the phylogenetic information, I hope to address several problems.

- To identify the key morphological attributes which are characteristic of the different functional groups, and necessary for their mode of life. Is the relationship between function and morphology a close one in scarabaeines?
- If the phylogeny identifies independent origins of functional types, can we identify convergence in body form (adaptation) from a similarity in morphology due to shared ancestry?
- To search for patterns of similarity in the morphological variation of dung beetle assemblages both within and between different habitat types and regions. Can we separate the influences of phylogenetic history from local environmental or intra-assemblage pressures?

- By investigating entire assemblages the relative positions of different species to each other in morphological and ecological space can be identified. By including information as to the breeding biology and feeding strategies of each species, further inferences as to the nature of competition and niche partitioning in assemblage structure will be made.

Chapter 2. A molecular phylogenetic analysis of the Scarabaeinae

2.1 Introduction

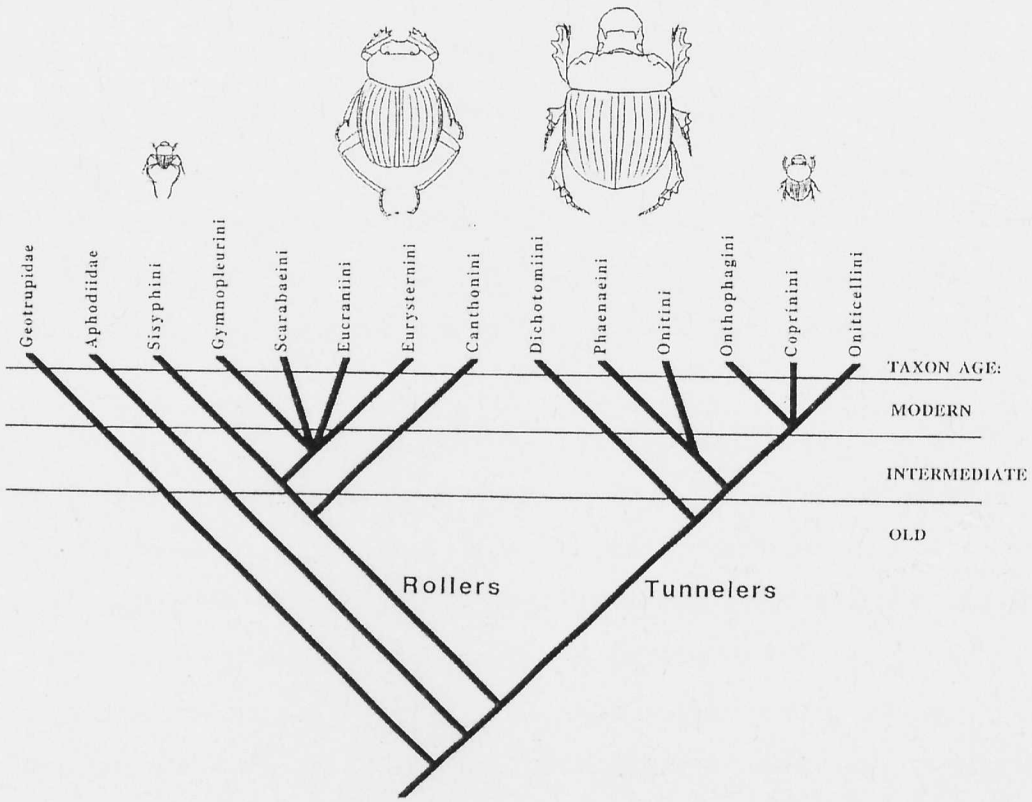
The use of molecular data in systematic studies has accelerated rapidly with the discovery of thermostable DNA polymerases such as *Taq*, isolated from the thermophilic bacteria *Thermus aquaticus*, and the polymerase chain reaction (PCR) (Mullis et al. 1986). With the addition of universal primers, the amplification and sequencing of DNA and RNA is now a comparatively simple operation, enabling access to a vast range of characters for use in phylogenetic analyses. Morphological similarity (and ecological function) between dung beetles on different continents is often striking and it can be difficult to distinguish homology (shared common ancestry) from convergence. Because I shall be investigating assemblage structure in this project, a phylogenetic hypothesis is of crucial importance in interpreting patterns in functional and morphological diversity. For example, using a phylogeny, Richman and Price (1992) were able to separate the effects of history and relatedness in a leaf warbler community and found that similarities in the species functional characteristics were adaptive.

2.1.1 Current hypotheses of dung beetle relationships

As discussed in chapter 1, the widely used Scarabaeinae classification of Balthasar (1963) divided the 12 tribes evenly into two clades based on their nesting behaviour, so that the rollers and tunnellers were placed into two sister groups. This reflects the notion that rolling is a highly derived and complex behaviour, and has only evolved once in the group. Rolling is thought to have evolved in the Canthonini before the break up of Gondwana (Cambefort 1991a), and to have subsequently been maintained in the major biogeographical regions (Africa, Australia and South America) by vicariance events and cladogenesis. This suggests that the Canthonini should be ancestral to all other rolling groups, the Scarabaeini, Gymnopleurini and Sisyphini in the Old World, and the Eurysternini and Eucraniini in the New World. In a similar way, the Dichotomiini are thought to be an old Gondwanian tribe which is ancestral to the other tunnelling groups, namely the Old World Onitini and Oniticellini, the globally distributed Coprini and Onthophagini and the Neotropical Phanaeini (Cambefort 1991a). This widely accepted theory has been challenged somewhat in the past

however. Using genitalic characters, Zunino (1984) postulated a relationship between the Afro-tropical Onitini and the Neotropical Phanaeini, which were then placed into a clade which included all of the rolling tribes, thus making this behaviour paraphyletic. Zunino found the rest of the tunnelling tribes formed a monophyletic sister clade. Figure 2.1 synthesizes and illustrates the key hypotheses put forward by the various authors who have been significant in our current understanding of dung beetle evolution. The suggested ages of the groups (Cambefort 1991a) are also presented. These are based on whether the tribes are thought to have arisen before the break up of Gondwana ('old' or "original" tribes), or in isolation in a region ('intermediate' or endemic tribes), or whether they are thought to be more recently derived groups which have only recently colonized wider areas ('modern' tribes).

Figure 2.1. Tree of the Scarabaeinae dung beetles summarizing the currently accepted hypotheses suggested by the current classification of Balthasar (1963) and evolutionary ideas suggested by Halffter and Matthews (1966), Cambefort (1991a) and Zunino (1984), and their relationship to other scarabaeoid dung beetles (Browne and Scholtz 1999). The tree is therefore rooted using the Geotrupidae and Aphodiidae as outgroups. Six tribes each of rollers and tunnellers have been classified as being of 'old', 'intermediate' or 'modern' age. Representatives of the main nesting groups are shown, which illustrate the differences in body size that exists in the group.



More recently, Montreuil (1998) examined 27 genera of Coprini and Dichotomiini, and using a range of morphological characters, determined that these two tribes were paraphyletic. However, this deals with only a small proportion of the total diversity, and it is unclear how these genera were selected. His findings were used to re-define two new monophyletic groups, the Ateuchini and Coprini. Whilst this evidence has been accepted by many already (Y. Cambefort pers. comm.), I have chosen to retain the use of Balthasar's 12 tribes here, since many genera are not included in Montreuil's analysis and would remain effectively 'unclassified'. Furthermore, the monophyly of most of the tribes has not been questioned, and the original tribes remain a useful framework in which to examine the Scarabaeinae. How this current classification represents the phylogeny is therefore of key interest in this chapter.

2.1.2 Methodological issues

Morphological studies on the group have suffered from two key problems: difficulties due to homoplasy, since many morphological characters are not independent of function, and character coding issues. Even within a single lineage, dung beetles exhibit highly diverse morphology, so the number of informative characters shared across the whole subfamily is potentially very low. Morphology in dung beetles is known to sometimes be influenced by environmental variation, for example by poor larval nutrition (Emlen 1997). Molecular sequence data on the other hand does not suffer from this sort of non-heritable variation and can be produced in a consistently objective manner, since the character state is unambiguous. The sheer abundance of molecular characters is an important consideration, and lends the data to more quantitative analysis than the more qualitative morphological information.

Molecular phylogenetics suffers from its own unique problems however. It requires fresh specimens, often limiting the scope of a study, and primers which amplify gene regions informative at the level being studied. Molecular data is plagued by background noise – for instance it is impossible to distinguish homology from analogy at individual base positions, especially since there are only 4 character states. Another serious drawback can be the problem of multiple substitutions (saturation). This is when older homologies are obscured by subsequent base substitutions, causing the data to be misleading. The completeness of taxon sampling can also affect the phylogenetic

accuracy obtained (reviewed by Caterino et al. 2000). One phenomenon often seen in studies such as this, where anciently diversified groups are included within the same data set, is that of long-branch attraction (Hendy and Penny 1989, Felsenstein 1978), commonly caused by very uneven rates of evolution along different branches on the tree - so two taxa have a greater chance of sharing a character by convergence than descent, and are falsely grouped together. By sampling the taxa densely however, the long branches can be broken up, circumventing this problem (e.g. Hillis 1996). Graybeal (1998) also found that by adding extra taxa, phylogenetic accuracy was enhanced more than by adding extra sequence data.

2.1.3 Choice of gene

Careful choice of the gene region to be studied can limit some of these problems and maximise the number of parsimony-informative characters produced. Mitochondrial DNA (mtDNA) has proven itself to be an invaluable tool in insect molecular systematics (see Simon *et al.* 1994 for a good review of its uses and limitations). It differs from nuclear DNA in that it is maternally inherited and clonal, does not undergo recombination and is single stranded. Mitochondrial genes generally go to fixation considerably faster than nuclear genes, decreasing the chance of amplifying ancestral alleles in paraphyletic groups (Birky *et al.* 1983). It also has a very high copy number - several hundred per cell in higher eukaryotes, which means that it is easy to recover a sufficient quantity for amplification, even from partially degraded specimens from which nuclear DNA may be irretrievable. This was an important consideration for some of the more uncommon dung beetle species for which fresh specimens were unavailable.

Different regions of the mtDNA molecule have been shown to evolve at different rates (reviewed by Moritz *et al.* 1987, Wolstenholme 1992) even within a single gene (Lunt *et al.* 1996). Consequently a faster-evolving region such as the 5' half of the 16S rRNA gene may be informative for recently diverged species, whilst it would likely be saturated with multiple substitutions and obscure any phylogenetic signal for more distantly related taxa. In such a case, the 3'half of the 16S gene would be of more use, as it has been identified as being highly conserved (Simon et al. 1994), as are the mitochondrial transfer RNA genes (tRNA), although these are less often employed, partly because of their small size.

Since the goal was to construct a phylogeny for the Scarabaeinae, informative of relationship at tribal and generic levels, it was apparent that no single gene region was likely to fulfil such requirements. Thus several different regions were investigated for their signal strengths. By combining data from more than one gene region, the intention was to produce a range of characters which would resolve groups at various taxonomic levels. Furthermore, this would enable us to identify discrepancies in topology and eliminate weaker signals from background noise.

Initial trials investigated the potential of a variety of nuclear gene loci, using 'universal' insect primers. Amongst those investigated were the Elongation Factor 1 alpha (EF-1α) locus, coding for a catalytic enzyme involved in the promotion of DNA polymerisation; Dopa decarboxylase (Ddc), involved in pigmentation and sclerotization of the cuticle, and in the synthesis of neurotransmitters; phosphoenol pyruvate carboxy kinase (PEPCK), a metabolic gene in gluconeogenesis; and the 'wingless' gene which is another regulatory gene involved in embryonic development. In each case, a number of different primer combinations were used but all these loci proved extremely difficult to amplify in the Scarabaeinae with the existing primers, and the results which were obtained appeared to lack sufficient levels of resolution to make further investigations worthwhile. Two promising mitochondrial gene regions were identified, including the 5' half of 16S rRNA (the major rRNA found in the small ribosomal subunit of prokaryote cells and mitochondria), and the protein-coding gene Cytochrome Oxidase I (a component of the respiration complex, and consequently the most conserved mitochondrial protein coding gene in terms of its amino acid evolution). COI has not proved informative in between-family and order analyses (Simon *et al.* 1994), but was considered likely to be more so at the generic and tribal levels with which we are concerned. The small nuclear ribosomal subunit gene 18S was also investigated thoroughly, and was amplified in four sections, as it is known to be an even more slowly evolving gene (Hillis and Dixon 1991). Analysis of all 12 tribes however showed this to be too uninformative, despite its 2000 base pairs. Instead, 28S was chosen as the third gene loci, the major rRNA of the large ribosomal subunit in eukaryotic cells. As a nuclear rDNA gene, this also occurs as multiple copies in the genome, improving the chances of amplification, compared to single copy genes. Whilst this introduces the risk of heterogeneous (paralogous) sequences within a single organism, the rDNA arrays have been found to evolve by a process of concerted

evolution so that the multiple copies are homogenised (Hillis and Dixon 1991, Page and Holmes 1998).

It should be noted that a significant reason for the choice of markers used was historical. As pointed out by Caterino et al. (2000), 16S and COI are amongst the most widely used genes in insect systematics, and indeed they advocated their use as standards, in order that different studies might complement one another, and wider scale analyses might be possible. Nuclear rDNA genes are also widely used, and the pre-existence of universal primers is an important consideration. In a similar study Mardulyn and Whitfield (1999) used the same combination of COI, 16S and 28S genes to examine the relationships between genera within the Microgastinae wasp subfamily, a group which is also species-rich and widespread. They concluded that the data appeared to exhibit the appropriate level of variation required for the relationships under examination.

2.1.4 Sequence alignment

In order to utilise sequence data for phylogenetic analysis, homology must be established between individual base characters in the sequences of each specimen. For protein-coding genes such as COI, this is fairly straight-forward, since the its function requires that sequence structure remains quite conserved. Mutations resulting in a frame-shift for example, would cause a major and deleterious change in the amino acid sequence, and so inversions or deletions in such genes are generally selected against. As ribosomal genes however, 16S and 28S are not subject to the same degree of conservation, and so they accumulate a much greater range of mutations. These can be point mutations (transversions or transitions), inversions, or base insertions or deletions (indels), due for example to movements of transposable elements, or replication and recombination errors such as slipped-strand mispairing (slippage). This is particularly evident in fast-changing regions of these genes in the hypervariable regions. Insect mitochondrial DNA is also subject to a strong A/T bias in its base composition, so that transitions are particularly frequent, and alignment of A/T rich regions, when length-variable, is increasingly more difficult.

Homology must therefore be established between sequences of different lengths. This is commonly done by the insertion of gaps into the sequences, signifying that an insertion or deletion event has taken place in one or more of the sequences in the

alignment, and thus all sequences are made to become the same length. This is the key to multiple sequence alignment, as performed by the program Clustal (Thompson et al. 1994, 1997). The alignments are therefore created in a separate step, prior to their use in phylogenetic analysis. However, any single alignment makes a statement about the homology of the nucleotides, and may be disposed toward particular topologies. In this method, no subsequent alteration of the alignment can be made to improve the topology in a more parsimonious way (discussed by Wheeler 2001). A method proposed to include topological information into the alignment procedure, is that of optimization alignment (Wheeler 1996), which creates a unique alignment in a dynamic fashion, using the branching structure of each topology tested.

With a significant proportion of the phylogenetic information potentially being contained in the length-invariable regions, this is therefore a critical issue for two of the three genes being amplified in this study. These two contrasting methods are described in greater detail below and their impact on the phylogenetic signal will be explored.

2.2 Materials and methods

2.2.1 Choice of specimens

To obtain the widest possible taxonomic and geographical coverage, taxa were collected from a range of locations and habitat types, with a view to obtaining a broad and even range of specimens from the twelve tribes, with particular emphasis on the two 'ancestral' groups of Canthonini and Dichotomiini, which are distributed on all major continents. In addition, detailed taxon sampling was considered important to try and circumvent the problem of long branch attraction, particularly as the age of some of these groups is thought to be over 100 million years old (canthonines and dichotomiines). Where available therefore, one or more species from each genus throughout the major biogeographic regions was included. Initially, an attempt was made to widely sample each of the 12 tribes, but subsequent sampling was guided by the tree topology itself (to clarify or challenge emerging patterns in the topology). Most taxa sequenced were collected in South Africa, Belize, Ecuador and Australia with additional material collected from the following regions:

Neotropical: Argentina, Brazil, Costa Rica, French Guiana
African: Ivory Coast, Madagascar, Namibia
Eurasian: Spain, UK
Oriental: Hong Kong, Kalimantan, Sabah Malaysia, Vietnam
Australian: New Caledonia, New Zealand

Specimens from which DNA was extracted were in many cases collected specifically for this purpose, although many specimens were obtained by the trapping protocols discussed in chapter 3. To limit degradation as much as possible, upon collection the beetles were placed into 96% ethanol, and stored at –70 °C when returned to the laboratory. Large-bodied specimens were also broken open between thorax and abdomen, to allow maximum penetration of the ethanol into the tissue. In some cases, where no fresh material was available, DNA extraction was attempted from dried, pinned material or 'wet' collections (specimens stored in alcohol). Amplification of COI, 28S and 16S was attempted in approximately 230 different Scarabaeinae species. For some taxa, COI proved especially problematic to amplify, most likely due to point-mutations within the primer binding site. Alteration of PCR conditions often resolved

this problem, but the locus could not be sequenced in a number of species. Similar problems were encountered with the amplification of 28S and 16S, but were successfully resolved in the majority of cases with the design of new primers.

2.2.2 Choice of outgroup

From initial investigations, it was found that the sequence data of more distantly related Scarabaeoidea, including the 'higher' scarabaeoids Dynastinae and Melolonthinae, and the more basal families Trogidae and Passalidae, were too saturated to be informative as outgroups (due to successive mutational events within a single base position obscuring previous and potentially phylogenetically-informative characters). The subfamily Aphodiinae were chosen as the primary outgroup with which to polarise character evolution, since morphological work (Browne and Scholtz 1999) and molecular analysis of the 18S gene carried out in our laboratory suggest that this is the sister taxa to the Scarabaeinae. Sequences were made available for this purpose from a simultaneous study on the evolution of the Aphodiinae (Maté 2002). Species from the aphodiine tribes Psammodiini and Eupariini were used in particular, as it was found that sequences from other groups such as *Aphodius* again suffered from multiple substitutions and saturation.

A complete list of the Scarabaeinae sequenced and the aphodiine outgroups utilised is given in table 2.1, which also presents their country of origin and the coding used in the cladograms presented below. Ten-letter codes were derived from the currently accepted nomenclature; the first two letters represent the tribe, the subsequent six letters correspond to the genus, and the final two letters represent the species. Taxa prefixed OG are outgroups.

Table 2.1. Scarabaeinae species sequenced. Species codes as used on the cladograms, and country of origin are given. Aphodiinae outgroups used in the analyses are also presented.

Tribe	Species Code	Species	Country of origin
Canthonini	CAamphisco	Amphistomus complanatus	Australia
	CAanachaco	Anachalcos convexus	South Africa
	CAanachasu	Anachalcos suturalis	Ivory Coast
	CAanonthti	Anonthobium tibiale	New Caledonia
	CAapteno01	Aptenocanthon sp. 1	Australia
	CAboletoco	Boletoscapter cornutus	Australia
	CAcanthodo	Canthon doesburgi	Ecuador
	CAcanthoin	Canthon indigaceus	Costa Rica
	CAcantholu	Canthon luteicollis	Ecuador
	CAcanthosm	Canthon smaragdulus	Ecuador
	CAcanthovi	Canthon viridis	Costa Rica
	CAcantsmca	Canthonosoma castelnaui	Australia
	CAcephalar	Cephalodesmius armiger	Australia
	CAcephalqu	Cephalodesmius quadridens	Australia
	CAcircelba	Circellium bacchus	South Africa
	CAcoproehe	Coproecus hemisphaericus	Australia
	CAdeltocam	Deltochilum amazonicum	Ecuador
	CAdeltocba	Deltochilum barbipes	Ecuador
	CAdeltocca	Deltochilum carinatum	Ecuador
	CAdeltocgs	Deltochilum gibbosum sublaeve	Belize
	CAdeltocps	Deltochilum pseudoparile	Belize
	CAdiorygsi	Diorygopyx simpliciclunis	Australia
	CAepirin01	Epirinus sp. 1	South Africa
	CAepirinae	Epirinus aeneus	South Africa
	CAepirinhi	Epirinus hilaris	South Africa
	CAepirinTM	Epirinus sp. 2	South Africa
	CAeudinody	Eudinopus dytiscoides	Argentina
	CAhansreaf	Hansreia affinis	French Guiana
	CAignambfa	Ignambia fasciculata	New Caledonia
	CAlepanuni	Lepanus nitidus	Australia
	CAlepanuus	Lepanus ustulatus	Australia
	CAmegathca	Megathoposoma candezei	Belize
	CAmonopl01	Monoplistes sp. 1	Australia
	CAmonoplcu	Monoplistes curvipes	Australia
	CAodonto01	Odontoloma sp.1	South Africa
	CAodontopu	Odontoloma pusillum	South Africa
	CAonthob02	Onthobium sp. 2	New Caledonia
	CAonthobco	Onthobium cooki	New Caledonia
	CApanelu01	Panelus sp. 1	Kalimantan
	CAparontsi	Paronthobium simplex	New Caledonia
	CAphacospu	Phacosoma punctatum	Kalimantan
	CApseudi01	Pseudignambia sp. 1	Australia
	CApseudi02	Pseudignambia sp. 2	Australia
	CApseudofr	Pseudonthobium fracticolloides	New Caledonia
	CApseudoSN	Pseudonthobium sp. 1	New Caledonia
	CAsaphobse	Saphobius setosus	New Zealand
	CAsaphobsq	Saphobius squamulosus	New Zealand
	CAscybalpy	Scybalocanthon pygidialis	French Guiana
	CAscypha01	Scybalophagus sp. 1	Argentina
	CAtemnopfi	Temnoplectron finnigani	Australia
	CAtemnoppo	Temnoplectron politulum	Australia
Coprini	COcatharca	Catharsius calaharicus	South Africa
	COcatharmo	Catharsius molossus	Kalimantan
	COcatharph	Catharsius philus	South Africa
	COcatharse	Catharsius sesostris	South Africa
	COcoprisae	Copris aeneus	South Africa

Tribe	Species Code	Species	Country of origin
	COcoprisag	Copris agnus	Kalimantan
	COcoprisam	Copris amyntor	South Africa
	COcoprisla	Copris laeviceps	Belize
	COcoprislu	Copris lugubris	Costa Rica
	COcoprisMI	Copris (microcopris) sp. 1	Kalimantan
	COcoprissi	Copris sinicus	Hong Kong
	COcoptodgl	Coptodactyla glabricollis	Australia
	COcoptodst	Coptodactyla storeyi	Australia
	COmetacaop	Metacatharsius opacus	South Africa
	COmetacatr	Metacatharsius troglodytes	South Africa
	COsynapsva	Synapsis vama	Vietnam
Dichotomiini	DIanomiopa	Anomiopus panamensis	Belize
	DIateuch01	Ateuchus sp.1	Ecuador
	DIateuchch	Ateuchus chrysopyge	Belize
	DIateuchec	Ateuchus ecuadorense	Ecuador
	DIbdelyrbo	Bdelyropisis bowditchi	Belize
	DIcanthigu	Canthidium guanacaste	Costa Rica
	DIcanthiha	Canthidium haroldi	Belize
	DIcanthiru	Canthidium rufinum	Ecuador
	DIcanthith	Canthidium thalassinum	Ecuador
	DIcoptor01	Coptorhina sp. 1	South Africa
	DIdemarzim	Demarziella imitatrix	Australia
	DIdemarzin	Demarziella interrupta	Australia
	DIdemarzmi	Demarziella mirifica	Australia
	DIdichot02	Dichotomius sp.2	Ecuador
	DIdichotbo	Dichotomius boreus	Ecuador
	DIdichotpa	Dichotomius parcepunctatus	Ecuador
	DIdichotyu	Dichotomius yucatanus	Costa Rica
	DIgromphae	Gromphas aeruginosa	Ecuador
	DIheliocan	Heliocopris andersoni	South Africa
	DIheliocha	Heliocopris hamadryas	South Africa
	DImacrod01	Macroderes sp. 1	South Africa
	DIontherdi	Ontherus diabolicus	Ecuador
	DIpedari01	Pedaria sp. 1	South Africa
	DIsarophco	Sarophorus costatus	South Africa
	DIsarophtu	Sarophorus tuberculatus	South Africa
	DItrichi01	Trichillum sp. 1	Costa Rica
	DIuroxys01	Uroxys sp. 1	Costa Rica
	DIuroxys02	Uroxys sp. 2	Ecuador
	DIuroxysmi	Uroxys micros	Belize
	DIuroxyspy	Uroxys pygmaeus	Ecuador
Eucraniini	ECanomoibi	Anomiopsoides biloba	Argentina
	ECanomoihe	Anomiopsoides heteroclyta	Argentina
	ECennearlo	Ennearabdus lobocephalus	Argentina
	ECeucranar	Eucranium arachnodes	Argentina
	ECglyphost	Glyphoderus sterquilinus	Argentina
Eurysternini	EReurystan	Eurysternus angustulus	Belize
	EReurystca	Eurysternus caribaeus	Belize
	EReurystha	Eurysternus hamaticollis	Ecuador
	EReurystin	Eurysternus inflexus	Ecuador
	EReurystpl	Eurysternus plebejus	Ecuador
	EReurystve	Eurysternus velutinus	Belize
Gymnopleurini	GYallogyth	Allogymnopleurus thalassinus	South Africa
	GYgarretni	Garretta nitens	South Africa
	GYgymnopvi	Gymnopleurus virens	South Africa
	GYparagy01	Paragymnopleurus sp. 1	Malaysia
	GYparagyma	Paragymnopleurus maurus	Kalimantan
	GYparagyst	Paragymnopleurus striatus	Kalimantan

Tribe	Species Code	Species	Country of origin
Oniticellini	OCcytocham	Cytochirus ambiguus	South Africa
	OCdrepanbe	Drepanocerus bechynei	South Africa
	OCdrepanki	Drepanocerus kirbyi	South Africa
	OCeuonitin	Euoniticellus intermedius	South Africa
	OChelictqu	Helictopleurus quadripunctatus	Madagascar
	OCliatonmi	Liatongus militarus	South Africa
	OConiticeg	Oniticellus egregrus	South Africa
	OConiticfu	Oniticellus fulvus	Spain
	OCtiniocsa	Tiniocellus sarawacus	Kalimantan
	OCtiniocsp	Tiniocellus spinipes	South Africa
	OCtragisdi	Tragiscus dimidiatus	South Africa
Onthophagini	OPcaccobbi	Caccobius binodulus	Kalimantan
	OPcaccobni	Caccobius nigritulus	South Africa
	OPcaccobsc	Caccobius schreberi	Spain
	OPcleptoco	Cleptocaccobius convexifrons	South Africa
	OPdigitodi	Digitonthophagus diabolicus	Kalimantan
	OPdigitoga	Digitonthophagus gazella	South Africa
	OPeuonthca	Euonthophagus carbonarius	South Africa
	OPhyalonal	Hyalonthophagus alcyon	South Africa
	OPmilichap	Milichus apicalis	South Africa
	OPonthopba	Onthophagus batesi	Belize
	OPonthopbb	Onthophagus babirussoides	Kalimantan
	OPonthopbi	Onthophagus bidentatus	Ecuador
	OPonthopca	Onthophagus capella	Australia
	OPonthopch	Onthophagus championi	Costa Rica
	OPonthopcl	Onthophagus clypeatus	Ecuador
	OPonthopco	Onthophagus consentaneus	Australia
	OPonthopcp	Onthophagus crinitis panamensis	Belize
	OPonthopfi	Onthophagus fimetarius	South Africa
	OPonthopfu	Onthophagus furcaticeps	Australia
	OPonthopgl	Onthophagus glabratus	Australia
	OPonthopha	Onthophagus haematopus	Ecuador
	OPonthopin	Onthophagus incensus	Costa Rica
	OPonthopla	Onthophagus laminatus	Australia
	OPonthopmi	Onthophagus mije	Australia
	OPonthopml	Onthophagus mulgravei	Australia
	OPonthopmt	Onthophagus muticus	Australia
	OPonthopob	Onthophagus obscurior	Kalimantan
	OPonthopqu	Onthophagus quadripustulatus	Australia
	OPonthopro	Onthophagus rorarius	Kalimantan
	OPonthopru	Onthophagus rubicundulus	Australia
	OPonthopSA	Onthophagus sp. 1	South Africa
	OPonthopse	Onthophagus semiareus	Malaysia
	OPonthopsi	Onthophagus similis	Spain
	OPonthopvu	Onthophagus vulpes	Kalimantan
	OPphalopar	Phalops ardea	South Africa
	OPproagobi	Proagoderus bicallossus	South Africa
	OPproagosc	Proagoderus schwaneri	Kalimantan
Onitini	OTbubasbis	Bubas bison	Spain
	OTbubasbub	Bubas bubalus	Spain
	OTcheiroho	Cheironitis hoplosternus	South Africa
	OTheteroca	Heteronitis castelnaui	South Africa
	OTonitisal	Onitis alexis	South Africa
	OTonitisca	Onitis caffer	South Africa
	OTonitisfa	Onitis falcatus	Hong Kong
	OTonitisfu	Onitis fulgidus	South Africa
Phanaeini	PHcoprop01	Coprophanaeus sp.1	Ecuador
	PHcopropla	Coprophaneus lancifer	French Guiana

Tribe	Species Code	Species	Country of origin
	PHcoproptc	Coprophaneus telamon corythus	Belize
	PHdendroba	Dendropaemon bahianum	Ecuador
	PHdiabromi	Diabroctis mimas	Brazil
	PHoxysteco	Oxysternon conspicillatum	Ecuador
	PHphanaeca	Phanaeus cambeforti	Ecuador
	PHphanaede	Phanaeus demon	Costa Rica
	PHphanaesa	Phanaeus sallei	Belize
Scarabaeini	SCdrepapco	Drepanopodus costatus	Namibia
	SCkheperni	Kheper nigroaeneus	South Africa
	SCpachylfe	Pachylomerus femoralis	South Africa
	SCpachys01	Pachysoma sp. 1	South Africa
	SCscarabga	Scarabaeus galenus	South Africa
	SCscarabSA	Scarabaeus sp. 1	South Africa
	SCsceliabr	Sceliages brittoni	South Africa
	SCsceliahi	Sceliages hippias	South Africa
Sisyphini	SIneosisco	Neosisyphus confrater	South Africa
	SIneosisfo	Neosisyphus fortuitus	South Africa
	SIneosismi	Neosisyphus mirabilis	South Africa
	SIneosisru	Neosisyphus ruber	South Africa
	SIsisyphcr	Sisyphus crispatus	South Africa
	SIsisyphfa	Sisyphus faciculatus	South Africa
	SIsisyphga	Sisyphus gazanus	South Africa
	SIsisyphse	Sisyphus seminulum	South Africa
Outgroup (Aphodiinae)	OGafrodias	Afrodiastictus sp. 1	Namibia
	OGairaphen	Airapus henriettae	Australia
	OGataenec	Ataenius sp. 1	Ecuador
	OGataenmon	Ataenius monteithi	New Caledonia
	OGataennb	Ataenius strigatus	USA
	OGataensci	Ataenius sciurus	USA
	OGaustrala	Australammoecius occidentalis	Australia
	OGleipsamca	Leiopsammodius caelatus	USA
	OGplatytom	Platytomomus calicollis	USA
	OGpleuroca	Pleurophorus caesus	Spain
	OGpsammopo	Psammodius porcicollis	United Kingdom

2.2.3 Laboratory protocols

DNA Isolation

DNA extraction followed the protocol set out by Hillis et al. (1996). Briefly, the tissue was crushed in a solution of 10mg/ml proteinase K, homogenisation buffer (providing optimal pH for the proteinase) and 10% SDS detergent (which aids cell lysis), and was then incubated at 65 °C. Thoracic flight muscle or leg muscle was most commonly used, but when body length was less than 5mm the whole specimen was used. Separation of DNA from the cellular protein was carried out by use of phenol/chloroform/isoamyl alcohol and microcentrifugation, a standard method where the more soluble DNA becomes suspended in a water solution. The DNA was then precipitated from solution with 10M ammonium acetate at low temperature, and then re-suspended in sterile distilled water for storage.

DNA amplification

A standard PCR protocol was adopted for amplification of 16S, 28S and COI genes (see Palumbi 1996 for a good review of PCR). The PCR reaction typically contained a cocktail of dNTPs, Taq DNA polymerase and buffer, to which the relevant primers and template DNA were added. Pre-existing universal insect primers were tested initially. These included 16Sa and 16Sb, and the COI primers 'Pat' and 'Jerry', all of which have been used successfully in studies of other Coleoptera groups in the past (Simon et al 1994). In addition a pair of 28S primers, 28Sdd and 28Sff were used. These were originally designed for *Drosophila*, but subsequently shortened for use in our laboratory on the Coleoptera family Pselaphidae.

Due to limited amplification success with the primer 16Sb, and also with the 28S primers dd and ff, several new and more group-specific primers were designed for the Scarabaeinae. These were named 16Sb2, a reverse primer for use with 16Sa, and the complementary pair 28SKa and 28SKb. Designed using the sequence information already obtained, these target more conserved gene regions. Primers were designed to be as long as possible, thus minimizing binding problems in instances where internal mismatching at the primer site occurs. The sequences of all of the primers employed are given in Table 2.2 below.

Standard PCR conditions consisted of a denaturation phase of 94 °C for 20 seconds, extension at 72 °C for 60 seconds, and an annealing temperature of 50 °C for the 16S and 28S primers, and 45°C for the CO1 primers. The conditions were cycled 35 to 40 times. The PCR products were separated out electrophoretically on a TAE agarose gel containing ethidium bromide, and then detected under UV light. Molecular weight of the products was determined by use of a molecular marker such as Bioline's 'Hyperladder'.

Table 2.2. Nomenclature and sequences of the primers employed. Standard primer nomenclature as given by Simon et al. (1994).

Standard name	Common name	Gene targeted	Sequence (5' to 3')	Size (bases)
C1-J-2183	Jerry	CO1	CAACATTTATTTTGATTTTTTGG	23
TL2-N-3014	Pat/ UEA 10	CO1	TCCAATGCACTAATCTGCCATATTA	25
LR-J-13417	16Sa	16S	ATGTTTTTGTTAAACAGGCG	20
LR-J-12887	16Sb	16S	CCGGTCTGAACTCAGATCACGT	22
	16Sb2	16S	TTTAATCCAACATCGAGG	18
	28S dd	28S	GGGACCCGTCTTGAAACAC	19
	28S ff	28S	TTACACACTCCTTAGCGGAT	20
	28S Ka	28S	ACACGGACCAAGGAGTCTAGCATG	24
	28S Kb	28S	CGTCCTGCTGTCTTAAGTTACC	22

'Geneclean' protocol

The PCR product was purified by use of BIO 101's Geneclean II kit (refer to manufacturers handbook for complete protocol). This involves the ionic binding of the DNA to silica 'glassmilk' at a high sodium iodide concentration. The product can then be washed of impurities such as unincorporated primers and dNTPs, and then resuspended into sterile distilled water.

Cycle sequencing

Nucleic acid sequencing was carried out using the Sanger dideoxynucleotide chain-termination method (Sanger et al. 1977). The ABI Prism 'Big Dye' terminator cycle sequencing ready reaction kit was used in the cycle sequencing of the PCR products. The kit includes all reagents, AmpliTaq F (a variant of Taq polymerase) and fluorescently labelled dye terminators, to which the appropriate primer and template DNA is added. Cycle sequencing conditions and the full protocol is fully explained in the manufacturers handbook (PE Applied Biosystems).

Purification of sequencing reaction

It is necessary to remove unincorporated dye terminators from the sequencing product prior to electrophoresis in order to obtain a noise-free signal. To do this, the product was passed through a spin-column containing sephadex gel. This retains small molecules including unincorporated ddNTPs and salts, whilst the labelled extension product passes through. Alternatively, the sequencing reaction was purified by precipitation of the DNA from solution with 7.5M ammonium acetate at low temperature, followed by microcentrifugation of the DNA strands, producing a pellet which was then washed in 70% ethanol and dried.

Automated sequencing and sequence editing

Automated DNA sequencing was carried out using ABI Prism instruments and software, where the fragments are separated electrophoretically and the fluorescent end-labels are identified with a stationary laser (see Tracey and Mulcahy 1991). The end product is a chromatograph, interpreted as the DNA sequence. The program Sequencher 3.0 (Gene Codes Corporation) was used to view and edit the ABI chromatographs, and then to align with each other the two complementary sequences generated for each specimen by the forward and reverse primers. The sequence data were then exported into the sequence editing program SeqPup (Gilbert 1999), whereupon the next step was the alignment of all species sequences with one another, for each locus.

All sequences from the three genes were submitted to the GenBank database at the National Center for Biotechnology Information (NCBI), using the Sequin 4.00 DNA sequence submission tool. All sequence data can therefore be retrieved from the NCBI on-line servers. GenBank accession numbers are as follows:

16S: AY131436 - AY131627
28S: AY131628 - AY131807
COI: AY131808 - AY131966

2.2.4 Alignment and phylogenetic analysis of sequence data

It was recognised that the most informative topologies were achieved by simultaneous analysis of the three genes, and that independently, each suffered from a lack of resolving power. A total evidence approach also avoids the problem of conflicting trees generated through separate analyses of the different data sets, and will potentially bring weak but informative signals together. Conflict between the sequences of the different gene regions was tested for using an Incongruence Length Difference Test (described below). The sequences of each gene were aligned independently however. Alignment of the COI sequences was simply achieved, using the program Clustal X (Thompson et al. 1997). This initially calculates a distance score between every pair of sequences through pairwise alignments. These scores are then used to construct a dendrogram, which is then used as a "guide tree" to direct the final multiple alignment. This crucial part comprises the alignment of two sequences or sets of aligned sequences, and is done progressively, using the branching order of the guide tree. Gap penalties can be set which control the 'cost' of opening a gap in the alignment, and of extending it (adding extra gaps to the alignment). Because COI contains no insertions or deletions and is of identical length in all species, gap penalty costs were not relevant and left at the default settings. Alignment within the ribosomal genes was more complex however, due to several A/T rich hypervariable regions present in each locus. Compared across the 93 scarabaeine genera sequenced, these areas exhibited a great number and variety of mutations, and considerable length variation, so that confidence in homologous alignment was low for these regions. A variety of different alignment and search strategies were thus adopted to tackle this problem, and are described below.

Alignments were carried out separately on the 28S and 16S data sets using Clustal, using a variety of parameters, and all three loci were then analysed together in a total evidence approach. Phylogenetic searches were conducted under parsimony using PAUP* version 4.0b10 (Swofford 1999). (See (G) however, in which optimisation alignments were performed). The different alignments produced were searched for optimal trees using PAUP* in the following way, unless stated otherwise: heuristic search, 1000 random addition replicates, tree bisection and reconnection (TBR) branch-swapping, all character positions weighted equally, gaps treated as missing data, saving 1 tree only at each step. The shortest tree saved was then used in a subsequent heuristic search, as a starting tree for TBR branch swapping, saving multiple trees (maxtrees set at 100). Strict consensus trees were then produced in each case. The methodology was adopted to maximise the number of replicates performed on these large data sets, to identify the most optimal tree 'islands' (Maddison 1991), followed by a more thorough search to determine the most parsimonious trees from the initial set. Transition : transversion weighting was applied in some cases, to reflect the greater A/T richness and higher transition frequency in the mitochondrial genes. The variety of methodologies employed to explore the data were as follows:

(A) The length-variable regions were removed from the 16S and 28S sequences to produce a single length-invariable data set. Two weighting schemes were tested regarding the substitution types: an equal weight for transversions and transitions, and assigning a higher cost to transversions relative to transitions of 1:2.

(B) The initial guide tree produced by the simple pairwise alignments in Clustal was substituted with the most parsimonious topology produced from the unweighted length-invariable data set (analysis A). This was because upon examination, the Clustal guide tree proved to make little phylogenetic sense. This approach is discussed in Caterino and Vogler (in press). Since Clustal requires a fully resolved topology for its guide tree, the strict consensus from (A) could not be used. However, since the consensus tree proved to be fully resolved for all but a few terminal taxa in the Phanaeini and *Onthophagus*, a single tree was chosen at random for this purpose. For Clustal's multiple alignments, a wide range of gap cost parameters were initially explored (gap opening and extension costs were held equal). The 16S and 28S genes were subdivided into regions (seven and six respectively), according to length-variable and invariable sequence areas, and these

were each examined for major 'inconsistencies' in their alignment. Sequences were often found to be well aligned within individual (usually monophyletic) clades, but observed to be poorly aligned between such clades. The implication of this would of course be a misleading phylogenetic signal. Therefore when such problems were observed throughout the sequence length, they were judged to be major internal inconsistencies, and when they were more isolated events in the alignment, they were deemed to be minor inconsistencies. Where alignments were particularly complex (for instance in the hypervariable regions of 16S), no judgement was made, to avoid unnecessary subjectivity. In this way, the parameter space was narrowed, and additional gap costs were then explored around the better alignments. Table 2.3 presents the inconsistencies found under a variety of costs. The most consistent alignments were produced for 16S by employing gap costs of 5 and 10, whilst costs of 10 and 15 produced the best alignments for 28S. All combinations of these alignments were searched using PAUP without weighting.

Table 2.3. Inconsistencies found in the length-invariable guide tree alignments (analysis B), using Clustal X under a variety of gap costs. The length invariable tree replaced the dendrogram from Clustal's initial pairwise alignments. Gap opening and extension costs were held equal for the multiple alignments. Sequences were examined by regions, which represent successive length-variable and invariable areas of the genes. X = major inconsistency, x = minor inconsistency. Definitions of inconsistencies are given in the text (analysis B).

	Clustal gap opening and extension costs						
	5	10	15	20	30	60	90
16S							
Region 1				X	X	X	X
Region 2	x					X	X
Region 3	x						
Region 4						X	X
Region 5							X
Region 6			X	X	X	X	X
Region 7							X
28S							
Region 1	X	x	x	X	X	X	X
Region 2	X			x	X	X	X
Region 3	x	x	x	X	X	X	X
Region 4					x		
Region 5	X				X	X	X
Region 6				X			

(C) Full Clustal alignments were performed under a variety of gap costs. Gap opening and extension costs were held equal, for both pairwise and multiple alignments. Alignments produced were examined by region, in the same manner as in (B), and parameter space was narrowed down once more. Table 2.4 displays the inconsistencies found under the different parameters. The most consistent alignments, as judged by the number of internal inconsistencies identified in the alignment, were produced for both 16S and 28S using gap costs of 20. A range of transition: transversion ratio weighting schemes were investigated through the PAUP searches, including equal weighting (1:1), and a higher cost of transversions relative to transitions by 0:1, 1:2 and 1:3.

Table 2.4. Inconsistencies found in complete sequence alignments using Clustal X under a variety of gap costs. Gap opening and extension costs were held equal, for both pairwise and multiple alignments. Sequences were examined by regions, representing successive length-variable and invariable areas of the genes.
X = major inconsistency, x = minor inconsistency. Definitions of inconsistencies are given in the text (analysis B).

	Clustal gap opening and extension costs				
	10	20	30	60	90
16S					
Region 1			x	X	X
Region 2	X		x	X	X
Region 3	X				
Region 4	X		X	X	X
Region 5					
Region 6	X			X	X
Region 7				X	X
28S					
Region 1			X	X	X
Region 2			X	X	X
Region 3	X		X	X	X
Region 4					
Region 5	X	x	X	X	X
Region 6					

(D) This analysis was identical to that described in analysis (C), but using a different Clustal alignment for 16S, produced using gap costs of 30. This was deemed the second-best alignment for this gene (see table 2.4), and was explored in the same way as (C) to test the extent to which the investigation of parameter space described

in (B) was subjective, and the degree to which a less optimal alignment would affect the topology of the cladogram.

(E) The best Clustal alignment was taken from analysis (C), and the hypervariable regions were excluded from the data set (a total of 117 characters), since these were the most questionable areas of alignment. Transition: transversion ratios were tested at equal weights, 1:2 and 1:3.

(F) Once again, the best alignment from analysis (C) was taken, and where possible, improvements in the alignment made by eye. In practice, only 28S was altered, since the A/T rich variable regions in 16S were too complex to be justifiably rearranged across over 200 taxa. Transition: transversion ratios were tested at equal weights and 1:2.

(G) Finally, in a departure from the previous analyses, the program POY (Gladstein and Wheeler 1997) was used for optimization alignments of the data. POY is designed to deal with homologous, length-variable sequences, and to avoid the problem of having to choose one multiple alignment over another. It does this by combining simultaneous sequence alignment with tree-building using parsimony. Very briefly, it works by constructing the putative sequence of every hypothetical ancestor at each node on a randomly proposed topology, starting with a pair of terminal taxa, and passing down the tree. The sequence of each hypothetical ancestor, at each internal node, is calculated according to the most parsimonious possibility (least cost according to specified gap and substitution costs), guided by the two sequences of its descendents (be they 'real' observed sequences or sequences hypothesized for another ancestral node). After determination of each node, the most parsimonious alignment for that tree topology has been identified. The least costly topologies are used to guide the next round of optimization, so that the group of proposed phylogenies migrate towards a more parsimonious alignment and corresponding tree structure. Optimization alignment is explained more fully by Wheeler (1996, 2001)

Since optimisation of raw sequences in POY is computationally expensive, the three gene loci were delineated as a series of characters, so that alignment of homologies would not be attempted between characters. COI was designated as a

single character, whilst 16S and 28S were divided into seven and six characters respectively, according to the same variable and invariable regions identified for examination under the Clustal alignments, described above. The analyses were conducted on a parallel computer cluster maintained at the Natural History Museum, to improve calculation efficiency. The data were explored under a variety of conditions (commands are given in the POY documentation), although primarily using the POY default parameters (gap = 2, change = 1) and 100 replicates. The effect was explored of assigning a lower cost to the two most hypervariable 16S regions (regions 2 and 4) to one-third in one search and to one-fifth in another, as was the effect of excluding these regions entirely and of equal weighting across all regions. In other searches, implied character weighting was also employed (Goloboff 1993). This is carried out during the analysis itself, using information from each tree examined, regarding the homoplasy of each character. It assumes that the more homoplasious a character is, the less reliable it is, and so it is assigned a lower cost accordingly. Two searches were conducted utilizing Goloboff weighting: the first with the 16S hypervariable regions 2 and 4 excluded, with the weighting calculated via the retention index (a function of the homoplasy for a character), and the second with the entire sequences, weighted using the constant *k*, which relates to the fit of a character to a tree. k = 4 was used, so that down-weighting of homoplasious characters was not so severe (see Goloboff 1993 for full explanation).

Finally parameter costs lower than the POY defaults were applied (gap = 3, change = 2), firstly in a simple search, and then using the parsimony ratchet (Nixon 1999), a method designed for large data sets such as this. This aims to improve searching efficiency by sampling a few trees from many tree islands, rather than many trees from just a few islands. As it searches the treespace, it finds tree islands, and moves between them by "warping" the treespace so that it can bridge the divide to another adjacent island, from where it continues its search (Sikes and Lewis 2001). In this way, it does not begin another iteration with a totally random tree, but utilises the searching it has already done, so that it minimises any loss in its search progress.

The number of parsimony-informative characters was recorded for each alignment produced, and all trees produced were evaluated regarding their fit to the data, using the

ensemble consistency index (CI) and retention index (RI). To examine the consistency of the topologies combined in the strict consensus trees, the number of resolved nodes and Rohlf's modified CI were calculated.

Upon examination of the trees produced by the various analyses (A) to (G), approximately 40 key groups were identified, reflecting a range of relationships amongst the ingroup taxa. Some groups were chosen to reflect the currently held theories of relationship such as the monophyly of the subfamily and that of the 12 tribes, whilst others were potentially biogeographically or functionally interesting clades. Each topology was then inspected to determine whether these key groups were found to be monophyletic, paraphyletic, polyphyletic or unresolved in each case. This allows us to establish the level of congruence between the topologies, and to determine the robustness of the different groupings under the various treatments applied. A single 'preferred' tree was chosen according to the manner in which the data were found to 'behave', and this was used as a best estimate of the Scarabaeinae phylogeny, given the data at hand.

In order to examine the level of phylogenetic congruence between the three gene regions, an Incongruence Length Difference (ILD) test (also termed a Partition-Homogeneity test) was carried out using PAUP* (Mickevich and Farris 1981, Swofford 1991, Sanderson and Shaffer 2002). This is a randomisation procedure which treats each gene as a separate data partition and then creates a large number of random data sets, of equal size to the partitions, which are then used for tree searching. By comparing the differences in tree length between the original and the random data partitions, the significance of any incongruence between the different gene regions can be assessed. Test parameters were as follows: characters partitioned by gene region, starting trees obtained by random addition, 100 random addition replicates, tree bisection and reconnection (TBR) branch-swapping, gaps treated as missing data, saving 1 tree only at each step, all character positions weighted equally, 'Maxtrees' set at 20.

Two different methods were utilised to evaluate the support for the different clades on the preferred tree. Bootstrap confidence values (Felsenstein 1985) were generated for the appropriate alignment for this tree: resampling all characters, 10,000 replicates, using the 'fast' stepwise addition search procedure in PAUP so as to maximise the

number of replicates for this large data set. Bremer support statistics (as described by Bremer 1988) were calculated for the nodes in the preferred tree using TreeRot version 2 (Sorenson 1999) and PAUP. Bootstrapping is a permutation technique which randomly resamples the data matrix with replacement, to create a 'new' data set to be analysed phylogenetically and determine the number of times each node or clade is found throughout the replicates. Bremer support is a decay index which reflects the difference in length between the nodes found in the preferred tree (as the shortest tree found for that data set) and the shortest tree which does not have that node.

Biogeographic region and nesting behaviour were mapped onto this tree using MacClade 4.0 (Maddison and Maddison 2000). Where ancestral states could not be resolved at nodes, the most parsimonious states were adopted, which may produce ambiguous results in some cases. Feeding behaviour was also traced onto the tree, but the availability of accurate data was so limited and widely spread across the tree, that the majority of the topology was equivocal and uninformative.

Evolutionary dating has not yet been attempted at this stage, since the necessary calibration for this group, and insects in general (although see Gaunt and Miles 2002) remains elusive. Attempts to apply a molecular clock to other Coleoptera DNA sequences (Vogler and Pons unpublished), suffered from a lack of accurate calibration, producing results which appeared to be highly misleading. This is possibly because a molecular clock assumes a constant rate of evolution within the gene in question across all species, and assumption frequently violated due to species differences in metabolic rate or generation time (Page and Holmes 1998).

2.3 Results

PCR sequencing proved successful in 195 species, spanning 93 genera from around the world and all 12 tribes sensu Balthasar (1963). The total number of characters under the different alignment parameters varied from 1763 with the hypervariable regions removed, to 2371 in one of the POY alignments. The mean overall A/T content of the loci across all taxa was 76 % for 16S, 72 % for COI, and 46% for 28S. For one arbitrarily chosen 'typical' alignment using all bases (length invariable topology used as guide tree, gap costs = 10 for multiple Clustal alignment, no weighting, 1926 characters in total), the proportion of character differences ranged from 0.6 % (12 base pairs) between two *Onthophagus* species, to 13.9 % (268 base pairs) between a *Cheironitis* species and a *Catharsius* species. The mean pairwise sequence divergence between taxa was 7.9 % (152 bases). The number of parsimony informative characters in each alignment is presented in table 2.5, and ranged from 786 to 630.

The tree statistics for each of the 25 different analyses conducted are also presented in table 2.5, including tree length, ensemble CI and RI, and Rohlf's CI for the strict consensus. Since the CI for a tree is calculated by summing the consistency indices of each character in a data set, it has been found to be negatively correlated with the number of taxa and characters (Siebert 1992), and also to be affected by weighting the data (Carpenter 1988). For a large (200 taxon) data set such as this, the RI scores probably reflect more accurately the fit of the trees to the data. RI attempts to score the levels of true synapomorphies in a data set, and excludes autapomorphies and symplesiomorphies (Farris 1989), but this is also apparently affected (inflated) by weighting the data, so that the unweighted data receives the lowest RI (table 2.5). Generally, the RI remains quite consistent at around 0.40 to 0.44, and does not really assist in choosing a preferred tree. Rohlf's CI suggests that the trees produced by some analyses (such as 14, 15 and 17), are quite variable and should be afforded less confidence, but otherwise this statistic remains consistently quite high. It is clear that the more characters used, the more homoplasy will be encountered, since few characters are likely to be perfectly indicative of phylogeny. The low CI and RI do suggest this data set to contain considerable levels of homoplasy.

Table 2.5. A summary of tree statistics for combined analysis under the different alignment parameters and weighting schemes for 16S and 28S

Analysis	Sequence regions excluded from analysis	Clustal alignment gap opening/extension costs	PAUP transition:tranversion weighting / gap : change cost	POY Number of steps	Number of trees	Total number of characters	Number of informative characters	Consistency Index	Retention Index	Number of resolved nodes (strict consensus)	Rohlf's consistency index (strict consensus)	Number of nodes congruent with length-invariable (guide) tree	Rohlfs CI with length invar. trees
(A) Length invariable regions only													
1	16S and 28S length variable regions		TS1 :TV1	12838	100	1763	630	0.1065	0.4028	188	0.897	N/A	N/A
2	16S and 28S length variable regions		TS1 : TV2	19667	24	1763	634	0.0956	0.4216	196	0.988	112	0.05
(B) Guide tree alignment + Clustal multiple alignments													
3		28S = 15, 16S = 10	TS1 : TV1	15647	69	1926	766	0.1095	0.4393	194	0.89	130	0.11
4		28S = 15, 16S = 5	TS1 : TV1	15365	100	1957	786	0.101	0.4386	181	0.876	138	0.329
5		28S = 10, 16S = 10	TS1 : TV1	15600	100	1926	759	0.1089	0.4374	189	0.956	125	0.111
6		28S = 10, 16S = 5	TS1 : TV1	15311	100	1957	779	0.1134	0.4369	187	0.905	128	0.124
(C) Pairwise and Multiple alignments using Clustal													
7		28S = 20, 16S = 20	TS1 : TV1	16837	24	1928	765	0.1013	0.3977	198	0.967	102	0.043
8		28S = 20, 16S = 20	TS0 : TV1	9957	23	1928	736	0.0656	0.4415	195	0.983	80	0.032
9		28S = 20, 16S = 20	TS1 : TV2	26918	100	1928	770	0.0876	0.4139	185	0.937	84	0.019
10		28S = 20, 16S = 20	TS1 : TV3	36924	2	1928	770	0.0816	0.4214	202	0.998	80	0.029
(D) Pairwise and Multiple alignments using Clustal													
11		28S = 20, 16S = 30	TS1 : TV1	16322	8	1926	774	0.1059	0.4208	200	0.996	97	0.052
12		28S = 20, 16S = 30	TS0 : TV1	9434	100	1926	738	0.0709	0.4754	180	0.916	75	0.026
13		28S = 20, 16S = 30	TS1 : TV2	25846	6	1926	778	0.0928	0.4422	200	0.916	90	0.031
14		28S = 20, 16S = 30	TS1 : TV3	35327	14	1926	778	0.0868	0.4512	191	0.705	84	0.029
(E) Clustal alignment, hypervariable regions excluded													
15	Hyper-variable regions in 16S and 28S	28S = 20, 16S = 20	TS1 : TV1	13254	336	1811	663	0.1098	0.4042	189	0.575	108	0.036
16	Hyper-variable regions in 16S and 28S	28S = 20, 16S = 20	TS1 : TV2	20366	9	1811	668	0.0985	0.4231	189	0.812	102	0.026
17	Hyper-variable regions in 16S and 28S	28S = 20, 16S = 20	TS1 : TV3	27432	16	1811	668	0.0932	0.4321	181	0.626	101	0.042
(F) 16S Clustal alignment, 28S alignment by eye													
18		16S = 20	TS1 : TV1	16607	12	1932	754	0.1013	0.4002	199	0.983	96	0.038
19		16S = 20	TS1 : TV2	26514	100	1932	762	0.0878	0.416	192	0.975	84	0.038
(G) POY optimization alignments													
20			2:1 (default)	16740	1	2269	734	0.156	0.472	204	n/a	91	0.035
21			3:2 Ratchet method	32877	1	2371	744	0.157	0.469	204	n/a	89	0.031
22	16S2 and 16S4 (hypervariable) regions		2:1,	14422	2	2050	677	0.143	0.455	199	n/a	106	0.026
23			2:1, 16S2 and 16S4 regions downweighted to 1/:	43584	2	2286	747	0.143	0.45	202	n/a	107	0.04
24	16S2 and 16S4 (hypervariable) regions		2:1, Goloboff weighting = RI	14410	1	2040	675	n/a	n/a	204	n/a	101	0.038
25			2:1, Goloboff weighting K factor = 4	16750	1	2285	744	n/a	n/a	204	n/a	101	0.038

Table 2.6 gives a summary of each topology from all analyses conducted, regarding the state of the 40 key groups chosen. A summary of the key groups recovered by the different analyses is also given, for an arbitrarily chosen 75% majority. Thus if a group is consistently found to be monophyletic in three-quarters of the topologies produced, it is given here as *M* (or *P* if polyphyletic/paraphyletic). If the resolution of the group is less certain, it is given as *M/P* or *P/M* (the first letter indicating the greater majority recovered). The topologies are found to be overwhelmingly congruent regarding the status of the Scarabaeinae, the 12 tribes, and the three functional groups, but there is considerably more incongruence between the various topologies regarding the other key groups examined. This is a further indication of the lack of robustness within the trees, and of these clades in particular, since the topology is clearly sensitive to the assumptions made. It was apparent that the use as a guide tree, of the length-invariable topology from analysis (A1), gave greater stability to the tree structure under differing multiple alignment costs than Clustal's simple pairwise dendrogram, since the topology remained relatively consistent under the different multiple alignment gap costs applied. The full Clustal alignments proved to vary significantly in topology upon application of the different weighting schemes applied. Subsequent removal of the hypervariable regions and alignment of 28S by eye also altered the topologies. Both proved to alter the estimation of relationships at a deep level in the trees. Some alignments failed to recover clades which were otherwise found in the topologies of the majority of other alignments, and which are clearly monophyletic, such as the tribe Sisyphini in analysis (D). This suggests that alignment is poor, and also likely to be unreliable for the other groups. POY analyses produced the most variable topologies within any single analysis type, although this is partly due to the greater number of parameter choices applied. Nevertheless, it serves to illustrate further the critical effect that alignment is playing upon the tree produced. Because of this instability under different alignment parameters and character type assumptions, and the inadequacy of the tree statistics to indicate one or more robust trees, the decision was made to choose the most conserved alignment possible as that conveying the most reliable phylogenetic evidence, that is the use of the length invariable regions only (analysis A).

Table 2.6. Summary of topologies for the most parsimonious cladograms produced by the different analyses. The parameters of each analysis are decribed in table 2.5.
M = monophyletic, P = Paraphyletic/Polyphletic, U = Unresolved

Analysis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	*75% majority*
Tree length	12840	19667	15647	15365	15600	15311	16837	9957	26918	36924	16322	9434	25846	35327	13254	20366	27432	16607	26514							
Scarabaeinae	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Canthonini	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Coprini	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Dichotomiini	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Eucraniini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Eurystemini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Gymnopleurini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Oniticellini	M	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Onthophagini	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Onitini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Phanaeini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Scarabaeini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Sisyphini	M	M	M	M	M	M	M	M	P	M	P	P	P	P	U	M	M	M	M	M	M	M	M	M	M	***M***
Rollers	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Tunnellers	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Dwellers	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Oniticellini + Onthophagini	M	M	M	M	M	M	M	M	P	M	M	M	M	M	M	P	M	M	M	M	M	P	M	M	M	***M***
Oniticellini + Onthophagini + Onitini	P	M	P	P	P	P	M	P	P	P	P	P	P	P	M	M	M	M	P	P	M	M	P	P	P	***P/M***
Sisyphini + Onitini	M	P	P	M	M	M	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
Onitini + *Macroderes*	M	P	M	M	M	M	M	P	M	P	P	P	P	P	P	P	P	M	M	M	P	P	P	P	P	***P/M***
Oniticellini + Onthophagini + Onitini + Sisyphini	M	M	M	M	M	M	P	P	P	P	M	P	M	P	P	M	P	P	P	P	M	M	P	P	M	***P/M***
Sisyphini + *Epirinus*	P	P	P	P	P	P	M	P	M	P	P	P	P	P	P	M	M	M	M	M	P	P	P	P	P	***P***
Epirinus + *Panelus*	M	P	M	M	M	M	M	M	M	M	M	M	M	M	P	M	M	M	M	P	P	P	P	M	P	***M***
Oniticellini + Onthophagini + Onitini + Sisyphini + *Epirin*	M	M	P	M	M	M	P	P	M	M	P	P	P	M, a	P	M	P	P	P	P	P	M	P	P	P	***P/M***
Gymnopleurini + *Anachalcos* + *Heliocopris*	M	M	M	M	M, e	M	M	P	M	M	M, e	P	M	P	M	M	M	M	M	M	P	M	M	P	P	***M***
Phanaeini + Eucraniini	M	M	P	M	M	M	P	M	M	P	P	P	M, e	M	U	M	M	M	P	M	M, e	M,e	M, e	M, e	P	***M***
Phanaeini + Eucraniini + *Ateuchus* + *Dichotomius*	M, g	M	P	M	M	P	P	M	P	P	M	P	P	M, g	M, g	P	M	M, g	P	P	P	P	M	P	P	***P/M***
American Canthonini	P	P	M	P	M	P	M	P	M	P	P	P	P	P	P	P	P	M	M	M	M, a	M	P	M	M	***P/M***
Small' American Canthonini (1)	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Large' American Canthonini (2)	M, a	M	M	M	M	M	M	P	M	P	M	P	P	P	P	P	P	M	M	P	P	P	P	M	P	***P/M***
Eurystemini + American Canthonini	M, f	M, f	M, d	P	P	P	P	M, f	P	P	P	P	P	P	P	M, f	M, a	P	M	M	P	P	P	P	M	***P/M***
Australian Canthonini	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***
New Caledonian Canthonini	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Coptodactyla + Australasian Canthonini	M	M	M	M	M	M	M	P	P	P	M	P	M	M	M	M	U	M	P	M	M	P	M	P	M	***M/P***
Demarziella + Australasian Canthonini	M	M	M	M	M	M	P	P	M	M	M	M	M	M	M	P	M	M	M	M	P	M	M	P	M	***M***
Coptodactyla + Demarziella + Australasian Canthonini	M	P	M	P	M	M	P	P	P	P	P	P	M	M	P	P	U	P	P	M	P	P	M	P	M	***P/M***
New Caledonian Canthonini + Australian Canthonini	M	M	M	M	M	M	M	M	M	M	P	M	M	M	M	M	U	M	M	M	M	M	M	P	M	***M***
New Zealand Canthonini + Australian Canthonini	M	P	M	M	M	M	M	P	M	M	M	M	P	P	M	M	U	M	M	M	M	M	M	M	M	***M***
New Caledonian + New Zealand + Australian Canthonin	P	P	M	P	M	M	M	P	M	M	P	P	P	P	P	P	U	M	M	M	P	M	P	P	M	***P/M***
Coptorhina + *Sarophorus*	M	P	M	M	M	M	M	P	M	P	P	P	P	P	M	P	P	M	M	P	M	M	M	M	M	***M/P***
Coptorhina + *Sarophorus* + *Odontoloma*	P, b	P, b	P, b	P, b	P, b	M, b	P, b	P, b	P, b	P, b	P, c	P, b	P, b	P, b	P, b	P, b	P, b	P, b	P, b	P, b	M	P, b	M	P, b	P, b	***P***
Copris	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	***M***
Uroxys + Australian Canthonini	P	P	P	P	P	P	M	P	M	M	M	P	P	M	M	M	P	M	M	M	P	P	P	M	P	***P/M***
Uroxys + *Copris*	M	M	M	M	M	M	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	***P***

(1) = *Canthon* (not *smaragdulus*) + *Scybalocanthon* + *Anomiopus*
(2) = *Deltochilum* + *Megathoposoma* + *Hansreia* + *Canthon smaragdulus*
a = monophyletic with inclusion/exclusion of stray group
b = basal groups
c = basal groups, excluding one genus
d = monophyletic with inclusion of *Canthidium haroldi*
e = monophyletic with inclusion of *Gromphas*
f = monophyletic with small American canthonines
g = monophyletic with *Ateuchus* only

Since the topology remains largely consistent for the closely-related species (such as within tribes), and is most affected by the different alignments at the deeper nodes, it is quite apparent that the difficult to align hyper-variable regions are causing this. It is also evident that because it is the more recently derived taxa which are best resolved, that the inclusion of the fast-evolving hyper-variable regions will hold little helpful phylogenetic information for resolution between the tribes, and are more likely in fact to be positively misleading and at odds with the rest of the data. Their exclusion therefore not only negates the need for choosing between the methods of analysis (the two-step approach of alignment generation by distance methods followed by a separate tree search, or the combined approach of optimization alignment), but also removes any subjectivity in choosing gap costs or in choosing a preferred topology. Although a length-invariable data set suffers from none of the problems of alignment, it is unfortunate to have to remove data in this manner, as it would seem likely to contain some phylogenetic signal for all taxa. However, the hypervariable regions of rRNA genes are in fact thought to converge in secondary structure due to the nature of the main mutational mechanism – slippage, which appears to be occurring in these regions, so apparent homology between taxa in these areas might be due to convergence of primary structure rather than shared ancestry (Hancock and Vogler 2000). This further supports the decision to not use these regions in the analysis, and suggests that this is the option most likely to allow the 'true' signal from the more conservative characters to filter through the 'noise' generated by the variable regions.

Both topologies produced by the weighted and unweighted analyses of the length-invariable data set proved to be equally representative of the clades recovered by the 25 different analyses (Table 2.6). A decision was therefore made to adopt the tree produced by the unweighted analysis as the preferred cladogram, since it was felt that a higher cost assigned to transversion type substitutions was less justified with the exclusion of the A/T rich length-variable regions. A further search using PAUP* (as described in section 2.2, but with 5,000 replicates) confirmed that this was the shortest tree found for the unweighted data at 12840 steps. The single tree chosen at random from the 100 shortest saved trees (as used in the guide tree alignment; analysis B) was selected as the 'preferred tree', since movement of a few rogue taxa within their monophyletic groups tended to collapse the Phanaeini and part of the *Onthophagus* clade in the strict consensus tree, producing two unresolved nodes. As such these are minor changes and do not alter the topology at any of the more significant levels. The

preferred tree, as the best phylogenetic estimate found using this methodology, is displayed below in figure 2.2, with bootstrap support and Bremer support values mapped on. If we accept this tree, it can be used to investigate the evolutionary trends with regard to nesting strategy and biogeography. The Incongruence Length Difference test returned a p-value of 0.99, representing no significant incongruence between the three data partitions (gene regions).

Figure 2.3 displays the length-invariable tree with the character of nesting strategy traced onto the topology. Designation follows that discussed above, and in chapter 1, with the Eurysternini, *Oniticellus* and *Tragiscus* (and the Aphodiinae outgroups) categorized as dwellers, and the Eucraniini as 'carriers'. Several nodes in the tree were found to be equivocal when resolving them under parsimony.

Figure 2.4 illustrates the same tree with the biogeographic region of each species mapped on. There are again a few equivocal nodes.

Figure 2.2. Most parsimonious tree obtained using PAUP, from the length-invariable data set. Bremer support values are displayed above the branches, whilst the bootstrap support is given below. Species codes are given in table 2.1.

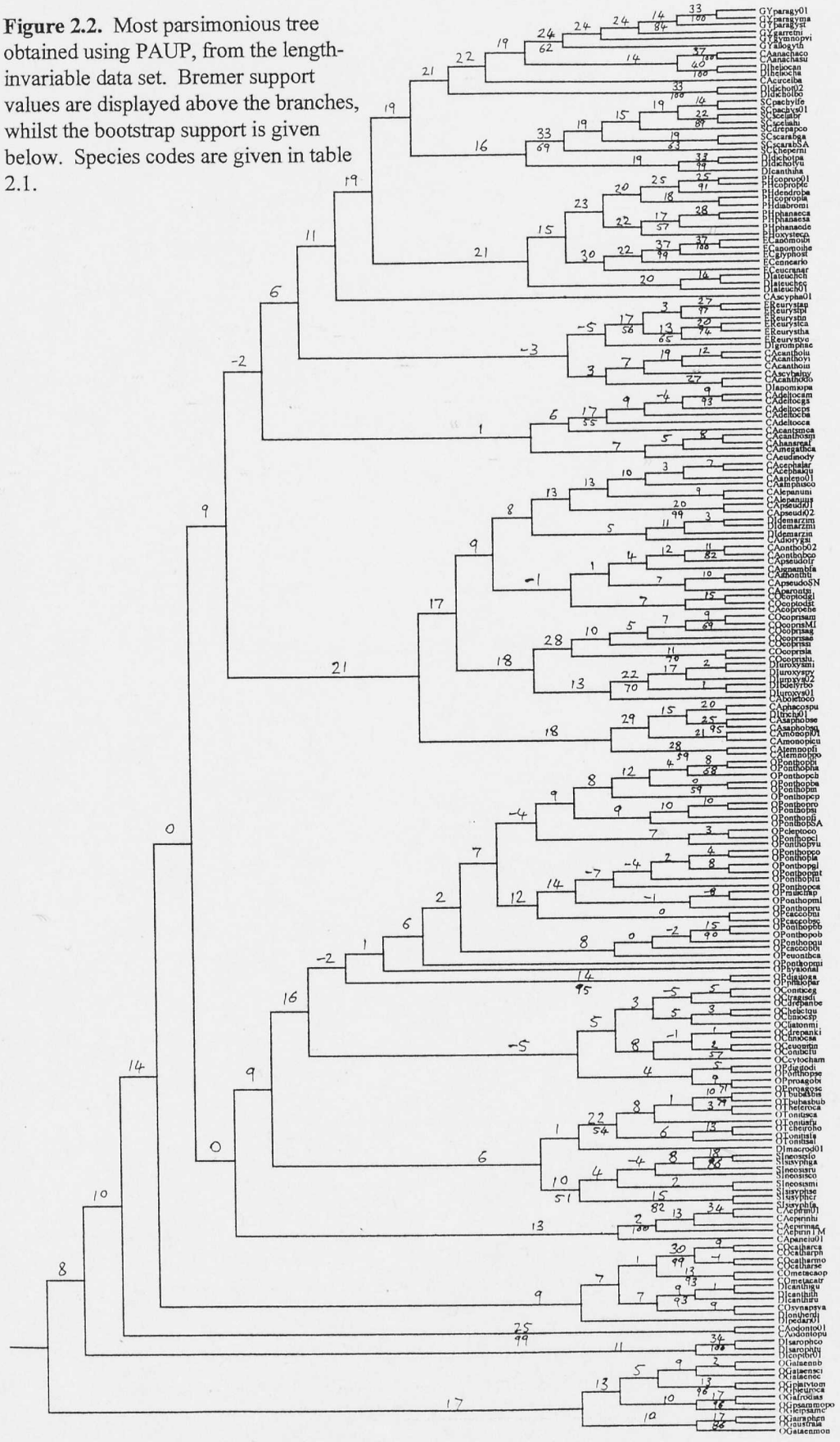


Figure 2.3. Length-invariable cladogram displaying species nesting behaviour

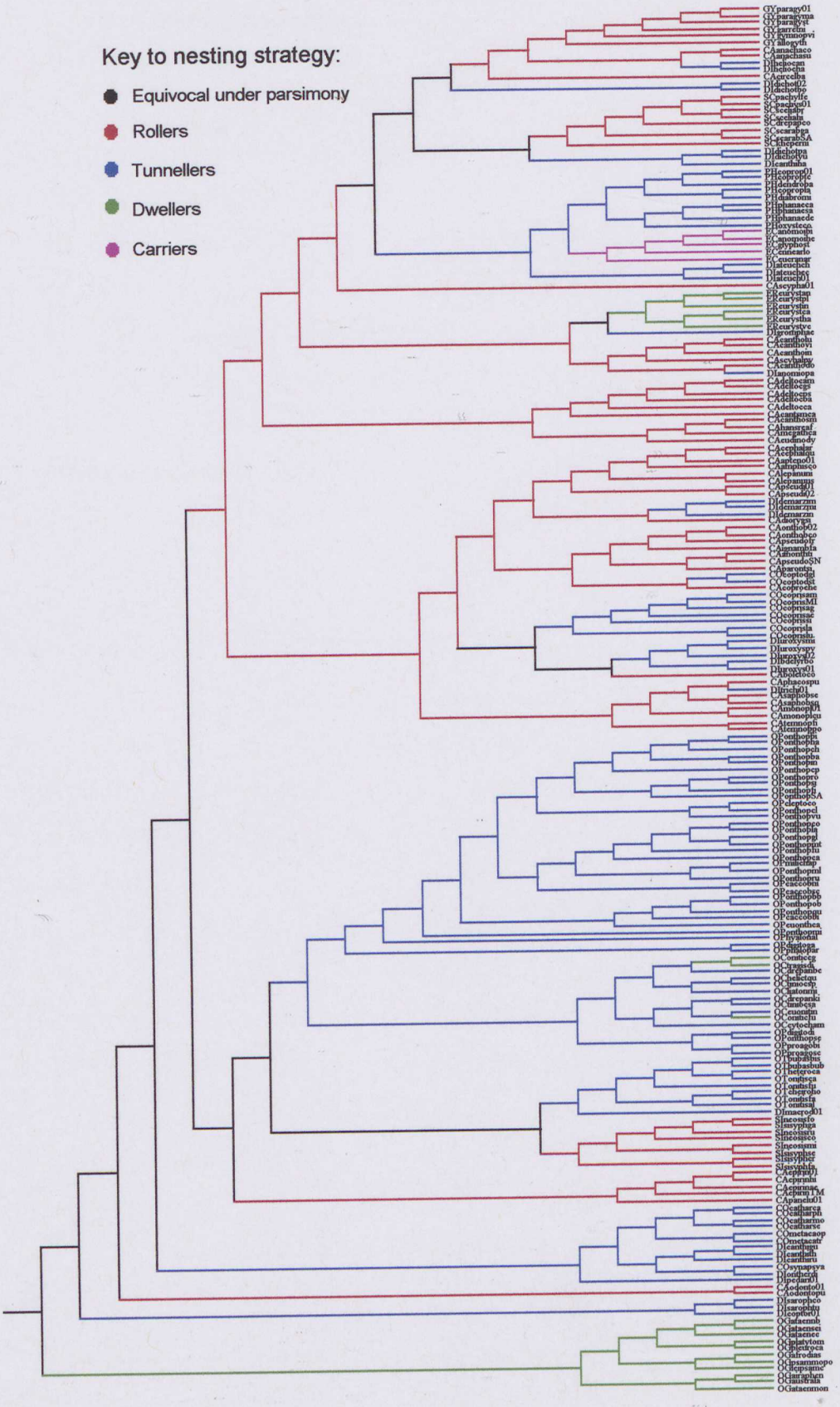
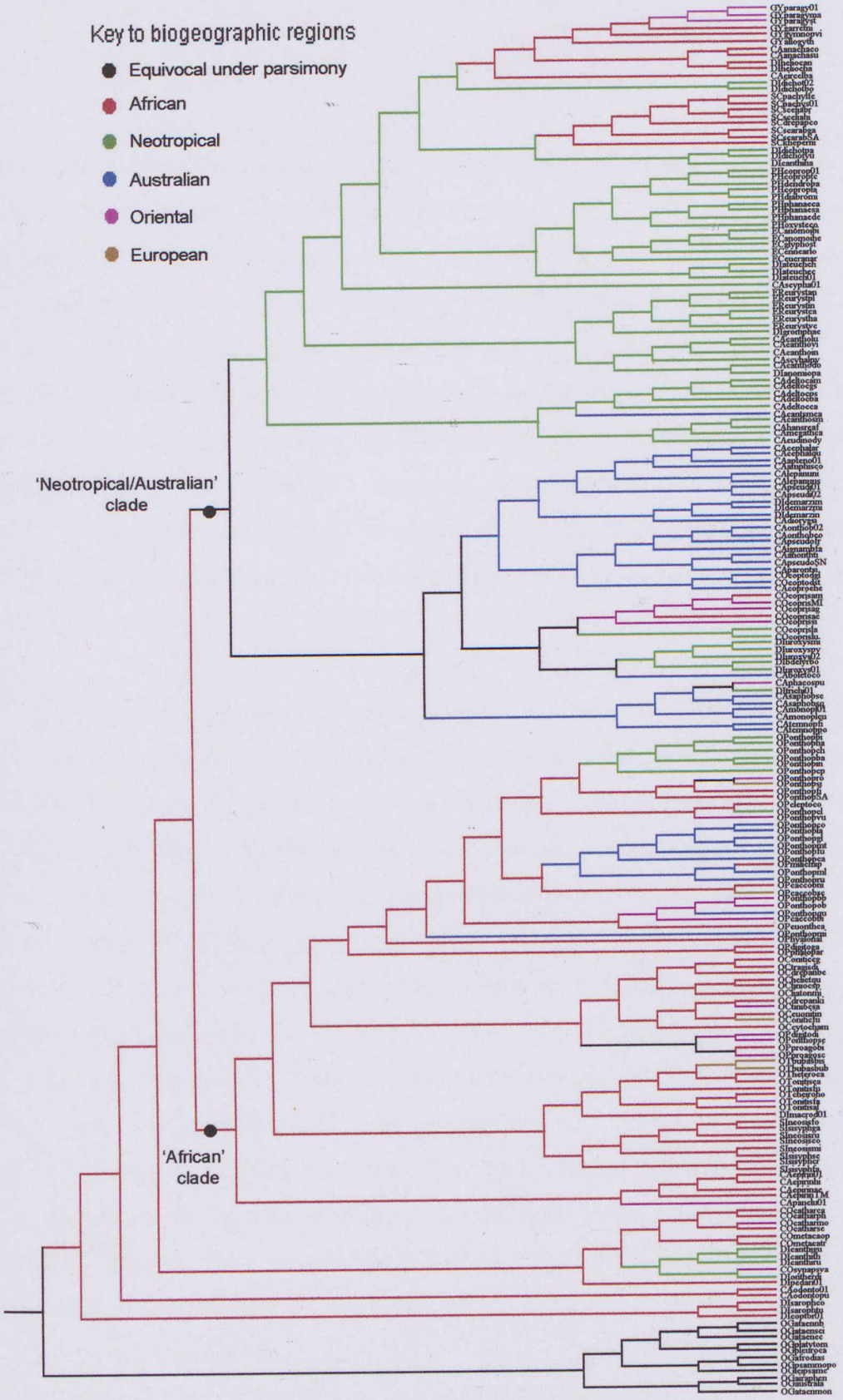


Figure 2.4. Length-invariable cladogram displaying biogeographic region of species



2.4 Discussion

2.4.1 The nature of the data set

Within the scope of this thesis, I have basically tested for a single key parameter of uncertainty, alignment, since this has been the most critical and influential component affecting the topology. As discussed by Hall (2001), the examination and modification of penalties is the single best means of improving the alignment and ultimately obtaining the most unambiguous phylogenetic signal from the data. We have seen that the support values remain low for the length-invariable tree, and it is clear that many of the relationships found are rather weakly supported. It is for this reason that attempts were primarily made to obtain a more accurate alignment of the length invariable regions, since the signal that is contained within these regions is potentially considerable and might have increased support and helped in the resolution of the topology.

However, a poor alignment of many characters will affect a number of assumptions. For instance, parsimony can fail if the assumption that change is rare is inappropriate, and when the evolutionary rates vary greatly across different branches (between taxa) (Felsenstein 1978). With the inclusion of the length-variable regions, the assumption that all characters are evolving at the same rate is clearly not the case, since different regions within the rRNA genes are clearly evolving at different rates. By excluding the hypervariable regions from the data where alignment is highly questionable, a strategy advocated by Hillis and Dixon (1991) to reduce unnecessary noise in a data set, a large number of the homoplastic characters distorting the resolution of the tree were removed. Unfortunately this has resulted in the removal of some 180 characters from the data (table 2.5) and may also cause the loss of some phylogenetic information. However, the ILD test result supports the decision to combine the three genes into a single 'total evidence' analysis, since no significant incongruence was found between the different partitions.

Future work on the data set is likely to include a fuller investigation of the effects of noise and saturation on the length-invariable alignment, but more character data is ideally required to improve the resolution at deeper levels of the topology. This is further complicated as distantly related taxa are apparently being pulled together

through long-branch attraction. An attempt has been made to compensate for this by the inclusion of as many taxa as possible, since this becomes less problematic where taxa are widely separated (Hillis 1996). However, it is also possible that new long branches can be introduced with new taxa, and that additional character sampling would have increased the phylogenetic signal more successfully (Mitchell et al. 2000).

Despite these problems, the congruence displayed by the different topologies lends important support for many clades which are consistently recovered, regardless of the tree-estimation methods, parameter choice and data included/excluded (table 2.6). Wenzel and Siddall (1999) suggest that even high levels of noise are generally insufficient to contravene good synapomorphies; when 40% of their experimental data matrix was noise, the majority of clades were still recovered. Whilst noise has caused the support levels to be lower, the consistent recovery of many scarabaeine clades is reassuring. The unstable nature of some of the other groups however suggests character saturation, and requires these relationships to be viewed with some scepticism.

The more consistent patterns will be discussed here alongside some of the biogeographical and behavioural patterns implied by the preferred tree. Due to the size of the phylogeny, one can draw a lot of conclusions of interest to a dung beetle 'specialist' from the relationships inferred between the ingroup taxa; for instance regarding which species within a genus are most closely related to one another, or how two taxa might be morphologically or behaviourally similar. I will however restrict myself here to discussing the higher-level and main evolutionary trends evident in the topology, with reference to a few relevant examples for illustrative purposes. This phylogeny is of course a best estimate given the available data, but would benefit from an increased number of informative characters.

2.4.2 The relationship of the Scarabaeinae and Aphodiinae

All analyses conducted have found the Scarabaeinae to be a monophyletic ingroup, albeit with the necessary exclusion of some more distant and substitution-saturated outgroup sequences. To investigate the relationship between the Scarabaeinae and their hypothesised sister group, the Aphodiinae (Browne and Scholtz 1999), we took a subset of the 16S and 28S data presented here and combined it with a similar sized Aphodiinae data set (Maté 2002) for alignment and phylogenetic analysis. Although the base of the

Scarabaeinae + Aphodiinae tree is weakly supported, we found evidence to suggest that the Scarabaeinae are in fact nested within the Aphodiinae, making this group paraphyletic (Maté, Inward and Vogler, in prep). The aphodiine tribes Psammodiini, Eupariini and Aegiliini were all closer to the Scarabaeinae than *Aphodius*, which supports their role as outgroups for the Scarabaeinae phylogeny. The tree also suggests that the Scarabaeoidea family Aulonocnemidae are located basally to the Scarabaeinae, and also within the Aphodiinae. This is an interesting finding, as the Aulonocnemidae were once considered to be members of the Scarabaeinae, due to them sharing such characters as a single meta-tibial spur, rather than the two found in the Aphodiinae (Maté 2002). More recently, this enigmatic group was tentatively placed ancestral to the aphodiines and scarabaeines, due to mouthparts intermediate in structure between the filtering mouthparts of the dung beetles and the grinding mandibles of the other scarabaeoids (Cambefort 1987).

2.4.3 The basal taxa and the origin of dung feeding

The genera *Coptorhina*, *Sarophorus* and *Odontoloma* were found to form the paraphyletic basal group within the Scarabaeinae in 23 of the 25 analyses. Whilst the two lineages of *Odontoloma* and *Coptorhina* + *Sarophorus* occasionally switched positions, it was the latter clade that was generally recovered to be the single most basal, as in our preferred tree. *Coptorhina* is a fungus-feeding genus, and its position in the phylogeny is supported by the work of Zunino (1984), who considered the group to be ancestral and primitive. Its feeding behaviour also lends some support to the suggestion that the Scarabaeinae arose from mycetophagous ancestors (Scholtz and Chown 1995). It is also noteworthy that the Aulonocnemidae, suggested by us to be immediately ancestral to the group, are also saprophagous (Cambefort 1987). This might suggest that the transition of dung feeding within the Scarabaeinae occurred at a basal node, implying an independent acquisition of coprophagy in the Aphodiinae. This possible multiple origin of coprophagy would tend to indicate that this group of Laparostict scarabaeoids has a certain predisposition towards dung feeding, which may be linked to the evolution of filtering-type mouthparts (discussed by Cambefort 1991c). The evidence for the evolution of dung feeding early on in the history of the Scarabaeinae lends significant credibility to the idea that has it has played a vital role in the subsequent radiation and diversification of the group, but it is interesting that the presumed primitive state still exists in the extant taxa.

We can deduce that the Scarabaeinae were certainly around before the Tertiary period – a suggestion of a Gondwanan origin for the group (discussed below) would imply a minimum age of around 100 million years. We find good phylogenetic evidence that the isolated dung beetle lineages in New Zealand and New Caledonia are derived from Australian taxa, and which seem likely to have separated from their Australian ancestors when the island groups broke away at the end of the Cretaceous period some 60 to 80 million years ago (Cox and Moore 1993). Although in itself, this is not proof of a vicariant origin, it suggests that some Scarabaeinae might even have lived alongside, and fed upon the dung of dinosaurs as has been previously suggested (Jeannel 1942, Chin and Gill 1996). Prior to the Cretaceous extinction event, around 65 million years ago, herbivorous dinosaurs were huge (Wing and Tiffney 1987), and there would therefore have been a considerable dung resource available to the group. If the highest global diversity and abundance of Scarabaeinae today (found in the African savannas) is indeed due to an abundance and diversity of dung types, it would seem likely that the megaherbivores of the Cretaceous period would have provided an even richer resource, and the diversity of the associated dung beetle assemblages might have exceeded anything on earth today. No equivalent large dung types would have been available to replace the dinosaur dung upon their extinction, since the mammals of the period were almost all small, probably insectivorous or frugivorous animals (Cox 2000). A rich dung beetle fauna may well have gone extinct, along with so many other groups at that time, and the small-bodied taxa identified as basal in the phylogeny may have been amongst the few groups to persist by utilising the small dung resources of the mammals (or other surviving organisms) of that period. Subsequent diversification of the Scarabaeinae with the radiation of mammal in the Tertiary may have simply paralleled an earlier coevolution with dinosaurs.

2.4.4 The 12 tribes

There is good support for the monophyly of many of the tribes, both from high bootstrap and Bremer support values and from the congruent topologies from the independent analyses. We can be confident therefore that the Eucraniini, Eurysternini, Gymnopleurini, Onitini, Phanaeini, Scarabaeini and Sisyphini are all monophyletic groups. In contrast, the Canthonini, Dichotomiini and Coprini are all clearly polyphyletic, and cannot be considered at the same taxonomic level as the other tribes.

The Canthonini and Dichotomiini each tend to be split into 6 or so distinct groups distributed throughout the cladogram, although many of these groups are weakly supported at the node joining them to a sister group, so their precise position in the tree is uncertain, and was frequently found to vary with the analytical assumptions made. The Dichotomiini are the most disjointed of all the tribes, with the support for sister relationships being particularly poor, and leading to particular uncertainty for these groups, so that little comment can be made about them with any great confidence. The tribe Coprini is seen to be clearly composed of three independently derived clades, which do not come together in any form under any analysis. Once more, the position in the tree of the two clades of *Catharsius* + *Metacatharsius* and *Copris* is debatable, but *Coptodactyla* is always found amongst the Australian Canthonini (discussed below). The genus *Copris* is interesting however in its well-supported monophyly, despite its worldwide distribution (equalled only by *Onthophagus*). Unfortunately, few well-supported conclusions can be drawn as to the validity of the morphological cladistic analysis by Montreuil (1998) upon the Dichotomiini and Coprini, in which he reclassified many genera into two new monophyletic groups, the Ateuchini and the Coprini. I would certainly concur with his assessment of the two groups being paraphyletic, but the molecular data suggests much greater polyphyly than he found, and gives no support for the monophyly of the new groups. This is probably due to both poor resolution of the molecular data at the required level, and (perhaps even more) to a lack in the morphological study of a sufficient diversity of other scarabaeine 'outgroups' for his analysis (rollers for instance). Wider taxon sampling might have returned a result more similar to those of this molecular analysis.

Although similarly polyphyletic, the various canthonine clades retain a more distinctive structure which is not significantly altered by the choice of parameters. Two important and substantial clades can be identified as the 'South American canthonines' and the 'Australian canthonines', which both have a tendency to be paraphyletic.

There appears to be a lack of sufficient information from the sequence data for these older 'tribes' (which may be due in considerable part to the saturation of many potentially informative characters with multiple substitutions, so masking homologies). It seems likely that these groups probably diverged early in the group's history, and are as distant from one another as the data suggests. Given that the diverging lineages would probably have then given rise to the more 'recent' tribes, the extant taxa

representing the more ancient lineages would become paraphyletic and consequently widely distributed on the tree. If the dichotomiine and canthonine groups are as basal as they are believed to be, it is perhaps unsurprising that these loci which are proving informative at a sub-tribal level (despite being carefully chosen to maximise their potential), are only weakly placing the older groups.

One other tribe has consistently been found to be paraphyletic; the Onthophagini, since the tribe Oniticellini are apparently nested within it. The Oniticellini are possibly a paraphyletic group themselves, since many of the analyses found two or three onthophagine species within this tribe, but this is more likely due to the close relationship between the two groups - exclusion of the length invariable regions recovered the Oniticellini as a monophyletic group in the preferred tree. This close relationship is unsurprising from a morphological viewpoint; Zunino (1984) concluded that the two tribes were sister taxa, and even Fabre (1918) noted that the larvae of *Oniticellus flavipes* have "*a magnificent horn on his corselet and a row of four spikes on each side of his abdomen, as is the rule among the Onthophagi*".

2.4.5 The evolution of nesting behaviour

One of the most important findings to emerge from this study is our estimation that neither rolling, tunnelling nor dwelling is a monophyletic behaviour. No evidence has been found to support the hypothesis that rollers and tunnellers exist as two sister clades, but rather that these strategies are thoroughly polyphyletic. It is particularly noteworthy that there exists two different nesting behaviours within the two basal lineages, for *Coptorhina* and *Sarophorus* are tunnellers, and *Odontoloma* is a roller. Rolling therefore not only evolved on multiple occasions, but apparently arose very early on in the group's history. Considering the length-invariable tree, rolling may have arisen a total of four or five times, and in some cases may even be an ancestral behaviour from which some of the tunnelling clades have arisen, depending on how one interprets the equivocal nodes in figure 2.3. Either way, it is clear that rolling is not the highly derived, single-origin event that it has been postulated to be. Rather the Scarabaeinae appear to be extremely plastic in terms of their nesting strategies, with frequent switching between rolling and tunnelling.

To include the Eucraniini in with the true rollers seems to be unjustified. Of the different analyses conducted, 18 out of the 25 found the tribe to be the sister group to the Phanaeini which are tunnellers. No support at all is found for the suggested sister relationship between the American Phanaeini and the African Onitini (Zunino 1984). The unique eucraniine behaviour of carrying dung with the forelegs and clypeal prongs appears to be an adaptation unrelated to true rolling, but once more illustrative of the plasticity of dung beetle functionality. It is interesting that the eucraniine sister group contains various species which are well known to move portions of dung with their heads by butting (Halffter et al. 1974, Gill 1991). Perhaps the Eucraniini strategy of dung translocation can be explained by a transition from simply using the head, to employing the front legs also.

The Eurysternini were only found to be a true sister group to the American canthonines in less than half of the analyses (including the preferred tree), but were just as frequently recovered to be in the same paraphyletic group as them. There is a strong indication that the tribe is closely related to the American canthonines, which would explain their roller-type morphology, ability to make balls and consequent traditional classification as rollers.

In a similar way, the other dwellers within the Oniticellini have seemingly undergone a reversal from tunnelling, as found in the other genera of the tribe and throughout the Onthophagini, to dwelling. The phylogeny (and supporting bootstrap values) suggests that this behavioural adaptation may even have evolved twice in the group, but importantly all analyses place the two main Scarabaeinae dweller groups in different parts of the tree, and we can be quite confident that the behaviour is independently derived in each case. The fact that *Oniticellus* and *Eurysternus* have independently switched to live functionally as dwellers does not merely represent another example of the functional plasticity of the group. This finding is of considerable importance to our understanding of dung beetle evolution, since it suggests that the possession of an apparently plesiomorphic and less competitive nesting behaviour is due to factors other than phylogenetic history. Whether this can be related to environmental pressures, empty niche space or competitive exclusion is only conjecture at this stage, but it provides an interesting viewpoint for the following chapters.

2.4.6 *The role of biogeography*

Another crucial, but unexpected result is the degree to which the dung beetle phylogeny corresponds to biogeographic regions. Figure 2.4 clearly shows the basal clades to be of Southern African origin, followed by a weakly supported intermediate clade of mixed regional affinities, and then two main sister clades which can be interpreted as an African clade and a Neotropical/Australian clade. Another implication of the relationships identified at the base of the topology, is the suggestion of an African origin for the Scarabaeinae. There are a number of early divergences involving African lineages, and the two most basal lineages are restricted to Southern Africa.

2.4.7 *The 'African clade'*

The 'African clade' (as indicated on figure 2.4) comprises four tribes with a few additional taxa. As discussed, the Oniticellini and Onthophagini form a monophyletic group, and sister to this in the length-invariable tree is a clade consisting of the Sisyphini and Onitini. It must be noted though that more typically in the other analyses, the Onitini and Sisyphini were found to be a paraphyletic group basal to the other two tribes. The African 'canthonine' genus *Epirinus* was usually recovered basal to this group, as in the preferred tree. Within the Onthophagini, most of the Neotropical and Australian species of *Onthophagus* form a distinct monophyletic group for each region (with a few notable exceptions), whilst the South East Asian *Onthophagus* seem to be more widely drawn from the group.

2.4.8 *The 'Neotropical/Australian' clade*

This group (figure 2.4) can be further divided into two sub-clades, the first of which includes the Australian (or more strictly Australasian) canthonines plus an additional group of *Copris* and *Uroxys*. Copris tends to group together with *Uroxys* when the variable regions are removed from the data, whilst its relationship with the Australian canthonines is rather variable, and may be an example of long-branch attraction between two widely divergent groups. *Uroxys* is more frequently recovered with these canthonines (table 2.6), but support is similarly poor. The Australian canthonines themselves were always found to be either paraphyletic, or even polyphyletic under some conditions, and the topology of the preferred tree illustrates this. The New

Caledonian taxa (*Onthobium, Anonthobium, Paronthobium, Pseudonthobium* and *Ignambia*) are always found as a monophyletic sub-clade within the group, as are the species of the New Zealand genus *Saphobius*. The most complete study of the Australian canthonines to date is that of Matthews (1974), who found the New Caledonian and New Zealand taxa to be closely related to the Australian groups. However, he also classified *Demarziella* and *Coptodactyla* as the sole representatives of two other tribes in Australia - the Dichotomiini and Coprini respectively, not least because both are functionally tunnellers. Conversely, the molecular data consistently recovered these taxa amongst the Australian canthonines. A tree search of the length-invariable data set, in which the Australian canthonines plus *Demarziella* and *Coptodactyla* were constrained to be monophyletic, only increased the length of the tree by 11 steps (out of a total of 12851 steps). This seems to be a more likely scenario than having *Copris* and *Uroxys* nested within the clade.

Sister to the Australian clade, is one which contains the majority of the endemic Neotropical groups, including the Phanaeini, Eucraniini, Eurysternini and all of the American canthonines. Additional to this are a few American dichotomiines and two monophyletic African roller clades, the Gymnopleurini and Scarabaeini, which probably do not truly belong in this clade, as they were found to switch positions in the topology frequently. There is no apparent morphological evidence to support a close relationship between the Gymnopleurini and Scarabaeini; if anything, they appear to be considerably morphologically diverged from one another (F. Genier pers. comm.). There is however good topological congruence within the molecular analyses suggesting that *Anachalcos* and *Heliocopris* are basal to the gymnopleurines. There is also good support for the sister relationship of the Eucraniini and Phanaeini, and of the proximity of the Eurysternini to the South American canthonines. The canthonines, which are paraphyletic in the preferred tree, were frequently recovered as a single monophyletic group (particularly by the POY optimisations), and certainly seem to all be closely related.

2.4.9 An interpretation

Despite the weak support afforded to many of the nodes in the phylogeny, the topological congruence provided by the numerous analyses and the length-invariable tree still provide us with a reasonable 'best estimate' of the key patterns in the evolution

of the Scarabaeinae. Based upon this available evidence, a number of hypotheses can be suggested.

The position of the African taxa *Coptorhina, Sarophorus* and *Odontoloma*, basal to both the African and Neotropical/Australian clades, suggests two possible alternative hypotheses regarding the origins of the group. Firstly, this might indicate an African origin for the group, with subsequent dispersal to the other continental land masses ultimately accounting for the distribution seen today. Many of the scarabaeine groups are endemic to particular regions, and relatively few monophyletic clades are widespread. The hypothesised close relationships between Neotropical and African taxa, such as the Phanaeini and Onitini (Zunino 1984), and the Eucraniini and Scarabaeini (Phillips and Scholtz unpublished morphological data) are not supported by this phylogeny, and with the notable exception of *Onthophagus*, it appears that more 'recent' dispersal between the continents was minimal until the formation of the Panama land-bridge. If the ancestral Scarabaeinae were indeed of African origin, it seems most likely that dispersal to South America (and Australia) must have been relatively early on across the widening Atlantic ocean. The relationship between the Australian and Neotropical taxa can then be explained by the continued links, via Antarctica, of these two continents (discussed below).

The second hypothesis, also consistent with the phylogeny, is that the basal African taxa were in evidence before the break-up of Gondwana, which began at the beginning of the Cretaceous era, with the separation of Africa from Antarctica and Australia, and its subsequent separation from South America some 100 million years ago (Duncan and Hargraves 1984). This would suggest a minimum age for the group of 100 million years, and a Gondwanan origin. Africa's separation from Eurasia in the early Tertiary means that it was in fact in an isolated position from the Late Paleocene (some 60 million years ago), until it collided with Eurasia in the middle Miocene (Morley 2000). Increased taxon sampling or the inclusion of additional gene loci, might reveal other similarly ancestral taxa still extant in the other Gondwanan-derived southern continents. Indeed the position of the mixed-origin clade, between the basal taxa and the principal African and South American/Australian clades, may be more meaningful in this capacity than its support suggests. We might expect to find examples such as this intermediate clade, where extant groups on opposite sides of the Atlantic are more closely related to one another than to other groups coexisting in the same localities, due

to a sharing of common ancestors over 100 million years ago before the division of the continents.

We can assume that the earliest Scarabaeinae were tunnellers, as the ancestral habitat of the Scarabaeoidea is soil (Scholtz and Chown 1995), but it seems that the translocation of dung by rolling might already have evolved as a strategy before the break-up of Gondwana. Cambefort (1991a) theorised that the continents derived from Gondwana would have "possessed an initial fauna of Canthonini and Dichotomiini". This hypothesis suggested that the two divergent lineages subsequently gave rise to all rollers and tunnellers in each region. Whilst the ideas of monophyletic functional groups have clearly been falsified, it seems likely that basal rollers and tunnellers would have been present on the separating biogeographical regions, given the nesting behaviours of the basal groups. Through a process of vicariant cladogenesis, the Old World and New World taxa subsequently evolved in isolation from one another. Whilst South America separated from Africa around 100 million years ago (Duncan and Hargraves 1984), it remained in contact with Antarctica via the Drake passage up until about 40 million years ago. Antarctica also remained in contact with Australia until 90 million years ago although the two continents are believed to have remained close until around 45 million years ago (Cox 2000). This meant that there was a link between South America and Australia for a considerable time after Africa split away. During this period, the earth's climate was remarkably warm; the poles were ice-free and there was a belt of moist forest reaching across from southern South America, through Antarctica and into Australia throughout the Palaeocene and into the Eocene periods (Morley 2000). This allowed great scope for interchange between the southern continents. The fossil record indicates that various South American placental mammals had spread into Antarctica during the Eocene, and that marsupials had probably moved from Antarctica into Australia at some point (Cox 2000), and an interchange of ratite birds and higher plants also occurred (Stehli and Webb 1985). It does not seem unreasonable that the dung beetle fauna might have followed the radiation of the mammals. The apparent close relationship between the Australian canthonines and the Neotropical canthonines is almost certainly a reflection of this sustained link between the continents after the initial break-up of the Gondwana land mass. Taxa in the two continents probably only really diverged after the Antarctic link was severed and gene flow ceased. Comparing my own data from Ecuador with an assemblage in French Guiana (Feer 2000) suggests that a high generic and even species overlap can be sustained between distant assemblages

when a continuous corridor of forest exists between them – in this case, the two Neotropical moist forest sites are over 2600 km apart (chapter 3). It is noteworthy that the Isoptera (termites) of Australia are also most closely related to those of South America (Donovan et al. 2000), also thought to be due to their one-time linkage via Antarctica.

The apparent relationship between the Australian canthonines and the American genus *Uroxys* may also be a remnant of this link, but topological congruence is low, and any conclusions would be weakly supported. *Copris* has diversified primarily in the Old World, and is probably artificially placed due to insufficient evidence to place it elsewhere. I suggest that the position of the Gymnopleurini and Scarabaeini clades with the Neotropical taxa should similarly be treated with suspicion; like *Copris* their unstable behaviour under different analytical parameters makes it hard to determine where they might belong on the topology. They probably diverged from all other taxa long ago, and may well have no close extant sister taxa, compounding the problem of long-branch attraction, and their spurious placement with other taxa. The gymnopleurines seem to have quite a strong affinity with *Anachalcos* and *Heliocopris* however. *Anachalcos* is believed to be another relictual (possibly Gondwanan) canthonine, and as such provides evidence of the rolling ancestry of the Gymnopleurini tribe. This suggests also that *Heliocopris*, a group which includes the worlds largest tunnelling species, has undergone a reversal in its nesting behaviour, from rolling to tunnelling, a shift which is also supported by morphological evidence (F. Genier pers. comm.).

2.4.10 The Australian taxa

From the Australian canthonines we see a monophyletic radiation (to a total of 10 different genera) within New Caledonia, which divided from Australia at the beginning of the Tertiary period, and is thought to be home to some of the earth's oldest rainforest flora (Morley 2000). This gives us a likely age of the split between the Australian and New Caledonian dung beetle taxa at around 60 million years ago. New Zealand, which has only two genera of Scarabaeinae today, would have divided from Australia at about the same time as New Caledonia, sometime in the Late Cretaceous or early Palaeocene (Cox and Moore 1993), and the two groups of islands have similar histories, and the only native mammals which each has are bats. Correspondingly in New Zealand, the

13 species of *Saphobius* (represented in the phylogeny), and the mono-specific *Saphobiamorpha*, feed on decaying fruits and leaves (Halffter and Matthews 1966). The breeding biology of the New Caledonia fauna is less well known, but the adults are attracted to standard dung-baited traps, and are therefore thought to feed on bird or reptile dung (G. Monteith pers.comm.). New Zealand may have once had a higher diversity of scarabaeines similar to that of New Caledonia, as it supported a similar rainforest vegetation until a deterioration of climatic conditions during the middle Pliocene when much of it became extinct (Morley 2000). It seems reasonable that a decline in the Scarabaeinae may have also occurred. The more northerly position of New Caledonia, at a similar latitude to the remaining rainforests in Queensland, presumably allowed the rainforest to persist here, and perhaps maintained its greater diversity of scarabaeines.

Two other groups of interest are nested within the Australian canthonines, are the genera *Demarziella* and *Coptodactyla* – until now considered to be members of tunnelling tribes through morphological studies. The lack of other tunnellers in Australia, apart from the genus *Onthophagus*, is certainly curious, when South America and Africa each have such a high diversity. Whether there were once more tunnellers present in Australia, which were subsequently lost as the climate altered and the prevailing habitat type changed from forest to the more arid environments which predominate today, we can only speculate. This phenomenon parallels the lack of placental mammals in Australia, when it is known that marsupials and placentals existed concurrently in South America before the break-up of the southern route between the continents (Keast 1972). Regardless as to the causes of Australia's unique assemblage structure, it seems likely the evolution of *Demarziella* and *Coptodactyla* is an adaptive radiation (sensu Schluter 2000), where ecological and phenotypic diversity has evolved within a multiplying lineage from a common ancestor. The presence of an empty niche (tunnelling) has been exploited by species from a group of rollers, which have apparently altered their rolling morphology (relative to that of their sister taxa) in order to take advantage of it. This will be further explored in chapter 5.

2.4.11 The Neotropical taxa

The American canthonines which are the apparent sister taxa to the Australian canthonines are seemingly paraphyletic, and include two main clades, one of

predominantly large species (including *Deltochilum* and *Megathoposoma*) and the other of small species (including *Canthon*). The small aphodiine-like 'dichotomiine' *Anomiopus* was consistently recovered within the group of small canthonines, a finding also supported by recent morphological examination (F. Genier unpublished); another example of reversal within a rolling clade to a more fossorial lifestyle. Equally intriguing, and more successful in terms of subsequent levels of speciation, is the evident reversal of the Eurysternini from the rolling strategy of the American canthonines in which they are nested, to that of dwelling.

The relationship between the American canthonines and dichotomiines is uncertain, as is the precise position in the topology of the Eucraniini + Phanaeini clade. There is a clear biogeographic basis to the relationship however. There is reasonable congruence to suggest that the Neotropical dichotomiine *Ateuchus* (and perhaps *Dichotomius*) are basal to this clade, and the relationship found between the two tribes is particularly well supported. It seems reasonable that the phanaeines retained a tunnelling behaviour from a dichotomiine ancestor, and that the Eucraniines evolved their unique carrying behaviour independently from any other rolling taxa.

Given the continuous forest coverage of the Amazon region of South America from the early Cretaceous to this day, and across most of Australia until the Eocene cooling event around 36 million years ago (Morley 2000), it seems highly likely that most of the taxa represented in the Neotropical/Australian clade would have evolved and speciated in rainforest habitat. With the subsequent loss of forest from southern South America and most of Australia, we still see that the greatest diversity of extant Scarabaeinae groups endemic to these continents is found in the forested areas. The one exception to this trend is the Eucraniini, which are restricted to the arid regions of Argentina, unlike their sister group, the Phanaeini which are forest-dwellers. The eucraniines presumably evolved in the drier non-forest habitats that persisted between the equatorial and southern bands of rainforest in South America prior to the Eocene cooling event, and which remain today south of the Amazon rainforest.

2.4.12 The Old World taxa

The basal taxa within the African clade (*Epirinus*) are also 'canthonines', and may indicate the origin of the rolling behaviour in the Sisyphini, the only other group of

rollers consistently recovered in the clade. A relationship between the Sisyphini and *Epirinus* is also supported by morphological data (F. Genier unpublished), as is the basal position of *Epirinus*. This would require a 'reversal' from this rolling ancestry to tunnelling in the other tribes in the clade. The Onthophagini and Oniticellini were found to be a single monophyletic group in all cases (excepting one where they were paraphyletic through the inclusion of Onitini). The exact nature of the relationship of the Onitini to the Onthophagini clade is harder to determine, although the three tribes were estimated to be monophyletic in 8 of the 25 analyses, which would require only a single reversal event. The switch to dwelling in two genera of Oniticellini has clearly occurred later on. The preferred tree illustrates the other less commonly found sister-relationship of the Onitini and the Sisyphini, in which it is equally parsimonious to postulate two transitions from rolling to tunnelling in the clade, or vice-versa. The higher-level relationship of these tribes does seem to be quite well established in any case (see table 2.6).

The dung beetle taxa present in Asian and European assemblages are predominantly drawn from the same clades which make up the African communities, but the enormous species and generic richness of these groups in sub-Saharan Africa (some 2000 of the world's 5000 species) strongly suggests that this is where the greater part of their evolution and cladogenesis took place. This is often linked to the great diversity of mammals which occur in the grasslands (e.g. Cambefort 1991a). No explanation however has been offered for the restriction of many of the apparently basal taxa, such as *Epirinus*, *Odontoloma*, *Macroderes* and *Circellium* to the Cape region. There is evidence that this region would have been occupied, at least during moister climatic periods, by a band of forest across the southern part of Africa (Coetzee 1978). These scarabaeine groups may have therefore originated in forest habitat (like the Australian and South American canthonines), which persisted in Southern Africa until the middle Miocene (Coetzee 1978). Small areas of evergreen forest persists in gulleys in the mountains of the Cape region which are thought to be refugial patches from this period (Morley 2000). In support of this forest origin, the sister taxa to *Epirinus* have been consistently found in the phylogenetic estimates to be *Panelus,* a south-east Asian genus restricted to tropical forest.

The Western Cape today however has a mediterranean climate and sclerophyllous vegetation. The establishment of the cold offshore Benguela current in the middle-

Tertiary, resulting from the development of ice in the Antarctic (van Zinderen Bakker 1975), has had a cooling effect on summer temperatures, and produces mild, wet winters. The more arid conditions to the north are thought to have isolated the plants of the region, and led to the high endemism seen in the Cape today (Dallman 1998). It may be that the once forest-dwelling Scarabaeinae of southern Africa, which were able to adapt to the changing conditions, have also become isolated by this climatic gradient.

The other African taxa, including the clade of Sisyphini, Onitini, Oniticellini and Onthophagini, as well as Gymnopleurini and Scarabaeini, all reach their greatest levels of diversity in the grasslands of southern Africa, and from here presumably spread into Asia and Europe. Since Scarabaeus, Sisyphus and Onthophagus have been recorded from the late Eocene period (around 40 million years ago) from French and Swiss deposits (Cambefort 1991a), they must have spread into Eurasia before the collision of Africa in the middle Miocene, when the main mammal interchange occurred between the regions (Van Couvering and Van Couvering 1975). This suggests that at least some taxa had moved northwards into the Iberian Peninsula after it came into close proximity with North Africa in the late Eocene (Smith et al. 1994). The phylogeny shows that the Oriental gymnopleurines clearly form a monophyletic group nested within the African genera, and although Onthophagus presents a more complex pattern, they appear to be African in origin, with early dispersals into Europe and Asia seeming likely. Subsequent colonisation events by *Onthophagus* probably occurred more recently; into Australia after its northwards drift had brought it close to South East Asia, and into the Americas via the Bering bridge during a warmer period of the earths history - in the Oligocene for example, with a number of Asian mammals which crossed into North America (Cox 2000). Movement into South America probably only occurred after the formation of the Panama bridge, perhaps explaining the decreasing species richness of *Onthophagus* moving south through the continent (Gill 1991, Cambefort 1991a).

2.4.13 Conclusions

Contrary to the widely held beliefs that rolling in the Scarabaeinae is a derived behaviour that originated only once, it seems that it evolved early on in the history of the group, and is in fact a functional strategy which has played a central role in the radiation of the subfamily. The molecular data suggests that rolling has arisen independently on multiple occasions, and that switching between rolling and tunnelling

has occurred often, and unexpectedly in both directions. It is this same plasticity which has allowed the nesting behaviour of dwelling to have arisen independently from both tunnelling and rolling ancestry, as well as unique adaptations such as carrying in the Eucraniini. The pressures which cause this switching have yet to be explained.

A second key element is also evident in the structure of the phylogeny, that of biogeographical history. Two hypotheses regarding the group's origin are supported, either African or Gondwanan, with subsequent cladogenesis taking place primarily within three primary continental theatres, Africa, South America and Australia, which has resulted in the distinct faunas seen today. Whilst the precise relationships between the taxa might still remain open to debate, and the phylogeny would benefit from the addition of more loci, this is the first time that such a strong biogeographic pattern has been identified as an elemental factor in the evolution of the group.

Chapter 3. An investigation into the structure of dung beetle assemblages in the Neotropics using a multidimensional approach

3.1 Introduction

The primary aim of this chapter is to characterize the structure of a 'typical' Neotropical dung beetle assemblage, and identify the key processes involved in its organization. Yet no single assemblage can really be considered typical, since it will always reflect the local ecological factors and inter-species interactions, as well as the phylogenetic and biogeographic history of its component taxa. However, by comparing systems which have different ecologies or histories, any similarities in structure which are identified can be interpreted as 'typical' for the given region, and the deviations interpreted in the light of the known historical or ecological variation. Thus the assemblages of a region can be characterized by the limits of their variance. Once we understand the structuring processes at a local and regional level, it should be more straight-forward to interpret and understand processes at the global level.

3.1.1 The need for a standardized trapping protocol

An assemblage must be characterized in such a way that is equally applicable to all clades, and any local or biogeographic area, so that two independent sites with different histories, taxa, habitat types or even on different continents can be compared in exactly the same manner as two sites adjacent to one another. The first goal then is to collect all the species in an assemblage in a way which allows the results to be directly compared between sites, even in different parts of the world. Whilst many dung beetle surveys have been conducted around the world, the data collection methods employed are as numerous as the studies. Trap types, bait usage, time period, spatial layouts and general trapping effort vary enormously, which means that direct comparisons between different assemblages are difficult and uncertain. The need for a standard sampling protocol is therefore clear, enabling comparisons to be made between independent assemblages, so that differences can be attributed to ecological and historical influences, without the confounding effects of sampling discrepancies.

3.1.2 Ecological morphology

Once the dung beetles of an area have been inventoried in a replicable way, each species can then be classified according to the functional role that it plays in that assemblage. Each species within a dung beetle community can therefore be placed into one or more different functional groups that reflect the manner in which it feeds, nests, and interacts with other species of the same assemblage. Feeding behaviour can be measured by empirical evidence and nesting behaviour is reasonably well known for most genera. The interactions between species are much harder to detect and measure however. One way by which this can be addressed is through morphology, since this will reflect important aspects of the lifestyle of an organism. The evolution of form will be governed by a fusion of a species ancestral history, ecology and functional role, and so we can expect to see the association between the organism and its environment reflected in its morphology (Wainwright and Reilly 1994). This is the assumption upon which the field of ecological morphology relies. Interspecific competition is believed to be an important structuring process in dung beetle communities (see Hanski and Cambefort 1991b), and yet it is an extraordinarily difficult phenomenon to measure or demonstrate. However, there is a general perception that dung beetle evolution has been strongly affected by competition, which has led to the diversification of species to occupy ecologically different niche space, by a variety of means such as body size, resource use, diel activity, reproductive strategies or behaviour. Morphology is generally agreed to reflect this (e.g. Richman and Price 1992, Ricklefs and Miles 1994), and reflect ecomorphological parameters relevant to competitive interactions. Hutchinson (1959) first suggested that linear measurements could be used to examine ecological similarity, and in this way, a range of measurements can be taken for every species in an assemblage, which are related to various facets of the organisms life. These can then be transformed, often by a multivariate analysis, to reduce the redundancy and dimensionality of the morphological space (Ricklefs and Travis 1980). Each species can be plotted onto a diagram of 'morphological space', where its positioning relative to other species, will help to inform us about its ecology or relationship to the other members of the assemblage with which it potentially interacts. Communities can then be examined for patterns in the use of morphological space, such as total volume occupied (range of morphological diversity), or distance between species (species packing). For example, a regular and non-random distribution of species throughout morphological and hence ecological space, would suggest that the species are evolving

to reduce the effects of competition within the community (Ricklefs and Miles 1994). Findings can be interpreted in the light of phylogenetic, historical, or environmental data. This may inform us as to how an assemblage is affected by such factors, and, where phylogenetic independence can be established, allows evidence of convergence to be recognized.

3.1.3 The evolution of functional morphology in dung beetles

The morphology of all animals reflects the most important demands of its way of life – namely locomotion, feeding and reproduction. In general, dung beetles share a compact form which seems to reflect their needs to excavate burrows and bury dung, but morphological variation within the Scarabaeinae is apparent, and the most striking variability is related to the functional differences in their nesting behaviour. Table 3.1 illustrates a few morphological traits that can be generally attributed to the different nesting groups, such as the curved and elongated hind legs seen in many rollers, with which they shape and roll dung balls. The tunnellers in turn seem to have more stocky and powerful shovel-like legs for digging. It is less clear whether or not the morphology of dwellers might be specialized for their lifestyle but the total variation across the different functional groups is likely to be considerable. Just as the shape and size of aquatic beetles was found to reflect their lifestyle and mode of feeding (Ribera and Nilsson 1995), and to reflect diet and diel activity in ground beetles (Ribera *et al.* 1999), so the various behavioural, nesting and feeding strategies of dung beetles are likely to influence their morphology to a considerable extent, since these must be subject to strong selective pressures. Suggestions have been made that there might be a trade-off effect between the ability of rollers to form and roll balls and the ability to burrow efficiently (Hanski and Cambefort 1991d). However, dung beetle morphology has never been quantified in a way that enables categorization and comparisons of entire clades or functional groups. By taking a range of quantitative measurements to record the size and shape of the beetles, the morphology of different functional groups (rollers, tunnellers and dwellers) can be compared and related to their various lifestyles.

Table 3.1. Some morphological differences between rollers and tunnellers

Rollers	Tunnellers
No front tarsi (some exceptions)	Front tarsi present
Slender legs	Short legs
Small pronotum	Wide pronotum
Some wingless groups	No wingless groups (one exception)
Shallow thorax	High thorax
No horns (some clypeal prongs)	Horns on head and thorax
Thin cuticle	Thick cuticle
No obvious sexual dimorphism	Sexual dimorphism common

Another important functional distinction found within the Scarabaeinae is the range of resource use. Is a difference in diet choice reflected in the functional morphology of the insects? It may be that one or other of the nesting strategies is either better suited or more poorly adapted for necrophagous feeding. Conversely, a change in diet may impact upon the morphology. By examining how the different nesting and feeding behaviours are distributed in the morphospace of a dung beetle assemblage, we hope to learn something of the extent of morphological adaptation present in the different functional groups.

3.1.4 Morphological variation between sites

Direct comparison of the morphospace occupied by three different assemblages will be carried out on a local scale, between two different habitats in Belize, and on a regional scale between these Central American sites and one in South America (Ecuador). Thus the roles of habitat, biogeographic history and species pools will be examined. This is intended to determine the regularity in the occupation of morphospace by dung beetle assemblages and to investigate which functional traits are the most critical for community composition. Ultimately, by comparing how the morphospace is used in different groups and assemblages, we hope to learn something of the key processes which structure and facilitate coexistence in a dung beetle community.

3.1.5 Placing the study sites into context

To determine whether local and regional trends of assemblage structure are applicable across a wider continental scale, or are restricted to the particular communities investigated, Scarabaeinae assemblages throughout North, Central and South America will be examined for broad patterns in functional composition. To do this, pre-existing

data sets from the literature must be employed, raising the comparability issues associated with non-standard data. Trapping regimes are likely to be different as are the range of bait types used. Therefore only assemblages for which an inventory is as complete and as comparable as possible to my own data will be used. This part of the chapter will therefore concentrate on elucidating overall structural differences between dung beetle assemblages across the Americas. By compiling data on the environmental conditions existing at each site, we will investigate how assemblage structure is correlated with changing ecological gradients throughout the region, and with the help of the phylogenetic and morphometric information, attempt to describe the factors which are responsible for the patterns of diversity across the area.

3.1.6 Biogeographic History of the Americas

In order to understand the patterns of dung beetle evolution in the Neotropics, it is important to first understand the biogeographic history of the region, so that this can be considered with any phylogenetic and ecological patterns identified. Until the beginning of the Cretaceous period, some 120 million years ago, South America was a part of the Gondwana land mass. Around this time, it began to separate from Africa, and from North America, which itself had been gradually dividing from Africa since the Jurassic (Wood 1993). As the two American continents moved apart, a volcanic chain arose between them (Duncan and Hargraves 1984), and it is thought that this acted as a kind of intermittent 'filter bridge', enabling some movement of organisms between the continents for much of the Cretaceous period (Wood 1993, Stehli and Webb 1985). South America would have remained linked to Australia via Antarctica until around 90 million years ago, when Australia split away. After its final separation from North America in the late Cretaceous, South America maintained a link with Antarctica until the late Eocene, but was otherwise isolated. Parrish (1993) suggested that terrestrial connections with North America may also have been re-established during brief periods of the Eocene, via volcanic island arcs. Palaeontological evidence supporting any organismal movements across this filter bridge is however very weak (Lavocat 1993).

After the split from South America at the start of the Cretaceous, North America would have been isolated from Africa apart from a likely connection via western Europe in the Late Cretaceous and Oligocene periods, when the land masses were in relatively close proximity and the intervening seas were shallow (Parrish 1993). In the Late Cretaceous,

North America was bisected by a shallow sea (the Mid-Continental Seaway) into two separate land masses. The western part was connected to Asia via Alaska and Siberia, and the eastern part to Europe, via Greenland (Cox 1993). By the end of the Eocene, Europe had separated from Greenland (and thereby from the Nearctic region), but the bridge between Asia and North America remained on and off, until only about 14,000 years ago. However the fluctuations in the climate of the Bering bridge region are believed to have influenced the exchange of organisms between the two continents. The greatest diversity of mammals would have entered the Nearctic during the mild periods of the Oligocene, with cooler periods both before this, and especially afterwards during the Miocene limiting movement to more cold-adapted groups only (Cox and Moore 1993). Eventually the glaciations of the Pleistocene were to seriously reduce the richness of mammal groups in North America.

Around 7 to 9 million years ago, an island chain arose between North and South America, which probably allowed a few taxa to move in either direction, by island hopping or rafting (Stehli and Webb 1985). It was not until some 3 million years ago that a continuous connection was established, with the emergence of the Panamanian land bridge. It was this geological event that enabled what has been termed the 'Great American Biotic Interchange' to occur (Marshall *et al.* 1982, Stehli and Webb 1985), where species from the two continents met and migrated in either direction across the bridge.

3.1.7 Relationships of the Flora

Of the three Neotropical sites inventoried in this chapter, the one in Ecuador has the most stable floral history. Due to its persistent position in the warm and humid equatorial belt, much of the Amazon region has had a continuous rainforest cover since the development of the angiosperm-dominated 'megathermal' rainforests (where plant distribution is restricted by the action of frosts or climate), in the Cretaceous period. Even during periods of contraction such as the global cooling at the end of the Eocene, and the last glacial maximum, the area has retained a significant closed-canopy rainforest distribution (Morley 2000).

The modern flora and fauna of Central America has a more complex history. It has long been noted that it has close affinities to that of the South American rainforests (see

Wallace 1876), although until the advent of the land bridge, most of its land mass was originally part of North America. At the time of its opening, the predominant habitat is thought to have been savanna-like or open forest (Webb 1985). Thus the great majority of species movement into the area must therefore have occurred in the last few million years. For plants and animals restricted to non-forest environments, the rainforest covering the Panama bridge once it had been formed, were as effective a barrier to trans-continental movement as the ocean had been beforehand (Simpson and Neff 1985). The Central American rainforest today consists primarily of South American organisms, with some Laurasian elements remaining (Simpson and Neff 1985).

Most of the plants of Belize have a wide distribution along the Atlantic coast of Central America, extending up to southern Mexico and down into northern South America (Standley and Record 1936), yet there is an important difference in the biogeographic affinities of the flora in the two adjacent forests of Las Cuevas and Mountain Pine Ridge which are under study here. Las Cuevas is a broadleaf forest flora, which is more closely related to that of the tropical forests of South America. Species found in the moist forests of Central America, which occur mostly on the Caribbean side, are a mixture of local endemics and widespread forest species which often have a distribution down to Amazonia and the Atlantic coast of Brazil (Prance 1989). Indeed, the moist forests of the Neotropics reach their northern limits around Veracruz in Mexico, a region termed the 'Mexican transition zone', at a latitude not too far north of the forests in Belize.

Mountain Pine Ridge, as its name suggests is an area of pine-oak forest. The coniferous forests in Central America have something of a different history to the rainforests. The genus *Pinus* is believed to have evolved in the middle latitudes of the Northern Hemisphere, and to have been widespread there by the late Cretaceous period. However, increasingly warmer climates and replacement by a better adapted angiosperm flora during the Eocene, reduced the distribution of the pines into refugial areas, including Mexico and Central America (Millar 1993). The pine forests probably developed here after the mountain-building volcanism of the Miocene period, from which they later diversified. Mirov (1967) suggests that the pine forests of Guatemala are an extension of the Mexican forests, since they bear close resemblance to the pine forests of the Rockies in New Mexico. Furthermore, pollen records from Mexico and Central America would seem to suggest a south-easterly migration of many northern

tree genera during the tertiary period, including *Abies, Picea, Alnus* and *Pinus* (Sauer 1988), and it is noteworthy that no fossil pines have been found anywhere south of the United States-Mexico border. The genus *Quercus* (oak) is also a northern hemisphere group, and has similar ecological requirements to *Pinus*, and so often invades pine forests as an understorey plant (both *Pinus* and *Quercus* have fire-resistant bark), where it can eventually succeed the pine in some cases (Spurr and Barnes 1973). *Quercus oleoides*, the species found in the Mountain Pine Ridge, is a member of the series *Virentes*, and is the only one occurring south of northern Mexico (Muller 1942).

In Mexico, pines are restricted to the mountains, whilst in Central America, *Pinus caribaea* occurs from sea level up to 800m along the Caribbean seaboard of Belize, Guatemala, Honduras and Nicaragua (Vidakovic 1991). It also grows in the Bahaman Islands and in western Cuba. This large and rather disjointed distribution may be due to land bridges between Central America and the Antilles during the Tertiary period (Mirov 1967). Its continuing presence at Pine Ridge, despite the vicinity of the broadleaf forest, is most likely due to the difference in soil types and underlying bedrock.

3.1.8 Biogeographic hypotheses for the Neotropical Scarabaeinae

The importance of biogeographic history to the taxonomic composition of the Neotropical fauna was established in chapter 2. The best estimate produced from the molecular data suggested the presence of a (paraphyletic) Neotropical clade within which the American canthonines were basal to the three tribes endemic to the region, the Eurysternini, Phanaeini and Eucraniini. I suggested that it was most likely that these groups had evolved after the separation of America from Africa, and with the exception of the Eucraniini, within a tropical forest environment. The position of the American dichotomiines was unclear from the data, but a Gondwanan origin, or early dispersal from Africa, similar to that of the canthonines, is possible.

Two other groups are found in Central American fauna, *Copris* and *Onthophagus*, of which only the latter extends into South America. Both of these genera are widely dispersed around the world, and it has been suggested that these taxa colonised North America from Eurasia via the Bering bridge (Cambefort 1991a). The clear African origin of *Onthophagus* makes this seem quite likely. After the opening of the Panama

land bridge some 3 million years ago, the 'Great American Interchange' apparently also included the Scarabaeinae, with the endemic Neotropical taxa moving northwards into Central and North America, and the Palaearctic immigrants *Onthophagus* and *Copris,* moving southwards. This was therefore a relatively recent event, and taxa (and assemblages) may still be becoming established, particularly due to the intermingling of independently derived lineages.

3.2 Materials and Methods

3.2.1 Study Sites

Field work in Central America was conducted around the Las Cuevas Field Research Station, Belize (16° 44' N; 88° 59' W), run by the Natural History Museum, London, in conjunction with the Belize Forestry Department. This is situated in the Chiquibul forest reserve, Cayo district, an area of some 77, 000 hectares of broadleaf moist forest which extends to the Guatemalan border. The reserve covers a hilly area ranging from 500 to 700 m above sea level, and is situated upon a Cretaceous limestone geology (Johnson and Chaffey 1973). The forest typically includes tree species such as breadnut (*Brosimum alicastrum*), sapodilla (*Manilkara zapota*), nargusta (*Terminalia amazonia*) and mahogany (*Swietana macrophylla*). It is essentially a secondary forest in structure since it has been subject to frequent disturbances in its history. Human impact has been present in the form of timber extraction and hunting, since the area has been populated for many hundreds of years, as evidenced by the abundance of Mayan ruins all around Las Cuevas. Extensive natural fires seem to have occurred every 10 - 15 years this century (Bird 1994), and tropical storms also affect the region regularly. In 1961 Hurricane Hattie devastated vast areas of the forest, meaning that the canopy height is presently relatively low in most areas, except in the lee of some hills. Plate 3.1 shows a typical internal view of the forest around Las Cuevas. Despite these disturbances, the area remains species-rich, at least botanically. At a nearby site also in the Maya mountains, Brewer and Webb (2002) found the tree species richness to be comparable or higher than other Central American forests of this type.

The second site in Belize, 'Mountain Pine Ridge', also in the Cayo District (17° 03' N, 88° 56' W), is approximately 35 miles north of Las Cuevas. This site is at a similar elevation of 400 - 600 m above sea level, but by contrast is situated on an outcrop of granite (Furley 1989), and has sandy soils which dry out very quickly. The greatest difference however, lies in its vegetation structure. Mountain Pine Ridge is an area of natural pine forest, although it is now managed and subjected to logging on a rotational basis. It is also subject to natural fires, but they are more frequent and extensive here. The dominant tree type here is *Pinus caribaea*, growing to a height of about 15 m. The understorey vegetation includes *Quercus oleoides*, various Rubiaceae, and *Miconia* and *Clidemia* species (Furley 1989). The vegetation is quite variable in its density, probably

due to the irregular pattern of fires and of logging in the area, creating areas at different stages of recovery. This is a significantly more open forest than the broadleaf. Plate 3.2 shows a view of the area in which trapping was conducted at Pine Ridge.

Field work was carried out in South America at the Tiputini Biodiversity Station (0° 38' S, 76° 09' W), which is run by the Universidad San Francisco de Quito, Ecuador and Boston University, USA. It is located in the Napo Region of Ecuador in the Yasuni Forest Reserve, an area of 680,000 hectares of tropical rainforest which is afforded National Park status. This is an area continuous with the Amazon rainforest, which makes up half of the worlds tropical moist forest at some four and a half million square kilometres (WCMC 1992). The largest continuous region of forest in the world, it also has the greatest species diversity. One study in Amazonian Ecuador recorded 473 species of tree in a single one-hectare plot (Valencia *et al.* 1994).

The Tiputini site is characterised by tree species such as *Macrolobium acacifolium*, *Coussapoa trinervia*, *Licania* sp. and *Eugenia* sp. It was chosen for its remoteness, which has afforded it protection from all unnatural disturbances such as logging, hunting or oil extraction, which threaten other more accessible areas of the Amazon forest. This means that the dung beetle assemblage can be considered to be in a pristine and 'natural' condition and the site to represent an 'archetypal' Neotropical assemblage. Plate 3.3 shows a view of the forest in this area.

Lying within the tropics, these sites are largely aseasonal and all receive a fairly high and regular rainfall throughout the year, particularly in Ecuador which is sited on the equator. In each case however, field work was timed to coincide with the start of the rainy season, since dung beetles world-wide are strongly affected by rainfall patterns (e.g. Janzen 1983, Davis 1987), with a peak in abundance of individuals a few weeks after the rains begin. This must still be recognized as a 'snapshot' of the assemblage, and the species dominance may vary slightly throughout the year. Indeed on an earlier trip to Belize, I collected most of the same dung beetle species (using a non-standard protocol), but in some cases, relative abundances of some species were markedly greater or lower than observed in the subsequent collection. For this reason, we are primarily interested in the presence or absence of species in an assemblage rather than their relative abundance.

Plate 3.1. The forest around Las Cuevas, Belize.

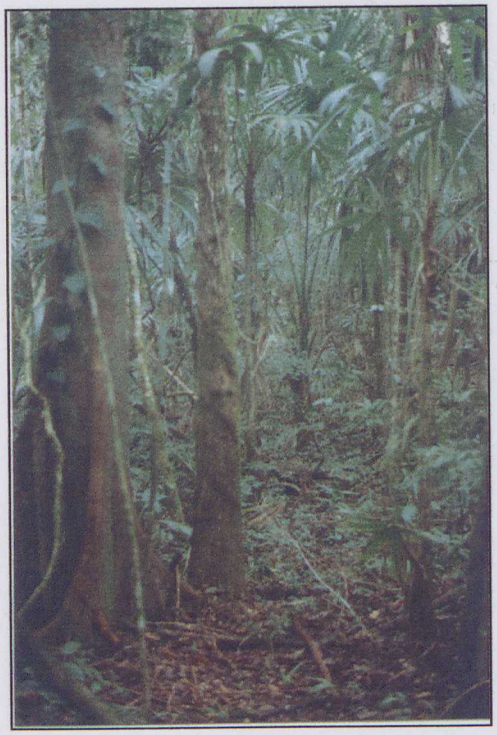


Plate 3.2. The forest at Mountain Pine Ridge, Belize.

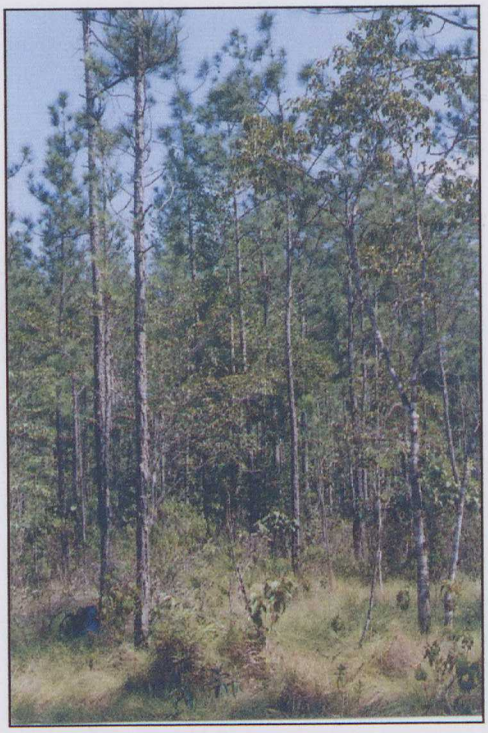


Plate 3.3. The forest around the Tiputini Biodiversity station, Ecuador.



The predominant trap type employed to capture dung beetles are baited pitfall traps, a simple but effective design, which actively attract the insects in the vicinity by odour. Since dung beetles detect their food by air-borne odour, using two types of chemoreceptive sensillae in the antennae (Warnke 1934), they can be passively trapped by employing attractants – a great advantage when attempting to sample an entire assemblage. This method was chosen as the primary means of capture in the study in which the bait was wrapped in a piece of muslin, or fine-mesh fabric, and suspended over the mouth of a half-litre vending-style cup sunk into the ground. Since the traps are liable to flooding, a plastic plate held by stakes above the trap served as a rain roof whilst still allowing access by the insects. The siting of the trap was also carefully chosen, to avoid flooding from run-off. In order to produce an optimally efficient and effective protocol, initial trials at Las Cuevas employed a variety of baits, namely ruminant (cattle) dung, non-ruminant (horse and tapir) dung, carrion (meat and fish) and decomposing fruit (banana). Each trap contained approximately 150 ml of a solution of chloryl hydrate (or salt), detergent and water. The detergent reduces the surface tension of the water, so any insects entering the trap are quickly drowned, whilst the chloryl hydrate or salt act as anti-bacterial agents, helping to preserve specimens in the warm temperatures.

Flight interception traps were also used to collect specialist feeders such as mycophagous beetles, which may not have been attracted to any of these baits. These comprise a baffle of nylon mesh placed across a likely insect flight path, with preservative-filled foil trays beneath to collect insects. A length of tarpaulin is placed above to keep rain out of the trays (see Davies and Stork 1996 for further information). Flight intercept traps are particularly effective at collecting beetles, since they tend to fall downwards when they strike the netting in flight. They are also most effective when employed amongst dense vegetation, since the black netting seemingly becomes invisible to flying insects in dull light conditions (pers. obs.), hence they operate especially well in rainforest.

For comparative purposes, three transects consisting of ten pitfalls each were run for two days for each bait type. Both horse and tapir (non-ruminant) dung and decomposing fruit were found to be much less effective baits for trapping dung beetles

than cattle dung. Additionally, these baits failed to attract any species which were not also attracted to ruminant dung (in greater numbers). The cattle dung was found to retain its moisture for longer than the non-ruminant dung types, and thus remain more attractive for longer. Since cattle dung is a globally available resource, it is an obvious choice for use in a standard trapping protocol.

In contrast, the carrion traps were found to attract many species that were not caught by the dung traps. Meat was found to be a better alternative to fish, attracting a greater number of species.

3.2.3 The standardardized protocol

After these initial trials, a standard protocol was settled upon so that subsequent collections at any site would be directly comparable, with identical trapping effort, bait types and spatial layout. Furthermore, it would ideally collect a high proportion of the dung beetle species at a site, as verified by a species accumulation curve. Two bait types, carrion and cattle dung, were each used in six transects (12 in total), set up at least 500 - 1000 meters apart. Each transect consisted of 10 pitfall traps laid approximately 10m apart in a straight line. The traps were baited, left for 2 days and the catch collected and preserved in alcohol. Since the dung-baited traps lose their attractiveness quickly, they were re-baited with fresh dung at this point. The carrion bait did not need changing, as it only loses its attractiveness if it becomes desiccated, and actually improves as an attractant as its odour increases (pers. obs.). The traps were then run for a further 2 days. Overall this gave a total trapping effort of 240 trap days for each bait type.

Two flight intercept traps were set up and run for approximately two weeks, and only re-sited if they proved ineffective in their initial positions. The captured insects were collected approximately every 3 days.

The protocol was subsequently conducted at Pine Ridge, Belize and Tiputini, Ecuador. Apart from hand-collecting, one other additional form of collecting was carried out at Las Cuevas and Tiputini where canopy-foraging dung beetles were targeted. This was done using baited pitfall traps mounted onto a simple platform and suspended on a line which was hoisted into the forest canopy. Traps were placed at 5m vertical intervals,

and baited with human-dung as a substitute for primate dung. A control (conventional baited pitfall trap) was placed on the ground beneath.

All specimens initially sorted into numbered morpho-species, recorded and pinned. They were subsequently identified using the collections of the Natural History Museum London. In some cases the Biologia Centrali-Americana (Bates 1890) proved useful to aid identification. Mean body length (measured from clypeus to elytral apex) was calculated for each species from three randomly chosen specimens. All species were then assigned to a functional nesting group (rollers, tunnellers and dwellers) based upon Cambefort's (1991a) generic-level scheme, with additional behavioural information regarding the Eurysternini (Halffter *et al* 1980). Feeding preference was assigned using the empirical data collected (abundance attracted to each bait type). A species was designated as a specialist, either coprophage or necrophage, if 90% or more of the individuals collected were attracted to dung or carrion respectively. If less than 90% of the individuals were collected at a single bait type, the species was deemed to be a generalist. Hanski and Cambefort (1991a) and Feer (2000) each applied the same arbitrary 90% cut-off between specialist and generalist feeders. Some species were collected only in the flight intercept traps, so such a designation was not possible.

The data from Las Cuevas was examined in order to determine whether the standardized sampling protocol was sufficient to collect the majority of species in a local population. Species richness curves were constructed from the data using a beta version of the program Biodiversity Professional (McAleece *et al.* 1997). This randomly pools the samples and tests species accumulation. Jaccard similarity coefficients (Sj) were also calculated in a pair-wise manner to examine local species turnover between transects.

$$Sj = a/(a+b+c)$$

Where a = the number of species occurring in both sites 1 and 2; b = number of species occurring in site 2 only; c = number of species occurring in site 1 only.

Feeding preferences of the species in the three assemblages were investigated by comparing the levels of coprophagy in the diets. A Chi-squared test was applied to determine the randomness of the feeding structure in each site. Finally, the recorded

feeding behaviour of each species was plotted against its mean body size on a scatter graph, to test for any correlation between diet and size.

3.2.4 Morphometric variables

For each species from the three Neotropical dung beetle assemblages, nineteen different linear quantitative measurements were taken. The measurements were chosen to best represent the total diversity in morphological shape and structure of all the species within an assemblage, and included dimensions of the body and hind leg in particular. Many of the body dimensions were chosen by virtue of their success in quantifying the morphospace occupied by different functional groups in other Coleoptera, namely water beetles and ground beetles (Ribera and Nilsson 1995, Ribera *et al.* 1999). The range of hind leg measurements were developed to quantify differences between dung beetle rollers and tunnellers in particular, based on the differences discussed in Chapter 1. The measurements taken are given in Table 3.2, and illustrated in plates 3.4 – 3.6. In order to make the measurements comparable between species, only female beetles were measured, to avoid any potential interference in shape caused by sexual dimorphism. This is particularly evident in the species bearing cephalic or pronotal horns (primarily tunnellers), and it has been shown that horn size can directly affect the size of surrounding structures such as wings, antennae or eyes, depending on their position, in a 'trade-off' type situation (Emlen 2001).

Table 3.2. Description of linear morphometric measurements

	Dorsal Measurements
1	length of pronotum
2	length of elytra
3	maximum width of pronotum
4	distance from (3) to anterior of pronotum
5	maximum width of elytra
6	distance from (5) to posterior apex of elytra
7	width of head (interocular distance)
	Lateral Measurements
8	length of head from clypeus to carina of temple
9	maximum height of elytra from side margin
10	distance from (9) to posterior apex of elytra
11	height of abdomen, measured from (9)
	Hind Leg Measurements
12	length of femur
13	maximum width of femur
14	inside length of tibia (curved)
15	inside length of tibia (straight line)
16	maximum distance from (14) to (15)
17	width of tibia across apex
18	area of tibia (mm square)
19	length of tarsi (not including claws)

Three specimens of each species in an assemblage were measured using a digital video camera with macro lens, linked to a computer. Images were taken from three angles, dorsally, laterally and a close-up view of the hind leg. In each case, a piece of graph paper was placed beneath the specimen for calibration of the photograph. Due to the nature of macro-photography, where focal depth is very shallow in close-up situations, a series of images were captured at different focal lengths. Using the program Auto-Montage (Synoptics Ltd 2000), these were overlaid to create a single 'in-focus' image. The same program also enables the user to take digital measurements directly, using a calibration taken from the graph paper in the image. The measurements were then exported for analysis.

Plates 3. 4, 3.5, 3.6. Illustration of morphometric measurements taken from dorsal, lateral and hind leg angles.

Plate 3.4.
Dorsal measurements



Plate 3.5.
Lateral measurements

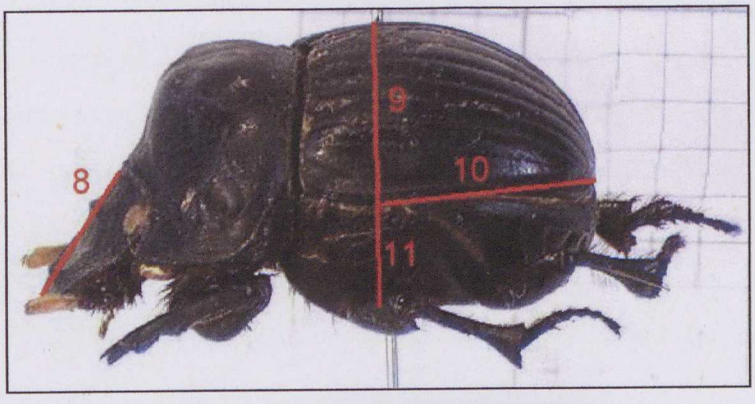
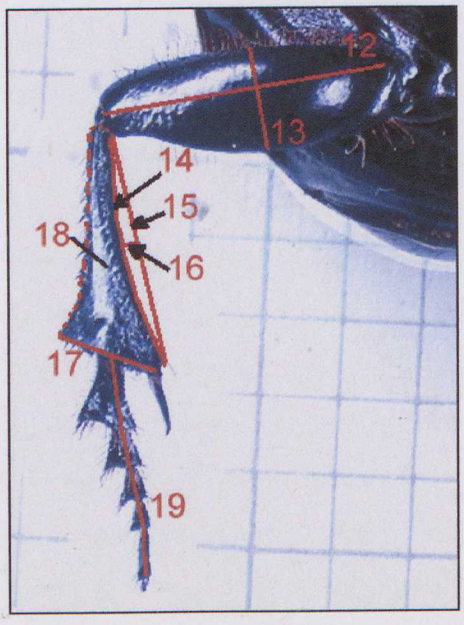


Plate 3.6.
Hind leg measurements



Dichotomius carolinus colonicus (Belize)

3.2.5 Analysis of morphospace

A form of ordination was used to describe the morphological space occupied by the species of the three assemblages. By using ordination we assume that there is an underlying 'latent' structure to the data, determined by one or more unknown environmental variables, and corresponding to a certain response model (Ter Braak 1995). Ordination attempts to elucidate that structure, by constructing one or more theoretical 'latent' variables which best fit the given data. It is designed to reduce the variation in a data set to a simplified ordination diagram which can be interpreted using external (usually environmental) data, and is an extremely powerful and user-friendly method for identifying and explaining patterns in large and complex data sets.

In this case, where no environmental data is available, only the species measurements, Principal Component Analysis (PCA) was used, sometimes termed 'factor analysis'. This is an indirect method of ordination using a linear response model. This form of ordination is similar to regression analysis, except that it creates and uses theoretical variables for explanatory purposes rather than 'real' external data. In using a linear response model, it attempts to explain variation in the data in terms of a linear 'straight-line' relationship between the data and the theoretical latent variable (here for example, we would expect a body part such as a femur to become wider or narrower in a linear manner, so we use a straight-line response curve rather than a symmetric unimodal one). Thus straight lines are first fitted to the data for each measurement by a 'least-squares' regression - carried out to obtain a residual distance between the fitted line and the observed values. PCA then constructs the theoretical variable that best minimizes the total sum of the residual values (Ter Braak 1995). The variable is then plotted as the first axis of the ordination diagram (also termed the first principal component or factor). This is subsequently repeated for the remaining variation, and the second axis is produced, which is entirely uncorrelated with the first. Each axis explains a certain amount of the variance in the data, the first axis explaining the greatest amount of variance, the second explaining the next greatest amount, and so on, with subsequent axes becoming less and less 'meaningful'. In themselves, these axes have no environmental basis, but they enable each species to be plotted onto the diagram in such a way that displays the relationships between them, and the location of each species in the diagram therefore relates to the general manner in which the species vary relative to the ordination axes.

3.2.6 PCA analysis of morphological measurements

The log value of each raw measurement was calculated to normalize the data, and the mean log value was found from the 3 specimens measured for each species. Many ecomorphological studies fail to take into account the confounding effects of body size (Reilly and Wainwright 1994). Each measurement by its nature must reflect the overall size of the beetle - for instance a larger beetle would generally have a longer femur. However, although size is considered to be an important factor in niche partitioning in a dung beetle assemblage, this approach is intended to primarily reflect shape differences. In initial tests, axis 1 of the PCA result (the greatest axis of variation) was seen to correlate exactly with body size. This however, does not mean that axes 2, 3 and 4 will be free from the effects of body size, and it can remain as an obscuring effect. To be confident that size had been totally removed from the pattern in evidence, residual values were calculated from a regression of the log measurement values against mean body size for each species. This was carried out in STATISTICA for Windows (StatSoft Inc. 1999). A body size substitute was calculated by adding together two of the measurements already taken (lengths of elytra and pronotum). These residual values were then used in the PCA analysis, performed using CANOCO for Windows version 4.0 (Ter Braak and Smilauer 1998). The species from Mountain Pine Ridge, Las Cuevas and Tiputini were all included in the same analysis so that the ordination diagrams for each could be directly compared.

The feeding behaviour and functional group of each species were mapped onto the resultant ordination diagram, using Cano Draw 3.1 (Smilauer 1997). For the first four axes, a series of MANOVA tests (multivariate analysis of variance) were performed using STATISTICA for Windows (StatSoft Inc. 1999), to test for significant differences between the occupation of morphospace by the different nesting and feeding groups within and between assemblages. This is a form of ANOVA which determines significant differences between the means of two or more specified groups (such as rollers and tunnellers) along multiple dependent variables (the axes scores). Where significant overall differences in morphospace were found, a Tukey HSD (Honest Significant Difference) test was performed. This is a post-hoc, multiple pair-wise comparison test, applied after an analysis of variance, to determine the significant

differences between group means (in other words to identify more accurately where the significance exists between the different functional groups).

3.2.7 Interpretation of the PCA ordination (morphospace) diagram

The total morphospace occupied by each assemblage can be visualized on a PCA 'biplot'. This plots the position of each species relative to the theoretical variables which are represented by the x and y axes. Each measurement is represented as an arrow, the direction of which indicates the maximum positive increase of that variable across the species under consideration. The length of the arrow represents the extent of this change. The species furthest from the origin of the biplot have undergone the greatest change relative to the others, and if their position is correlated with one or more particular arrows, it is these which best explain the variation in morphology indicated. Similarly, a theoretical axis (which as discussed has no direct environmental basis in a PCA) can be correlated with one or more arrows, which best explain the changes seen in the species distributed along that axis.

3.2.8 Assessing patterns in functional group composition of dung beetle assemblages across the Americas

In order to examine the composition of the three assemblages in a wider context, and to identify any broad patterns in assemblage structure throughout the Neotropics, a data set was compiled to include a number of additional dung beetle assemblages, ranging geographically from the southern USA to Uruguay, and encompassing a variety of different habitat types. The localities and habitat types of these sites are given in Table 3.3, and displayed on a map in Figure 3.1. These independent data sets were drawn from the limited number of studies available in the literature to date. Each was deemed to be as complete and truly representative a picture of the dung beetle assemblage of that region as is currently available, and as comparable to my own data sets as possible. Each study comprises a suitably equivalent level of trapping effort, though there is variation in the trapping techniques employed. Thus where available, multiple data sets were included for each region, to help identify any anomalies in the results due to incomparable data. The most undisturbed sites were also chosen, since they were deemed more likely to reflect the 'natural' composition and structure of the dung beetle assemblages. Generally, the individual studies had targeted such areas for the same

reason, but in some localities no completely undisturbed habitat remains, due to loss of mammals or a history of grazing by domestic animals. Feeding behaviour was only available for some of the sites, and was therefore omitted from the analysis.

Figure 3.1. Map illustrating the distribution of the dung beetle assemblages compared throughout North and South America. Sites in BLUE are those collected by the author. See Table 3.3 below for site names.



Table 3.3. The localities of the dung beetle assemblages used in the regional-level multivariate analysis. Site numbers refer to localities in figure 3.1.

Site number	Country	Site	Habitat type	Author
1	USA	Skeleton Canyon, S.E. Arizona	Arid semi - desert	Dajoz 1994
2	USA	Welder refuge, South Texas	Temperate chapparal	Nealis 1977
3	Mexico	Los Tuxtlas, Veracruz	Rainforest	Moron 1979
4	Belize	Pine Ridge, Cayo	Open dry forest	Inward
5	Belize	Las Cuevas, Cayo	Rainforest	Inward
6	Panama	Barro Colorado Island	Rainforest	Gill 1991
7	Colombia	Leticia, Amazonas	Rainforest	Howden & Nealis 1975
8	Ecuador	Tiputini, Napo	Rainforest	Inward
9	French Guiana	Nouragues	Rainforest	Feer 2000
10	Uruguay	Cerro Colorado (Estacion Experimental)	Temperate chapparal	Morelli et al. 1997

3.2.9 Environmental data

For each of the 10 sites, environmental data were collected for a range of variables. These were chosen to represent a variety of processes that might play a role in the structuring of a dung beetle assemblage, and to range from large scale (continental) to small scale (local) environmental data. Each variable and its collection method is

explained below, and all of the environmental data compiled for the assemblages is given in Table 3.4.

1. Biogeographic region. Sites were classified as to whether they were in the Neotropical or Nearctic region, following the generally recognized zoogeographic regions (which in the Americas, also reflect the currently accepted scheme of floral kingdoms (Cox 2001). These are also the regions used by Cambefort (1991a) in his biogeographic categorisation of dung beetles.

2. Longitude. This was decimalized, and calculated as the number of degrees from the international date line.

3. Latitude 1. Also decimalized, this was coded as the number of degrees latitude from the North Pole, and thus serves as a positional measure of latitudinal distance between the sites.

4. Latitude 2. This was decimalized, and calculated as the number of degrees distant from the equator, regardless of northerly or southerly heading.

5. Net primary productivity (NPP). NPP information (tonnes of dry weight per hectare per year, local maximum) was obtained from the data set compiled by Eggleton *et al.* (1994). They allocated NPP data scores from Uchijima and Seino (1987) to equal-area geographic regions (based on 10° longitude intervals) as defined by the WORLDMAP system (Williams 1999).

6. Rainfall. Mean annual rainfall data (mm/yr) was collected for each site, from the source literature and from international public domain records amassed by The Global Historical Climatology Network (GHCN) at the National Climatic Data Center, Oak Ridge National Laboratory, and available online (http://www.worldclimate.com/).

7. Temperature. Mean annual temperature (°C) was obtained from the source literature where available, or calculated from the temperature records of the GHCN as above.

8. Seasonality of rainfall. This was calculated in two steps in order to avoid a biased measure of increased seasonality towards environments with higher rainfall, such as rainforest, where total monthly rainfall figures may vary greatly through the year, even though it remains a moist habitat. Firstly the range of precipitation was determined for the site using the mean monthly values, and then seasonality was calculated from this value as a percentage of the total mean annual rainfall:

$$(\text{wettest month} - \text{driest month}) \times \frac{100}{\text{mean annual rainfall}}.$$

9. Seasonality of temperature. This was similarly calculated, so as to best directly compare sites under different temperature regimes.

10. Altitude. Data were extracted from the source literature where available, and from a global gazetteer (available online at http://www.calle.com/world, supported by Falling Rain Genomics, Inc.) where not given.

11. Vegetation type. This was designated in a broad fashion (i.e. rainforest, temperate chaparral, open dry forest, arid semi-desert) since detailed habitat attributes are difficult to assess without the necessary information being available for each site. These were coded as categorical variables.

12. Disturbance. This was coded as a continuous variable, but was another difficult variable to categorize, since it involves the application of a single scheme to different habitat types, each potentially suffering from very different forms of disturbance. As previously stated, sites were chosen to be as undisturbed as possible. Some still suffered from various forms of interference however, and based on the information given in the literature, the following continuous coding scheme was applied:

    1.Primary/climax - undisturbed by logging, hunting or agriculture.

    2.Near primary - disturbed in the past (e.g. by hunting), but showing minimal impact today.

    3.Old secondary - not disturbed for at least 25 years, but still bearing signs of disturbance (e.g. loss of mammal diversity).

4.Young secondary - recently disturbed (in last 25 years).

5.Reclaimed habitat - cleared for unnatural plantation/agriculture, and since allowed to re-naturalize.

6. Highly disturbed - unnatural monoculture or equivalent (e.g. plantation / crops).

13. *Mammal species richness.* Since it is generally believed that dung beetle evolution and radiation has been closely linked to that of mammals (e.g. Hanski and Cambefort 1991), data were collected on the species richness of terrestrial mammals present in the locality of each site. Since the accuracy and availability of mammal species records varies greatly between sites, mammal distribution data were taken from a common source (Corbet and Hill 1986) to enable direct comparison. Although this source usually takes account of the habitat type in which individual mammal species are found, the data reflects the overall mammal diversity of a region, rather than an accurate inventory of the site in question. The data excludes any marine mammals found in the locality.

14. *Mammal body size.* Using the data collected from Corbet and Hill (1986), each species of mammal was assigned to a body size class, based on the average body mass of the family in which it is classified. Body mass information was obtained from Nowak (1991). Size classes were as follows: Small (<2kg) , medium-small (2-20 kg), medium-large (20-200 kg) and large (>200 kg).

15. *Mammal feeding behaviour.* This is used as a substitute for the availability of different dung types, since the diet of the mammal influences the structure of its faeces. As mentioned in Chapter 1, dung beetles often specialize on particular dung types. Using the data collected from Corbet and Hill (1986), each species of mammal was similarly assigned to a feeding group (omnivore, herbivore or carnivore) based on information obtained from Nowak (1991), regarding the diets of the different mammalian genera and families in question.

3.2.10 Functional groups as a means of classifying assemblage structure

The most appropriate way to classify and compare each of the dung beetle assemblages, is at the functional level, since different taxa in different areas may still occupy similar

functional roles to one another. There are however a variety of ways which one might classify dung beetle species functionally. Cambefort (1991a) suggested body size for such a purpose, and used an arbitrary 13mm cut-off length between large and small beetles. This was however devised from a study of a West African tropical forest assemblage (pers comm.), and might prove inappropriate for other regions or habitat types. To investigate this, body size categories were initially included in the analysis, but subsequently omitted. They were found to add little to the resolution of the results, and were considered to be potentially misleading, as the arbitrary nature by which body size is partitioned does not truly reflect the continuous gradient of body sizes in an assemblage. It is perhaps noteworthy that peaks in body size are clearly seen within the dung beetle assemblages investigated. These patterns were investigated for a way to partition the dung beetles by body size, but within a single assemblage, these peaks were seen to often occur at different body sizes in different nesting groups. Furthermore, even within nesting groups, the distribution of the peaks was found to vary between sites. Feeding behaviour was also omitted from this analysis, because this is assigned using empirical trapping data, and the comparability of trapping methods used in the other studies was poor. Feeding behaviour is examined further in chapter 4 however.

Consequently, the most conservative means of categorizing the beetles was adopted; by simply using nesting behaviour, which has been well documented for all dung beetle genera in the 10 sites. The functional group composition of each assemblage is presented in table 3.5.

Table 3.4. *Environmental data collected for ten American dung beetle assemblages. See main text for full details of each variable.*

Country	*Site*	*Biogeographic region*		*Longitude*	*Latitude 1*	*Latitude 2*	*NPP*	*Mean annual*	*Mean annual*	*Seasonality of*	*Seasonality of*	*Altitude*	*Vegetation Type*				*Disturbance*
		Nearctic	*Neotropical*	(decimalized)	(decimalized)	(decimalized)	(Tonnes/ha/yr)	Rainfall (mm/year)	Temperature (°C)	Rainfall	Temperature	(m)	*Rainforest*	*Temperate Chaparral*	*Open, dry forest*	*Arid semi-desert*	
USA	Skeleton Canyon, S.E. Arizona	1		70.77	58.29	31.71	5	350	17	24.8	114.7	1300				1	4
USA	Welder refuge, South Texas	1		82.7	61.92	28.08	20	966	21.5	11.2	79.1	15		1			3
Mexico	Los Tuxtlas, Veracruz		1	84.94	71.65	18.35	19	2475	23.1	17.4	21.6	325	1				2
Belize	Pine Ridge, Cayo		1	91.44	72.97	17.03	20	1560.5	24.6	12.3	18.7	450			1		4
Belize	Las Cuevas, Cayo		1	91.41	73.56	16.44	20	1560.5	24.6	12.3	18.7	500	1				3
Panama	Barro Colorado Island		1	100.49	80.91	9.09	24	2425	27.9	16.6	5.7	100	1				2
Colombia	Leticia, Amazonas		1	110.43	94.09	4.09	27	2812.3	25.6	6.1	3.1	85	1				2
Ecuador	Tiputini, Napo		1	103.91	90.38	0.38	27	2546.8	25.3	6.9	9.5	225	1				1
French Guiana	Nouragues		1	127.6	85.95	4.05	25	2880	26	10.6	5	300	1				1
Uruguay	Cerro Colorado (Estacion Experimental)		1	124.68	123.52	33.52	17	1249.6	16.5	3.7	73.3	210		1			4

Country	Site	Total mammal species	Mammal body size				Mammal feeding behaviour		
			Large (>200 kg)	*Medium-large (20-200 kg)*	*Medium-small (2-20 kg)*	*Small (<2kg)*	*Herbivores*	*Carnivores*	*Omnivores*
USA	Skeleton Canyon, S.E. Arizona	72	0	9	9	54	15	38	19
USA	Welder refuge, South Texas	62	0	8	14	40	10	34	18
Mexico	Los Tuxtlas, Veracruz	176	1	11	24	140	16	123	37
Belize	Pine Ridge, Cayo	145	1	10	17	117	12	104	29
Belize	Las Cuevas, Cayo	145	1	10	18	116	12	104	29
Panama	Barro Colorado Island	187	1	12	28	146	17	131	39
Colombia	Leticia, Amazonas	246	2	15	45	184	39	145	62
Ecuador	Tiputini, Napo	213	1	14	38	160	30	123	60
French Guiana	Nouragues	127	0	7	35	85	19	68	40
Uruguay	Cerro Colorado (Estacion Experimental)	31	0	2	9	20	3	16	12

Table 3.5. Assemblage composition data for the ten American sites.

Country	Site	Tunnellers	Rollers	Dwellers
USA	Skeleton Canyon, S.E. Arizona	9	4	0
USA	Welder refuge, South Texas	8	8	0
Mexico	Los Tuxtlas, Veracruz	16	8	3
Belize	Pine Ridge, Cayo	7	2	2
Belize	Las Cuevas, Cayo	21	9	6
Panama	Barro Colorado Island	43	13	3
Colombia	Leticia, Amazonas	33	10	6
Ecuador	Tiputini, Napo	39	20	9
French Guiana	Nouragues	54	19	8
Uruguay	Cerro Colorado (Estacion Experimental)	7	2	0

3.2.11 Multivariate ordination analysis

In order to interpret the species composition data in the light of the environmental data collected for each site, ordination was again considered to be the most appropriate technique. As previously discussed, ordination is an exploratory method of data analysis, to detect a simple structure underlying the data, or to reduce its dimensionality into a form that can be understood. The indirect ordination method of PCA, used to analyse the morphometric data, employs only species data to construct theoretical variables. Since indirect ordination was not designed to detect the effect of a measured environmental variable upon a species, it cannot account for such effects. Therefore, when we wish to understand the role that particular environmental measurements have upon the structure of our data, we use a direct method of ordination, which uses both the species and environmental data to arrange the sites under examination on the ordination diagram. This is also called constrained ordination, since it uses the environmental variables to interpret the ordination axes, which are therefore constrained to be a function of the environment (Ter Braak 1994). In this way, the axes are directly related to environmental variation, unlike the indirect methods of ordination.

Constrained ordination techniques arose as non-linear regression models (Ter Braak 1996), in which the species response to a changing environmental gradient was described by the classic Gaussian response curve. There are two main methods of constrained ordination which could be used to deal with this type of data. The first is a Canonical Correspondence Analysis (CCA), described as a unimodal method, since it uses the bell-shaped parabolic curve as its model of species response. The second is a Redundancy Analysis (RDA) which conversely uses a linear response 'curve' (a straight line) as its model of species response, and is therefore termed a linear method, like the PCA.

The unimodal response curve describes the effect upon a species of a widely varying environmental variable, with a peak in species abundance occurring at its optimum where it responds most favourably to the variable, and a tailing-off at greater and lesser extremes. The CCA technique therefore tends to be used when there are considerable differences in species composition between samples, and the variables measured are deemed to vary enough to have caused the observed compositional differences. The linear response model of the RDA is applied when the differences between sites are

primarily in the relative abundances of species. This is because species can be seen to react in a more linear fashion through a short section of an environmental gradient (for instance along one part of a unimodal curve).

In this data set, the data are species richness within functional group, and therefore show a more linear response than individual species abundance, hence the linear response model is more suitable in this case, and a redundancy analysis was therefore chosen as being the more appropriate technique for this data set.

The algorithm used in RDA, is essentially a modified version of that used in PCA, with a series of regressions and the calculation of residual values from a linear combination of the environmental variables (Ter Braak 1995). The combination which minimizes the sum of the total residuals is chosen as the first axis. Additionally, with direct ordination the statistical significance of the relationship between the species and each environmental variable can be tested using a Monte Carlo permutation test. This is similar to the bootstrap test used in chapter 2, in that it creates a specified number of random data sets from the species data, under the null hypothesis that any of these is equally likely to have been found (in other words that there is a random structure to the data).

The RDA was performed using the program CANOCO for Windows, version 4.0 (Ter Braak and Smilauer 1998). The significance of correlation between each variable and the species (functional group) data was tested using a Monte Carlo permutation test. 'Marginal' significance was examined, whereby each variable is tested in isolation, to determine its effect on the variance explained. Each Monte Carlo permutation test was run with 999 random replications, to give a minimum possible p-value of 0.001. 'Conditional' significance can also be examined, where each variable is ranked in order of its importance in explaining the variance in the data. This technique adds the variables one at a time, starting with the one with the highest eigenvalue, until no significance remains. In this way, it produces a 'model', which suggests which variables are most important in explaining the variance, and which are unimportant. However, if two or more variables are autocorrelated (explain the same variance in similar ways), the removal of the significance explained by one of the variables will also remove that explained by the other variables. This gives the misleading impression that the remaining variables explain much less of the variance seen than they

actually do. The technique was initially performed on the data, but subsequently rejected for this reason. Marginal significance is more predictable, in that it ranks all variables in order of their importance of explaining the variance, regardless of any covariation.

The ordination diagrams from the RDA were plotted for the ten American sites using Cano Draw 3.1 (Smilauer 1997). Only significant marginal variables were plotted.

3.2.12 Interpretation of the RDA ordination diagram

Redundancy analysis diagrams can be interpreted in a similar manner to the PCA biplots. Here sites (assemblages) are plotted, and the variation in functional group composition between them can be best explained by the direction of the arrows representing the environmental variables. Where the centroid of a site falls in close proximity to an environmental arrow, the functional group composition can be said to be positively correlated with that variable. Arrows pointing directly away from a site, conversely indicate a negative correlation. Arrows which point at a perpendicular (90°) angle away from a site, indicate no correlation with that variable. The length of the arrow is similarly representative of the strength of correlation between the variable and the site. Sites clustering together in one part of the biplot are similar in terms of functional group composition and in terms of the variables which best explain their position. Note that a relationship identified between an environmental variable and a site does not imply a causal effect, but merely a correlation between them. There is always the possibility of variables not accounted for being more important. (For further discussion on the interpretation of ordination diagrams see Ter Braak and Prentice 1988).

3.3 Results and Discussion

Because of the variety of fields and analytical techniques involved in this study of Neotropical assemblages, a broad range and considerable quantity of results have been generated. The results and discussion are presented here as a single integrated section, in an attempt to clarify the major findings of each component as it is presented. These are then synthesised, and broad conclusions are drawn in the final sections.

3.3.1 Species inventories

The species inventories for Mountain Pine Ridge, Belize; Las Cuevas, Belize; and Tiputini, Ecuador are presented in Tables 3.6, 3.7 and 3.8 respectively. For each species, results from the various trapping methods are given, and feeding strategies are assigned using the empirical evidence collected. The mean body lengths are also given for each species. In total, some 12,000 specimens were collected across the three study sites, representing 104 species, with abundance for individual species ranging from 1 to 3,300 individuals.

Although total abundance of individuals is similar at Las Cuevas and Tiputini, this clearly fluctuates temporally (over 2400 individuals of *Bdelyropsis bowditchi* were collected at Las Cuevas, compared to only two from an earlier collection). Individual abundance should therefore only be viewed as a snapshot at single point in time, and should not be compared between sites, collected at different times.

Table 3.6. Dung beetle species inventory from Mountain Pine Ridge, Belize, with trapping results and feeding strategy. Feeding strategy codes: N=Necrophagous; C=Coprophagous. Tribal abbreviations follow those given in Table 1.1.

Tribe	*Species*	Dung Pitfalls	Carrion Pitfalls	Flight intercept traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding strategy
CA	Canthon cyanellus		1		1	9.4	0	N
	Deltochilum lobipes		1		1	24.5	0	N
CO	Copris laeviceps	1			1	12.4	100	C
DI	Canthidium puncticolle		5	1	6	5.1	0	N
	Canthidium sp.1		1		1	5.8	0	N
	Dichotomius carolinus colonicus	2			2	25.2	100	C
ER	Eurysternus caribaeus	1			1	12.5	100	C
	Eurysternus magnus	2			2	16.7	100	C
PH	Coprophanaeus telamon ssp.corythus		6		6	23.1	0	N
	Phanaeus endymion		7		7	15.1	0	N
OP	Onthophagus crinitis v. panamensis	1			1	8.5	100	C

Table 3.7. Dung beetle species inventory from Las Cuevas, Belize, with trapping results and feeding strategy. Feeding strategy codes: N=Necrophagous; C=Coprophagous; G=Generalist. Tribal abbreviations follow those given in Table 1.1.

Tribe	Species	Dung Pitfalls	Carrion Pitfalls	Flight intercept traps	Canopy traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding Strategy
CA	Canthon cyanellus			6		6	9.7	Unavailable	
	Canthon euryscelis		1	8		9	6	0	N
	Canthon lamprimus	8	6	11		25	7.3	57.1	G
	Canthon subhyalinus		1		115	116	6.5	99.8	C
	Deltochilum gibbosum ssp.sublaeve	8	22	5	10	45	26.7	26.7	G
	Deltochilum mexicanum	1	2		1	4	22.9	33.3	G
	Deltochilum pseudoparile	21	170	11		202	13	11	G
	Deltochilum scabriusculum	4	2	4		10	27.9	66.7	G
	Megathoposoma candezei	18	1	2		21	21.5	94.7	C
CO	Copris laeviceps	41				41	12.2	100	C
DI	Anomiopus panamensis	1		10		11	4.7	100	C
	Ateuchus chrysopyge	2970	332	9		3311	8.3	89.8	G
	Bdelyropsis bowditchi	1715	737	1		2453	6	69.9	G
	Canthidium ardens	2	4	3		9	5.3	33.3	G
	Canthidium haroldi	5	15	1		21	10.1	25	G
	Canthidium sp.1	5		1		6	5.5	100	C
	Dichotomius amplicollis	1		2		3	19.8	100	C
	Dichotomius satanus	11				11	20.4	100	C
	Uroxys micros	15	39	1		55	4.2	27.8	G
ER	Eurysternus angustulus	285	21	7		313	9.8	93.1	C
	Eurysternus caribaeus	129	7	3		139	16.4	94.9	C
	Eurysternus foedus	7				7	18.6	100	C
	Eurysternus magnus	13				13	15.7	100	C
	Eurysternus mexicanus	1		1		2	12.1	100	C
	Eurysternus velutinus	5		4		9	18.4	100	C
PH	Coprophanaeus telamon ssp.corythus		55	17		72	23	0	N
	Phanaeus endymion	13	72	11		96	17.2	15.3	G
	Phanaeus sallei	15	1	3		19	19.4	93.8	C
OP	Onthophagus anthracinus	8		1		9	4.7	100	C
	Onthophagus batesi	489		1		490	8.8	100	C
	Onthophagus crinitis panamensis	381	36	7		424	9.8	91.4	C
	Onthophagus longimanus	118		1		119	6.4	100	C
	Onthophagus rhinolophus	10	79	11		100	9.2	11.2	G
	Onthophagus sp.2 (nr. sharpi)		1	14		15	7.8	0	N
	Onthophagus sp.4	1		1		2	6.3	100	C
	Onthophagus sp.5	1		1		2	5	100	C

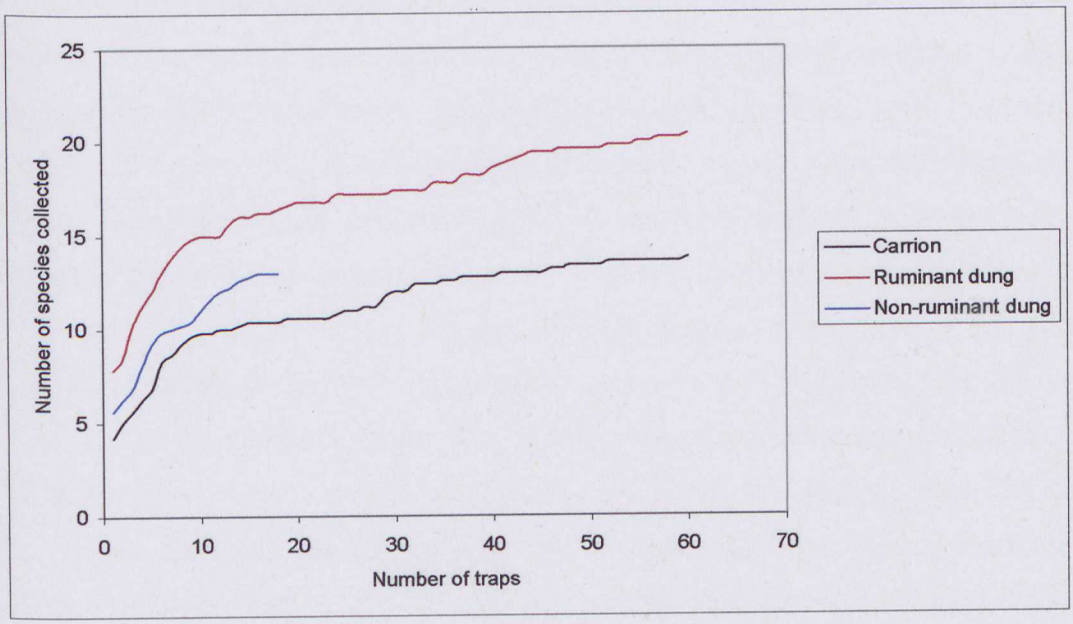
Table 3.8. Dung beetle species inventory from Tiputini, Ecuador, with trapping results and feeding strategy. Feeding strategy codes: N=Necrophagous; C=Coprophagous; G=Generalist. Tribal abbreviations follow those given in Table 1.1.

Tribe	Species	Dung Pitfalls	Carrion Pitfalls	Flight intercept traps	Canopy traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding Strategy
CA	Canthon aequinoctialis	54	1	3		58	14.6	98.2	C
	Canthon angustatus	5	1			6	7.1	83.3	G
	Canthon doesburgi	1	7	5		13	6.1	12.5	G
	Canthon luteicollis	118	39	1		158	8.7	75.2	G
	Canthon politus	1	4			5	8.2	20	G
	Canthon quadriguttatus				47	47	6.1	100	C
	Canthon smaragdulus	45	5	3	1	54	14.5	93.9	C
	Canthon sp. 1	30	71			101	8.9	29.7	G
	Canthon sp.2	13	16	3		32	11.3	44.8	G
	Canthon sp.3				83	83	7.6	100	C
	Canthon sp.4		1		4	5	7.3	80	G
	Canthon sp.5				1	1	7.7	100	C
	Canthonella sp.1	1	6			7	4.1	14.3	G
	Cryptocanthon sp.1	3	1			4	3.3	75	G
	Deltochilum amazonicum	1	51	1		53	21.4	1.9	N
	Deltochilum barbipes	3	56	5		64	12	5.1	N
	Deltochilum carinatum		22	4		26	16.2	0	N
	Deltochilum sp.1	13	220	21		254	13.7	5.6	N
	Deltochilum sp.2	1	54	7		62	9.7	1.8	N
	Scybalocanthon sp.1		2	1		3	6.3	0	N
DI	Ateuchus (romani?)			2		2	6.8	Unavailable	
	Ateuchus ecuadorense	108	26	32		166	7.6	80.6	G
	Ateuchus sp.1	143	85	10		238	5.8	62.7	G
	Ateuchus sp.2	1	13			14	8.6	7.1	N
	Ateuchus sp.3			1		1	6.3	Unavailable	
	Ateuchus sp.4	1				1	5.6	100	C
	Bdelyrus sp.1	2	1			3	6.8	66.6	G
	Canthdium onitoides			1		1	7.3	Unavailable	
	Canthidium centrale	4	3			7	8.9	57.1	G
	Canthidium gerstaeckeri	4	10	3		17	9.6	28.6	G
	Canthidium rufinum	2	2	1		5	7.3	50	G
	Canthidium sp.1			2		2	6.1	Unavailable	
	Canthidium sp.2	3	1	2		6	4.5	75	G
	Canthidium sp.3	1	4			5	5.4	20	G
	Canthidium sp.4		1	1		2	4.8	0	N
	Canthidium thalassinum	19	1	12		32	4.5	95	C
	Dichotomius boreus	5	4			9	26.5	55.6	G
	Dichotomius lucasi	5	8	2		15	13.3	38.5	G
	Dichotomius ohausi	30	73	1		104	16.7	29.1	G
	Dichotomius parcepunctatus	95	58			153	13.5	62.1	G
	Dichotomius problematicus	22	3			25	15.5	88	G
	Dichotomius sp.2	31	9			40	19.9	77.5	G
	Dichotomius sp.3	18	17			35	19.6	51.4	G
	Ontherus diabolicus	7	8			15	12.4	46.7	G
	Trichillum sp.1		1			1	3.3	0	N
	Uroxys latus		1			1	5.4	0	N
	Uroxys minutus		2			2	3.8	0	N
	Uroxys pygmaeus	1	3			4	3.4	25	G
	Uroxys sp.1	7				7	6.6	100	C
ER	Eurysternus caribaeus	133	82	2		217	14.8	61.9	G
	Eurysternus confusus	160	210	17		387	10	43.2	G
	Eurysternus foedus	7	13	2		22	17.6	35	G
	Eurysternus hamaticollis	39	13	1		53	19.5	75	G
	Eurysternus hirtellus	5	17	2		24	7.2	22.7	G
	Eurysternus inflexus	105	37	2		144	11.3	73.9	G
	Eurysternus plebejus	27	17	2		46	8.5	61.4	G
	Eurysternus sp.1	4	3	4		11	8.3	57.1	G
	Eurysternus velutinus	8	39	12		59	18.8	17	G
PH	Coprophanaeus sp.1		88	4		92	17.4	0	N
	Coprophanaeus telamon	1	82	3		86	23.7	1.2	N
	Oxysternon conspicillatum		1			1	22.1	0	N
	Phanaeus cambeforti	3	8	4		15	13.3	27.3	G
	Phanaeus chalcomelas	8	2	3		13	17	80	G
OP	Onthophagus bidentatus	2	3			5	6.2	40	G
	Onthophagus clypeatus	6	4	2		12	7.1	60	G
	Onthophagus coscineus	1	2	4		7	4.1	33.3	G
	Onthophagus haematopus	318	18	28		364	6.3	94.6	C
	Onthophagus marginicollis	1	5			6	6	16.6	G

3.3.2 Success of the trapping protocol

The minimum number of traps required for obtaining a comprehensive sample of the local dung beetle assemblage was determined from the data collected at Las Cuevas. A species richness curve for each bait type (Figure 3.2) demonstrates an initially sharp increase of species with the number of pitfall traps, with subsequent species accumulation more gradual. Note that although a curve is also included for non-ruminant dung (collecting a total of 18 species), its use was discontinued after the initial trials as all of these species were also collected with cattle dung (discussed above). Both the carrion and cattle dung baited traps were seen to collect 75 % of their species totals in the first 18-20 traps. Carrion attracts 90% of the specialists (necrophages) with some 35-40 traps, and cattle dung attracts 90% of coprophages with 40-45 traps. The final 10% of the species trapped therefore requires between one-third and one-half of the total trapping effort.

Figure 3.2. Species richness curves illustrating the rate of species accumulation for three different bait types



There was substantial variation in the number of species encountered in the various pitfall transects. The pair-wise Jaccard similarity coefficients (Sj) showed a pair of transects 5500 metres apart to have the lowest similarity (Sj = 0.48), whilst a pair only 1000 metres apart had the greatest similarity (Sj = 0.95). The mean similarity coefficient value was 0.67. Whilst there does appear to be a general trend between

increasing dissimilarity and distance, there is still a random element to the results. It does however suggest that complementarity would be greatest when two or more distant sites are combined and that multiple transects within a site are important. Although non-ruminant dung (both horse and tapir) was found to be an unnecessary bait when using cattle dung in a Neotropical forest, the carrion baited traps caught three strict necrophagous species. Using a combination of bait types also elucidates the feeding preferences of each species. The flight intercept traps caught one species at Las Cuevas not caught with a baited pitfall trap, and also a number of other species in greater numbers than were trapped using bait.

From this initial trial of the protocol, the total number of specimens obtained varied widely between different species, from many hundreds for the most abundant taxa to only two or three specimens for others. Abundance also varied between individual pitfall traps, within and between sites. There was a clear correlation between abundance of a species at a single site and its representation in other sites but a few species that were dominant in one area were poorly represented in another. For example 120 individuals of *Eurysternus angustulus* were trapped in one transect compared to only one in another, 1500 metres away. *Bdelyropsis bowditchi* similarly varied from only one individual to over 300, in two transects 2500 metres apart. This indicates some ecological heterogeneity in the study area, a common finding in dung insect communities due to the ephemeral and patchy nature of dung and carrion distribution (Shorrocks 1990). We also tested the dynamics of trapping success over a four day period. As expected, the number of specimens caught in the pitfall traps was seen to decline slightly after the first 2 days. The total number of specimens trapped in days 3 and 4 in the cattle dung was 82% of that trapped in the first 2 days, with carrion catching 89%. However, the trapping-out effect is relatively small. Using mark and recapture techniques, Peck and Forsyth (1982) estimated an 80 hectare study plot of South American forest to hold some 170,000 dung beetle individuals. They also recaptured individual beetles up to 1 km away after two days, and this high abundance and mobility both serve to reduce the impact of trapping .

The flight intercept traps did not work well in the forest at Pine Ridge, due most likely to both the low abundance of individuals present and to the openness of the habitat type. However, they performed well in the two rainforest sites, catching 4 dung beetle species not collected with any bait type at Tiputini, and a wide variety of other species. Hill and

Cermak (1997) tested flight intercept traps at different heights in a rainforest in Queensland, Australia, and found that the majority of dung beetle species and individuals fly below 5m and probably below 2m from the ground. Due to the foraging behaviour of many dung beetles, ground-based flight intercept traps are therefore optimally placed to capture dung beetles foraging on the wing. They are an extremely useful passive method of collecting and providing the most efficient means for catching species specialized to a particular but unknown resource.

3.3.3 Attractiveness of the bait types

In the initial trials, cattle dung proved a better attractant than horse or even tapir dung which is the only one of these endemic to the area. Janzen (1983) also notes that tapir dung did not appear attractive to dung beetles in a Costa Rican forest. Non-pelleted ruminant dung however has a consistency which retains moisture for longer than the more fibrous dung of non-ruminant herbivores and would therefore remain more attractive for a longer period. Dry dung is well-known to be unattractive to most Scarabaeinae. Since the majority of microbes in dung are contained in the liquid component (Hanski 1991b), it is likely that this most nutritious part of the resource is lost rapidly as the dung dries, which is why it becomes increasingly less attractive to the beetles. Therefore, cattle dung being moister, will have a greater microbial fauna and flora, and from their by-products, a greater odour by which the beetles are attracted.

Of the carrion baits, it was also evident that meat (pork or beef in this case), was more attractive than fish, even though this has been used quite extensively in the past for trapping dung beetles in the Neotropics (e.g. Halffter and Arellano 2002, Morón 1987). The corpses of large animals are known to provide a wider variety of resources, and thus attract a greater diversity of carrion feeders (Doube 1987). Smaller resources on the other hand, such as snail and slug carrion have been shown to attract fewer invertebrate carrion feeders (Kneidel 1984), although some dung beetles are clearly attracted to decomposing invertebrates. Various African species of *Onthophagus* and *Sceliages* specialize on the carcasses of large millipedes, attracted by particular defensive secretions (quinones) (Krell *et al.* 1997). In general however, it is likely that the decomposing odour of mammalian carrion attracts a wider range of beetles with the promise of a larger resource.

In summary, use of cattle dung and carrion baited pitfall traps, in conjunction with flight intercept traps were seen to provide the greatest level of complementarity, and to sample a Neotropical forest assemblage in the most efficient and comprehensive manner. The species richness curves suggest that a high proportion of species are collected in this manner, even though species accumulation is still gradually increasing. The Jaccard similarity coefficients also suggest that use of widespread transects also sample the heterogeneous population effectively, and that a straight-line transect of traps, covering approximately 100m, should maximise the area covered. A considerable proportion of the trapping effort appears to be required to collect the 'final' 10% of species, which suggests that the trapping regimes which are not so extensive should be more or less comparable, assuming that multiple bait types and trapping localities are used. Nevertheless, the extra 10% of species was deemed important enough to warrant the continuation of the full protocol described here, and it was therefore adopted unchanged for use in Ecuador and Pine Ridge, and with only slight modification of bait types in later work conducted in Africa (described in Chapter 4).

3.3.4 Species composition of the three assemblages

A summary of the total number of species, genera and tribes collected at each of the three Neotropical sites is given in Table 3.9. The numbers of species assigned to each of three nesting and three feeding groups is also shown.

Table 3.9. A summary of dung beetle assemblage composition of the three Neotropical sites sampled. The numbers of species in each nesting group is given with the percentage of the total species that each represents. Feeding groups have been assigned based on the pitfall trap data, although not all species can be assigned to a particular strategy.

	Pine Ridge, Belize	Las Cuevas, Belize	Tiputini, Ecuador
Species	11	36	68
Genera	9	14	17
Tribes	6	6	5
Nesting groups			
Rollers	2 (18.2%)	9 (25%)	20 (29.4%)
Tunnellers	7 (63.6%)	21 (58.3%)	39 (57.4%)
Dwellers	2 (18.2%)	6 (16.7%)	9 (13.2%)
Feeding groups			
Coprophages	5	20	9
Necrophages	6	3	14
Generalists	0	12	41

In total, 36 species of Scarabaeinae were collected at Las Cuevas, and only 11 species at Pine Ridge. From the inventories presented (Tables 3.6 and 3.7), it is apparent that the species in the assemblage at Pine Ridge are essentially a subset of the species at Las Cuevas, with the addition of three species not collected at Las Cuevas, which are apparently better suited to the drier and more open conditions of this habitat type. *Canthidium puncticolle* frequents dry forests as far south as Costa Rica (pers. comm. B.D. Gill), and locality data from specimens of *Deltochilum lobipes* held at the Natural History Museum tend to suggest that it is found also in dry, shrubby open areas. *Dichotomius carolinus colonicus* is widespread across a variety of habitats, throughout Central America.

Despite the species-poor nature of the Pine Ridge fauna - less than one-third the number of species at Las Cuevas, all six tribes found at Las Cuevas are represented, and 9 of the 14 genera. Considering only 11 species are present in the assemblage, this is a striking finding – most species therefore are sole representatives of their genus.

Tiputini, Ecuador has almost twice as many species as Las Cuevas with 68 species in total. There is only a very small species overlap between the two sites – three species, all of the genus *Eurysternus*. Generic overlap on the other hand is high – only 4 of the genera occurring at Las Cuevas were not also collected at Tiputini. Eight of the nine genera occurring at Pine Ridge were also collected at Tiputini. This means that the broad taxonomic and phylogenetic composition remains the same across the three different assemblages, in spite of local differences in the habitat, and of regional differences in the species pools. All of the major clades present in Tiputini are also found at Las Cuevas and Pine Ridge, and so we can assert that taxa are not 'selected' at random from the phylogeny, but that each assemblage consists of a particular selection of 'compatible' taxonomic groups.

3.3.5 Functional group composition

Some interesting trends can also be identified regarding the proportions of the nesting groups at the three sites. Firstly, the tunnellers make up approximately two-thirds of the species in each assemblage (see Table 3.9). Rollers represent an equal proportion of the assemblage to dwellers at Pine Ridge, but increase in proportion in Las Cuevas, and again in Tiputini. This increase is accompanied by a relative decrease in the proportions

of dwellers in the latter two sites, although actual species richness still in fact increases for this functional group. If Eurysternus were treated as rollers, as is often the case (and which is clearly their ancestral nesting behaviour, as seen in figure 2.3), then the roller-tunneller proportions would be the same in each site. There is clearly a phylogenetic component to this, but the pattern is suggestive of inherent structure in Neotropical dung beetle assemblages, which is maintained throughout differences in habitat and species richness.

3.3.6 Biogeographic affinity of the assemblages and the relevance of Aphodiinae

The two Belizean sites have one clade represented which is not found at Tiputini, in *Copris laeviceps*. Although we now know that the tribe Coprini is polyphyletic (chapter 2), the position of the genus *Copris* is uncertain, but is probably not closely related to any of the endemic Neotropical clades. As discussed above, it is thought to have entered Central America from North America along with *Onthophagus*, upon the formation of the Panama bridge (Cambefort 1991a), but to have encountered sufficient competition from the endemic Neotropical groups, so that its distribution remains restricted, and it has so far been unable to colonise assemblages as far south as that in Ecuador. *Onthophagus* is more speciose in the Neotropics than *Copris*, and from its global distribution and species richness, is clearly a competitively successful species. There are however, fewer species of Onthophagus in Ecuador than Las Cuevas, which may reflect either the speed of colonisation of the group, or the levels of competition encountered from other Scarabaeinae in the two regions.

The majority of species in the three assemblages are members of clades which evolved in South America, in isolation from the other continental faunas for tens of millions of years. With the exception of *Onthophagus* and probably also *Copris*, these taxa are endemic to this continent.

Although the trapping results of Aphodiinae species have not been recorded here, since they are generally a minor part of the dung insect community in Neotropical forests, and are therefore rarely considered in dung beetle studies of the region. However, it is perhaps noteworthy that this group was in fact relatively more abundant in Pine Ridge than Las Cuevas. Whilst only a few specimens of a single small, unidentified *Aphodius* species were collected at Las Cuevas, two species of *Ataenius* were found to reach

considerably greater abundances than any scarabaeine dung beetle at Pine Ridge. This is interesting since it may represent a northern element within the community, as they are a dominant part of the dung beetle fauna in the northern temperate regions where numbers of Scarabaeinae are lower.

In a study along an altitudinal transect in the 'Mexican Transitional Zone' in Veracruz, passing through various vegetation types, Halffter *et al.* (1995), found that Aphodiinae became abundant only in the pine forests at higher altitudes, where they were not in the tropical wet forests below. They also reported a general dominance of northern elements at these higher altitudes, with a reduction in the numbers of Scarabaeinae accompanied by an increase in numbers of Geotrupidae and Palaeoamerican genera. Whilst the forest at Pine Ridge shows no such increase in geotrupids, and its dung beetle fauna is clearly dominated by Neotropical elements, this increased abundance of aphodiines may reflect the competition experienced within dung beetle communities. In this case, a forest type which is unfavourable to one group, appears to be giving the other a competitive advantage, which they would not otherwise enjoy at this latitude. In general, Aphodiinae are thought to suffer from less inter-specific competition in the temperate dung insect communities where they reach their maximum species richness (Hanski 1991a), and there appears to be a negative correlation between the abundance of Scarabaeinae and of Aphodiinae along a latitudinal gradient.

3.3.7 Feeding behaviour in the different assemblages

The feeding behaviours of the dung beetles within each assemblage are illustrated in Figures 3.3, 3.4 and 3.5 (drawn to the same scale for comparative purposes). The recorded resource (bait) preference for each species is represented by the percentage of coprophagy in the diet (Tables 3.6, 3.7, 3.8). To assess the randomness of the distribution of diet within each site, a χ^2 test was conducted. The null 'expected' value (a random and equal distribution of species across each of the 10 equal feeding categories) is represented on the histograms as a straight line, and the results of the χ^2 test are presented in Table 3.10. They confirm a highly significant departure from the expected structure for both Belizean assemblages, whilst the observed feeding behaviour in Ecuador is not significantly different from the random distribution. Pine Ridge has an approximately equal number of coprophagous and necrophagous species, but no generalists. Conversely, 34% of the species at Las Cuevas are classified as

generalists, although the majority of species in the assemblage are coprophagous (57%). The pattern of feeding behaviour at Tiputini is different again, since here the greatest number of species are generalists (64%), with the next most speciose feeding group being the necrophages (22%). In contrast to Las Cuevas, coprophages are the least abundant feeding group with only 14% of species.

Table 3.10. Results from the χ^2 test on the distribution of feeding behaviour in each assemblage (df = 9). Significant results are coded as: $*P<0.05$; $**P<0.01$; $***P<0.001$

	MPR, Belize	Las Cuevas, Belize	Tiputini, Ecuador
χ^2	44.45 ***	83.06 ***	14.75
P value	1.16^{-6}	3.98^{-14}	0.098

Whilst the low numbers of individuals captured at Pine Ridge makes the assignment of feeding preference more questionable, only one of the species collected here was assigned as being a generalist at Las Cuevas. One other collection of dung beetles from a Central American pine forest was made by Halffter *et al.* (1995), in Mexico, and compared to other nearby habitats along an altitudinal gradient. They report a predominance of coprophages over generalists (and necrophages) in the pine forest, but it should be noted that few taxa were collected there, and these were predominantly northern in origin (*Onthophagus*), and so might not accurately represent a Neotropical assemblage. Halffter and Matthews (1966) however describe a shift in feeding behaviour in two unrelated genera, *Canthon* and *Phanaeus*, from being almost exclusively coprophagous in the USA and Mexico, towards an approximately equal level of carrion and dung feeding in the Central and South American species. Thus it would seem the increasing levels of generalism in the assemblages from Pine Ridge to Las Cuevas to Ecuador is following this trend. Even leaving aside Pine Ridge, the difference between the two moist forests is a striking one.

Figures 3.3, 3.4, 3.5. Histograms illustrating the distribution of feeding behaviour of the dung beetle assemblages in the three Neotropical forest sites. The mean 'expected' value is shown as a straight line for each instance.

Figure 3.3. Feeding preferences at Mountain Pine Ridge, Belize

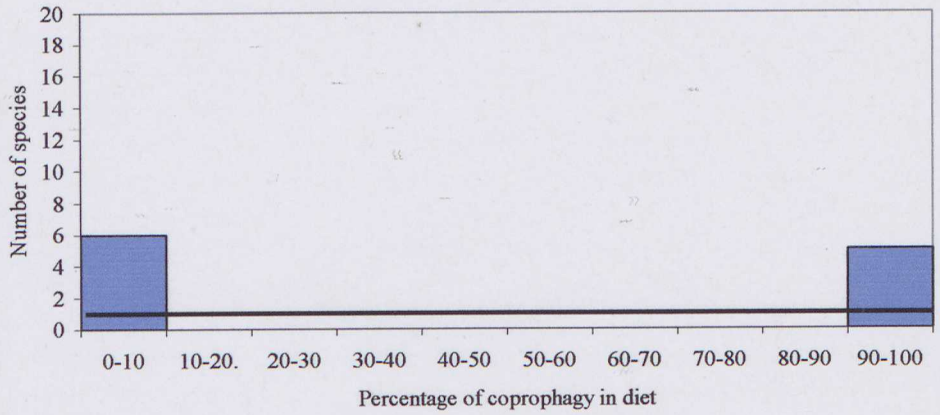


Figure 3.4. Feeding preferencest at Las Cuevas, Belize

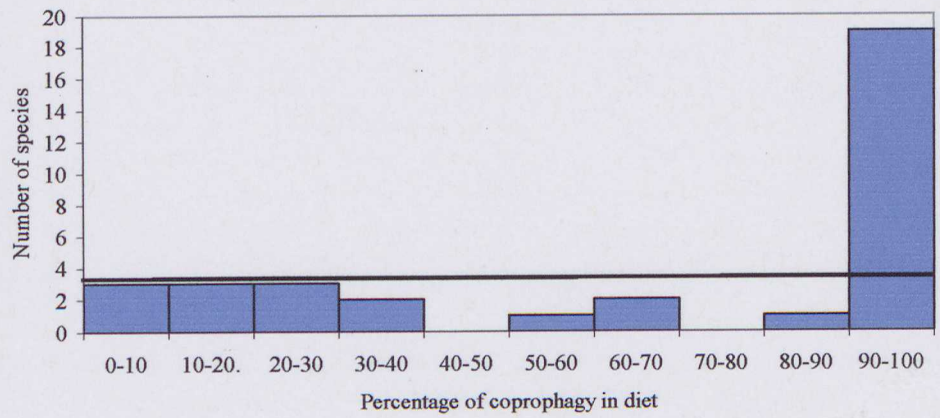
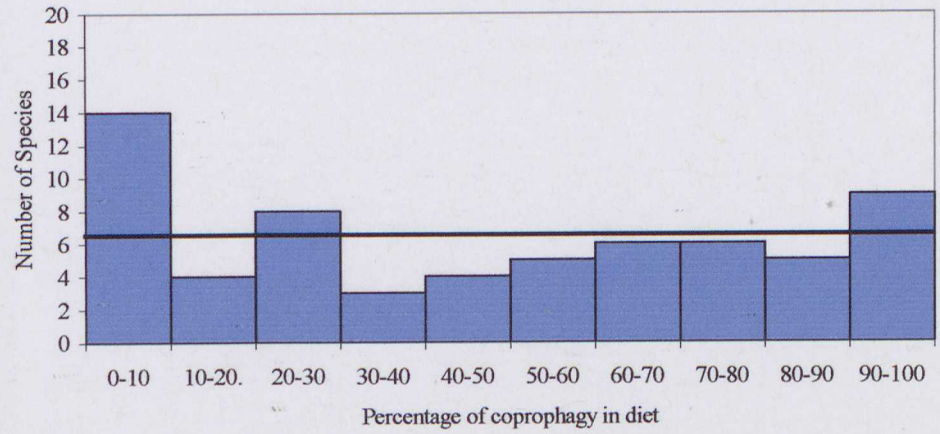


Figure 3.5. Feeding preferences at Tiputini, Ecuador



Figures 3.6, 3.7, 3.8. Plots showing the distribution of feeding behaviour throughout the size range of the three Neotropical dung beetle assemblages.

Figure 3.6. Feeding preferences and body size distribution of the Mountain Pine Ridge dung beetle assemblage

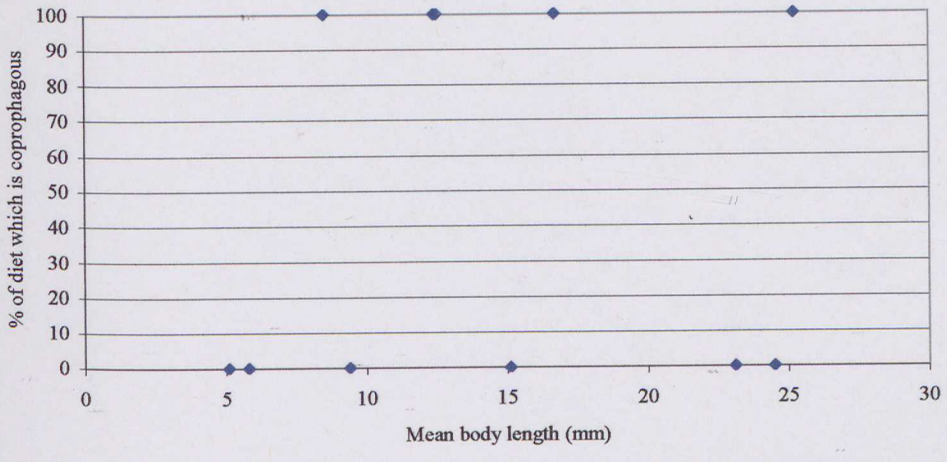


Figure 3.7. Feeding preferences and body size distribution of the Las Cuevas dung beetle assemblage

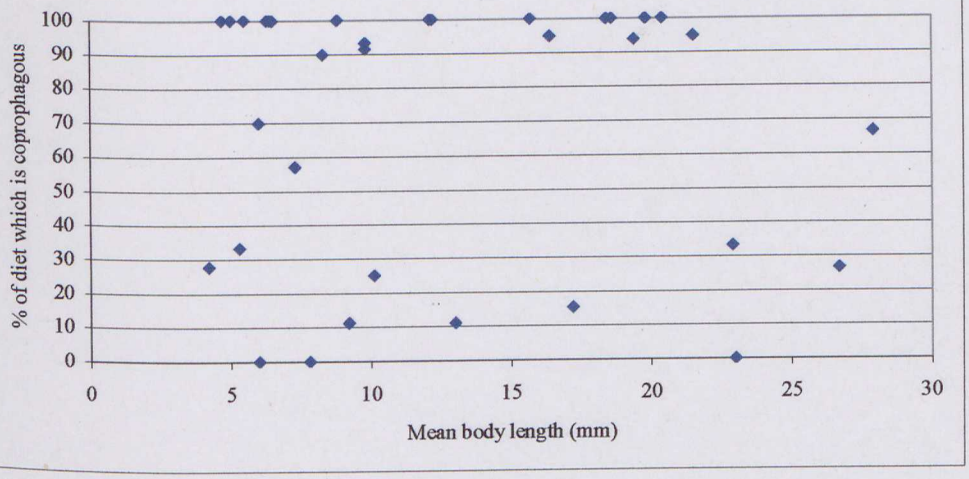
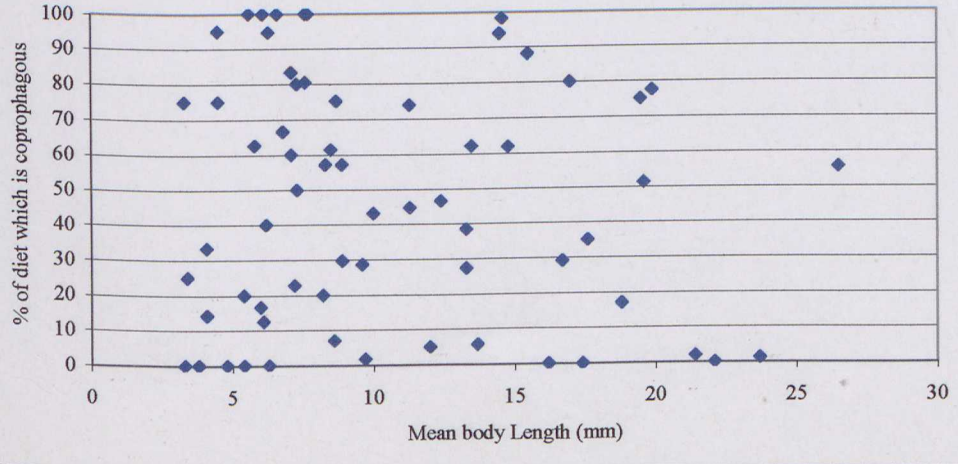


Figure 3.8. Feeding preferences and body size distribution of the Ecuador dung beetle assemblage



The feeding preference of each species has been plotted against its body length in Figures 3.6 (Pine Ridge), 3.7 (Las Cuevas) and 3.8 (Tiputini). These diagrams show the range of body sizes in the three assemblages to be similar (each figure is drawn to the same scale), and that it is primarily the species packing within that size range that is increasing as the assemblages become more species rich, particularly amongst the smaller sizes. Hanski and Cambefort (1991a) suggest that body size may affect food selection, since in Africa at least, larger species tend to be coprophagous. However, these results indicate no tendency for any feeding group to be of a particular size range, as the size distributions of each group reflects the overall size distribution of the assemblages. Most importantly however, the figures clearly show the trend of an increasingly generalist diet preference from the Pine Ridge to the Tiputini assemblage.

Pine Ridge represents one of the most southerly intrusions of pine-oak forest habitat into Central America, and as such, the pattern of increasingly generalist feeding behaviour could be interpreted as a latitudinal gradient. However, apart from habitat differences, such an effect could potentially be confounded by phylogenetic relationships amongst the species within the assemblages, since closely related species would be more likely to share the same characteristics (Harvey and Pagel 1991). Thus if one or more particular clades with generalist diet types were more speciose at Tiputini, the pattern could simply be due to the different abundances of these groups. To investigate this, the feeding behaviour for a range of monophyletic genera, from different areas of the phylogeny (chapter 2), has been tabulated in Table 3.11 for comparison across the three sites.

Table 3.11. The diet choice of the species in six monophyletic genera found in the three Neotropical sites. Feeding behaviour: Cop. = Coprophage, Nec. = Necrophage, Gen. = Generalist

	Pine Ridge Belize			Las Cuevas Belize			Tiputini Ecuador		
	Cop.	Nec.	Gen.	Cop.	Nec.	Gen.	Cop.	Nec.	Gen.
Eurysternus	2	0	0	6	0	0	0	0	9
Onthophagus	1	0	0	6	1	1	1	0	5
Dichotomius	1	0	0	2	0	0	0	0	7
Canthon	0	1	0	1	1	1	5	0	7
Deltochilum	0	1	0	0	0	4	0	5	0
Canthidium	0	2	0	2	1	5	5	6	9

From this data, increasing levels of generalism can be seen in most clades. One example, *Eurysternus,* represents an entire functional group – the dwellers. They are generally believed to be predominantly coprophagous (Halffter and Matthews 1966, Gill 1991), and in both Pine Ridge and Las Cuevas, all species collected were dung-feeders. In Ecuador however all species, including the three shared with Las Cuevas, *Eurysternus caribaeus*, *E. foedus* and *E. velutinus* (Table 3.7 and 3.8), adopted a generalist feeding strategy. Similarly, all species of the unrelated genus *Dichotomius* were coprophagous at Pine Ridge and Las Cuevas, whereas all 7 species found at Tiputini were generalists. This trend is therefore not only phylogenetically widespread and seen in all functional groups, it is occurring within monophyletic clades and even within individual species. The conclusion from this must therefore be that there is one or more ecological determinants involved.

3.3.8 Historical patterns affecting diet choice

As discussed previously, it is known that necrophagy is more predominant in the Neotropical forests compared to African ones, and also that within Africa, generalism is more common in forests compared to savannas (Hanski and Cambefort 1991a). This however is the first study to report a trend of changing levels of generalism *within* Neotropical forest assemblages, and thus poses the question, what are the prevailing factors that are generating this apparently latitudinal pattern?

It has long been suspected that there exists a general pattern of increasing levels of ecological specialization in the tropics (e.g. MacArthur 1969), whereby a narrower niche width allows greater species packing, or even leads to greater levels of speciation (Cracraft 1985). Resource partitioning is one possible mechanism by which species could achieve niche separation. For example, the much-studied endemic diversity of cichlid fish in Lake Malawi (over 500 species), has been suggested to be at least partly related to a fine-scale partitioning of food resources observed between sympatric species (Genner *et al.* 1999). However, the trend seen in these three assemblages presents the opposite situation, in that resource use is becoming less partitioned. Macroecological patterns cannot of course be applied to all situations, and Price (1991) argues that such specialization in the tropics has never been successfully invoked in any herbivorous insect group. Gaston (2000) concludes that latitudinal patterns in diversity are likely to be related to a range of contributory factors, which will vary according to

the group involved. So it is that an explanation for the variation in dung beetle resource use is likely to be quite complex and possibly unique.

It is important to consider the local resource availability of the sites, since this would seem likely to be highly influential upon the diet choice of a dung beetle. Since we believe that coprophagyevolved quite basally in the group (chapter 2), it is necessary to examine the availability of dung to each assemblage. It is apparent from the fossil record that there were originally many more large herbivorous mammals endemic to South America before the interchange with North America. In fact throughout the Tertiary period, the mammal fauna of South America was dominated by endemic ungulate groups, all of which are now extinct (Simpson 1965). One of the most diverse groups, the Notoungulata, comprised 13 families and over 100 genera (Cifelli 1983). Their demise is thought be related to a number of factors, including increasingly arid conditions and changing habitat associated with the uplift of the Andes, and ultimately to perhaps being out-competed by advanced Holarctic herbivores or devoured *en-masse* by placental carnivores (Cifelli 1983, Gould 1980). Hunting by man may also have played a significant part in their extinction (Patterson and Pascual 1972, Martin 1967). Regardless of the causes, this implies that a greater abundance of large dung would have been previously available to the Amazonian dung beetles, and would have gradually declined or have been replaced by the dung of smaller omnivorous mammals which dispersed southwards across the bridge (Marshall *et al.* 1982). This suggests that dung feeding might have been a more widespread strategy than today in a Cenozoic Amazon assemblage. It is even possible that the loss of the primitive ungulates may have been accompanied by a related loss in dung beetle taxa specialist to ungulate dung, with the more plastic and adaptive groups remaining.

In contrast, the pre-Pleistocene mammal fauna of Central America had no affinity with any of the South American forms, and were instead members of the North American groups (Rich and Rich 1983). Upon its opening, the Central American bridge is thought to have supported predominantly savanna or open-woodland types of habitat, since 22 of the 31 mammalian genera which were exchanged between the continents were savanna-adapted groups (Webb 1985). The situation at Pine Ridge may reflect the type of open vegetation and the depauperate nature of the dung beetle fauna that might have existed at this time. The lowland tropical forest which now covers the great majority of Central America is believed to have only arisen there late in the Pleistocene era (Cox

and Moore 1993), meaning that it would only have been an available habitat to Neotropical forest dung beetles quite recently. The assemblage at Las Cuevas is still therefore a relatively young community of beetles compared to the Tiputini site. Neotropical taxa may still be dispersing northwards into Central America, in the same manner that *Onthophagus* and *Copris* are thought to still be dispersing southwards into South America (Cambefort 1991a). The implications of this are that the Las Cuevas assemblage may still be below the expected carrying capacity for a Neotropical rainforest. This may or may not also be true of Pine Ridge, but its vegetation type has had a more consistent history, which has probably made it available to immigrant species for a longer period of time.

3.3.9 A possible explanation

The increasing levels of generalism in the more species-rich sites (Las Cuevas and Tiputini) was an unexpected finding. Increased species richness is thought to be accommodated by ecological specialization (Price 1980, Farrell and Mitter 1993), so we might expect the Ecuadorean assemblage in particular to have more specialists than were found. Conversely, generalists tend to be more tolerant of harsh environmental conditions (Pianka 1988), and under an optimal foraging theory, food-scarce environments should lead to increased generalization (MacArthur and Pianka 1966). Hence we might expect there to be more generalists at Pine Ridge. To find the opposite of the expected trend therefore requires some explanation.

By virtue of its high species richness, the Ecuadorean assemblage appears to be a more environmentally favourable environment for Scarabaeinae, particularly compared to Pine Ridge. This greater species richness does not seem to be a function of greater large mammal abundance however, as it does in Africa (e.g. Doube 1991). Tiputini and Las Cuevas both have only one large mammal species (the tapir), and five or six smaller artiodactyls each (deer and peccaries), all of which are in fact members of immigrant families from North America (Marshall *et al.* 1982). The high total number of mammal species at Tiputini is due mainly to smaller bodied groups such as Rodentia and Chiroptera, which from the empirical evidence, do not seem to support a high abundance of strictly coprophagous species. Therefore the resource availability of large mammal dung is likely to be quite similar in Las Cuevas and Tiputini. Inter-species competition for such resources may consequently be greater in the Amazonian

assemblage, which could potentially select for greater levels of generalism, although in an otherwise favourable environment this seems unlikely. It is more likely that differences in the environmental or ecological pressures between the sites are of greater importance.

Since we have seen that necrophagy/generalism is not only widespread phylogenetically, and that even individual species will alter the relative levels of dung and carrion they consume according to their environment, there is evidently considerable plasticity in the feeding behaviour of the Neotropical scarabaeines. I suggest that the favourable conditions of the Amazon rainforest site represent the 'normal' or ancestral situation for a Neotropical assemblage, and that feeding generalism may be a characteristic of many taxa in a benevolent habitat. Conversely, the less favourable conditions at Pine Ridge (and to a lesser degree at Las Cuevas) are driving the diet choice of taxa towards specialization.

Generalism is likely to reflect the degree of plasticity of a species towards use of alternative resource types. Given the patchy, ephemeral nature of both dung and carrion as resources, under favourable conditions it seems that an individual of a generalist species is more able to utilise whatever type of resource it encounters. It is not actually known which elements of carrion provide the nutrition for dung beetles, but it seems likely that the microbial fauna involved in decomposition would be an important part of the diet, in the same way that the microbes in dung are to coprophages. This would also explain the widespread ability to switch between resource types. The proportion of carrion which a generalist species consumes may of course depend on its ability to process different components of the resource, and indeed varies from species to species (tables 3.6-3.8). The species found to have a higher proportion of necrophagy in their diet may be able to process more than just the microbial content of the carrion, and may digest the decomposing tissue to some extent. This would certainly seem to be the case for *Canthon virens*, a Brazilian species which actively predates live ants of the genus *Atta* (Halffter and Matthews 1966 and references therein).

It seems likely that under harsh environmental conditions, taxa are forced to revert towards their ancestral, 'typical' or preferred feeding behaviour (before the widespread extinction of the Neotropical mammalian megafauna for example). One can view the environmental pressures of a habitat as a series of 'filters', through which species must

pass, in order to be a member of a community (Lawton 2000). In a harsh habitat such as Pine Ridge, these environmental filters are more severe, restricting the richness of species present. For example, the more open nature of the habitat in Pine Ridge will present the species with conditions of low humidity, high insolation and greater aridity, which will desiccate dung or carrion rapidly, killing the microbial fauna and making the resource unusable. I suggest that under such conditions, a species is driven to feed on the resource type which it is best adapted to process efficiently, namely its ancestral feeding behaviour. Thus a species from a coprophagous lineage will presumably be best adapted to process dung, and derive the greatest nutrition from it, perhaps through more efficient handling or digestion of the resource. Apparent examples of this are illustrated by the switch of all species of *Eurysternus* and *Dichotomius* from generalists in Ecuador, to strict coprophages in Las Cuevas and Pine Ridge. In contrast, in a rainforest habitat such as Tiputini's, where humidity is higher, and conditions more stable, the resource remains usable for longer (pers. obs.), and the environment does not demand specialization in the same way.

One way to test this hypothesis might be to investigate the feeding distribution of an Amazonian assemblage in a harsher, non-rainforest environment, or perhaps along an altitudinal gradient. We might also expect to see similar patterns of reduced generalism in harsher environments in other regions, compared to favourable habitats.

3.3.10 Canopy specialists – another example of niche partitioning?

At Las Cuevas, one species, *Canthon subhyalinus*, was trapped in large numbers in the dung-baited canopy pitfall traps, and was not trapped at ground level in the control pitfalls below. In fact, only a single specimen of this species was trapped at ground level (at carrion) in the entire trapping protocol carried out at the same time. This species can therefore be considered a 'canopy specialist'. Similarly at the Tiputini site, four species of canopy specialist were collected, of the same genus (*Canthon*), which were not caught at ground level in the control traps or in the main trapping protocol. It is striking that only species of *Canthon* were collected in the canopy at the two sites. Although these specialists probably do not constitute a monophyletic group within *Canthon*, since they are from at least two different subgenera, the genus does seem to have a predisposition towards such behaviour, as it has not developed in any other genus in the Neotropics. Many other dung beetles are attracted to primate dung at

ground level in these forests, as evidenced by the control traps sited on the ground, but only these few species forage in the canopy. Canopy specialists have been collected in tropical forests on other continents, including *Sisyphus* and *Onthophagus* in Africa (Walter 1984), *Arachnodes* in Madagascar (Vadon 1947) and *Onthophagus* in Borneo (Davis *et al.* 1997). Whilst the behaviour of the African Onthophagus is uncertain, all the other species share a feature in common with the *Canthon* collected in Belize and Ecuador – that is they are all capable of rolling dung. Incredibly, even the two groups of South East Asian Onthophagus species observed by Davis *et al.* (1997) in Sabah, have evolved a method of rolling dung with the hind legs. One group utilises curved metatarsi, whilst the second has a pair of tibial spurs; both mechanisms serving to shape balls of dung and roll them off the leaf surfaces to the ground below, in a manner similar to that employed by the 'true' rollers. Rolling is therefore clearly an important strategy for specializing in this way. A tunneller would be restricted to feeding on the dung in situ, and would still need to find dung at ground level for breeding purposes, by rolling the dung out of the canopy down to the forest floor, a roller can then bury it and continue its life cycle as normal. Primate dung caught in the canopy offers a rich resource not available to non-specialists, and in some cases selects for extreme morphological adaptation, presumably when the niche is not already occupied. Whilst the species collected in the Neotropics are already suited to such a lifestyle, and do not seem to be morphologically different from other non-specialist *Canthon*, their foraging behaviour is clearly modified. I suggest that this is another example of niche partitioning in a dung beetle assemblage, taking advantage of an otherwise untapped resource. The greater species richness of canopy specialists at Tiputini compared to Las Cuevas may be correlated with a higher primate species richness in Ecuador (14) compared to Belize (2), and a greater availability of dung resources.

3.3.11 Morphometric analysis of the three assemblages

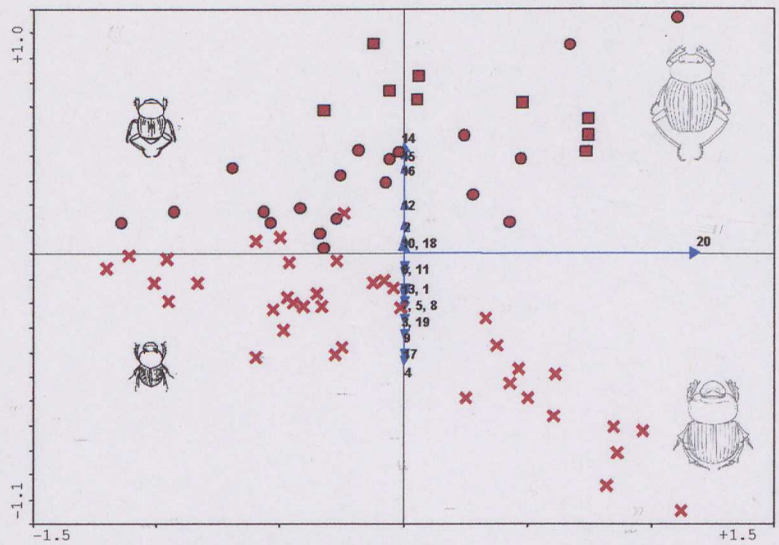
The first three axes constructed by the PCA analysis of the quantitative morphological measurements are presented in a series of figures (3.9 to 3.14). Although all species from the three sites were included in a single analysis, each assemblage is displayed separately to allow ease of comparison. For each set of figures (representing the three assemblages), the axes and the direction of increase of each measurement is therefore identical and directly comparable between sites. Eigenvalues calculated for each axis explain the level of variation in the data that can be accounted for by each of those axes.

Thus the eigenvalue of 0.398 for axis 1 suggests that 39.8% of the variance in the morphospace is explained here. Axis 2 accounts for 20.5% of the variation, and axis 3 for 14.8%. Axis 4 still accounts for 7.5% of the variance, but is not displayed as this is a relatively small amount compared to the 75% explained by the first three axes.

Axes 1 and 2 are presented in figures 3.9 (Tiputini), 3.10 (Las Cuevas) and 3.11 (Pine Ridge), whilst axes 2 and 3 are presented in figures 3.12 (Tiputini), 3.13 (Las Cuevas) and 3.14 (Pine Ridge). All species in each assemblage have been coded according to their nesting group.

Figures 3.9 – 3.11. PCA biplots illustrating the morphospace occupied by three Neotropical dung beetle assemblages. Axes 1 and 2 are presented, and nesting behaviour is mapped on.

Figure 3.9. Tiputini, Ecuador, Axes 1 & 2



Key to nesting behaviour

- ○ Roller
- × Tunneller
- □ Dweller

Figure 3.10. Las Cuevas, Belize, Axes 1 & 2

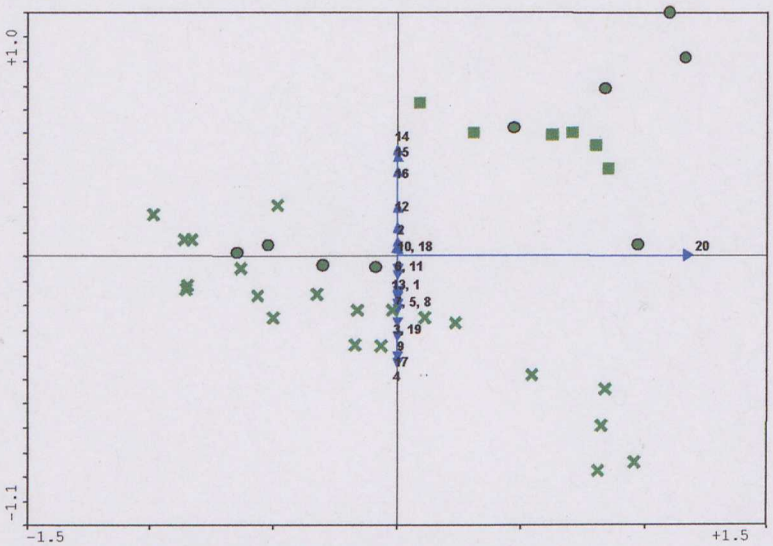
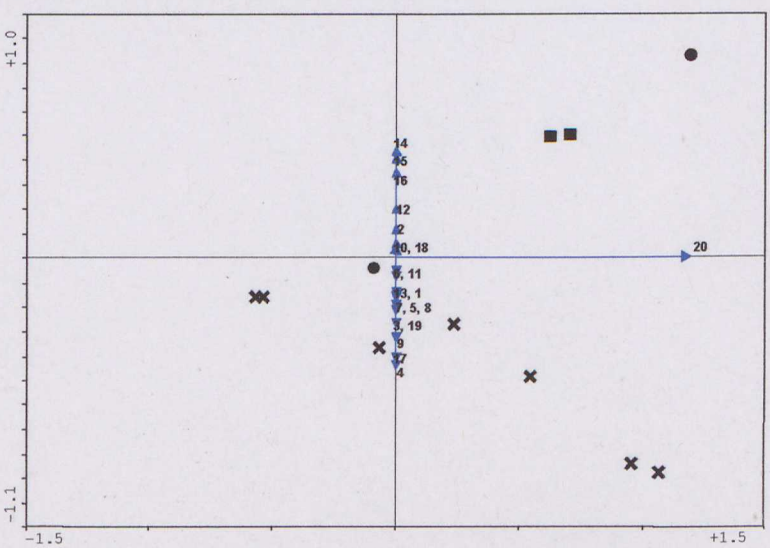
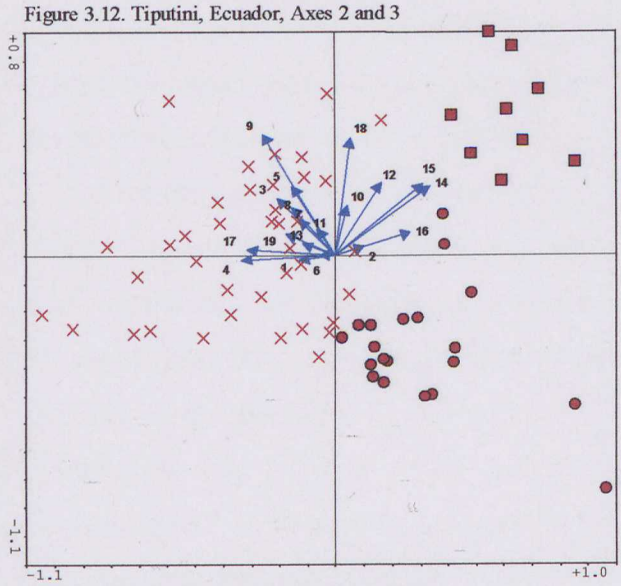


Figure 3.11. Mountain Pine Ridge, Belize, Axes 1 & 2



Figures 3.12 – 3.14. PCA biplots illustrating the morphospace occupied by three Neotropical dung beetle assemblages. Axes 2 and 3 are presented, and nesting behaviour is mapped on.

Figure 3.12. Tiputini, Ecuador, Axes 2 and 3



Key to nesting behaviour

- ○ Roller
- × Tunneller
- □ Dweller

Figure 3.13. Las Cuevas, Belize, Axes 2 and 3.

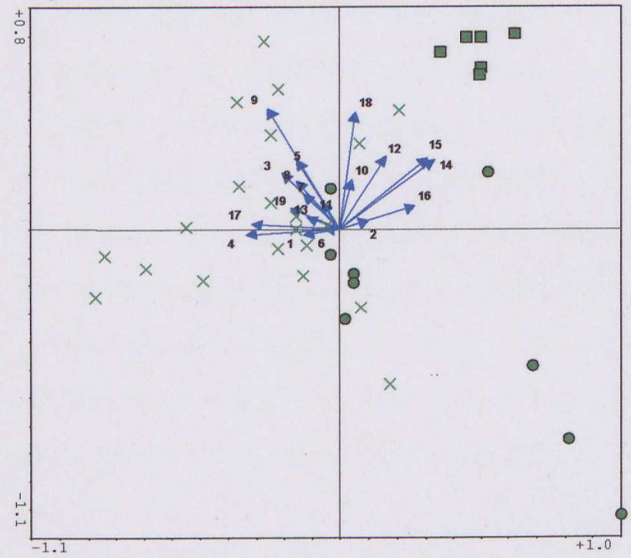
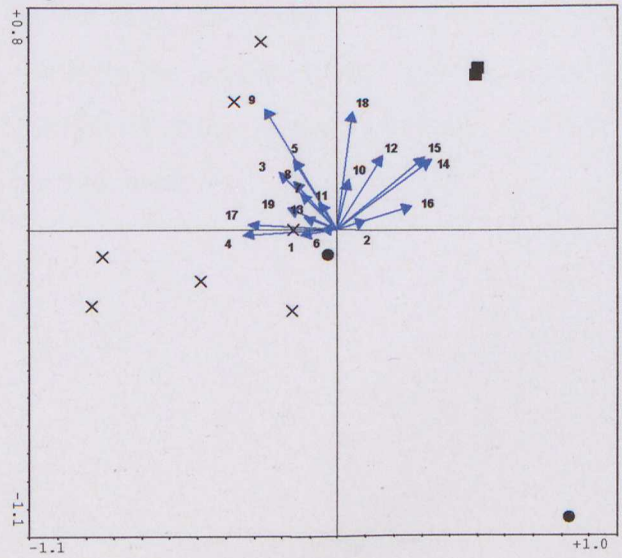


Figure 3.14. Mountain Pine Ridge, Belize, Axes 2 and 3.



3.3.12 Correlation of the first three axes with beetle morphology

By virtue of the methodology employed in this analysis (whereby the residual values of each measurement were calculated from a regression with body length - discussed above), we expect axis 1 to be directly correlated with body size. This is confirmed by the direction of the arrow for measurement 20 (body length) along the first (x) axis, and a fit of 1.0 (this means that 100% of the variance from this measurement is explained in axis 1. Table 3.12 presents the fit of each linear measurement to the first three PCA axes. Since all the remaining measurements have had this element of body size 'removed' from them, they are oriented at right angles to measurement 20, indicating no correlation between them. For figures 3.9 – 3.11 we can therefore be confident that axis 1 represents only the size of the beetle species and axis 2 represents only 'shape' measurements. Consequently the species of each assemblage are spread across the morphospace from left to right in order of increasing body size. It is evident from these diagrams that for all sites, both the rollers and the dwellers are almost exclusively restricted to the upper half of each diagram (having positive y-axis values), whilst the tunnellers have primarily negative values and are below the x-axis. The length of the arrows along axis 2 and the values of fit (Table 3.12) suggest greatest positive correlation ('roller characteristics') between this axis and measurements 14, 15 and 16, which represent a greater relative length and degree of curvature of the hind tibiae. The greatest negative correlations with axis 2 ('tunneller characteristics') are indicated to be with measurements 4, 17 and 3, which are indicate greater proportions of the pronotum and a greater width of the tibia at its apex.

There is a clear overlap of morphospace between the rollers and the dwellers in each assemblage, measured in terms of area (table 3.13). There is also a slight overlap between the smaller rollers and tunnellers at Las Cuevas and Tiputini, but the two functional groups diverge in morphospace as the species become larger, moving apart on both axes.

Table 3.12. Fit of the morphological measurements to the first three PCA axes as a fraction of the total variance of each measurement. A negative value indicates a negative correlation with that axis. Eigenvalues for each axis are also presented. Descriptions of each of the measurements are given in Table 3.2.

	Axis 1	Axis 2	Axis 3
Axis eigenvalue	0.398	0.2056	0.1483
Measurement			
1	0	-0.2567	-0.0031
2	0	0.2253	0.0176
3	0	-0.507	0.3491
4	0	-0.6036	-0.0008
5	0	-0.2763	0.5195
6	0	-0.1144	0.0002
7	0	-0.396	0.2957
8	0	-0.3774	0.3145
9	0	-0.2259	0.4697
10	0	0.0139	0.1891
11	0	-0.0438	0.0569
12	0	0.148	0.283
13	0	-0.3771	0.0431
14	0	0.6541	0.2633
15	0	0.6159	0.2999
16	0	0.4687	0.0324
17	0	-0.6441	0.0017
18	0	0.0165	0.6488
19	0	-0.3009	0.048
20	1	0	0

Table 3.13. Two-dimensional morphospace areas and nearest neighbour distances for PCA Axes 1 and 2, and PCA Axes 2 and 3, for three Neotropical assemblages. Area values are given for the total assemblage, for each functional and nesting group and for areas of overlap. Mean, maximum and minimum nearest neighbour distances are given for the whole assemblage in each case.

	Tiputini, Ecuador		Las Cuevas, Belize		Pine Ridge, Belize	
	Axes 1 & 2	*Axes 2 & 3*	*Axes 1 & 2*	*Axes 2 & 3*	*Axes 1 & 2*	*Axes 2 & 3*
Morphospace areas						
All species	3.039	2.083	2.28	1.91	1.454	1.679
Rollers	0.835	0.38	0.876	0.582		
Tunnellers	1.013	0.806	0.759	0.85	0.215	0.397
Dwellers	0.227	0.134	0.059	0.021		
Overlap between rollers and tunnellers	0.046	0	0.053	0.116		
Overlap between rollers and dwellers	0.134	0	0.056	0		
Overlap between tunnellers and dwellers	0	0	0	0		
Coprophages	0.316	0.118	1.655	0.886	0.728	0.422
Necrophages	2.255	1.011	0	0.139	1.373	0.419
Generalists	2.417	1.608	1.453	1.161		
Overlap between coprophages and necrophages	0.316	0.118	0.007	0.134	0.712	0.014
Overlap between coprophages and generalists	0.316	0.118	1.046	0.555		
Overlap between necrophages and generalists	1.879	0.732	0	0.101		
Nearest neighbour distances						
Mean nearest neighbour distance	0.913	0.705	1.008	0.76	1.056	0.936
Maximum nearest neighbour distance	2.55	2.01	2.253	2.126	2.027	2.042
Minimum nearest neighbour distance	0.006	0.016	0.011	0.026	0.031	0.026

PCA axes 2 and 3 are presented in figures 3.12 to 3.14, with axis 2 now represented as the x-axis. By excluding axis 1, body size has been removed and these figures should therefore illustrate only the shape element of morphospace occupied. Furthermore, by removing axis 1, the distribution of shape within the assemblages can be seen more clearly, and correlated more accurately with the various functional groups. With size factored out, the rollers, tunnellers and dwellers all occupy even more distinct areas of the morphospace, with only a small overlap seen between rollers and tunnellers in Las Cuevas. The orientation of the arrows in these morphospace diagrams indicate the measurements by which the different groups are primarily divided. As in the previous figures, the tunnellers are separated from the other functional groups by measurements related to increased width across the apex of the tibia and relatively greater pronotal length. However, the tunnellers can also now be seen to be specifically separated from the rollers by a positive correlation with measurements 9, 5 and 3 (greater height and width of elytra, and greater pronotum width), and to a lesser extent with measurements 8 and 7 (width and length of head). The tunnellers can similarly be said to be separated in morphospace from the dwellers by correlations with low values of measurements 14, 15 and 16 (meaning that the tunnellers have shorter and less curved hind tibia). Finally, whilst the previous figures tended to suggest that the rollers and dwellers occupied similar areas of the morphospace, they are in fact well separated along axis 3. Their division can be seen to correlate most closely with measurements 18 and 12. These suggest that the dwellers have longer hind femora and a greater area to the hind tibia.

MANOVA tests performed upon the species coordinates in morphological space, followed by Tukey HSD tests, reveal that all three functional groups in the Neotropical forests occupy significantly different positions in morphospace along both axis 2 and 3. On axis 2, significance was greatest between the distributions of the tunnellers and rollers, and tunnellers and dwellers ($F_{1,2} = 86.74$, $p < 0.0001$), (Tukey HSD tests $p = 0.0001$), whilst rollers and dwellers were more similar in distribution to one another, although still significantly different (Tukey HSD test $p = 0.03$). All functional nesting groups were found to be equally different to each other group on axis 3 ($F_{1,2} = 57.62$, $p < 0.0001$), (Tukey HSD tests $p = 0.0001$).

3.3.13 The importance of body size in niche separation

Whilst axis 1 has been constrained to represent size alone, we have confirmed from preliminary analyses that it accounts for the single greatest amount of variation in morphology. The manner in which all three assemblages demonstrate a similar size range, regardless of the total species richness present, suggests an underlying pattern which is consistent across different habitats and local species pools. Regular spacing of body sizes in different communities was first noted to reflect their associated resource sizes by Hutchinson (1959). Size is clearly an important component of niche separation in dung beetle assemblages. Cambefort (1991a) classified the rollers and tunnellers into small and large categories based on their mean lengths, and Doube (1991) used size as an integral part of his classificatory system of Southern African dung beetles. The body size of an organism will impact upon many aspects of its ecology and life history, and in dung beetles, has been suggested to affect the selection of the food resource and the way in which it is handled (Hanski and Cambefort 1991a). Even within an apparently functionally homologous assemblage such as a northern temperate *Aphodius* guild, a wide range of body sizes from under 2 mg up to 40 mg, have been recorded (Hanski 1991a). Within the Scarabaeinae, body mass ranges from about 1mg in *Panelus* up to 10g in *Heliocopris* (Hanski and Cambefort 1991a), and such differently sized taxa are seen to occur together in many assemblages. The sites of the present study have a wide spread of sizes ranging from 3.3 mm (*Trichillum* sp in Ecuador) to 28 mm (*Deltochilum scabriusculum* in Las Cuevas), although there are no very large species here, perhaps due to the low availability of large dung resources. The smaller species on the other hand, should have a wider range of resources available to them, such as rodent pellets, which would not support the breeding requirements of larger beetles. As diet choice may be related to dung availability (amongst other factors), it does not seem unlikely that the size of the species present might also be affected. Upon examination of the species inventories for Las Cuevas and Tiputini (Tables 3.7 and 3.8), it is intriguing that the dozen or so most numerically dominant species are of a considerable size range, and are also drawn from a variety of different phylogenetic clades and functional groups, and they are seen to be correspondingly widespread throughout the morphospace also. Hanski (1982) predicts that the most dominant species in a community should be more evenly spaced in size than would be expected from a random selection of species, so as to facilitate coexistence of competing species. As well as being considered to be competitively superior, there is a trend in large dung beetles towards a lower fecundity,

countered by provision of higher parental care. Smaller dung beetles on the other hand might be inferior competitively, but generally produce more offspring and can utilise resources which are too small for the larger beetles (Hanski and Cambefort 1991d).

Coexistence between potentially competing species was examined in a number of dung beetle assemblages from the USA and Mexico. Kohlmann (1991) examined pairs of coexisting *Phanaeus* species, and found that they tended to consist of one larger and one smaller species. When they were of similar sizes, one of the species tended to be rarer. My own data shows both Las Cuevas and Tiputini to each have two Phanaeus species coexisting together. In each case, they appear close to each other in the morphospace, indicating that they are ecologically similar, which is perhaps unsurprising given their monophyly. The pair at Las Cuevas are of similar sizes, but one is distinctly more abundant (*P. endymion*) than the other. Furthermore, the rarer species is strictly coprophagous, whilst the other is more often captured at carrion. The pair at Tiputini were conversely found to have similar abundances to one another, but also appear to have different diet preferences, and are more different in mean size (with a ratio of 1.28 compared to 1.13 at Las Cuevas). It seems therefore that niche partitioning is achieved in a variety of manners between just these species, including different relative abundances, resource use and body size.

3.3.14 The separation of functional groups in morphospace

From the results presented, we can see that the functional groups do not merely represent artificial boundaries within a continuous morphological distribution. The most striking division of morphospace is between the rollers and tunnellers. The occupation of distinct areas reflects their different functional roles in the dung community, and the measurements which best explain their separation can be interpreted in terms of their lifestyles. The similarity in the morphospace occupied by the dwellers and the rollers along axis 2 seemingly reflects the confounding effects of phylogeny, namely that the Eurysternini are derived from a lineage of rollers (chapter 2), and have subsequently undergone a reversal in their nesting strategy. Whilst we can not know when the behavioural shift occurred, it has apparently not greatly affected the characters which so strongly separate the rollers from the tunnellers along axis 2, the relative length and curvature of the hind tibiae. These are evidently the adaptations needed for ball formation and rolling as discussed previously, and these dwellers have

retained this ability to form balls even if they do not actually roll them (Halffter and Matthews 1966). The dwellers are subsequently seen to be separated along axis 3 from the rollers, a split correlated with greater area of the hind tibia and longer hind femora. I suggest that these traits are specifically related to the subsequent shift away from the necessity to roll dung balls: the legs remain curved and functional for making balls, but perhaps need not be as slender for rolling them, and thus have developed a greater surface for moving soil or dung. The rollers presumably have shorter hind femora than the dwellers, for the greater power which a shorter lever would provide when pushing a ball or digging (tunnellers have the shortest femora to provide the greatest possible digging power). As the eurysternines neither bury nor roll their dung balls, the hind legs presumably do not need to be so powerful, and are perhaps more efficient for simple locomotion.

The morphology of the tunnellers divides them from the other two functional groups on axis 2 and 3, based primarily on a relatively larger, wider pronotum; deeper, wider elytra, and greater width of the hind tibia. There was also a correlation with longer, wider heads. All of these can be related to requirements of burrowing. The pronotal size most likely reflects the greater musculature in the thorax that is required for digging the often extensive burrows made by this group. The elytral measurements also reflect this, simply being in proportion with the thorax; pronotal depth was not measured, but presumably would have been found to be autocorrelated with elytral depth. The large surface area of the legs and greater proportions of the head are both related to the beetles mechanism of soil movement – the head is used shovel-like to both loosen earth and remove it from the burrow (Halffter and Matthews 1966), and so a larger head would be more efficient, as would wider legs.

3.3.15 A functional trade-off?

Thus whilst axis 1 illustrates the size separation of species in an assemblage, axes 2 and 3 reflect the functional separation of the different groups. This manifests itself as a trade-off (an ecological cost) between rolling and digging characteristics. It therefore seems reasonable to assume that a greater positive or negative value on axis 2, indicates that a species will display either the rolling or digging characteristics more strongly, and will be a more efficient roller or tunneller accordingly. Thus the more morphologically extreme rollers might form or roll balls faster, or be able to make and deal with larger

dung balls. More 'extreme' tunnellers might conversely be able to burrow more quickly. Presumably there is an optimum morphology for a tunneller, in a balance between digging efficiency and the energetic cost of the digging structures. For a roller, the trade-off is likely to be one of functionality – according to the orientation of the measurements in morphospace, a more proficient roller seems to suffer a reduction in its digging capabilities. Although rollers do not generally nest as deep as tunnellers (Hanski and Cambefort 1991b and references therein), there must still be a need to bury the balls as efficiently as possible to avoid theft from other individuals, desiccation of the dung, entry by kleptoparasite species, or indeed predation of themselves. Whilst there is probably an optimum strategy for a roller, the range of morphologies may be suggestive of further niche separation within the functional group, although there is a phylogenetic component to their relative positioning (for instance the species furthest along axis 2 are predominantly of one genus, *Deltochilum*). In the same way, separation of the tunnellers might also be exhibited in their range of morphologies, perhaps reflecting the different depths to which they burrow; even closely related species are known to excavate nests at different depths (Edwards and Aschenborn 1987).

If such ideas are correct, we can predict that we would see a similar trade-off in the functional morphology of other, independently evolved, rolling and tunnelling lineages. These ideas will therefore be explored further in chapter 5, where regional patterns in the occupation of morphospace are considered.

What then does the small area of overlap in morphospace between the rollers and tunnellers suggest? If we accept that the morphospace reflects the ecology of the beetles, then perhaps those species with less differentiated morphologies may also have less differentiated lifestyles, or perhaps be more primitive. Given that the overlapping species are members of functionally different groups and unrelated phylogenetic clades, this is unlikely, although they may be more in competition with each other than with other species more distantly placed in the ecomorphological space. The convergence of roller and tunneller morphologies is most apparent in the smaller insects (see figures 3.9 – 3.11), and it may be that there is an allometric effect in both groups, where larger bodied species require more extreme morphologies to achieve the equivalent functional role, and that further measurements are needed to separate them further. As we have seen with the *Phanaeus* example, there are various ways by which species can be separated ecologically, other than by functional morphology. Unfortunately, much of

this type of natural history information is unavailable for the species in these assemblages.

3.3.16 The distribution of feeding behaviour across morphological space

Figures 3.15 to 3.20 illustrate once more the total morphospace occupied by each assemblage across the first three PCA axes, but the species are coded here by their diet preferences. At Tiputini (Figures 3.15 and 3,18), non-coprophagous beetles (necrophages and generalists) are spread across the entire space, whereas the strict coprophages are much more limited in their distribution and occupy a central 'core' area of the morphospace (particularly on axes 1 and 2), which is approximately one-tenth the area of the non-coprophages (Table 3.13). Although they comprise both rollers and tunnellers, from a variety of clades, with the removal of size, the coprophages are seen to remain clustered along axis 2, but less so on axis 3 (Figure 3.18). MANOVA and Tukey HSD tests show that the morphospace filled by coprophages in the Ecuadorean site is significantly different to that of the coprophages in either of the two Belizean sites on axis 3 ($F_{1,2} = 4.7$, $p = 0.016$), (Tukey HSD tests $p < 0.05$).

Figures 3.15 – 3.17. PCA biplots illustrating the distribution of feeding behaviour in morphospace, Axes 1 and 2.

Figure 3.15. Tiputini, Ecuador, Axes 1&2

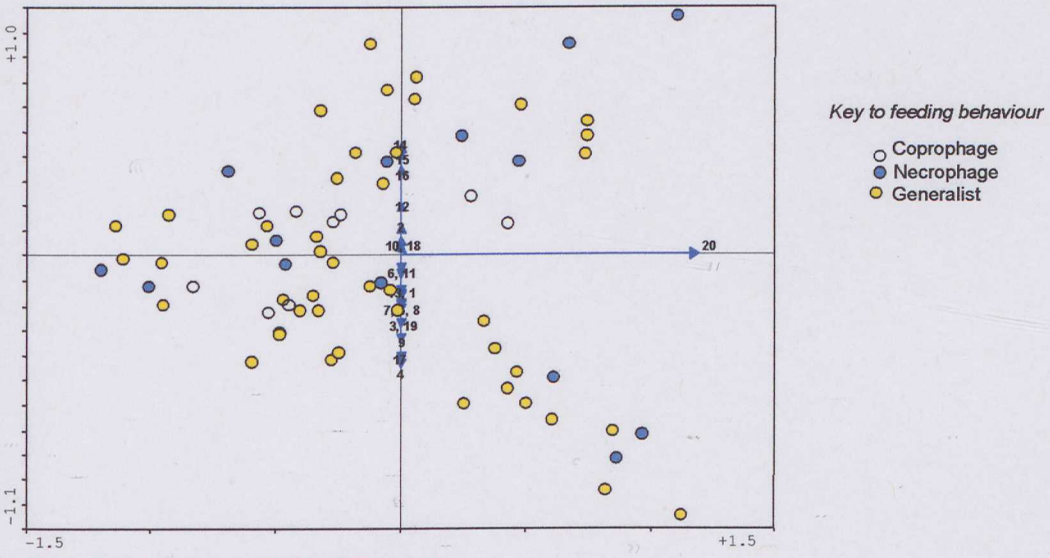


Figure 3.16. Las Cuevas, Belize, Axes 1 & 2

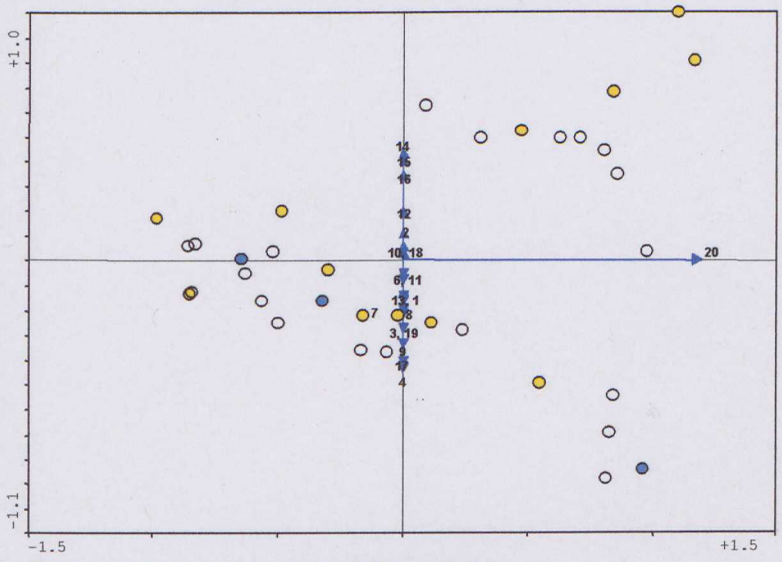
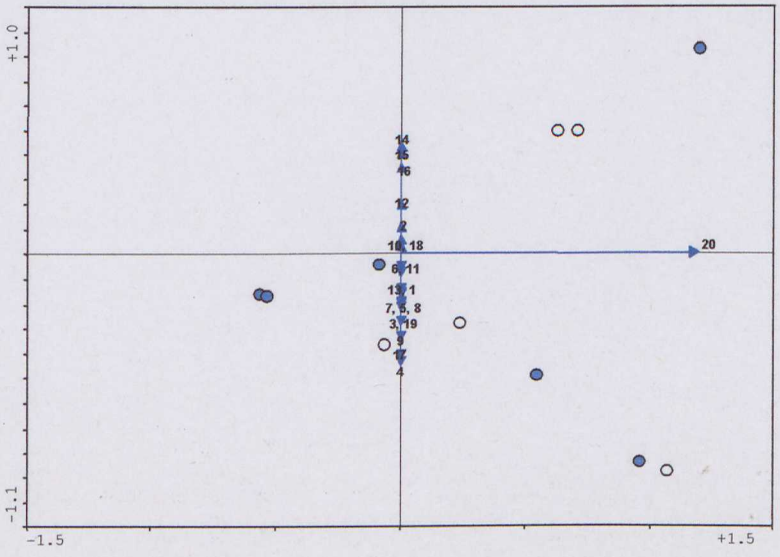
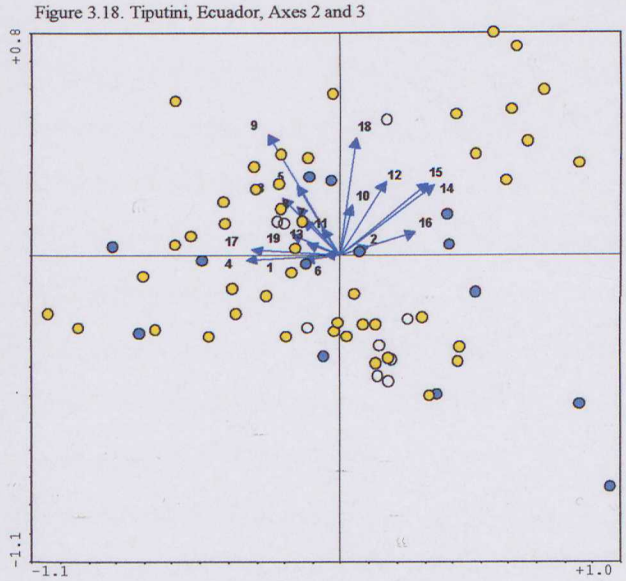


Figure 3.17. Mountain Pine Ridge, Belize, Axes 1 & 2



Figures 3.18 – 3.20. PCA biplots illustrating the distribution of feeding behaviour in morphospace, Axes 2 and 3.

Figure 3.18. Tiputini, Ecuador, Axes 2 and 3



Key to feeding behaviour

- Coprophage
- Necrophage
- Generalist

Figure 3.19. Las Cuevas, Belize, Axes 2 and 3

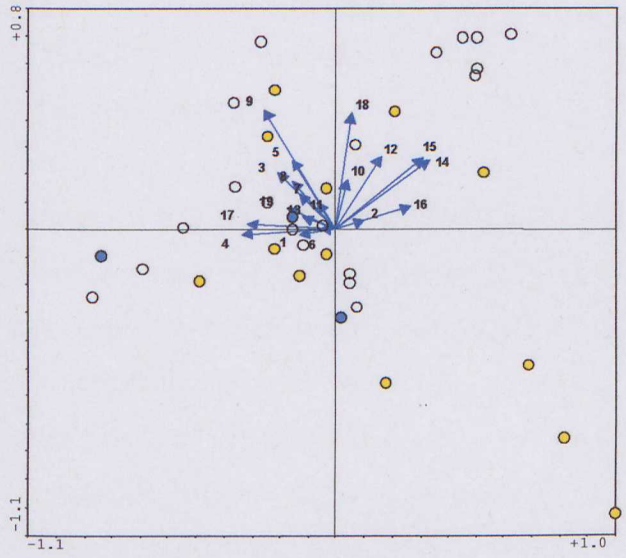
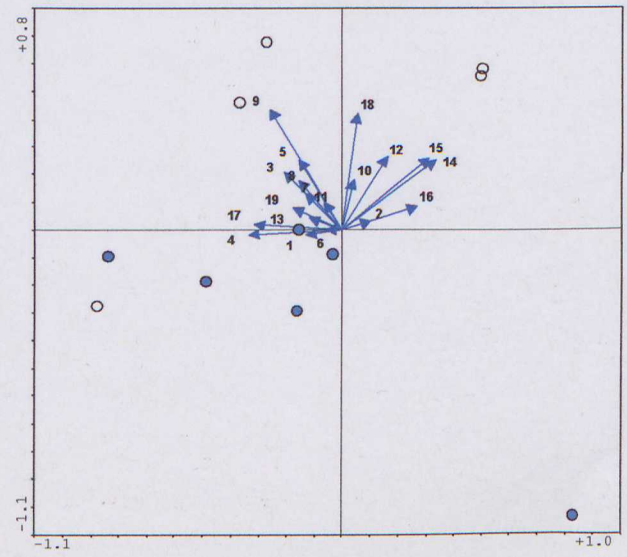


Figure 3.20. Mountain Pine Ridge, Belize, Axes 2 and 3



Las Cuevas has a greater abundance of coprophagous beetles, and they are well distributed across the morphospace, as are the generalists (Figures 3.16 and 3.19). The coprophages at Las Cuevas have a much higher mean nearest neighbour distance than do those at Tiputini (0.723 compared to 0.444), which reflects their broader distribution across the morphospace on axes 1 and 2. The three necrophagous species at Las Cuevas are well distributed along axis 1, as they are of differing body sizes, but do cluster particularly along axis 3 (Figure 3.19). Similarly, with no generalists at Pine Ridge, both coprophagous and necrophagous beetles are well spread along axis 1 and 2 (Figure 3.17), but appear to be distinctly separated along axis 3, with a small overlap (Figure 3.20). As mentioned previously, axis 3 is most closely correlated with measurement 18, tibial area (see Table 3.12). A MANOVA and Tukey HSD test found no significant difference between the positions in morphospace occupied by the different feeding groups in the Neotropical areas. However, a comparison of all Neotropical coprophagous and necrophagous species returned a p-value of 0.054 for axis 3, ($F_{1,2}$ = 2.96, p = 0.056), a very nearly significant difference between the space occupied by the two feeding behaviours.

These are interesting findings, since there is no tendency for the functional groups to adopt any particular feeding strategy. In the Ecuadorean assemblage however, most of the coprophages are located at or around the region where the morphospace occupied by the rollers and tunnellers comes closest and even overlaps, a trend observed on all three axes (Figures 3.15 and 3.18). As discussed, this area of overlap in less differentiated morphology may represent a set of species which are potentially most in competition with one another. In terms of their feeding behaviour, this region not only includes the coprophages, but also necrophages and generalists, so that the widest range of diets has been retained in the area of least morphological differentiation. Whilst it appears that generalism is a more successful strategy in Neotropical forests with conditions of high species richness, as discussed above, specialist coprophages seem to persist where morphological specialisation does not. In a manner similar to that found in the cichlid communities (Genner *et al* 1999), this suggests that niche separation is being achieved in a resource-limited environment, between ecologically and morphologically similar species, by means of diet preference.

Why the coprophagous and necrophagous species should show a tendency to separate along axis 3, as illustrated particularly at Pine Ridge requires a different explanation.

The inference from the diagram is that the coprophages have a greater tibial area than the necrophages, which as discussed, relates to increased digging abilities. There is some correlation with measurement 9 also, which relates to depth of the elytra, and as suggested, is indicative of a deeper pronotum, housing more muscle for more powerful digging. Why would coprophages need to be better diggers, and more so at Pine Ridge than Tiputini, where this separation is not really seen?

Pine Ridge is a much more open habitat, with considerably less canopy cover than the moist forests. This means that dung would dry up and be rendered unusable for dung beetles considerably faster than in the deep shade and higher humidity afforded by a rainforest. Consequently, there is likely to be a need to bury dung as quickly as possible to avoid desiccation, and so more efficient digging might be selected for, regardless of functional group. In open conditions, the soil is also likely to dry out and become harder, which might also require a more powerful body form. In the trapping protocol, carrion was not found to lose its attractiveness as quickly as dung, as its consistency is quite different. This probably also explains why most of the necrophages were trapped in greater abundances than the coprophages at Pine Ridge. Therefore rapidity of digging might not be as important in necrophages, as the need to process the resource will be less pressing. They may also tend to feed directly from a carcass rather than immediately burying it. Adults of the necrophagous species *Anachalcos convexus* occur in open bushveld habitat in Southern Africa, and can be observed feeding directly on carrion (pers. obs.), although this species reportedly still breeds in dung (C.H. Scholtz pers. comm.). Cambefort (1984) found that immature adults in the Ivory Coast often fed on the more nitrogen-rich omnivore dung, even though they bred in herbivore dung. This was suggested to help in their maturation; the building of muscle and egg development in females. Such could be the situation in the Neotropics, but unfortunately it is not really known whether or not the Neotropical generalist and necrophagous species breed predominantly in dung or carrion.

Overall, the divergence seen in morphology between the different feeding groups (coprophages and necrophages) at Pine Ridge, suggests that morphological separation might be more important in a harsher environment. The unexpected lack of generalism at Pine Ridge, compared to the high levels in Las Cuevas and Tiputini (discussed above), might be explained by a divergence in morphology, and supports the hypothesis that greater specialization in diet is being driven by ecological or environmental factors.

3.3.17 Assemblage- level comparisons of morphospace

All three assemblages occupy broadly similar areas of morphospace, particularly when size is removed in axes 2 and 3. Total morphospace area does decrease slightly from Tiputini to Las Cuevas, and again to Pine Ridge, but is accompanied by an increase in mean nearest neighbour distance (Table 3.13). This indicates that the species packing in morphospace is greatest in Tiputini, and falls as species richness decreases. MANOVA tests revealed no significant differences between the total morphospace occupied by the three different assemblages, on any axis. Similarly, the morphospace occupied by the individual functional groups (rollers, tunnellers and dwellers) is also not significantly different between any of the sites.

When considering that the species richness of Tiputini is over six times greater than that at Pine Ridge, the consistency of the total morphospace and that occupied by the functional groups in the three assemblages is remarkable. This finding is contrary to similar studies on avian communities (e.g. Ricklefs and Travis 1980, Travis and Ricklefs 1983), where increasing diversity in an assemblage tended to be added to the periphery of its morphospace, and that the nearest neighbour distance remained relatively constant. The uniformity seen in these dung beetle assemblages seems to reflect the occurrence of all of the main clades in each site (see species inventories). Thus the morphospace occupied by a number of different monophyletic groups in the three sites was investigated with MANOVA tests. No significant differences between assemblages were found for the Phanaeini, *Dichotomius*, *Onthophagus*, or *Deltochilum*.

Because of the incidence of the same phylogenetic clades in the three assemblages, the similarities are apparently not due to convergence, but to shared ancestry. The consistency of morphospace usage provides a clue as to why, even in a depauperate community such as Pine Ridge, the phylogenetic composition remains so consistent. Taxa seem to be progressively lost, from all phylogenetic groups, until only those which are sufficiently spaced from one another remain. Species which are more closely related are believed to be more likely to compete with one another (Richman and Price 1992, Genner *et al.* 1999), since they tend to be more ecologically similar. In this case, it certainly seems that phylogenetically, morphologically and thus ecologically separated species are more likely to be retained, or are better able to coexist in a

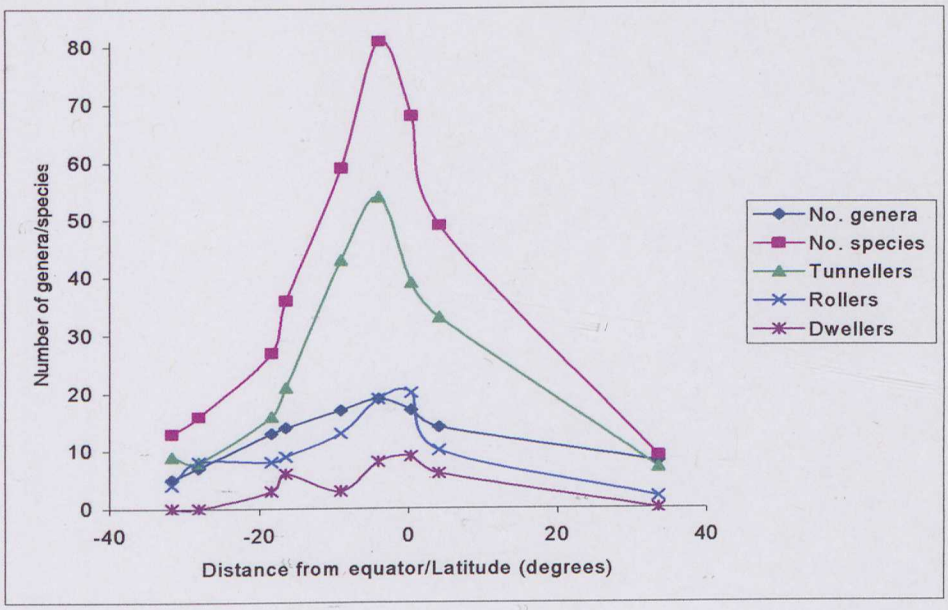
depauperate assemblage, than species which are more similar in these respects. Figure 3.17 for Pine Ridge also shows how species are further separated on axes 1 and 2 by diet choice.

Since the overall pattern of morphospace occupation is by and large independent of the local species pool and forest-type, we can assume that it is structured in a manner common to all Neotropical forests, and is presumably constrained by phylogeny to a considerable extent. Would the same pattern be seen in other forests on different continents with independently derived dung beetle lineages? Whether this is a model applicable only to the Neotropical lineages, to tropical forests in general or to a wide range of dung beetle assemblages worldwide, will be addressed in chapter 5, where the influence of phylogenetic history upon morphology will be more fully explored.

3.3.18 Functional diversity of assemblages across the Americas

For each of the ten American sites, the generic and species richness are plotted on Figure 3.21, along with the numbers of species in each functional group. In an attempt to elucidate any pattern 'typical' of the American dung beetle assemblages (moving south along a latitudinal and habitat gradient), Pine Ridge was excluded in favour of Las Cuevas as a more representative habitat for that latitude. There is a clear peak in numbers of species and genera at the sites closest to the equator, which can be primarily attributed to a considerable increase in the numbers of tunnellers at these sites. Both generic and species richness of rollers and dwellers also increase at lower latitudes, but in a more gradual manner. Such diversity patterns can be attributed to a variety of environmental and historical factors however, so integration of environmental data for the sites is clearly important, and was achieved in a multivariate analysis.

Figure 3.21. Dung beetle assemblage structure across a latitudinal gradient in the Americas.



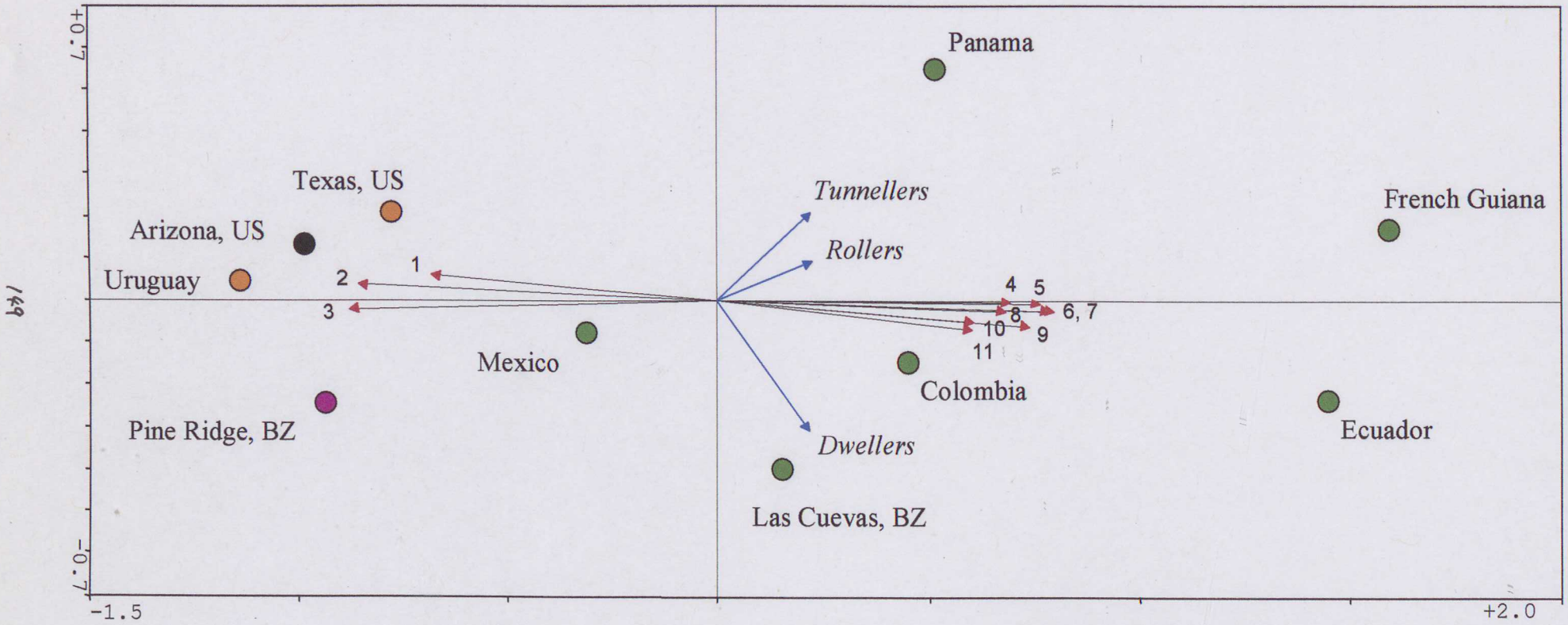
3.3.19 Findings of the multivariate analysis

The results of the ordination are presented in Figure 3.22, which displays all significant environmental variables. Table 3.14 displays the significance level (p-values) for each of the variables plotted, as obtained from the Monte Carlo tests. It also gives the λ values (eigenvalue, or fit) of the variables when considered independently ('marginal' significance).

Table 3.14. Environmental variables found to be significantly correlated with the variation in assemblage structure of the ten American sites. p-values were obtained for each variable by examining its marginal significance with a Monte Carlo test (999 permutations). λ1 values denote the eigenvalue (fit) of each variable when treated on its own.

Environmental variable	λ1	Monte Carlo test p-value
Disturbance	0.78	0.001
Latitude 2	0.74	0.001
Medium-small mammals	0.64	0.003
Rainforest	0.62	0.01
Mean annual rainfall	0.59	0.004
Omnivorous mammals	0.55	0.002
Mean annual temperature	0.48	0.013
NPP	0.47	0.016
Seasonality of temperature	0.47	0.024
Herbivorous mammals	0.37	0.035
Total mammal species	0.37	0.051

Figure 3.22. Ordination diagram for the comparison of functional diversity across ten American dung beetle assemblages. Marginal significant variables are presented. Variables: *(1) Seasonality of temperature, (2) Latitude 2 (distance from equator), (3) Disturbance, (4) Mean annual temperature, (5) Mean annual rainfall, (6) Tropical forest, (7) Medium-small mammals, (8) NPP, (9) Omnivorous mammals, (10) Herbivorous mammals, (11) Total mammals.* Habitat type: *Green = Rainforest, Pink = Open, dry forest, Orange = Temperate chaparral, Black = Arid semi-desert*



Axis 1 has an eigenvalue of 0.899, meaning that it accounts for almost 90% of the variance in the data. It is therefore considerably more important in explaining assemblage structure than any subsequent axis. Nevertheless, axis 2 explains a further 7% of the variance, and so a total of 97% of the total variation is accounted for in figure 3.22. The ten sites are clearly spread out along axis 1, and all of the significant environmental variables are closely correlated with this axis. Three 'clusters' of sites can be identified relative to axis 1, and will be discussed as such here due to the overwhelming significance of this axis.

The first cluster comprises the two sites of Nouragues in French Guiana and Tiputini, Ecuador. Both are situated in areas of primary Amazon rainforest, and share a high species richness of 81 and 68 species respectively. Despite the fact that they are over 2,600 km apart - one on the Guiana Shield and the other in the far western Amazon basin, 25 % of the species and 80 % of the genera at Tiputini also occur at the French Guiana site. Furthermore, their clustering indicates a close similarity of the functional group structure existing in each.

The second group includes the two most northerly sites - Arizona and Texas, USA; the Central American site of Pine Ridge, Belize and the most southerly site in the analysis, Cerro Colorado, Uruguay. These comprise a mixture of habitats: chaparral, a brush-grass complex (Texas and Uruguay), semi-desert grassland (Arizona), and an open pine-oak forest (Pine Ridge). Although diverse, these habitats are all open in structure, unlike the rainforest habitat sites of all the other sites.

The final group of assemblages represents a more loose affiliation, but one that is primarily intermediate between the first two clusters. It includes Barro Colorado Island, Panama; Leticia, Colombia; Las Cuevas, Belize and Los Tuxtlas, Mexico. All can be considered as rainforest, but have suffered from a certain level of disturbance.

3.3.20 Correlations with the environmental data

Of the significant environmental variables, most are positively correlated with axis 1. These can be classified into two types of variable for ease of interpretation, although they are auto-correlated, and therefore confound each other to a certain extent. The first type are the 'rainforest attributes', and comprise rainforest vegetation, mean annual

rainfall, mean annual temperature and NPP. The second type relate to the 'mammal fauna attributes' of the region, including medium-small, omnivorous and herbivorous mammal richness, and the total mammal species richness. Three significant variables are negatively correlated with axis 1, including disturbance, latitude 2 (distance from equator), and seasonality of temperature.

The direction of increase of the rollers and tunnellers is similar, indicating that these two functional groups increase and decrease in a similar manner across different sites. The orientation of the dwellers however (at right-angles to the rollers and tunnellers) suggests that any increase in this group is totally uncorrelated with changes in the other two nesting groups. There is a general correlation between all functional groups and the rainforest and mammal variables, but this is strongest for the rollers. In terms of absolute numbers however, the species richness of tunnellers increases more (figure 3.21).

The positioning of French Guiana and Ecuador, with high axis 1 values, indicates a positive correlation with the rainforest and mammal fauna attributes, and a negative correlation with higher latitude, disturbance and seasonality. At the other extreme lies the cluster of the non-rainforest sites, which conversely display the opposite characteristics. The various intermediate sites are placed at points along axis 1 depending upon how well the two opposing sets of variables explain their functional group structure; Mexico is apparently more affected by disturbance and higher latitude, whilst Panama and Colombia are more correlated with the rainforest attributes.

The gradient of functional group (and species) richness along axis 1 of the ordination diagram can be clearly associated with habitat. The assemblages of French Guiana and Ecuador cluster together because they represent the most diverse and functionally rich assemblages in the data set. They also occupy two areas of primary Amazonian rainforest, one of the oldest continuously forested areas on the planet, with tropical rainforests first appearing there some 90 to 100 million years ago (Morley 2000). Dung beetle evolution in the Neotropics appears to have been closely associated with the rainforest. Indeed, given their predominant restriction to forests, most of the endemic clades, such as the South American Canthonini, Phanaeini, Eurysternini and various "Dichotomiini" groups, most likely evolved and radiated in such an environment. Very few South American scarabaeine taxa display a greater (or even equal) species richness

in more open areas. The tribe Eucraniini are one such group, restricted to arid areas of Argentina (Halffter and Matthews 1966), where they specialize on Lagomorph dung (Cambefort 1991a). The Ecuadorean and French Guianan assemblages therefore most likely provide the best representation of an 'ancestral' or typical Neotropical dung beetle assemblage. This would explain why they are strongly correlated with the rainforest attributes - a higher mean rainfall and temperature, greater net primary productivity and reduced seasonality are most likely to be just a few of the properties of a rainforest to which the dung beetles of the region are best suited. The correlation with a greater abundance of omnivorous and herbivorous mammals may be confounded by a similarly higher propensity for mammal richness in Amazon forest. However, since the extinction of much of the Tertiary mammal fauna, it is these groups which must be the primary dung providers in the region, and thus a correlation between greater mammal and dung beetle richness seems reasonably likely.

The two other groups are affected to varying degrees by non-rainforest characteristics. One of the most significant of these is disturbance ($p<0.001$), be it from human (logging, hunting of mammals) or natural effects (hurricane, fire). Only French Guiana and Ecuador are undisturbed by human impact. Dung beetle assemblages in tropical forests are known to be particularly adversely affected by deforestation, and a resulting reduction in species abundance and increased dominance of disturbance-tolerant species has been clearly demonstrated in Neotropical, African and South East Asian forests (e.g. Howden and Nealis 1975, Klein 1989, Nummelin and Hanski 1989, Davis 2000). Canopy loss leads to various detrimental changes in the physical habitat of the dung beetle assemblage, such as alteration of soil structure and increased temperature fluctuations. Of the indirect effects, one of the most important is likely to be a reduction in the forest-dwelling mammals, which will of course affect the dung availability of the area. Thus the position of the Colombian assemblage in the intermediate group is most likely due to the relatively recent selective logging and hunting occurring in the area. Given time to recover, it would probably be very similar to Tiputini, given its position in Amazonia. It seems that forest-dwelling dung beetles can be quite resilient to disturbance, and can return to a similar functional structure within a relatively short time span, provided there is a nearby source or reservoir from which they can re-colonise an area (Gathorne-Hardy and Inward, in prep.).

The Central American moist forest assemblages have also suffered varying levels and types of disturbance, which will have undoubtedly adversely affected the species composition. However, the forest structure will also be different by virtue of its relatively young age compared with the Amazon forest, and a latitudinal effect which is linked with increasing seasonality. Presumably, the forest around BCI, Panama is more 'Amazonian' in its affinity than the Mexican forest, which being the most northerly is more seasonal, has fewer mammals, and is evidently more similar to the non-rainforest sites.

Disturbance is clearly strongly correlated with the clustering of the non-rainforest sites (Uruguay, Texas, Arizona and Pine Ridge), as all four sites suffer considerably, although from different types of disturbance. However, this is confounded by a number of other variables. Firstly, the strong negative correlation with rainforest attributes clearly suggests that an open vegetation type and its associated influences on the physical and biotic environment, also plays a critical role in assemblage structure. The stark differences observed between Las Cuevas and Pine Ridge, which share the same regional species pool, serves to illustrate the considerable differences in functional group composition that open and closed forest types produce. The latitudinal distance from the equator is also a strong correlate ($p<0.001$), and yet Las Cuevas is at the same latitude as Pine Ridge (and shares the same weather patterns), but neither this nor Mexico falls into the group.

I therefore suggest that it is habitat type which is the most critical component in the functional structure of a Neotropical assemblage, and specifically the division between open-type vegetation (in various forms) and closed-canopy forest. This appears even more influential on structure than any long term historical effects. This is suggested by the positioning of the Uruguayan assemblage, which has a totally different biogeographic history (as part of continental South America) to the other sites with which it clusters, whose taxa have primarily colonized the sites only after the advent of the Panama land bridge.

The three Neotropical forest assemblages sampled for this study (Pine Ridge, Las Cuevas and Tiputini) are widely distributed across the ordination diagram, with one found in each of the three clusters. This suggests that each site is affected by very different environmental factors, and that the patterns identified in their ecology and

morphospace are likely to be amongst the most wide ranging and fully representative of this larger data set. Thus Ecuador seems to be an example of a 'typical' Neotropical assemblage, and Pine Ridge an example of an assemblage depauperate in its composition, due primarily to less favourable habitat, but illustrating the patterns in morphospace and taxonomic organization, which are apparently inherent to most Neotropical assemblages.

3.3.21 Conclusions

In setting out to characterize the way in which Neotropical dung beetle assemblages are structured, a variety of techniques have been employed, leading to a number of inferences. Firstly, there appears to be an inherent structure underlying all Neotropical assemblages, which manifests itself in a variety of ways. It can be seen in the consistency of the functional group ratios and body size ranges in the three different study sites. It is also apparent in the uniformity of phylogenetic composition and in the total morphospace occupied, despite differing habitat, species pool and species richness (species packing). Where carrying capacity of a habitat is lower, this structure persists and species are well separated morphologically, ecologically and phylogenetically, which seems to be important for the coexistence of species. The major environmental variable to affect functional composition in the Neotropics appears to be habitat type (specifically closed forest versus open-type vegetation), although there is likely to be a variety of other factors associated with this, such as resource availability and disturbance. Habitat effects seem to play a greater role in functional composition than historical effects (phylogenetic or biogeographic), at least with respect to within-region history. This begs the question whether this is a pattern intrinsic to dung beetle communities both within other regions, and between them. Such ideas will be investigated in the subsequent chapters.

The influence of competition in dung beetle assemblages is suggested by the wide variety of niche separation which has been found. Partitioning has been seen here to occur through differential body size, foraging behaviour, resource use, nesting strategy, and morphology. A trade-off has been seen in the Scarabaeinae between rolling and digging characteristics, and there is even some evidence for morphological partitioning between different feeding behaviours. Conversely, where species are morphologically

and ecologically similar, niche partitioning has been achieved through the retention of an otherwise much diminished feeding behaviour.

Finally, a standardised trapping protocol has been developed and tested, and its use is advocated for all future dung beetle community investigations in the Americas, since it appears to be an optimally efficient way to sample a complete assemblage. The method also serves to identify the feeding behaviour of the species collected, which has been identified as clearly being an important mechanism of functional niche separation, whilst plasticity of feeding behaviour is evidently an important characteristic of a Neotropical scarabaeine assemblage.

Chapter 4. The functional diversity of dung beetle assemblages: a continental comparison.

4.1 Introduction

From the molecular data in chapter 2, it is evident that the great majority of the taxa which make up the dung beetle assemblages in Africa and South America, diverged from one another tens of millions of years ago, and may have been evolving independently since the separation of the two continents over 100 million years ago. Whether the origins of the group lie in a Gondwanan or African origin, the assemblages of the two continents are ideal for a comparative faunal analysis. There is evidence of functional convergence between the taxa of the two regions (the independent origin of dwelling for instance), but is there any evidence that the entire functional structure of the assemblages have converged? Now that it is apparent that biogeographic history has had a strong influence on the phylogenetic composition of each continent, can we identify trends in the ecological structure of assemblages? Will they be similarly influenced by biogeographic history, or will assemblages in comparable habitats, but different regions, be ecologically similar? In short, this chapter will address two issues regarding dung beetles at an assemblage level: what are the ecological patterns, both within and across biogeographic regions, and what are the mechanisms driving them?

4.1.1 Local and regional effects upon species diversity

The diversity of a local community will be influenced by a range of factors, from regional level processes such as climate, biogeographic events and speciation, down to more localized processes such as disturbance, habitat type, and various inter-species interactions (predation, competition, mutualism) (Ricklefs 1987). Dung beetle ecology often focuses on the local processes that determine the presence or absence of a species in a community, in particular habitat and competition (see for example the various studies in Hanski and Cambefort 1991). This is perhaps too simplistic an approach, considering the multiplicity of factors that can play a part in assembling a community. To fully understand the origins of an assemblage, we must first take into account the most large-scale regional events, which determine for instance whether or not a clade even exists in the regional pool, before considering the small-scale interactions between competing individuals. Local and regional diversity are closely inter-linked (Caswell

and Cohen 1993), since local communities are assembled from the regional pool, via a series of filters (Lawton 2000), both historical and environmental (discussed below). We therefore need to first understand the origin of the regional pool before we can examine the processes acting at the local level. To achieve this for the Scarabaeinae, I will compare the biogeographic, floral and mammal histories of Africa and the Neotropics (which represent the greatest taxonomic dichotomy), before considering the local processes, to try and gain an insight into the structuring of assemblages across the globe. Building upon the findings of the Neotropical assemblage structure, I will therefore investigate and compare the functional diversity (particularly nesting behaviour and feeding strategy) of various assemblages around the world, both within different geographic regions and particular habitat types. I shall then search for evidence of convergence in the community structure of independent lineages, and try to identify the key environmental variables which play the greatest role in scarabaeine diversity.

4.1.2 The key African habitat types

In chapter 3, the central role of habitat type to dung beetle evolution in the Neotropics was highlighted. There is a strong suggestion that the extant taxa of the region have primarily originated in the rainforest of South America where the greatest diversity and species richness is found. In comparison, the open habitats of the Americas have a considerably more depauperate fauna.

The dung beetle assemblages of Africa can be broadly placed into three main habitat types – rainforest, savanna and Mediterranean-type habitat. Sub-Saharan Africa has the richest Scarabaeinae fauna of any continent in the world, being home to over 2000 of the worlds 5000 total species (Doube 1991). Within this region, the savanna assemblages have been most widely studied (e.g. Kingston 1977, Walter 1978, Doube 1987, 1991 and references therein, Cambefort 1984, 1991b and references therein, Krüger and Scholtz 1998), with somewhat less attention devoted to the rainforest habitats (Numellin and Hanski 1989, Cambefort 1985, Cambefort and Walter 1991) and Cape fynbos habitats (Davis 1987). This is primarily due to the considerably greater species richness of dung beetles to be found in the savannas, a trend which is generally perceived to be due to the greater species richness of large ungulate mammals there, and the consequently higher diversity and quantity of dung (e.g. Cambefort 1991b). This

has naturally led to the assumption that the African savannas have been the main theatre of radiation in the Old World. The grass-dominated savannas of today have only been in existence since the mid-Eocene period (Van der Hammen 1983), although other open, arid environments would have been present before this time. It is not unlikely that the grassy savannas might have coevolved with the large grazing herbivorous mammals; the Perissodactyla (rhinos, zebras) and Proboscidea (elephants) would have been the predominant groups from the Eocene onwards, until the much more recent radiation of the Bovidae (buffalo, wildebeest, antelope etc.) in the Miocene. By this time the conditions in southern Africa were more arid and suitable for grassland, and the bovid ability to digest cellulose efficiently by rumination would have proved a competitive advantage (Langer 1974).

The forests of Central Africa are much older, having been present since the late Cretaceous period, (as they have in Amazonia) by virtue of their position in the equatorial belt (Morley 2000). Unlike the Neotropical forests however, the African rainforests have had a more turbulent history, with several substantial extinction events since this time, associated with periodic climatic cooling and drying. Periods of considerable diversification and expansion are also evident, particularly during the early Miocene, when forest extended as far south as the Cape region. However, the late Miocene and early Pliocene saw the expansion of savanna across much of the African tropics, whilst the rainforests contracted and suffered their final widespread extinctions, leaving the current flora quite depauperate in comparison to rainforests of other regions (Morley 2000).

Occupying an apparently important if enigmatic role in African dung beetle history, is the Cape region, with its distinctive mediterranean-type vegetation structure unique to southern Africa. Just as its flora has a high endemicity and comprises an entire floral kingdom (Cox 2001), so various groups of dung beetles, quite basal in the cladogram, are restricted to this region (chapter 2). Whether this represents a relictual distribution has yet to be confirmed.

One of the initial aims of this chapter then, is to compare and quantify the similarities or differences in the functional structure and diversity of a number of African dung beetle assemblages across a range of vegetation types (savanna, tropical forest, fynbos). Furthermore, because of the generally-held belief that dung beetle diversity is closely

associated with the rich African mammal fauna (e.g. Cambefort 1991a), trapping, although often extensive, has very rarely included carrion within its sphere of interest. Although trapping with carrion has been carried out in an African forest (Cambefort and Walter 1991), no data comparable to that employed in chapter 3 exists for either African savanna or mediterranean-type assemblages. This means that whilst preference towards particular dung types may be known, necrophagy is perceived to be virtually nil in these assemblages.

4.2 Materials and methods

4.2.1 Trapping of savanna and Mediterranean-type assemblages

The first part of the field work was conducted in the southern part of the Kruger National Park, South Africa (24° 50'S; 31°35'E) in January 1999 during the early part of the summer rainy season. The Scarabaeinae of the southern African savannas are subjected to long, arid periods each year, and are therefore strongly seasonal (e.g. Doube 1991), reaching a peak in abundance and species richness during the wetter months of the year. Kruger is an elongated area of 19,500 square km (8000 square miles) of savanna, woodland and riverine forest and its protected status means that it has a relatively intact and undisturbed community of large herbivores, probably the richest in southern Africa. Kruger reportedly has 147 species of mammal (Braack 1996), although some 65% of these are small (hares, mice and bats for example). The vegetation type in the southern part is predominantly thornveld savanna, comprising a mix of drought-resistant plants dominated by knobthorn (*Acacia nigrescens*), marula (*Sclerocarya birrea*) and bush-willow (*Combretum* spp). African savannas are extremely floristically rich, having only slightly lower richness (c. 1750 species/ 10,000km^2) than the African rainforests (c. 2020 species/ 10,000km^2) (Menaut 1983).

The standard trapping protocol was employed as described in chapter 3; six transects of 10 baited pitfall traps set up and run for 4 days each for all bait types. Here in the Kruger though, in addition to baiting with cattle dung and carrion, elephant dung was also used to reflect the high diversity of dung types present in this habitat. The relative unattractiveness of non-ruminant dung observed in Belize can not be extrapolated to an African savanna. Cattle dung was found to have a very similar consistency to the buffalo dung found in the area (both animals being ruminant bovids), whilst the coarse elephant dung was used to represent the coarser non-ruminant dungs. It was also a plentiful resource and observed to support a particularly high species richness of scarabaeines. After two days, the dung bait was changed, and the carrion was moistened to prevent desiccation. An abundance of scavengers in the African savannas (such as hyaena, civet, jackal), is probably another significant reason why carrion-baited traps have been so infrequently used in this habitat type. To prevent the theft of the bait, each carrion-baited pitfall trap was covered by a section of wide-mesh metal grille, which was staked into the ground at each corner. These proved largely effective at

deterring would-be scavengers, although some bait was still lost when traps were dug up.

In order to take account of the somewhat heterogeneous vegetation type of the locality, trapping was conducted in two vegetation types of slightly differing structure. Three transects of each bait type and a flight intercept trap were deployed in two adjacent areas; the first classified as 'mixed savanna woodland' and the second 'acacia woodland' (National Parks Board, unpublished). In practice, the latter had a sparse tree cover (approximately 15-20 feet tall) interspersed with large acacia bushes above a scrubby ground cover, and was generally more closed than the first, which consisted of regularly spaced bushes and small trees with a tall grass-dominated herbaceous layer. In total, this gave a trapping effort of 240 trap days for each bait type. Some hand-collecting was also conducted in the area from the abundant dung resources.

Assemblages of a second habitat type were trapped in three sites in the Western Cape province, South Africa in August 2000, during the winter rainfall period. This region is subject to unusual climatic patterns whereby the region has cooler summers and mild, wet winters during which the adult dung beetle fauna reaches its peak richness (Davis 1987). Trapping was carried out in two National Parks and a nature reserve, in order to sample indigenous fynbos vegetation which had suffered minimum disturbance. The sites included the Cape Peninsula National Park (34° 20'S; 18° 25'E), Paarl Nature Reserve (33° 47'S; 18° 53'E), and the West Coast National Park (33° 10'S; 18° 05'E). At each site the standardised trapping protocol was applied in the same manner as at the Kruger National Park. The bait types of cattle dung and carrion were used, whilst elephant dung was substituted for horse dung (as the only regularly available large non-ruminant mammal dung). These areas have all suffered from a general loss of large mammals in the area, but both the West Coast and Cape Peninsula National Parks have re-introduced some species such as zebra, springbok (*Antidorcas marsupialis*) and bontebok (*Damaliscus dorcas*). Paarl has only small bovid species such as klipspringer (*Oreotragus oreotragus*). The fynbos vegetation in these areas is dominated by Mediterranean-type sclerophyllous shrubs such as *Protea* and *Erica,* and is marine-influenced in the more coastal areas of the two National Parks, where shrubs such as *Euclea* start to predominate. The largest of the three reserves, with the richest mammal fauna, is the West Coast National Park. Reclaimed from grazing farmland, this now covers some 380,000 hectares, and was recently designated as UNESCO biosphere

reserve. It has another habitat type not found in the other reserves, a form of dune thicket, situated on sandy coastal dunes, and a transect of each bait type was placed here. At this site, one additional bait type was used, millipede carrion (family Spirostreptidae) to target a particular genus, *Sceliages* of the tribe Scarabaeini, which specialize exclusively on this resource, even making brood balls from them (Bernon 1981).

Specimens collected from the Kruger National Park were sorted to morpho-species and identified using the collections of the Transvaal Museum and the University of Pretoria, South Africa, and the Natural History Museum, London. Material collected from the Cape sites was identified using the collections of the Natural History Museum, London. As for the Neotropical assemblages in chapter 3, mean body length was calculated for each species collected, and they were assigned to a functional nesting group according to Cambefort's generic-level scheme (1991a), and using behavioural information on the Oniticellini (Davis 1989, Cambefort 1982). The empirical data collected was also used to classify the species by feeding group (coprophage, necrophage or generalist), according to the same system as used in chapter 3. The recorded feeding behaviour of each species was plotted against its mean body length as a way of visualising the distribution of feeding behaviour throughout the assemblages in a manner that can also be easily compared with the equivalent data from the three Neotropical sites. This would also elucidate any correlation between body size and diet type.

4.2.2 Examining functional composition of African dung beetle assemblages

The functional composition of the assemblages at Kruger and the three Cape sites was compared with a number of other sites from a range of habitat types throughout Africa, using redundancy analysis (RDA) techniques as described in chapter 3. As before, other sites were drawn from the literature, and used only if a similarly extensive trapping regime had been observed, employing multiple bait types, which could be considered comparable to my own data. The localities and habitat types of the 10 sites are presented in table 4.1. Savanna, rainforest and mediterranean-type vegetation types were included, and the sites ranged in latitude from Morocco to South Africa. Replicate data sets were included for each habitat type, so that anomalies due to incomparable data might be identified. Sites were chosen which were as undisturbed as possible, and therefore as representative of that habitat type and region as was available. Functional

(nesting) groups were again used to categorise the resident species of each site in the RDA. This was necessary due to a minimal species overlap between the assemblages and a lack of more complex behavioural information for the species. It also makes the analysis more comparable with the Neotropical RDA. Feeding behaviour data were only available for some of the assemblages, and were omitted from this analysis.

Table 4.1. Localities and habitat types of the African regional-level multivariate analysis

Country	Site	Habitat type	Author
Morocco	Fes-Sais	Mediterranean-type	Janati-Idrissi et al. 1999
Niger	Niamey	Arid semi-desert	Rougon & Rougon 1991
Ivory Coast	Kakpin	Tropical savanna	Cambefort 1991b
Ivory Coast	Tai National Park	Rainforest	Cambefort & Walter 1991
Gabon	Makokou Research Station	Rainforest	Cambefort & Walter 1991
South Africa	Kruger National Park	Tropical savanna	Inward
South Africa	Mkuzi game reserve	Tropical savanna	Doube 1991
South Africa	West Coast Nat.Pk, Western Cape	Mediterranean-type	Inward & Wilsher
South Africa	Paarl Nature Reserve, Western Cape	Mediterranean-type	Inward & Wilsher
South Africa	Cape Peninsula Nat. Pk, Western Cape	Mediterranean-type	Inward & Wilsher

Environmental data were collected for each of the African sites. The variables measured were identical to those collected in chapter 3: longitude, latitude 1 (measured as the distance from the North Pole), latitude 2 (measured as the distance from the equator), net primary productivity, mean annual rainfall, mean annual temperature, seasonality of rainfall, seasonality of temperature, altitude, vegetation type, disturbance, mammal species richness, mammal body sizes, and mammal feeding behaviour. The environmental data collected for the ten African assemblages are presented in table 4.2, and functional composition of each assemblage is given in table 4.3.

An RDA was performed using CANOCO for Windows 4.0 (Ter Braak and Smilauer1998), as described in chapter 3, testing the marginal significance of each variable using a Monte Carlo permutation test. Ordination diagrams were plotted with CanoDraw 3.1 (Smilauer 1997), plotting only significant marginal variables.

Table 4.2. *Environmental data collected for ten African dung beetle assemblages. See main text for full details of each variable.*

Country	Site	Longitude (decimalized)	Latitude 1 (decimalized)	Latitude 2 (decimalized)	NPP	Mean annual Rainfall (mm/year)	Mean annual Temperature (°C)	Seasonality of Rainfall	Seasonality of Temperature	Altitude (m)	Vegetation Type			Disturbance
											Rainforest	*Tropical Savanna*	*Mediterranean type*	
Morocco	Fes-Sais	184.59	56.42	33.58	7	546	17.2	15.2	99.4	570			1	4
Niger	Niamey	182.05	76.68	13.32	3	556.2	29	33.7	32.4	200				6
Ivory Coast	Kakpin	176.53	81.61	8.39	21	1178	26.8	18.3	14.6	300		1		2
Ivory Coast	Tai National Park	173.45	84.74	5.26	21	1771	25.6	14.9	11.7	200	1			1
Gabon	Makokou Research Station	192.52	89.66	0.34	22	1736.2	23.7	17	13.5	500	1			1
South Africa	Kruger National Park	211.35	114.5	24.5	20	547	21.8	15.9	50	240		1		2
South Africa	Mkuzi game reserve	211.53	117.37	27.37	15	623	21.2	14	33	140		1		2
South Africa	West Coast Nat.Pk, Western Cape	198.05	123.1	33.1	6	270	16.5	17.5	45.5	5			1	3
South Africa	Paarl Nature Reserve, Western Cape	198.53	123.47	33.47	6	936	16.5	15.5	58.2	120			1	3
South Africa	Cape Peninsula Nat. Pk, Western Cape	198.25	124.2	34.2	6	613	16.8	14.7	51.8	5			1	2

Country	Site	Total mammal species	Mammal body size				Mammal feeding behaviour		
			Large (>200 kg)	*Medium-large (20-200 kg)*	*Medium-small (2-20 kg)*	*Small (<2kg)*	*Herbivores*	*Carnivores*	*Omnivores*
Morocco	Fes-Sais	61	0	3	6	52	6	40	15
Niger	Niamey	56	4	17	8	27	15	31	10
Ivory Coast	Kakpin	140	4	20	20	96	22	89	29
Ivory Coast	Tai National Park	149	3	16	22	108	36	78	35
Gabon	Makokou Research Station	129	2	15	21	91	35	64	30
South Africa	Kruger National Park	144	6	33	29	76	39	78	27
South Africa	Mkuzi game reserve	136	6	33	26	71	37	74	25
South Africa	West Coast Nat.Pk, Western Cape	146	5	35	24	82	42	77	27
South Africa	Paarl Nature Reserve, Western Cape	146	5	35	24	82	42	77	27
South Africa	Cape Peninsula Nat. Pk, Western Cape	146	5	35	24	82	42	77	27

Table 4.3. Assemblage composition data for the ten African sites.

Country	Site	Tunnellers	Rollers	Dwellers
Morocco	Fes-Sais	19	4	0
Niger	Niamey	19	0	1
Ivory Coast	Kakpin	109	21	2
Ivory Coast	Tai National Park	69	5	1
Gabon	Makokou Research Station	61	4	1
South Africa	Kruger National Park	71	16	3
South Africa	Mkuzi game reserve	89	29	2
South Africa	West Coast Nat.Pk, Western Cape	6	9	0
South Africa	Paarl Nature Reserve, Western Cape	1	4	0
South Africa	Cape Peninsula Nat. Pk, Western Cape	2	2	0

4.2.3 Examining functional composition of assemblages at a global level

In order to compare and contrast the structure of dung beetle assemblages from around the world, a data set of 28 globally-distributed sites was compiled. Data sets existing in the literature, comparable to my own, were sought from all biogeographic regions where dung beetle assemblages occur, although particular emphasis was placed on the three regions which were identified in chapter 2 as key areas in dung beetle evolutionary history; namely Africa, South America and Australia. An attempt was also made to include assemblages from as wide a range of vegetation types as possible from the different areas, to reflect the diversity of habitats occupied by dung beetles across the world. Therefore all sites previously compiled for the ordination analyses within Africa (above), and North, Central and South America (chapter 3) were included, since each encompassed a range of native habitat types and geographic regions. In addition, assemblage data from Australia, Europe, India and South East Asia were also incorporated. Many more data sets from past studies exist, but the majority were not comparable, due to inferior trapping efforts, a single (often poorly chosen) bait type, or because the site in question had suffered so much disturbance as to be non-representative of the region. All those chosen were deemed to be as comparable as is currently available, but where available, replicate data sets from each region were incorporated to help identification of anomalous results through the inclusion of poor or biased data. The localities and habitat types of all 28 assemblages is presented in table 4.4, and the world-wide coverage is illustrated by figure 4.1, a map of the locations of all sites.

Table 4.4. Localities and habitat types of the global-level multivariate analysis. Sites marked * were included in an additional analysis regarding the feeding behaviour of the resident species.

Country	Site	Habitat type	Author
USA	Skeleton Canyon, S.E. Arizona	Arid semi-desert	Dajoz 1994
USA	Welder refuge, South Texas	Temperate chaparral	Nealis 1977
Mexico	Los Tuxtlas, Veracruz	Rainforest	Morón 1979
Belize	Pine Ridge, Cayo *	Open, dry forest	Inward
Belize	Las Cuevas, Cayo *	Rainforest	Inward
Panama	Barro Colorado Island *	Rainforest	Gill 1991
Colombia	Leticia, Amazonas *	Rainforest	Howden & Nealis 1975
Ecuador	Tiputini, Napo *	Rainforest	Inward
French Guiana	Nouragues	Rainforest	Feer 2000
Uruguay	Cerro Colorado (Estacion Experimental)	Temperate chaparral	Morelli et al. 1997
France	Cazevielle, Herault	Mediterranean-type	Kirk & Wallace 1990
India	Singur, West Bengal	Open, dry forest	Oppenheimer 1977
Thailand	Sakaerat, Nakhon Ratchasima Prov.	Open, dry forest	Hanboonsong 2000
Sabah, Malaysia	Danum Valley Research Station	Rainforest	Davis 1993
Sulawesi	Dumoga-Bone Nat.Pk *	Rainforest	Hanski & Krikken 1991
Morocco	Fes-Sais	Mediterranean-type	Janati-Idrissi et al. 1999
Niger	Niamey	Arid semi-desert	Rougon & Rougon 1991
Ivory Coast	Kakpin	Tropical savanna	Cambefort 1991b
Ivory Coast	Tai National Park *	Rainforest	Cambefort & Walter 1991
Gabon	Makokou Research Station *	Rainforest	Cambefort & Walter 1991
South Africa	Kruger National Park *	Tropical savanna	Inward
South Africa	Mkuzi game reserve	Tropical savanna	Doube 1991
South Africa	West Coast Nat.Pk, Western Cape *	Mediterranean-type	Inward & Wilsher
South Africa	Paarl Nature Reserve, Western Cape *	Mediterranean-type	Inward & Wilsher
South Africa	Cape Peninsula Nat. Pk, Western Cape *	Mediterranean-type	Inward & Wilsher
Australia	Kirrama forest, Queensland *	Rainforest	Hill 1996
Australia	Hartleys Creek, Queensland	Open, dry forest	Monteith unpublished
Australia	Southwestern Australia *	Mediterranean-type	Ridsdill Smith et al. 1983

Figure 4.1. The global distribution of the dung beetle assemblages compared with multivariate ordination. Sites: (1) Skeleton Canyon, Arizona, USA (2) Welder refuge, South Texas, USA (3) Los Tuxtlas, Veracruz, Mexico (4) Pine Ridge, Cayo, Belize (5) Las Cuevas, Cayo, Belize (6) Barro Colorado Island, Panama (7) Leticia, Amazonas, Colombia (8) Tiputini, Napo, Ecuador (9) Nouragues, French Guiana (10) Cerro Colorado (Estacion Experimental), Uruguay (11) Cazevielle, Herault, France (12) Singur, West Bengal, India (13) Sakaerat, Nakhon Ratchasima Province, Thailand (14) Danum Valley Research Station, Sabah, Malaysia (15) Dumoga-Bone National Park, Sulawesi (16) Fes-Sais, Morocco (17) Niamey, Niger (18) Kakpin, Ivory Coast (19) Tai National Park, Ivory Coast (20) Makokou Research Station, Gabon (21) Kruger National Park, South Africa (22) Mkuzi game reserve, South Africa (23) West Coast National Park, Western Cape, South Africa (24) Paarl Nature Reserve, Western Cape, South Africa (25) Cape Peninsula National Park, Western Cape, South Africa (26) Kirrama forest, Queensland, Australia (27) Hartleys Creek, Queensland, Australia (28) Southwestern Australia, Australia.

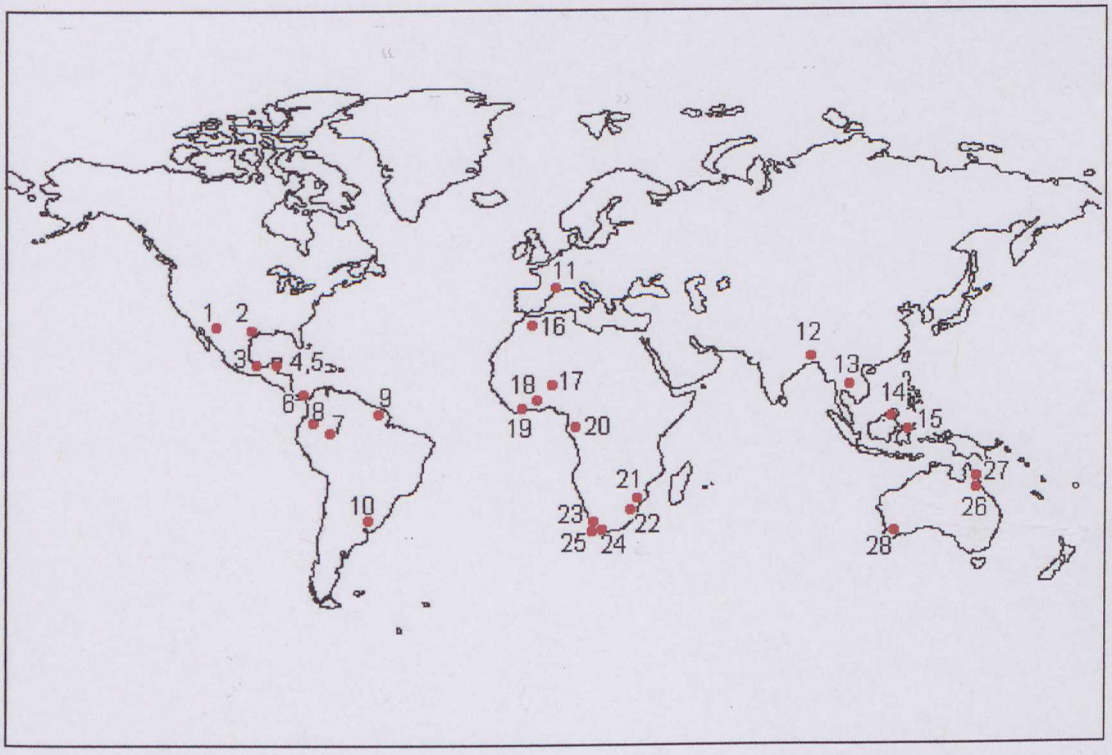


Table 4.5. *Environmental data collected for a range of dung beetle assemblages from across the globe. See main text for full details of each variable.*

Country	Site	Biogeographic region						Longitude (decimalized)	Latitude 1 (decimalized)	Latitude 2 (decimalized)	NPP	Mean annual Rainfall (mm/year)	Mean annual Temperature (°C)	Seasonality of Rainfall	Seasonality of Temperature	Altitude (m)
		Nearctic	*Neotropical*	*Eurasian*	*African*	*Oriental*	*Australian*									
USA	Skeleton Canyon, S.E. Arizona	1						70.77	58.29	31.71	5	350	17	24.8	114.7	1300
USA	Welder refuge, South Texas	1						82.7	61.92	28.08	20	966	21.5	11.2	79.1	15
Mexico	Los Tuxtlas, Veracruz		1					84.94	71.65	18.35	19	2475	23.1	17.4	21.6	325
Belize	Pine Ridge, Cayo		1					91.44	72.97	17.03	20	1560.5	24.6	12.3	18.7	450
Belize	Las Cuevas, Cayo		1					91.41	73.56	16.44	20	1560.5	24.6	12.3	18.7	500
Panama	Barro Colorado Island		1					100.49	80.91	9.09	24	2425	27.9	16.6	5.7	100
Colombia	Leticia, Amazonas		1					110.43	94.09	4.09	27	2812.3	25.6	6.1	3.1	85
Ecuador	Tiputini, Napo		1					103.91	90.38	0.38	27	2546.8	25.3	6.9	9.5	225
French Guiana	Nouragues		1					127.6	85.95	4.05	25	2880	26	10.6	5	300
Uruguay	Cerro Colorado (Estacion Experimental)		1					124.68	123.52	33.52	17	1249.6	16.5	3.7	73.3	210
France	Cazevielle, Herault			1				183.47	46.54	43.46	11	980	13.6	11.1	128.7	250
India	Singur, West Bengal					1		268.15	67.54	22.46	25	1600.8	26.6	20.4	40.9	15
Thailand	Sakaerat, Nakhon Ratchasima Prov.					1		281.55	75.7	14.3	23	1260	26	20.7	25.7	500
Sabah, Malaysia	Danum Valley Research Station					1		297.48	84.99	5.01	30	2745	26.7	11	5.6	185
Sulawesi	Dumoga-Bone Nat.Pk					1		303.54	89.66	0.34	30	2030	26	6.6	4.2	300
Morocco	Fes-Sais				1			184.59	56.42	33.58	7	546	17.2	15.2	99.4	570
Niger	Niamey				1			182.05	76.68	13.32	3	556.2	29	33.7	32.4	200
Ivory Coast	Kakpin				1			176.53	81.61	8.39	21	1178	26.8	18.3	14.6	300
Ivory Coast	Tai National Park				1			173.45	84.74	5.26	21	1771	25.6	14.9	11.7	200
Gabon	Makokou Research Station				1			192.52	89.66	0.34	22	1736.2	23.7	17	13.5	500
South Africa	Kruger National Park				1			211.35	114.5	24.5	20	547	21.8	15.9	50	240
South Africa	Mkuzi game reserve				1			211.53	117.37	27.37	15	623	21.2	14	33	140
South Africa	West Coast Nat.Pk, Western Cape				1			198.05	123.1	33.1	6	270	16.5	17.5	45.5	5
South Africa	Paarl Nature Reserve, Western Cape				1			198.53	123.47	33.47	6	936	16.5	15.5	58.2	120
South Africa	Cape Peninsula Nat. Pk, Western Cape				1			198.25	124.2	34.2	6	613	16.8	14.7	51.8	5
Australia	Kirrama forest, Queensland						1	325.44	108.4	18.4	22	1148	24.1	23.9	34.9	620
Australia	Hartleys Creek, Queensland						1	325.34	106.4	16.4	22	2151	24.6	19.5	26.8	5
Australia	Southwestern Australia						1	298.31	123.39	33.39	11	486	15.6	13.7	81.4	300

Country	Site	Vegetation Type						Disturbance	Total mammal species	Mammal body size				Mammal feeding behaviour		
		Rainforest	*Open, dry forest*	*Tropical Savanna*	*Mediterranean type*	*Temperate Chaparral*	*Arid semi-desert*			*Large (>200 kg)*	*Medium-large (20-200 kg)*	*Medium-small (2-20 kg)*	*Small (<2kg)*	*Herbivores*	*Carnivores*	*Omnivores*
USA	Skeleton Canyon, S.E. Arizona						1	4	72	0	9	9	54	15	38	19
USA	Welder refuge, South Texas					1		3	62	0	8	14	40	10	34	18
Mexico	Los Tuxtlas, Veracruz	1						2	176	1	11	24	140	16	123	37
Belize	Pine Ridge, Cayo		1					4	145	1	10	17	117	12	104	29
Belize	Las Cuevas, Cayo	1						3	145	1	10	18	116	12	104	29
Panama	Barro Colorado Island	1						2	187	1	12	28	146	17	131	39
Colombia	Leticia, Amazonas	1						2	246	2	15	45	184	39	145	62
Ecuador	Tiputini, Napo	1						1	213	1	14	38	160	30	123	60
French Guiana	Nouragues	1						1	127	0	7	35	85	19	68	40
Uruguay	Cerro Colorado (Estacion Experimental)					1		4	31	0	2	9	20	3	16	12
France	Cazevielle, Herault				1			4	48	0	6	8	34	6	29	13
India	Singur, West Bengal		1					5	91	1	11	16	63	14	61	16
Thailand	Sakaerat, Nakhon Ratchasima Prov.		1					3	162	2	14	32	114	37	86	39
Sabah, Malaysia	Danum Valley Research Station	1						1	190	2	12	35	141	52	97	41
Sulawesi	Dumoga-Bone Nat.Pk	1						1	99	0	6	6	87	30	33	36
Morocco	Fes-Sais				1			4	61	0	3	6	52	6	40	15
Niger	Niamey						1	6	56	4	17	8	27	15	31	10
Ivory Coast	Kakpin			1				2	140	4	20	20	96	22	89	29
Ivory Coast	Tai National Park	1						1	149	3	16	22	108	36	78	35
Gabon	Makokou Research Station	1						1	129	2	15	21	91	35	64	30
South Africa	Kruger National Park			1				2	144	6	33	29	76	39	78	27
South Africa	Mkuzi game reserve			1				2	136	6	33	26	71	37	74	25
South Africa	West Coast Nat.Pk, Western Cape				1			3	146	5	35	24	82	42	77	27
South Africa	Paarl Nature Reserve, Western Cape				1			3	146	5	35	24	82	42	77	27
South Africa	Cape Peninsula Nat. Pk, Western Cape				1			2	146	5	35	24	82	42	77	27
Australia	Kirrama forest, Queensland	1						2	93	0	1	17	75	14	44	30
Australia	Hartleys Creek, Queensland		1					2	93	0	1	17	75	14	44	30
Australia	Southwestern Australia				1			2	42	0	0	10	32	8	18	16

The same environmental data were collected for each of the additional sites, so that all 28 assemblages were coded for the same variables as given above and in full detail in chapter 3. The only additional data included here was the biogeographic region to which the sites belonged. All environmental data collected are presented in table 4.5. It was apparent that only a functional group level would be appropriate to compare such a great diversity of assemblages, as there was no species overlap between sites on different continents, and very little generic overlap. Use of a higher-level classification was considered, using the phylogenetic information obtained in chapter 2 since the current tribal-level classification is evidently inaccurate for some groups. This was however discarded due to the lack of unequivocal phylogenetic evidence for many groups. All species could however be coded according to functional nesting strategies. The functional composition of each of the assemblages from across the world are displayed in table 4.6.

Table 4.6. Functional composition data for the globally distributed dung beetle assemblages

Country	Site	Tunnellers	Rollers	Dwellers
USA	Skeleton Canyon, S.E. Arizona	9	4	0
USA	Welder refuge, South Texas	8	8	0
Mexico	Los Tuxtlas, Veracruz	16	8	3
Belize	Pine Ridge, Cayo	7	2	2
Belize	Las Cuevas, Cayo	21	9	6
Panama	Barro Colorado Island	43	13	3
Colombia	Leticia, Amazonas	33	10	6
Ecuador	Tiputini, Napo	39	20	9
French Guiana	Nouragues	54	19	8
Uruguay	Cerro Colorado (Estacion Experimental)	7	2	0
France	Cazevielle, Herault	9	3	0
India	Singur, West Bengal	32	2	1
Thailand	Sakaerat, Nakhon Ratchasima Prov.	52	5	0
Sabah, Malaysia	Danum Valley Research Station	49	12	0
Sulawesi	Dumoga-Bone Nat.Pk	35	4	0
Morocco	Fes-Sais	19	4	0
Niger	Niamey	19	0	1
Ivory Coast	Kakpin	109	21	2
Ivory Coast	Tai National Park	69	5	1
Gabon	Makokou Research Station	61	4	1
South Africa	Kruger National Park	71	16	3
South Africa	Mkuzi game reserve	89	29	2
South Africa	West Coast Nat.Pk, Western Cape	6	9	0
South Africa	Paarl Nature Reserve, Western Cape	1	4	0
South Africa	Cape Peninsula Nat. Pk, Western Cape	2	2	0
Australia	Kirrama forest, Queensland	10	11	0
Australia	Hartleys Creek, Queensland	12	4	0
Australia	Southwestern Australia	5	4	0

Data from the literature was only used when it was judged to be of comparable quality to the trapping effort of the standardised protocol (described in chapter 3). Several of the studies also included empirical data for the different bait types employed. Where available, this was used to calculate the feeding behaviour of each species present in that assemblage, in the same way as it had been for the various assemblages collected for this project. This produced a sub-set of 14 assemblages representing all major continents, with the species coded as coprophages, necrophages or generalists. These sites have been marked with an asterisk in table 4.4. An equivalent sub-set of environmental data was also created from table 4.5.

The two global-level data sets (representing functional composition and feeding behaviour) and the associated environmental data were then analysed by a redundancy analysis, using CANOCO, in the same manner as the African data above, and the American data in chapter 3 (examination of marginal significance with a Monte Carlo permutation test, 999 replicates). The species richness of the different feeding groups represented in each assemblage was also plotted on a bar chart, to help in the interpretation of the ordination diagram for diet choice.

4.3 Results

4.3.1 Trapping results

An inventory of the Scarabaeinae collected in the Kruger National Park by the standardised protocol is presented in table 4.7. This also gives the empirical data from each trapping method, as well as the mean body length and feeding strategy of each species. Some small differences in species composition were noted between the two sites sampled in the Kruger (mixed savanna woodland and acacia woodland), but these tended to reflect the presence or absence of rarer species.

Table 4.8 displays the species inventories and associated data for the three assemblages sampled in the Western Cape province. The protocol collected a total of 90 species at Kruger, compared to a maximum of 15 in one of the Cape sites, the West Coast National Park. Only five species were collected at Paarl, and four at the Cape Peninsula. There is no species overlap between the Kruger and the Cape assemblages, although a number of genera are shared between the regions. Table 4.9 summarises the composition of the assemblages in terms of taxonomic groups, nesting strategies and feeding behaviour. Two findings are particularly striking regarding the Cape assemblages, the relatively high proportions of rollers compared to Kruger (which has a particularly high tunneller richness), and the almost total lack of non-coprophagous feeding. The only necrophagy seen in the three sites was in *Sceliages brittoni,* a specialist on millipede carrion. The distribution of feeding behaviour with body size is displayed for the Kruger in figure 4.2, and for the West Coast National Park (as the most diverse and undisturbed of the three Cape assemblages) in figure 4.3. Whilst there is only the single occurrence of strict necrophagy and no evident generalism in the Cape site, 23% of the species at Kruger were found to be either necrophagous or generalist in their diet (21 species in total). This proved somewhat higher than expected, since the generally accepted perception of the dung beetle communities of the African savannas is that they are almost entirely coprophagous. Non-coprophagy is quite taxonomically widespread in the Kruger assemblage, occurring in six of the nine tribes present, although only seven of the 33 total genera. Two-thirds of the generalist/necrophage feeding behaviour are concentrated in the genus *Onthophagus*.

Table 4.7. Dung beetle species inventory from Kruger National Park, South Africa, with trapping results and feeding strategy. Feeding strategy codes: N=Necrophagous; C=Coprophagous; G=Generalist. Tribal abbreviations follow those given in Table **1.X.**

Tribe	Species	Ruminant dung pitfalls (cattle)	Non-ruminant dung pitfalls (elephant)	Carrion pitfalls	Flight Intercept Traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding strategy
CA	Anachalcos convexus	47	6	493	1	547	24.2	8.7	N
	Odontoloma sculpturatum	1			1	2	2.5	100	C
CO	Catharsius philus	6	4		1	11	18.1	100	C
	Copris amyntor	76	116			192	16.7	100	C
	Copris elphenor	14	7			21	24.5	100	C
	Copris mesacanthus	22	8			30	15.5	100	C
	Copris pandion			9		9	26	0	N
	Metacatharsius opacus	221	44	214		479	15	50.1	G
	Metacatharsius troglodytes	80	25	2		107	12.7	97.5	C
DI	Coptorhina nitidipennis	1		1	12	14	10.9	50	G
	Heliocopris marshalli					0	34.6	100	C
	Pedaria puncticollis	28	19			47	7.4	100	C
	Sarophorus costatus	26	20	1		47	8.9	96.3	C
GY	Allogymnopleurus thalassinus	333	44			377	13.8	100	C
	Garreta nitens	783	312	2	6	1103	18.1	99.7	C
	Gymnopleurus thelwalli	13	1	7		21	10.1	65	G
	Gymnopleurus virens	30		20		50	9.6	60	G
OC	Drepanocerus kirbyi	10	18			28	4.5	100	C
	Drepanocerus laticollis	1	2			3	5.3	100	C
	Euoniticellus intermedius	16	5			21	9.6	100	C
	Liatongus militaris	5	8			13	9.5	100	C
	Oniticellus egregius		1			1	13	100	C
	Oniticellus formosus	2	1			3	10.1	100	C
	Oniticellus planatus	6	3			9	11.2	100	C
	Tiniocellus sp.1	41	11			52	7.8	100	C
	Tiniocellus spinipes	57	41			98	6.9	100	C
OP	Caccobius histerinus		2		1	3	4.1	100	C
	Caccobius nigritulus	8				8	4.4	100	C
	Caccobius sp.3				1	1	4.2	Unavailable	
	Cleptocaccobius postlutatus	19	8			27	3.3	100	C
	Cleptocaccobius viridicollis		2			2	3.2	100	C
	Digitonthophagus gazella	33	14			47	11	100	C
	Euonthophagus carbonarius	37	19	1	1	58	9.4	97.4	C
	Hyalonthophagus alcyon	28	2			30	9.6	100	C
	Milichus apicalis	1	12			13	7.5	100	C
	Onthophagus aeruginosus	54	23	1	30	108	6.6	98.2	C
	Onthophagus bicavifrons			26	18	44	8.3	0	N
	Onthophagus croesulus				1	1	7.6	Unavailable	
	Onthophagus declivicollis	89	21	7	1	118	7	92.7	C
	Onthophagus depressus	16	1	26	1	44	9.5	38	G
	Onthophagus deterrens			5		5	6.4	0	N
	Onthophagus ebenicolor	17		65	4	86	7.1	20.7	G
	Onthophagus ebenus			12	1	13	9	0	N
	Onthophagus flavolimbatus	50	11	3		64	4	94.3	C
	Onthophagus ginelli		10			10	8	100	C
	Onthophagus lamelliger	36	55	13	3	107	5.1	80.1	G
	Onthophagus nanus			1		1	4.2	0	N
	Onthophagus obtusicornis	81	18	118	2	219	7.7	40.7	G
OP	Onthophagus omostigma	70	14	14	1	99	4.8	83.3	G
	Onthophagus pallidipennis	23	3			26	7.4	100	C

Tribe	Species	Ruminant dung pitfalls (cattle)	Non-ruminant dung pitfalls (elephant)	Carrion pitfalls	Flight Intercept Traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding strategy
	Onthophagus pauxillus	5	2			7	3.1	100	C
	Onthophagus rugulipennis	3	1			4	4.2	100	C
	Onthophagus semiflavus	211	69		5	285	4.2	100	C
	Onthophagus sp.11				1	1	8.1	Unavailable	
	Onthophagus sp.12				1	1	4.1	Unavailable	
	Onthophagus sp.13				1	1	4.4	Unavailable	
	Onthophagus sp.16 (sugillatus)	8		2		10	4.2	80	G
	Onthophagus sp.18	1			1	2	3.7	100	C
	Onthophagus sp.19	1		1		2	4.1	50	G
	Onthophagus sp.21			2		2	5.7	0	N
	Onthophagus sp.3	4			1	5	5.5	100	C
	Onthophagus sp.7	2				2	7	100	C
	Onthophagus sp.8	38	5			43	6.1	100	C
	Onthophagus sp.9	2	1	1		4	4.2	66.7	G
	Onthophagus teitanicus	29	19	2	23	73	7.2	93.5	C
	Onthophagus vinctus	26	26	6		58	6.2	81.3	G
	Phalops ardea	316	61			377	12	100	C
	Phalops boschas	37	8			45	10	100	C
	Phalops dregei	1				1	10.2	100	C
	Phalops flavocinctus	5	3			8	10.7	100	C
	Proagoderus bicallosus	3	3			6	14	100	C
	Proagoderus loricatus	20	6			26	14	100	C
	Proagoderus tersidorsis	69	16		2	87	12.9	100	C
OT	Cheironitis hoplosternus					0	15.2	100	C
	Heteronitis castelnaui					0	35.3	100	C
	Onitis alexis	13	5			18	15.4	100	C
	Onitis fulgidus	7	90	2		99	17.4	97.8	C
	Onitis mendax	1				1	19	100	C
	Onitis uncinatus	2	1			3	18.7	100	C
	Onitis viridulus	12	4		1	17	20.3	100	C
SC	Kheper nigroaeneus	56	16	1	2	75	30.5	98.2	C
	Kheper subaeneus	8	3			11	34	100	C
	Scarabaeus galenus	11		1		12	29.2	91.7	C
	Scarabaeus interstitialis			5		5	14.2	0	N
SI	Neosisyphus calcaratus	383	41	2		426	7.9	99.4	C
	Neosisyphus fortuitus	96	105		1	202	12.3	100	C
	Neosisyphus infuscatus	16	2			18	9	100	C
	Neosisyphus spinipes	33	12			45	10.1	100	C
	Sisyphus crispatus	206	72	9	6	293	7.6	95.8	C
	Sisyphus seminulum	15				15	4.4	100	C

Table 4.8. Dung beetle species inventory from three sites in the Western Cape, South Africa, with trapping results and feeding strategy. Feeding strategy codes: N=Necrophagous; C=Coprophagous; G=Generalist. Tribal abbreviations follow those given in Table **1.X.** * = Trapped with millipede carrion only.

Tribe	Species	Ruminant dung pitfalls (cattle)	Non-ruminant dung pitfalls (horse)	Carrion pitfalls	Flight Intercept Traps	Total	Mean body length (mm)	Percentage of coprophagy in diet	Feeding strategy
	West Coast National Park								
CA	Epirinus aeneus	15				15	6.9	100	C
	Epirinus sp. 2	1				1	8	100	C
	Epirinus sp. 3	1				1	6	100	C
	Odontoloma pusillum	41	10		4	55	2.8	100	C
CO	Copris anceus	24				24	19.7	100	C
DI	Sarophorus tuberculatus	1				1	8.8	100	C
OP	Onthophagus cameloides	1				1	9.5	100	C
	Onthophagus minutus	125	7		1	133	6.6	100	C
OT	Onitis confusus	1				1	18.9	100	C
SC	Kheper bonellii	46				46	27.6	100	C
	Pachysoma hippocrates	7	1			8	25.8	100	C
	Scarabaeus intricatus	5	1			6	12.6	100	C
	Scarabaeus rugosus	221			2	223	23.6	100	C
	Scarabaeus sp. 1	1				1	16.6	100	C
	Sceliages brittoni	0		6 *		0	21	0	N
	Paarl Nature Reserve								
CA	Epirinus comosus	29	17			46	5.6	100	C
	Epirinus sp.1	73	43	4		120	4.3	100	C
	Odontoloma dentinum	9	7			16	3.1	100	C
	Odontoloma sp.1	118	58	1		177	2.2	100	C
DI	Macroderes sp. 1	4	1			5	11.9	100	C
	Cape Peninsula National Park								
CA	Epirinus comosus	10	1			11	6.4	100	C
	Odontoloma pusillum	3				3	2.8	100	C
CO	Copris anceus	13				13	20.6	100	C
OP	Onthophagus giraffa	3				3	10.2	100	C

Table 4.9. A summary of dung beetle assemblage composition of the four South African sites sampled. Numbers of species present in each nesting group is presented with the percentage of the total that each represents. Feeding groups have been assigned according to the empirical trapping data, although not all species can be assigned to a strategy.

	Kruger National Park	West Coast National Park	Paarl nature reserve	Cape Peninsula National Park
Species	90	15	5	4
Genera	33	10	3	4
Tribes	9	6	2	3
Nesting groups				
Rollers	16 (17.8%)	10 (67%)	4 (80%)	2 (50%)
Tunnellers	71 (78.8%)	5 (33%)	1 (20%)	2 (50%)
Dwellers	3 (3.4%)	0	0	0
Feeding groups				
Coprophages	64	14	5	4
Necrophages	8	1	0	0
Generalists	13	0	0	0

Figure 4.2. Feeding preferences and body size distribution of the Kruger National Park dung beetle assemblage

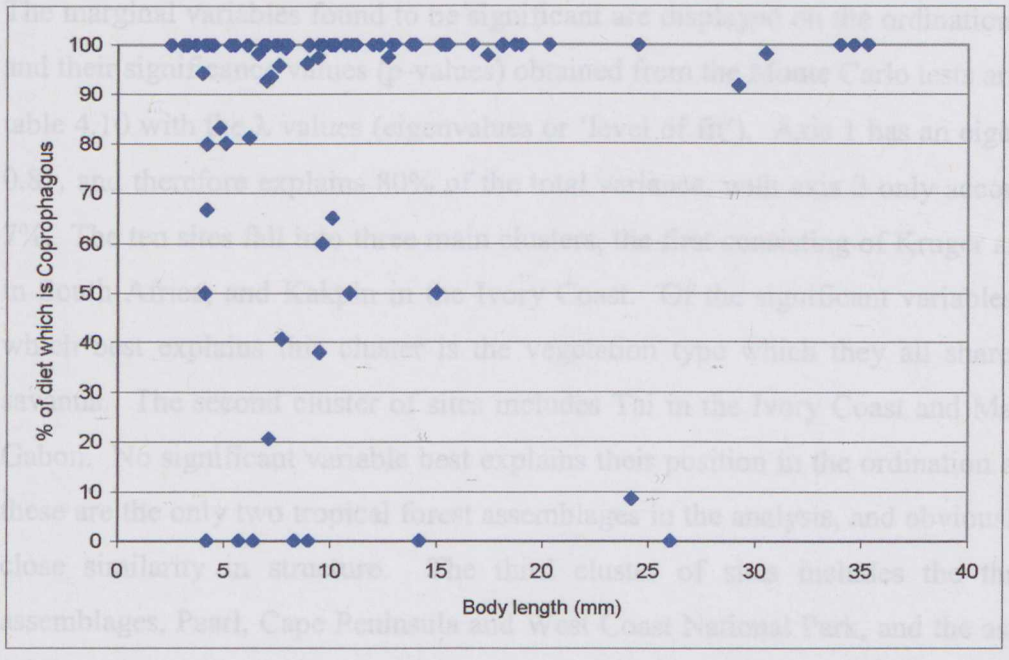
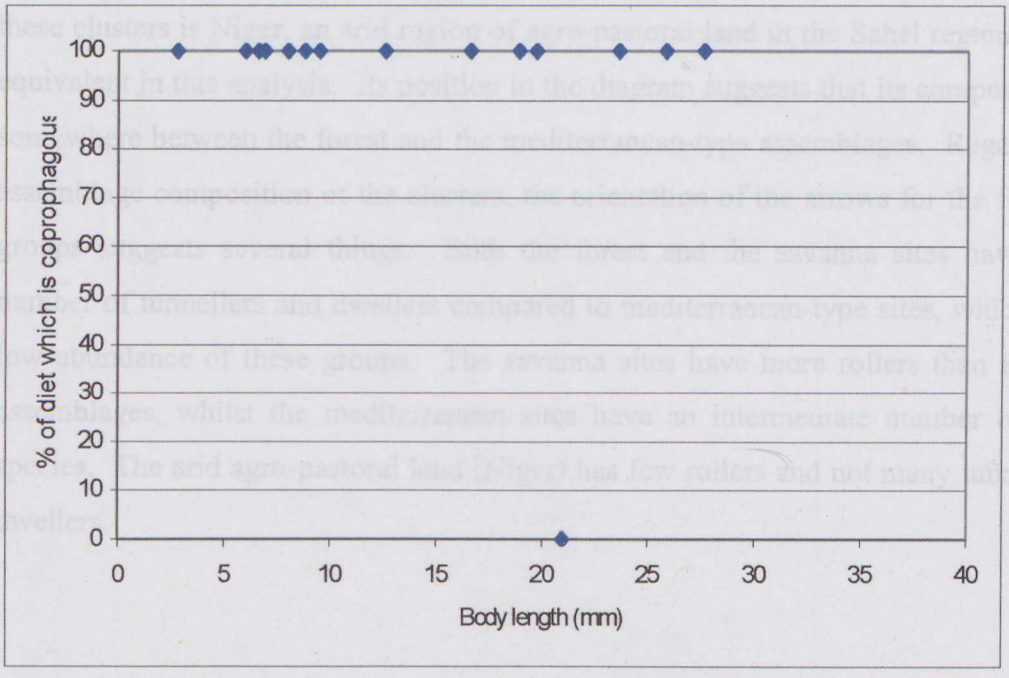


Figure 4.3. Feeding preferences and body size distribution of the West Coast National Park dung beetle assemblage



The results of the ordination of the African assemblages are presented in figure 4.4. The marginal variables found to be significant are displayed on the ordination diagram, and their significance values (p-values) obtained from the Monte Carlo tests are given in table 4.10 with the λ values (eigenvalues or 'level of fit'). Axis 1 has an eigenvalue of 0.81, and therefore explains 80% of the total variance, with axis 2 only accounting for 7%. The ten sites fall into three main clusters, the first consisting of Kruger and Mkuzi in South Africa, and Kakpin in the Ivory Coast. Of the significant variables, the one which best explains this cluster is the vegetation type which they all share, tropical savanna. The second cluster of sites includes Tai in the Ivory Coast and Makokou in Gabon. No significant variable best explains their position in the ordination space, but these are the only two tropical forest assemblages in the analysis, and obviously share a close similarity in structure. The third cluster of sites includes the three Cape assemblages, Paarl, Cape Peninsula and West Coast National Park, and the assemblage at Fes-Sais in Morocco. These assemblages also all occupy the same habitat type dominated by mediterranean sclerophyllous vegetation. This cluster also has a significant negative correlation with Net Primary Productivity (NPP), which means that these sites share in common a low NPP. The only site which does not fit into one of these clusters is Niger, an arid region of agro-pastoral land in the Sahel region, with no equivalent in this analysis. Its position in the diagram suggests that its composition lies somewhere between the forest and the mediterranean-type assemblages. Regarding the assemblage composition of the clusters, the orientation of the arrows for the functional groups suggests several things. Both the forest and the savanna sites have a high number of tunnellers and dwellers compared to mediterranean-type sites, which have a low abundance of these groups. The savanna sites have more rollers than the forest assemblages, whilst the mediterranean sites have an intermediate number of rolling species. The arid agro-pastoral land (Niger) has few rollers and not many tunnellers or dwellers.

Figure 4.4. Ordination diagram for the comparison of functional diversity across ten African dung beetle assemblages. Marginal significant variables are presented. Habitat type: *Green = Rainforest, Red = Tropical savanna, Blue = Mediterranean-type, Black = Arid semi-desert*

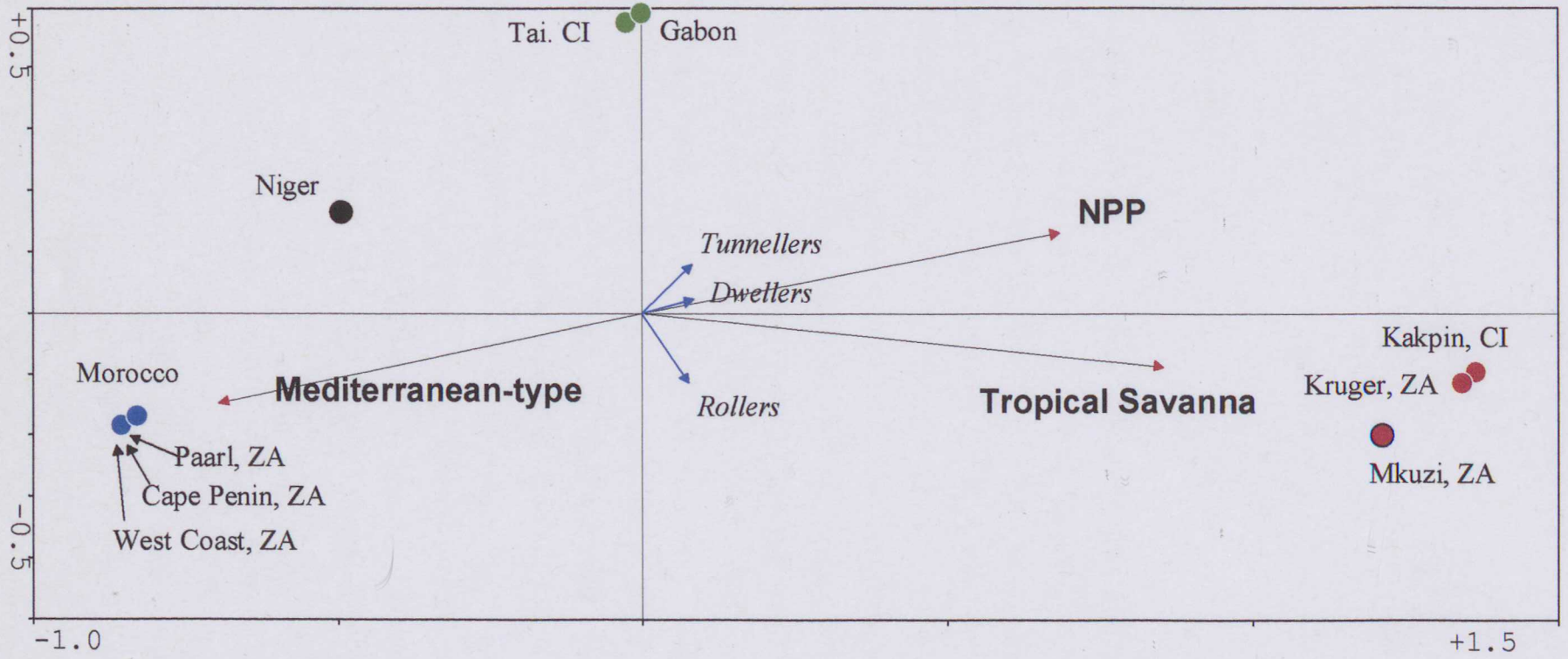


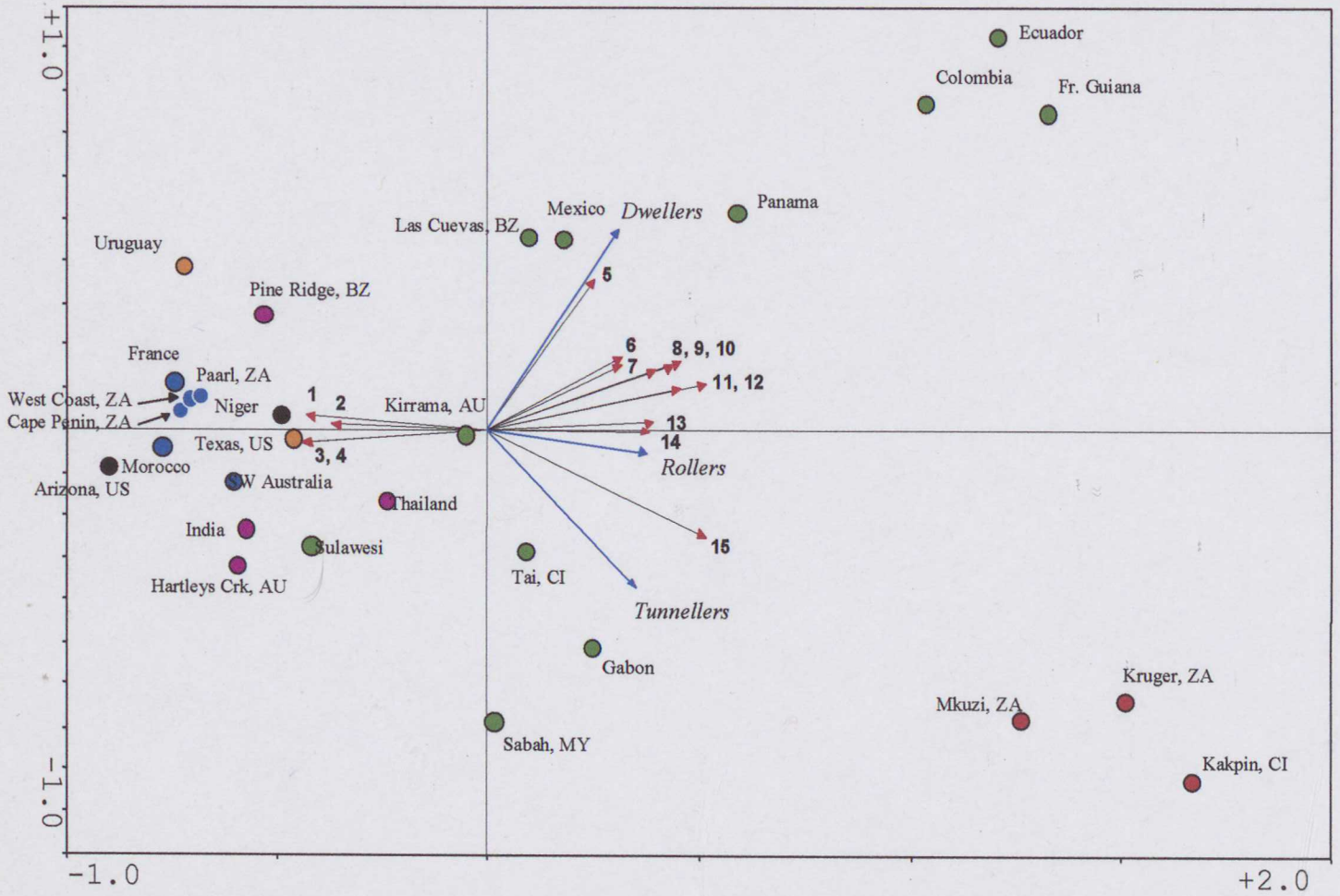
Table 4.10. Environmental variables found to be significantly correlated with the variation in assemblage structure of the ten African sites. p-values were obtained for each variable by examining its marginal significance with a Monte Carlo test (999 permutations). $\lambda 1$ values denote the eigenvalue (fit) of each variable when treated on its own.

Environmental variable	$\lambda 1$	Monte Carlo test p-value
Tropical savanna	0.74	0.01
Mediterranean type	0.5	0.01
NPP	0.48	0.017

4.3.3 The global-level analysis

The ordination diagram of the global-level assemblage comparison is presented in figure 4.5, and the associated marginal variables found to be significant are given in table 4.11, with their p-values and eigenvalues. Axis 1 of this diagram explains 57% of the variance, whilst axis 2 explains 17%, so that 74% of the total variance in the global data is accounted for in the ordination space presented. This is a more complex analysis than for the African assemblages alone, and accordingly, many more variables were found to be significantly correlated with the data. The majority of them are closely correlated with axis 1. Two clusters of sites stand out from the rest, each having a strong positive correlation with axis 1 and the associated significant variables, but they are also strongly separated along axis 2. The first cluster comprises Ecuador, Colombia and French Guiana, the group of Amazonian rainforest sites, whilst the other consists of the three African savanna sites, Kakpin, Kruger and Mkuzi. These two clusters each represent the most species-rich sites in the Neotropics and the Afrotropics respectively, as indicated by the positive correlation between axis 1 and the direction of increase of species richness of the functional groups. However, whilst the Neotropical forest assemblages have a greater abundance of dwellers, the African savannas have more tunnellers, as well as slightly more rollers. Axis 1 therefore tends to reflects the overall species diversity of the assemblages, with the least rich sites having the greatest negative values along the axis. Axis 2 on the other hand seems to separate the assemblages by biogeographic region. Consequently, the Neotropical sites tend to all have positive values, whilst the Old World sites (African and Asian) have a greater tendency towards negative values along this axis. The South American rainforest sites are seen to be strongly correlated with several habitat-specific variables, such as the biogeographic region (Neotropics), habitat type (rainforest) and a high mean annual

Figure 4.5. Ordination diagram for the comparison of functional diversity across a world-wide range of dung beetle assemblages. Marginal significant variables are presented. Variables: *(1) Disturbance, (2) Mediterranean-type vegetation, (3) Seasonality of temperature, (4) Latitude 2 (distance from equator), (5) Neotropical, (6) Mean annual rainfall, (7) Tropical rainforest, (8) Small mammals, (9) Carnivorous mammals (10) Omnivorous mammals, (11) Total mammals, (12) Medium-small mammals, (13) NPP, (14) Mean annual temperature, (15) Tropical savanna.* Habitat type: *Green = Rainforest, Red = Tropical savanna, Pink = Open, dry forest, Orange = Temperate chaparral, Black = Arid semi-desert, Blue = Mediterranean-type*



rainfall and the African savanna sites have a similarly strong correlation with habitat type, but more interesting and meaningful are the shared positive correlations with a number of the same significant variables. These comprise NPP and mean annual temperature, as well as a number of mammal categories, including small, medium-small and a greater total abundance of mammals, as well as more carnivorous and omnivorous mammals. These mammal categories are clearly autocorrelated, for instance many of the small and medium-small mammals will be carnivores or omnivores. Similarly, the correlation between greater dung beetle richness and mammal richness may also be expected along a latitudinal or disturbance gradient, but remains intriguing nonetheless.

Table 4.11. Environmental variables found to be significantly correlated with the variation in assemblage structure of 28 globally distributed sites as characterized by functional groups. p-values were obtained for each variable by examining its marginal significance with a Monte Carlo test (999 permutations). $\lambda 1$ values denote the eigenvalue (fit) of each variable when treated on its own.

Environmental variable	$\lambda 1$	Monte Carlo test p-value
Tropical savanna	0.33	0.002
Medium-small mammals	0.28	0.001
Omnivorous mammals	0.23	0.004
Total mammal species	0.22	0.003
Carnivorous mammals	0.22	0.003
Latitude 2	0.21	0.001
Seasonality of temperature	0.2	0.006
Neotropical	0.19	0.003
Disturbance	0.18	0.006
Small mammals	0.18	0.014
NPP	0.16	0.008
Mean annual temperature	0.16	0.019
Mediterranean type	0.14	0.021
Mean annual rainfall	0.14	0.024
Rainforest	0.13	0.027

Occupying intermediate positions along axis 1, are two more groups of sites. Again representing biogeographic regions, the first consists of the three Central American rainforest sites in the analysis: Las Cuevas, Belize; Los Tuxtlas, Mexico; and Barro Colorado Island, Panama. Paralleling these below axis 2, is a grouping of three Old World rainforest assemblages, Tai, Ivory Coast; Makokou, Gabon and Sabah, Malaysia. Their intermediate positions suggest a structure less strongly correlated with the variables described for the first two clusters, and as having a generally lower dung beetle species richness than the former sites. All of them still have positive values on

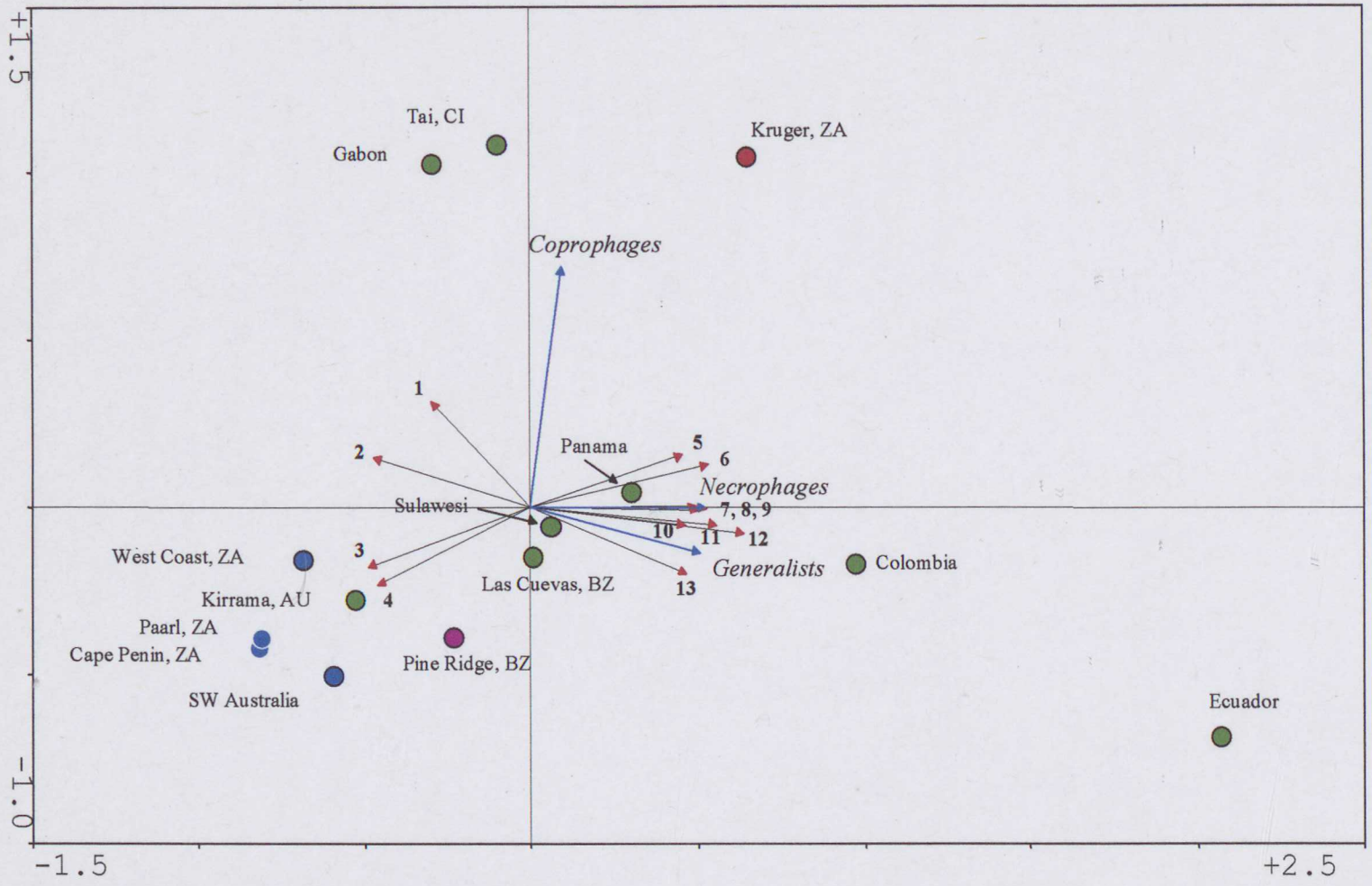
axis 1 however, although several of them clearly have a stronger association with axis 2. Unfortunately, the lack of variables correlated with axis 2 makes any relationship hard to interpret.

The remaining sites all have negative axis 1 values, and are therefore negatively correlated (to varying degrees) with the variables described above, and positively correlated with variables 1-4, which describe greater levels of disturbance and seasonality, as well as higher latitude. The sites could be described as forming a single loose cluster, since they all tend to share a general tendency towards a relatively lower species richness than the preceding clusters. However, this would oversimplify the structure present. Five sites can be identified as forming a core group, consisting of the Mediterranean-type sites (the three Western Cape assemblages, Southern France, Morocco, and south west Australia). This group then has the strongest positive correlation with variables 1-4, and the strongest negative correlation with the other variables (NPP, mammal abundance). Close to this group are the two arid semi-desert sites of Arizona, USA and Niger, as well as the chaparral assemblage of Texas. Occupying an intermediate position between the mediterranean-type sites and the Central American rainforest sites is the open, dry forest of Pine Ridge, Belize. In a remarkable parallel, all of the other open, dry forests occupy the same intermediate position below axis 1, between the mediterranean-type sites and the Old World forest sites, including Sakaerat forest reserve in Thailand, Hartleys Creek in Australia and Singur in India. The rainforest site of Sulawesi is also found in this group. Finally, the chaparral of Uruguay is found parallel to axis 1 with the mediterranean-type sites, but with a higher axis 2 value, as do the other Neotropical assemblages. The Kirrama forest of Australia is so close to the origin that it cannot be easily correlated with any of the variables, but seems to be intermediate between several clusters.

4.3.4 Global-level feeding behaviour

The ordination results exploring the feeding behaviour of assemblages across the world are displayed in figure 4.6. The significant marginal variables are again illustrated in the ordination space, and their p-values and eigenvalues are given in table 4.12. Quite striking is the finding from the ordination diagram that the direction of increase of coprophagous species is uncorrelated with the direction of increase of necrophages and generalists. It is also evident that the greatest abundance of coprophages are found in

Figure 4.6. Ordination diagram for the comparison of feeding groups across a world-wide range of dung beetle assemblages. Marginal significant variables are presented. Variables: *(1) African, (2) Seasonality of rainfall, (3) Latitude 2 (distance from equator), (4) Mediterranean-type vegetation, (5) Mean annual temperature, (6) NPP, (7) Total mammals, (8) Medium-small mammals, (9) Mean annual rainfall, (10) Carnivorous mammals, (11) Small mammals, (12) Omnivorous mammals, (13) Neotropical.* Habitat type: *Green = Rainforest, Red = Tropical savanna, Pink = Open, dry forest, Blue = Mediterranean-type*



the African savanna and forest assemblages, whilst the greatest number of necrophagous and generalist Scarabaeinae are seen to be in the Amazonian forests, with Ecuador having the greatest richness of these groups. The pattern identified in chapter 3 is seen here also; levels of coprophagy decline slightly from the Central American forests to the South American forests, but are accompanied by a considerable increase in non-coprophagous groups. The mediterranean type sites from the Cape and south west Australia are the most depauperate in all groups, although we also find the Kirrama forest, Australia and Pine Ridge, Belize being similarly species-poor.

Table 4.12. Environmental variables found to be significantly correlated with the variation in the feeding behaviour of 14 globally distributed sites. p-values were obtained for each variable by examining its marginal significance with a Monte Carlo test (999 permutations). $\lambda 1$ values denote the eigenvalue (fit) of each variable when treated on its own.

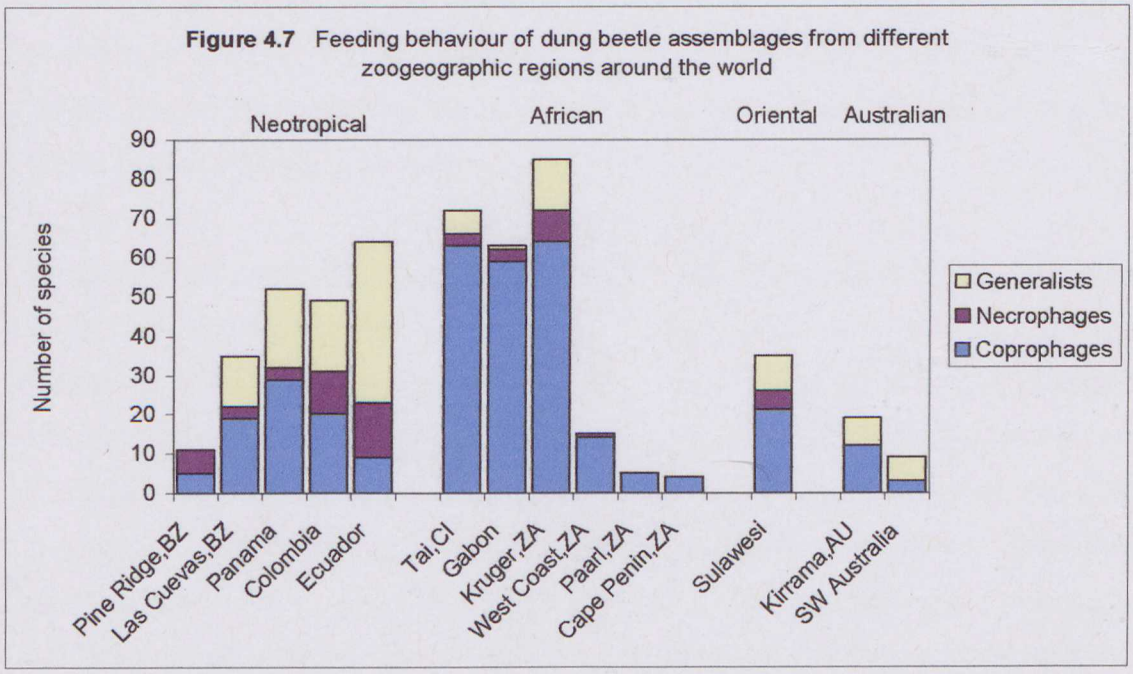
Environmental variable	$\lambda 1$	Monte Carlo test p-value
Omnivorous mammals	0.42	0.004
Small mammals	0.31	0.009
NPP	0.3	0.007
Mean annual rainfall	0.28	0.013
Latitude 2	0.27	0.011
Mediterranean type	0.27	0.018
Neotropical	0.26	0.01
Total mammal species	0.26	0.016
Seasonality of rainfall	0.25	0.018
Medium-small mammals	0.24	0.031
Mean annual temperature	0.23	0.028
Carnivorous mammals	0.21	0.044
African	0.19	0.048

The number of significant environmental variables suggests once more a complexity to the patterns seen, and the presence of autocorrelated or confounding variables. Nevertheless, there is a strong positive correlation between the abundance of non-coprophagous groups (necrophages and generalists) and an increasing number of small, omnivorous and carnivorous mammals. Whilst not found to be significant (and therefore not shown on the ordination diagram), it is still interesting that there is also the suggestion of a correlation between the abundance of coprophages and the abundance of herbivorous mammals, indicated by very similar directions of increase in ordination space. Axis 1 is also closely correlated with total species richness, so that the most species-rich assemblages have the highest positive values.

The distribution of the three main diet types (coprophagy, necrophagy and generalism) is presented for each of the 14 world-wide assemblages in a bar chart, figure 4.7. The African assemblages all have a high proportion of coprophagous species, with necrophagy and generalism only appearing as the species richness increases, and only reaching moderately large abundances when species numbers are high. Conversely, the Neotropical assemblages all have a substantial proportion of non-coprophagous feeding even at lower species richness. As the assemblages increase in size, both coprophagous and non-coprophagous feeding increases initially, but the largest assemblages have a considerably greater proportion of non-coprophagous diet types. There is an apparent latitudinal effect here also (discussed in chapter 3).

The assemblage in Sulawesi is alone in South East Asia, and therefore difficult to interpret. It supports however a moderately large proportion of non-coprophagy, and from figure 4.6, is similar in structure to Las Cuevas, Belize. The Australian sites also support quite a high abundance of generalism, although no strict necrophagy is observed. This is seen in both the rainforest and the mediterranean type vegetation types.

Figure 4.7 Feeding behaviour of dung beetle assemblages from different zoogeographic regions around the world



4.4.1 The Kruger assemblage

The high species richness of dung beetles in Southern Africa has long been thought to be linked to the high diversity of large, herbivorous mammals (Halffter and Matthews 1966), although suggestions of speciation driven by geographic isolation during periods of climatic fluctuations have also been made (Doube 1991). The assemblage trapped at Kruger clearly illustrates this rich Scarabaeinae diversity, with 33 different genera represented and 90 species. The importance of using the multiple bait types of the standardised trapping protocol to fully sample an assemblage was clearly proven here - the perception that the African savanna assemblages consist entirely of coprophagous beetles seems to have been dispelled, in this instance at least. With almost a quarter of the species collected at Kruger actually being classified as necrophagous or generalist in feeding behaviour, the niche space provided by mammal dung alone may not be as broad as expected. An interesting illustration of this was that each of the three most abundant species collected in the protocol, *Garreta nitens*, *Anachalcos convexus* and *Metacatharsius opacus,* fall into different feeding categories; coprophagy, necrophagy and generalism respectively. In chapter 3, niche partitioning was suggested to occur in a variety of ways in the Neotropical assemblages, including separation of species through nesting behaviour, feeding behaviour, body size and morphology. Might there also be similar patterns in the African taxa?

A variety of different soils are found under different climax savannas (Montgomery and Askew 1983), and it seems likely that soil type may be the cause of the subtle differences in vegetation structure seen between the adjacent sites sampled within the Kruger park (mixed savanna woodland and acacia woodland). These sites yielded very similar species compositions to one another, and any differences found were due to the presence or absence of rarer species. This is as likely to be due to the locally heterogeneous spatial distribution of individuals in a patchy environment (McLaughlin and Roughgarden 1993) as to any preference for soil type. Just as spatial variation was found between two sites, so it was between transects with apparently identical vegetation structure (chapter 3). If we examined each trap or dung pat individually, we would probably note even greater variation between them (pers. obs.), because the smaller the scale studied, the more patchy the environment becomes, due to

microhabitat differences and patch dynamics. Because of the spatial and temporal heterogeneity of dung and carrion distribution, comparisons of dung beetle assemblages need to be carried out at a landscape level, to take account of the variety of microhabitats and niches occupied, rather than being misled by localised fluctuations.

4.4.2 The assemblages of the Western Cape

Body size distribution is quite similar between Kruger and the West Coast (figures 4.2 and 4.3), but a comparison of the feeding groups and functional structure of Kruger with the Cape assemblages does not suggest the same similarities as were seen between the Neotropical assemblages. Instead, whilst generalism and strict necrophagy is quite abundant at Kruger (figure 4.2), the single example of *Sceliages brittoni* (the specialist on millipede carrion) from the Cape sites seems to be an exception to the rule. Furthermore, the predominance of tunnellers at Kruger is strongly contrasted by the predominance of rollers in the Cape sites.

Doube (1991) suggests that South African dung beetle communities are primarily structured by soil type, and to a lesser extent by vegetation type (and dung types). His examination of the distribution of different functional groups on alternative soil types found that large rollers often dominate on sandy soils (measured in biomass), although quite why is unclear. This seems to be the case in the West Coast National Park, where its coastal vicinity causes the soil to be sandy and allows dunes to persist. Here there is a high abundance of the tribe Scarabaeini (6 of the 15 species), including a species of the derived, flightless genus *Pachysoma*, which is restricted to arid coastal areas of south-western Africa (Scholtz 1989). Another four small rolling species were also collected at West Coast, taking the total number of rollers to 10 out of 15 species. I suggest that this unusual functional structure relates to the morphological trade-off identified in rollers in chapter 3, where an ability to roll balls was suggested to affect their ability to burrow. Whilst rolling presumably confers a competitive advantage in one way, the inability to burrow deeply or quickly would reduce this advantage in another. However, the coarse structure of sandy soils means that digging in them is much easier than in loam or clay type soils, which become hard-packed. This would negate much of the need for the specialised digging morphology identified in many of the tunnelling species (see chapter 3; discussed further in the African fauna in chapter 5). Thus the rollers would not only be able to minimise competition at the primary

resource by effective removal of dung, but would not be hindered by a poor ability to bury it, drastically reducing the period when their dung ball can be stolen, desiccated or invaded by kleptoparasites, as well as reducing their own exposure to predators. This may pass the competitive advantage firmly to the rollers, and explain their unusually high proportion of rolling species in the West Coast National Park. There may of course be alternative historical explanations for this pattern, since the Cape clearly has a unique history.

Of the other two Cape sites, both Paarl and the Cape Peninsula had very low dung beetle species richness, but their structure is still subtly different. Paarl has a predominance of small rollers, whilst it is more balanced in the Cape Peninsula, with two small rollers, one large tunneller and one small tunneller. Despite its status as a nature reserve, Paarl supports no large mammal species, and only a few small to medium sized mammals such as klipspringer. The dung resources present therefore appear to be small pelleted omnivore or carnivore dung only. The Cape Peninsula has a greater abundance of large mammals than Paarl, but these are relatively recent re-introductions, having been lost from the area long ago with the establishment of Cape Town as a permanent settlement. There does therefore seem to be a correlation between the richness of the mammal community and the dung beetle assemblages in the Cape sites. Two genera in particular, *Epirinus* and *Odontoloma*, comprise a considerable proportion of the species collected in the three Cape sites, and these taxa are largely restricted to the Cape region. This suggests they either have a competitive advantage over other groups in this region (and are better adapted to the habitat type than other groups), or that they have gone extinct elsewhere. From the molecular phylogenetic data (chapter 2), *Odontoloma* was found to be one of the basal groups to the entire Scarabaeinae, whilst *Epirinus* was basal to the 'African clade'. It seems likely therefore that *Odontoloma*, and perhaps *Epirinus*, were present before the radiation of the large mammals in Africa, perhaps even before the break-up of Gondwana. As older taxa, they may have originated in a less competitive environment than that of the African assemblages of today, where high species richness is likely to select against poorer competitors (although see the discussion regarding their possible persistence from an earlier period of megaherbivores; chapter 2).

Given the higher than expected levels of non-coprophagy at Kruger, it is perhaps surprising that the levels are so low in the three Cape sites, where all of the

Scarabaeinae trapped are coprophagous with the exception of *Sceliages*. Whether this is a function of low species richness, or an inherent trait to the Cape assemblages will be investigated below.

4.4.3 African assemblage structure

The regional level redundancy analysis of the ten African assemblages clearly partitioned the sites into tropical savanna, rainforest and Mediterranean-type habitats. Whilst different habitat types will clearly be affected and structured by environmental factors such as seasonality, rainfall and latitude, I have attempted to account for many of these, and they have not been found significantly correlated with the variance explained in the ordination. Presumably these variables have been measured at too crude a scale to predict habitat type. Instead, this data suggests that vegetation type is the most important factor in the functional structuring of African Scarabaeinae assemblages. One of the strongest pieces of evidence in support of this is the clustering of distant geographic localities, which share few, if any species, but occupy the same habitat type. Such similarities are seen with the mediterranean-type assemblages of Morocco and the South African Cape sites, and with the savannas of the Ivory Coast and South Africa.

'Habitat' can be viewed as a composite set of environmental variables, all of which will influence the assemblage structure in some way. Whilst the plants themselves are likely to have no direct effects on the beetles, vegetation structure will indirectly affect environmental factors such as soil moisture, humidity and microclimate, which will all impact in various ways on the Scarabaeinae. Of course vegetation is itself influenced by a range of factors; climate is the major determinant (e.g. Pianka 1988), but topography and soils will also play a significant role, as will the action of grazing animals. In turn, the soil type will be influenced by the vegetation above, the bedrock below and the climate; and the mammal fauna (and thus the availability of dung types) will also be affected by the vegetation and climate. Thus there is a close inter-relationship between edaphic, climatic and biotic factors, which are usually inextricably linked and self-sustaining. Since any or all of these factors may influence the dung beetle fauna of a locality, it is difficult to ascribe any single environmental factor to the pattern observed in figure 4.4, and correlations between patterns and particular habitat types are as near to an answer as we can hope for with this data. As discussed above, there seem to be situations where the functional structure of dung beetle communities is

directly influenced by soil type, but unfortunately I do not have the necessary data to test this further. Shallow soils are also known to contain a reduced scarab fauna (Halffter 1991), so a comparison of relative soil depths might prove informative. Although none of the variables describing mammal richness were found to be significant, the data source (Corbet and Hill 1986) provides the presence or absence of individual species at only a regional level, and is therefore likely to be misleading at local levels, since it cannot account for local spatial variation or particular habitat types. For instance, the mammal fauna of Paarl is low, and using regional-level data for this site is inaccurate, yet more precise data does not exist. I hypothesize therefore that given more accurate data, one would find a correlation between dung beetle diversity and mammal richness in Africa, allied with the differences in habitat type. Leaving aside mammal species richness, the habitat types support very different levels of mammal *biomass*. For instance, savanna vegetation in East African game reserves is estimated to have an ungulate biomass of 15 - 25 metric tons per km^2, and even the drier southern African thornveld savannas such as that sampled in the Kruger, are thought to still support some 2 - 5 tons per km^2 (Cooke 1972). Contrast this with the estimated total mammal biomass of only 0.1 tons per km^2 in tropical West African forest, of which most is comprised of rodents, primates and insectivores (Cooke 1972). The arid vegetation types of the Cape region probably supports an even lower biomass than this. Consequently, the biomass of dung produced will also vary between habitats, and an effect on the dung beetle richness seems likely.

4.4.4 Energetics considered

Whilst mammal biomass may explain the particularly high richness of the savannas, why should the forests have a lower relative abundance of rollers than the other habitat types? This may relate to the energetics of the different functional behaviours. It has been suggested that rolling is a more energy-costly strategy per unit time than slow tunnelling, with rollers requiring a higher temperature to roll efficiently, and that dwelling is the least energy consuming strategy (Krell et al. in press). Although a variety of dung beetle species thermoregulate, both in Africa (e.g. Ybarrondo and Heinrich 1996) and the Neotropics (Young 1984), they are essentially poikilothermic organisms, and in a shaded environment such as a forest where temperatures will be lower than in an open savanna, rollers may lose the competitive advantage of their nesting strategy. Temperature has been correlated with the distribution of scarabaeine

species richness across France (Lobo et al. 2002), and it may be that that the energy-expensive strategies of rollers and tunnellers may be the primary reason for their poor distribution in the more temperate regions of the world. In the UK for example, there are no rollers and only 8 small scarabaeine tunnellers. In contrast, there are 59 Aphodiinae species (Jessop 1986), all of which are dwellers and are therefore more energy-efficient, and so presumably require less external thermal energy, and can survive better the seasonal swings in temperature.

However, the energetic constraints of rolling do not seem to apply to the Neotropical fauna, where rollers are quite abundant. In Tiputini, Ecuador, 30 % of the species are rollers, compared to only 6-7% in the African forests. Different phylogenetic histories are likely to be responsible for this – if the African fauna is indeed reliant upon the sun's thermal energy, it is probably because it originated in a savanna habitat. If the Neotropical fauna is forest-adapted, the rollers have presumably compensated for the lower insolation levels in another way. Perhaps a higher energy diet, obtained through generalist or necrophagous feeding, compensates for the energetic constraints of rolling.

4.4.5 Global-level comparisons

Despite such differences in phylogenetic and biogeographic history, the regional-level ordination analyses of the Neotropical and African assemblages, have each suggested the strongest correlation to be between habitat type and functional diversity (figures 3.22 and 4.4). In the global-level analysis (figure 4.5), the richest assemblages in the New World and the Old World were seen to be of different habitat types, savanna in Africa and Amazonian rainforest in the Neotropics. The functional structure was clearly different too, but the total species richness was in fact very similar. From these two clusters of sites, the overall pattern is one of convergence, with functional structure becoming more similar through the intermediate habitat types (Central American rainforest above axis 1 and Old World forest below), and then depauperate open, dry forests in the two regions. The greatest convergence is seen in the open non-forested habitats, including sites from North America, North Africa, South Africa, Australia and Europe, all strongly correlated with greater seasonality, higher latitude and increased disturbance. A similar trend towards the convergence of feeding behaviour can also be seen in the smaller global data set, where the richest Neotropical and African sites are well separated, but the more species-poor assemblages are more similar to each other.

Examination of functional diversity (nesting groups) and feeding behaviour offers two different measures of dung beetle assemblage structure. Convergence is more distinctly seen in the functional diversity of the assemblages than feeding behaviour, but this may be related to the poorer sampling of the latter. Better replication of habitat types in different regions has been achieved in the functional diversity analysis, which reinforces the pattern by addressing potential problems of measurement accuracy of the non-standardized data points.

4.4.6 Regional convergence of assemblage structure

Given similar resources in independent systems, the expectation is that a community with analogous functional structure might evolve in each case (Schluter and Ricklefs 1993), therefore dung beetle communities in similar habitats might be expected to be similar in functional structure, regardless of history. However, the tropical rainforest communities of the Old World, New World and Australian rainforests are all quite different (figure 4.5). Instead, the convergence is seen between the relatively 'harsher' habitat types (at higher latitudes, more open or more disturbed), which poses the question: why? By its nature, the concept of convergence implies a historical change from an ancestral condition to the present condition. When considering convergence in species, a phylogenetic framework is required to judge the ancestral conditions (Wiley 1981). I have already implied convergence in the function and morphology of dung beetles using a phylogeny, for instance in the multiple originations of rolling. Entire communities however, are likely to have much more complex histories, since they consist of many species, from many different lineages. Schluter (1986) suggested that convergence in communities can be detected by finding differences in assemblage structure between habitat types which are consistent between regions. For example, rainforests all over the world are remarkably similar in structure and form, compared with drier forests within the same continents. Such a parallel would indicate convergence of these communities. Such an argument can be applied to the findings of the global-level analyses of functional diversity and feeding behaviour, and supports the suggestion of a convergence of dung beetle assemblages between regions.

4.4.7 Regional and local components of functional diversity

Habitat type (or vegetation type) appears to be the most important variable in determining the functional (nesting) diversity of Scarabaeinae assemblages on a local scale, both in Africa and the Neotropics. In Belize for example, the forests of Pine Ridge and Las Cuevas are geographically adjacent, and are therefore similarly affected by many large-scale environmental variables, yet it is the vegetational differences (and presumably the numerous associated, but unidentified small-scale factors) which apparently cause the assemblage structure to be so different. As such, habitat can be regarded as the key local factor influencing functional diversity. Similar effects of habitat upon the local species richness of bracken herbivores have been detected in Britain and New Mexico (Lawton *et al.* 1993).

However, when comparing regions, vegetation type does not predict where the highest species richness will be found. For instance, whilst Neotropical forests support the greatest richness and diversity of Scarabaeinae, in Africa the rainforests are clearly less rich than the savannas. Karr (1976) found a similar pattern in the bird assemblages of Africa and South America, where the forest fauna of the Neotropics was richer than the African forest fauna, but that the African savanna habitats were considerably more species rich than the Neotropical savannas. Thus habitat type is clearly important within a region, but it is the different histories of each region which are responsible for which habitat type provides the richest and most 'favourable' range of resources for the scarabaeine fauna. Because these resources evidently include a range of variables such as the mammal fauna (figure 4.5), in order to understand the differences between habitats within each continent, and globally, it is necessary to consider the histories of the mammals as well as the vegetation of each assemblage, in particular the more recent trends which are better understood and which have more directly influenced the dung beetle communities of the present day.

Beginning with the Neotropical region, the persistent forest cover of the Amazon region since the Cretaceous (Morley 2000), has probably meant that the majority of Neotropical taxa have evolved in a rainforest habitat (with the odd notable exception). The mammal fossil record of the Amazon is unfortunately poor compared to that of the Patagonian subregion, although it is known that the continent had a great richness of ungulates and other large herbivores during much of the Tertiary period. In the early

Pleistocene, the fauna was as rich in major categories as that of Africa today – the Pampas region alone supported some 8 orders, 22 genera and 34 species of large herbivorous mammal (Keast 1972b). The relatively recent large-scale extinctions at the end of the Pleistocene has left the Pampas region with only four species of large herbivore, two cervids (deer) and two camelids (guanaco and vicuna), although a variety of large rodents also occupy this herbivore niche (Keast 1972b). It seems highly likely that the early Pleistocene dung beetle fauna of the region would have been richer than that of today, and perhaps was even more diverse than in the tropical forests. The mammal fauna of the rainforests may not ever have been particularly abundant, in the same way that the African forests of today support considerably lower levels than the savannas. However, the Amazon forests have provided a more stable habitat throughout this period, certainly in terms of vegetational history. Pollen profiles from the Amazon suggest that a high plant diversity was maintained in the refugial areas during the glacial-cooling events of the Pleistocene, as these remained stable and moist, and extinctions were moderately low (Morley 2000). Perhaps the forest mammals also survived this period better than those of the Patagonian regions. Today, the Brazilian sub-region has a much richer mammal fauna than the Patagonian sub-region, with 195 genera compared to 76, whilst Central America has 138 genera (Hershkowitz 1972). This is reflected in figure 4.5 by the correlation of total mammal richness with the distribution of the Neotropical sites, from the depauperate site of Uruguay to the much richer sites of the Amazon, and it is quite possible that the habitats which suffered less mammal extinction may have more 'intact', and richer dung beetle faunas today.

This stability of the South American forest flora contrasts with the considerable extinction levels suffered by the flora of the African forests in the Miocene, when the savannas were expanding, and continuing into the Pliocene, when significant desiccation associated with the glacial maxima occurred (Morley 2000). This may also explain the current low levels of mammals in the African forests, and perhaps the forest-dwelling dung beetles suffered associated losses. Mammal and scarabaeine groups better adapted to a drier environment might have persisted, such as the savanna-adapted taxa (of which the present-day forest assemblages seem to be a sub-set), whilst various older, more basal groups may have been lost, leaving taxa such as *Odontoloma* and *Epirinus* and *Circellium* isolated in the Cape region where the climate is milder. The Pleistocene extinctions of mammals were more widespread – as well as the catastrophic decline in South America, Australia also lost most of its large grazing and browsing

marsupial herbivores (Owen-Smith 1987). The mammal fauna of Africa suffered some extinction also, but nothing like that of the Neotropics, which is why its current ungulate richness is unparalleled. Just as in South America, there is a similar correlation between the mammal diversity of the different habitat types in Africa and the associated dung beetle richness, and therefore whilst the richest scarabaeine faunas are forest-dwelling in South America, they are savanna-dwelling in Africa, where the greatest radiation of large herbivores occurred. The functional structure of the Neotropical forest assemblages is unique, whilst the Old Word forests (African and Oriental) are much more similar to one another. This reflects the sharing of taxa between them at a tribal and generic level, and illustrates how phylogenetic history influences the functional diversity of assemblages. It is noteworthy that the African mammals also have a strong phylogenetic affinity with those in the Oriental region (Bigalke 1972).

A local species assemblage can be viewed as an assortment of species drawn from the regional pool, via a series of ecological 'filters' (Lawton 2000). These work on various temporal and spatial scales from the regional to the local level (Angermeier and Winson 1998). Thus everything from the large scale effects of biogeography, such as how isolated the community is, to effects of the landscape, such as how patchy or available the habitat is, can act as a filtering mechanism, determining the composition of a local community or assemblage. Only at the lowest level do inter-species interactions impact upon the species which passed through the other filters (Lawton 2000). As Lawton points out, the effects of regional or landscape level processes will be much stronger upon a local pool, than the reverse effects of local interactions will be upon the regional pool.

Of the numerous filters which will have determined each and every scarabaeine local species assemblage, two or three have emerged from this study as playing the key roles in each region. The filter operating on the largest scale can be said to be the biogeographic history of the continents, since this has most strongly influenced the phylogenetic composition of each region (chapter 2). The next set of filters are of regional landscape history, including climatic history, the stability of the vegetation types and the history of the mammal fauna (although this will also have a biogeographic component to it). At this point, there is something of a dichotomy, where a regionally more favourable habitat type can be distinguished from an abiotically harsher habitat.

Within the species-rich habitat type (be it African savanna or Neotropical forest), we can assume that the environmental filters have been sufficiently benign for many dung beetles to pass through. Particular tropical environments are known to be major sources of origination and not simply refuges of diversity (e.g. Jablonski 1993). For the Scarabaeinae, the Neotropical forests and African savannas appear to be just such 'cradles of evolution'. At this point then, habitat suitability is not a limiting factor, but as a more 'benevolent' environment for many different species, the local species richness will be increasingly constrained by species interactions. Of the various potential forms of interaction, the most influential upon dung beetle assemblages is probably inter-specific competition for resources. In the Neotropical assemblages of chapter 3, I identified various ways in which niche-separation was achieved, but the competitive abilities of the species will ultimately govern which of them pass through this final filter, and the ultimate composition of the local assemblage.

In the case of the environmentally harsher habitats of each region, the environmental filters are particularly rigorous, so that relatively few species can pass through them. These filters might include arid conditions, low rainfall, strong seasonality, disturbance (such as deforestation) and a lack of suitable mammal dung. Most species fail to progress through these obstacles and are excluded, since the harsher the habitat, the greater the number or severity of the filters, compared with those of the more benevolent habitats (which appears to be where most scarabaeine taxa originate). Those species which can withstand the harsher conditions of a less favourable habitat (for example through adaptation or simply being tolerant) are usually relatively few in number, and their abundance may still be limited by the conditions. The final composition of the assemblage may be depauperate enough that competition for resources is lower, and inter-species interactions play only a minor filtering role.

Of course, the benevolence of the habitat will really be a continuous gradient. Using such ideas to explain the patterns of figure 4.5 (global-level ordination), I suggest that due to their distinct regional histories, the African savannas and the South American rainforests represent the most-favourable habitats, where the filters can be easily passed through by the species of the regional pool. This means that the assemblage structure of the favourable habitats is influenced relatively more by local species interactions than the less favourable habitats. Where habitat is harsher, such local interactions are less apparent.

The functional diversity of the assemblages in the two regions is different because of the phylogenetic independence of the taxa in each of the regional pools. Despite the convergent evolution of unrelated taxa towards functionally similar strategies (such as rolling, or necrophagy), the phylogenetic composition and the interactions occurring between the taxa in the favourable habitats of Africa and the Neotropics are sufficiently different to produce the divergent functional structures seen in figure 4.5. Conversely, where the environmental filters of a harsh habitat affect the assemblage composition to a greater degree than local-species interactions, similarly harsh conditions lead to similarly depauperate functional structures, regardless of different ancestral histories of the regional species pools. This environmental harshness may explain the convergence seen in functional diversity in the mediterranean type and other open, arid habitats from across the world. Two levels of intermediate habitats are seen, along the gradient of benevolent environmental conditions. Thus the Old World rainforests (African and Oriental) and the Central American rainforests each have a poorer functional diversity than the regional ideals, but are still strongly affected by different phylogenetic histories (which are most likely to have produced the lower functional diversities, as they are not really environmentally 'harsh' habitats). More similar to each other, and to the other more seasonal sites, are the open, dry forests which are presumably influenced more by the environmental filters, and are thus more convergent.

Similar patterns of convergence have also been observed in the termite faunas of different biogeographic regions. Using similar ordination techniques, equatorial forests in Africa, SE Asia and South America were found to be functionally different, yet in the higher latitude tropical forests of SE Asia, Madagascar and Australia, all relatively depauperate termite communities, they were more convergent in functional diversity, probably due to the harsher habitat effects associated with higher latitudes (Davies *et al.* in press). Schluter and Ricklefs (1993) compiled data from a number of studies to search for convergence of species diversity between similar habitat types in different continents. They found tentative examples in desert dwelling lizards, birds of Mediterranean habitats and plant diversity in warm deserts, and suggested that convergence at a community level may be commonplace in nature. It is interesting that all of these examples involve more arid or seasonal habitats, just as the dung beetle assemblages are seen to converge in Mediterranean-type and open, dry forest habitats.

All appear to have similar local processes, such as environmental instability, which appears to be an important element in ecological convergence.

4.4.8 Trends in feeding behaviour

Whether convergence in feeding behaviour can also be seen in the more depauperate assemblages of the world, is more difficult to judge. It would seem that environmental filters such as the abundance and quality of mammal dung, as well as interactions between species in terms of competition for resources, would all play a crucial role in the diversity of feeding behaviour in any assemblage. However, it is interesting that despite differences in functional composition between the savanna and forest dwelling assemblages in Africa, feeding behaviour remains very similar in these different habitats (figure 4.7), with high levels of coprophagy relative to non-coprophagous feeding. In the most depauperate sites of the Cape, coprophagy is also the most common feeding behaviour, since generalism is non-existent, and necrophagy extremely rare. Conversely, in the Neotropical assemblages, non-coprophagy is high, whilst the Australian sites (one rainforest and one mediterranean-type vegetation) each have relatively high levels of generalism, but uniquely, no strict necrophagy. Thus there appears to be a strong biogeographic factor (or regional historical effects) affecting the diet choice of all assemblages within the main biogeographic regions. Non-coprophagous behaviour is phylogenetically widespread, and seems to be quite plastic even within a species (discussed in chapter 3). Even amongst the African taxa, as demonstrated here, carrion seems to be a more important resource than generally believed. The great abundance of ungulate mammals in the African savannas, generally suspected to be responsible for the diversity of its associated dung beetle fauna, also seems likely to be the key to the high levels of coprophagy there, and perhaps through phylogenetic inertia, to a continued predominance of coprophagy where large mammal diversity declines, in the forests and fynbos habitats. The higher levels of necrophagy in the Neotropics has been attributed to the great extinctions of South American mammals (Halffter and Matthews 1966), and the accompanying reduction in dung resources, although the Neotropical dung beetles may have always retained a higher level of non-coprophagous feeding, particularly if the mammal richness was historically lower in the forests than the pampas (as discussed above).

However, convergence between independent lineages is seen under particular circumstances. The similar lack of generalists in the more species-poor, environmentally harsh sites, in both Africa (the Cape sites) and the Neotropics (Pine Ridge, Belize), is striking. As discussed in chapter 3, specialists have greater competitive abilities, but often have narrow tolerances for environmental conditions, are poorer dispersers across environmentally harsh habitats. Generalists might be less competitive, but have broader tolerances and are usually better dispersers and more opportunistic (Pianka 1988). A harsher environment should therefore select for greater generalism, since this gives a species greater flexibility to survive unpredictable conditions (e.g. Lapchin 2002). Regarding diet choice, the optimal foraging theory predicts that food-scarce environments should also lead to utilization of a broader range of resources (MacArthur and Pianka 1966). Only when food resources are concentrated does it usually pay to specialise, and in a patchy environment, where the energy involved in searching for a specific resource might be greater than the return, a generalist should have a competitive advantage. One might therefore expect greater levels of generalism in dung beetle diets in these harsher environments, particularly since diet choice is evidently quite plastic in Scarabaeinae, but instead, greater specialism is seen. For an unknown reason, presumably related to microclimate, generalist behaviour is apparently less competitive in a harsh environment and is being excluded in favour of specialization. This unexpected result supports the hypothesis proposed in chapter 3, that reduced levels of generalism might occur in harsher habitat types, where environmental filtering effects are stronger, and species are driven to specialise on the resource type to which they are most well adapted at handling or processing. Generalism is conversely more abundant in a more favourable environment, where ecological or environmental constraints do not demand specialization. The independence of the convergent sites suggests a general pattern which might be inherent to dung beetle assemblages, independent of their biogeographic and phylogenetic histories.

Finally, the correlation between the direction of increase of necrophagy and generalism and increasing number of smaller, omnivorous and carnivorous mammals is an intriguing one. It must be pointed out that the pattern could be an autocorrelation between the taxa involved, with mammal distribution being similarly affected by the biogeographic histories or environmental variables, and correlations should be treated with some caution. However, this does tend to support the hypothesis of a progression

towards necrophagy initially via the more nitrogen-rich dung of omnivorous animals (Cambefort 1991c), or perhaps that necrophagous or generalist scarabs are able to increase in diversity through the use of smaller omnivore or carnivore dung. Such a relationship might help to explain the abundance of non-coprophagous species in the South American forests for example. Conversely, there is no apparent link between the abundance of coprophagous species and these mammal categories, indicating that their dung may be of limited use to strict coprophages.

4.4.9 Conclusions

Unfortunately community ecology is characteristically complicated, due to a large number of case histories, and a lack of universality in local patterns (Lawton 1999). The phylogenetic history of the Scarabaeinae largely reflects the biogeographic history of the different regions. Habitat type is critically important to the assemblage structure within a region, but a comparison of the ecological structure of assemblages in similar habitats in different continents tends also to reflect the different phylogenetic and biogeographic histories of each region. This means that the richest scarabaeine fauna of different continents varies not only in structure but in the habitat it occupies. I have identified a variety of patterns, some of which appear to be unusual, and seem to depend upon the historical circumstances and unique ecological role of the Scarabaeinae, such as the contrasting diversity of feeding behaviour and apparently more favourable habitat types in Africa and the Neotropics. Other trends, such as the convergence of functional diversity in harsher habitats, are representative of more widespread ecological mechanisms, seemingly driven by a range of similar environmental filters acting from the regional to the local level.

Chapter 5. A regional comparison of morphospace, and the influence of phylogenetic history.

5.1 Introduction

In chapter 3, the ecomorphology of three Neotropical assemblages was compared, and several important trends were found. The different functional roles of the rollers and tunnellers were seen to be reflected by different morphologies, and there appears to be a morphological trade-off between the two groups, which probably translates to an ecological trade-off. The overall morphospace area and position was also seen to be quite consistent between the three assemblages, with species packing apparently being most affected by habitat quality.

However, in a local comparison of this sort, where taxonomic composition is similar, traits of organisms or assemblages are also likely to be similar due to shared history, and true convergence is hard to identify. Differences between the assemblages are likely to be due to physical environmental differences rather than different phylogenetic or biogeographic factors. This means that one cannot safely extrapolate the findings from one region to another, where different lineages, habitat types and biogeographic histories may be completely different. As regards the Scarabaeinae of the world, they can be divided taxonomically into three major biogeographic realms, the 'Old World' (including Africa, the Orient, and Eurasia), the Neotropics and Australia. The phylogenetic divergence of the Scarabaeinae in these regions is also now apparent. It is also apparent that the more favourable habitat types differ in these three regions. In the Old World, the African sub-Saharan fauna achieves the greatest richness and diversity, whilst the European, Asian and Oriental taxa appear to be largely derived from these African groups. The most species-rich dung beetle assemblages in Africa occur in savanna habitats. The Kruger assemblage therefore represents one of the richest and most diverse scarabaeine faunas of the entire Old World region. Similarly, the Ecuadorean assemblage can be said to represent one of the richest Neotropical assemblages, because here, rainforest is a more benevolent habitat for dung beetles. I have postulated that these different favourable habitats reflect the different vegetation types in which the various dung beetle faunas of each continent primarily evolved. The same is likely to be true for Australia; here, the area of wet tropical rainforest in northern Queensland has the highest overall biodiversity, and also has the richest

Scarabaeinae fauna (Matthews 1974). For much of its history since the Cretaceous, Australia was covered by rainforest, which only became as restricted as it is today during the mid to late Miocene, due to increased desiccation from the northwards drift of the continent (Morley 2000). The persistence of ancient lineages in the now relictual rainforests of northern Queensland is evident. For instance, 13 of the 19 recognised 'primitive' families of angiosperm plant occur there (Barlow 1981). These include a form of magnolia, *Austrobaileya scandens*, the pollen of which closely resembles the earliest flowering plant pollens from around 120 million years ago (Vandenbeld 1988). In the same way, many of the Australian canthonine species are restricted to the rainforest, and few are found in the arid, open scrub habitats of the interior. However, another important habitat exists for the group in Queensland, the open (sclerophyllous) forests dominated by *Eucalyptus* trees, in which the assemblages also reach quite a high relative richness.

5.1.1 The relationship between morphospace, phylogeny and ecology

Since morphospace is assumed to reflect the association between an organism and its environment, we might expect similar functional roles in different lineages to be reflected by a similar occupation of morphospace. For instance, comparisons of birds in Africa and South America have suggested that ecologically similar groups occupy similar morphological space (Keast 1972, Karr and James 1975). Nevertheless, we might also expect phylogeny to be an important component in defining the occupation of morphospace. We have already seen how phylogenetic composition apparently influences the functional diversity of an assemblage, causing divergence in the assemblage structure of the species rich habitats of South Africa and the Neotropics (chapter 4). A comparative study of the community structure of insectivorous bats in the Old World and the Neotropics showed different use of morphospace due to different evolutionary histories (Heller and Volleth 1995), and desert-dwelling lizards of Australia, Africa and North America have also been shown to occupy rather different morphological spaces (Ricklefs *et al.* 1981).

We have seen that there is a strong biogeographic component to the topology of the Scarabaeinae phylogeny. This distinctness of the lineages which populate each region has led to high levels of endemicity in the different continents. In fact, only a single genus, *Onthophagus*, is shared by all regions. Despite this phylogenetic divergence

however, the taxa appear to be performing similar functional roles within the different regions. Given this, we can ask a variety of questions related to the global patterns of assemblage structure. For example, is the assemblage structure (as characterised by morphospace) similar in the three different continents, regardless of phylogenetic history? Do the taxa of different regions with the same nesting strategy also occupy equivalent positions in morphospace? If they do, is this due to convergence or to shared ancestry? If we find convergence in function, does it require convergence in morphology (true functional adaptation)? Furthermore, can we identify 'convergent trajectories' in the different faunas, for example by identifying 'gaps' which have been filled by unrelated taxa? Finally, can we determine whether the morphospace structure of a dung beetle assemblage or regional pool is more strongly influenced by phylogenetic constraints, environmental factors or even by inter-species interactions?

5.2 Materials and Methods

5.2.1 Morphometric measurements

In chapter 3, a range of linear morphological measurements were collected from some 115 different species from three Neotropical dung beetle assemblages (Pine Ridge and Las Cuevas in Belize, and Tiputini in Ecuador). The 19 measurements included dimensions of the head, thorax, abdomen and hind leg, and are described fully in table 3.2, whilst the choice of measurements and methods of data collection are discussed in section 3.2.4. These measurements were used to investigate patterns in the local and regional usage of morphospace within the Neotropics. However, as a single data set, these measurements can be considered to effectively represent the scarabaeine fauna of the Neotropical forests, since it not only consists of a wide range of taxa, collected by standardized and thorough methods, but also includes assemblages from three different forest types from both South and Central America.

In two similar and related studies, species have been measured (using Auto-Montage) from a number of scarabaeine assemblages in South Africa (C. Wilsher unpublished) and Australia (R. Davies unpublished), to examine local and regional patterns of morphospace use across habitat type in these areas. Due to the collaborative nature of these studies, the measurements were taken in a manner identical to my own, making them directly comparable. Furthermore, the species measured in South Africa included those collected in the Kruger National Park and the West Coast National Park (described in chapter 4), so that morphological measurements are available for two distinct African assemblages collected using the standardised trapping protocol. The Australian data set includes the species from an extensive number of assemblages sampled in both open forest and rainforest in northern Queensland (G. Monteith unpublished). From the Australian species measured, I have extracted data for those of four different assemblages, so that the numbers of species from each continent might be broadly similar. Two open forest, and two rainforest assemblages were chosen from a total of 39 sites sampled across the northern Queensland subregion (G. Monteith unpublished). Each of the 39 localities had been classified into either lowland open forest, lowland rainforest, upland open forest or upland rainforest ('lowland' representing sites from 0-300 m above sea level, and 'upland' from 300-1500m above sea level). One assemblage (the most extensively sampled) was chosen from each of

these categories for inclusion in the 'Australian data set', so that a range of species from the two main habitat types in this region were represented. The Queensland sites include Hartleys Creek (lowland open forest; 16°40'S, 145°34'E), Ravenshoe (upland open forest; 17°38'S, 145°30'E), Bloomfield Road (lowland rainforest, 15°48'S, 145°19'E), and Charmillin Creek (upland rainforest, 17°42'S, 145°31'E). Sites were also chosen to cover as broad a geographical area as possible in the region.

In total, the global morphometric data set included approximately 110 Neotropical species (representing three complete assemblages), 100 African species (representing two assemblages), and 60 Australian species (representing four assemblages). Any species from the African or Australian assemblages, which had not already been measured, were added to the data set by myself. All African and Neotropical species were collected as part of the standardised trapping protocol for this project, and the Australian assemblages were considered to have been collected with equal trapping effort. An advantage of this approach is that all species from Belize, Ecuador and South Africa have associated information on feeding behaviour, collected by the standardised trapping. Using similar empirical data collected in Queensland (G. Monteith unpublished, Hill 1996), each of the Australian species was also assigned to a feeding group (discussed in chapter 3).

A morphometric analysis was then carried out in the same manner as described in chapter 3. Briefly, all measurement data were log-normalised, and the mean log value determined from the 3 female specimens measured for each species. Residual values were then calculated for each mean log value from a regression against mean body size of the species, using STATISTICA (StatSoft Inc. 1999). A Principal Components Analysis (PCA) of all species was then carried out using CANOCO (Ter Braak and Smilauer 1998), and biplot diagrams were produced. MANOVA tests (multivariate analysis of variance) were carried out on the species distribution along each PCA axis to test for significant differences in the occupation of morphospace by the Scarabaeinae of the three continents. Distribution was investigated regarding the total fauna of each region, as well as the different functional (nesting) and feeding groups. Where significant differences were found in the distribution along an axis, a post-hoc Tukey HSD (Honest Significant Difference) was performed using STATISTICA, to identify which of the regions was significantly different from the others.

5.2.2 A regional comparison of species rich and less rich habitat types

In chapter 4, convergence was identified in the functional structure of the environmentally harsh habitat-types of Africa and the Neotropics (West Coast National Park and Pine Ridge), whilst the species rich habitat types (Kruger National Park and Tiputini) were seen to diverge. To test for any similar, and perhaps related convergence or divergence in the morphospace occupied by these assemblages, further MANOVA tests were carried out on the species distribution within these four habitats. The distribution of the species in each site was tested against that of the other three sites, and species distributions from the two species rich habitats were tested against those of the species poor habitats.

5.2.3 Testing for a correlation between morphometric distance and phylogenetic divergence

In order to investigate the influence of phylogenetic history on dung beetle morphology, I tested for a correlation between the morphometric and phylogenetic distances between species, using a Mantel test. This is a method of evaluating the degree of correspondence between two distance matrices, and tests the null hypothesis that there is no relationship between them. Crucially, it does not assume that the data are independent; indeed cells in a distance matrix are not independent of each other. The significance of the correlation is tested by simultaneous random permutation of rows and columns with a Monte Carlo test. A standardized Mantel statistic (r) is produced, which can be interpreted as a measure of the strength of relationship between the matrices, and a p-value, indicating the level of significance. I used PC-ORD Version 2.0 (McCune and Mefford 1995) to conduct the Mantel test.

All species which were represented in both the global-level morphometric analysis, and the phylogeny (by at least two of the three gene loci sequenced) were included in the Mantel test. Because many of the specimens sequenced were collected from the assemblages included here in the morphospace investigations, there was a considerable overlap of 87 species from 54 different genera, from all three regions. This amounted to 45% of the species in the phylogeny, and 33% of the species in the analysis of morphospace represented. Species from all major tribes and phylogenetic clades were included, excepting only the Eucraniini. To calculate the phylogenetic distances

between species, I chose not to use branch lengths from a cladogram, since an incorrect topology, or insufficient taxon sampling, would give a biased and misleading distance value. Instead, using the length-invariable alignment (so as to minimise any equivocal influence from poor sequence alignment), the total number of base differences between sequences was calculated in a pair-wise manner, using PAUP. To account for the variation in the overall sequence lengths of the different species, the total number of pairwise base changes was calculated as a percentage of the total number of bases shared by each pair of sequences. This was then entered into a triangular 'dissimilarity' matrix. Because PC-ORD will automatically create a distance matrix from a table of quantitative variables, the morphometric data matrix consisted of a table of the species coordinates from the first three morphometric PCA axes. These were then converted to Euclidean distances by PC-ORD. A Monte Carlo test performed 9999 randomisations of the data to calculate the Mantel statistic.

5.2.4 The removal of historical influences from regional morphospace

If one can remove the historical (phylogenetic and biogeographic) processes which are structuring the morphospace, it might be possible to identify the key local components affecting the fauna of an assemblage or region. The tunnelling genus *Onthophagus* is shared by all three biogeographic regions however, and although the tribe Onthophagini is apparently made paraphyletic by the Oniticellini (chapter 2), the genus *Onthophagus* seem to be (by and large) a monophyletic group. In addition, *Onthophagus* species have a distinctive morphology which is easily recognisable, regardless of the region from which they originate. All species of *Onthophagus* from the three faunas were therefore examined independently from the other taxa, and MANOVA tests were conducted to search for any significant differences in the species distribution in morphospace in the different continents.

5.3 Results

5.3.1 The occupation of morphospace by the different regional faunas

The results of the global-level morphospace analysis are presented in a series of PCA biplots. Figures 5.1 to 5.4 display the first two PCA axes, with the species from all regions presented in figure 5.1, and then the fauna of each continent separated in the subsequent figures, for ease of comparison. In the same way, the results of axes 2 and 3 are presented for each fauna separately in figures 5.5 to 5.7. The eigenvalues of each linear morphological measurement for each of the first four axes are displayed in table 5.1. Axis 1 explains almost 40% of the variance in the data, axis 2 explains 19%, and axis 3 explains 11%. As in the Neotropical morphospace analysis (chapter 3), axis 1 represents size, whilst the subsequent axes represent only shape components of the morphology.

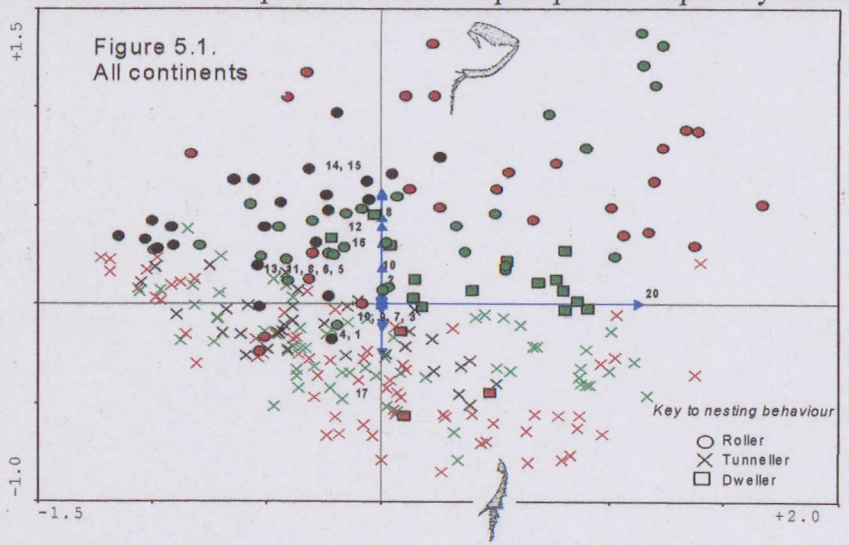
Table 5.1. Fit of the morphological measurements to the first four PCA axes as a fraction of the total variance of each measurement. A negative value indicates a negative correlation with that axis. Eigenvalues for each axis are also presented.

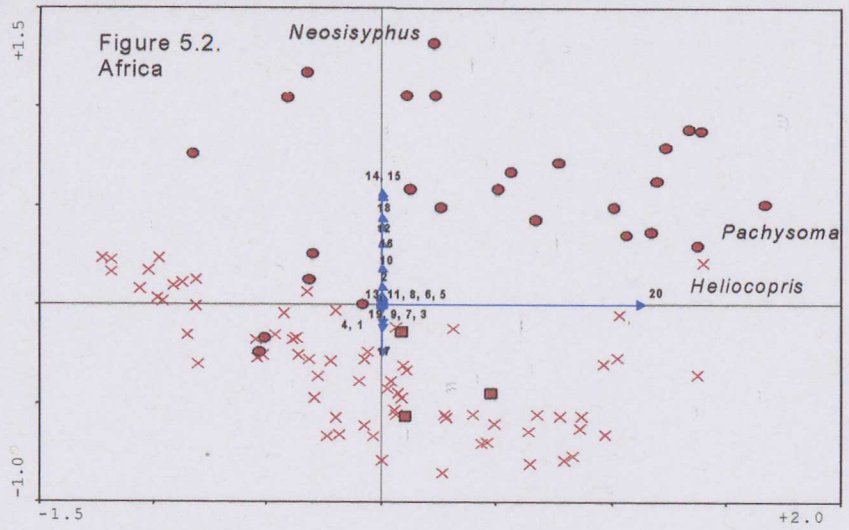
	Axis 1	Axis 2	Axis 3	Axis 4
Axis Eigenvalue	0.3994	0.193	0.1125	0.0929
Measurement				
1	0	-0.1123	0.222	0.3337
2	0	0.1668	-0.1249	-0.3148
3	0	-0.0086	0.5633	0.0104
4	0	-0.0572	0.2138	0.0886
5	0	0.0048	0.4073	-0.0668
6	0	0.02	0.0385	0.0448
7	0	-0.0273	0.2617	-0.1588
8	0	0.0018	0.3431	-0.143
9	0	-0.0002	0.2365	-0.6277
10	0	0.1317	0.0158	-0.459
11	0	0.0205	0.2071	0.3617
12	0	0.6513	0.0399	0.0306
13	0	0.0071	0.425	0.0042
14	0	0.9445	-0.0045	0.0078
15	0	0.9398	-0.0024	0.0098
16	0	0.5083	-0.0656	-0.0139
17	0	-0.2961	0.3621	-0.0003
18	0	0.4883	0.2554	0.0143
19	0	-0.0008	0.3973	0.0732
20	1	0	0	0

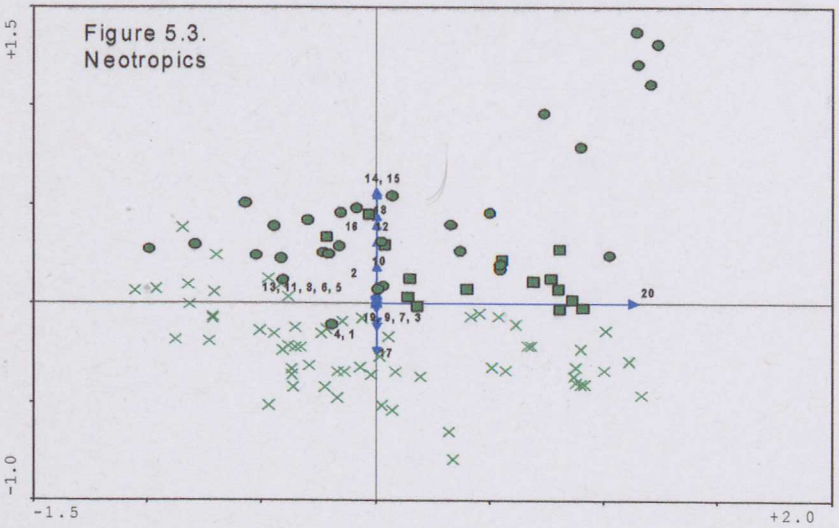
A MANOVA test found the species distribution of the Australian fauna to be highly significantly different to both the African and the Neotropical faunas on axis 1 ($F_{1,2}$ = 11.5, $p < 0.0001$), (Tukey HSD tests $p < 0.0001$). From figures 5.2 to 5.4, it is evident that there is a lack of species in Australia with high axis 1 values (in other words, there are no large species in this data set). No significant difference was found between Africa and the Neotropics on axis 1, as the body size distribution is evidently similar.

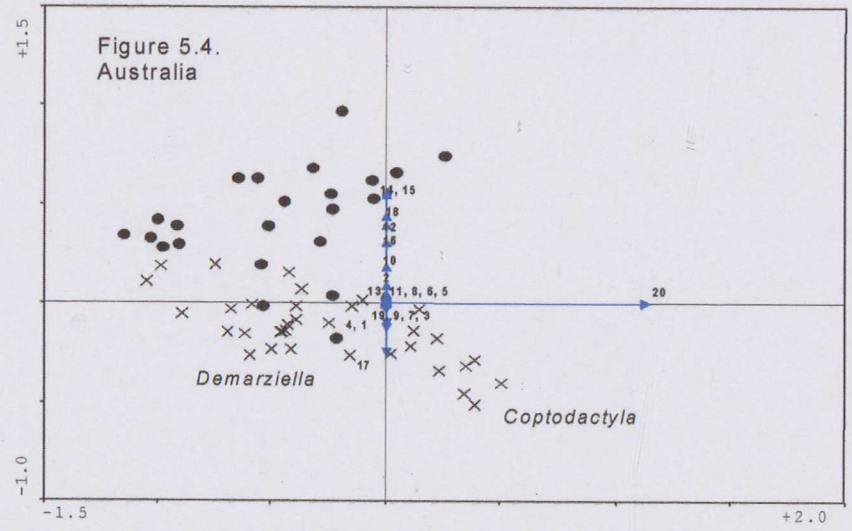
No significant difference was found between the three continents on axis 2 or 3, although there was a nearly significant difference between the distribution of Australian and African species along axis 2 ($F_{1,2} = 2.8$, $p = 0.061$), (Tukey HSD test $p = 0.054$). Table 5.2 presents the areas of morphospace of the various functional groups in the three continents, as well as the nearest neighbour distances calculated from the species coordinates. The total morphospace occupied by the African fauna is somewhat larger in area than the Neotropical morphospace (by 37%), but is three times the area occupied by the Australian fauna on axes 1 and 2. Species packing (indicated by the mean nearest neighbour distances) is greatest in the Australian fauna on all axes, with the African species being least closely packed, even with the removal of size in axes 2 and 3.

Figures 5.1 – 5.4. A comparison of the morphospace occupied by the dung beetle faunas of three different continents; PCA axes 1 and 2.

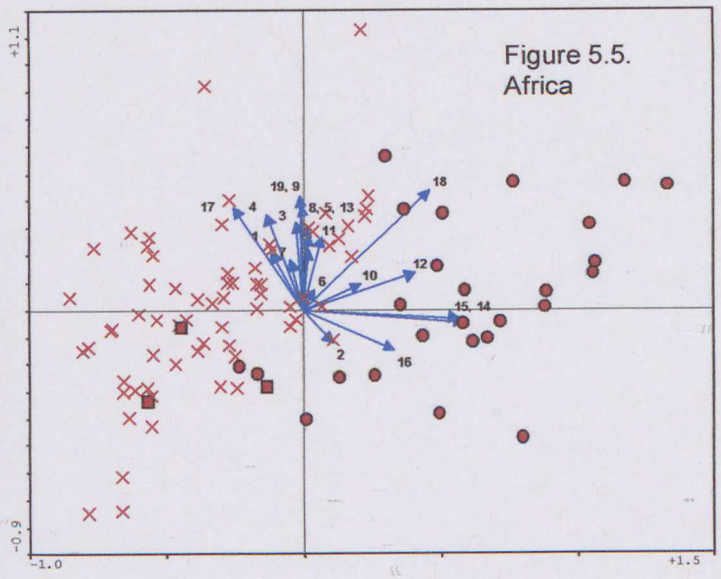






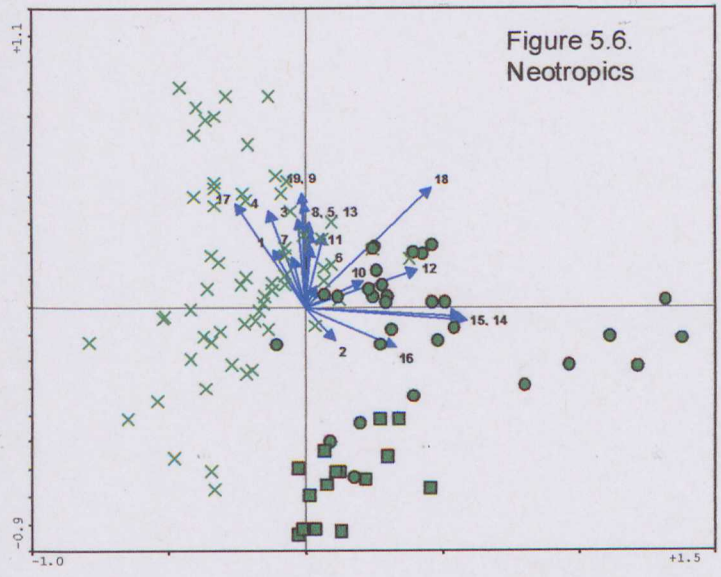


Figures 5.5 – 5.7. A comparison of the morphospace occupied by the dung beetle faunas of three different continents; PCA axes 2 and 3.



Key to nesting behaviour

○ Roller
× Tunneller
□ Dweller



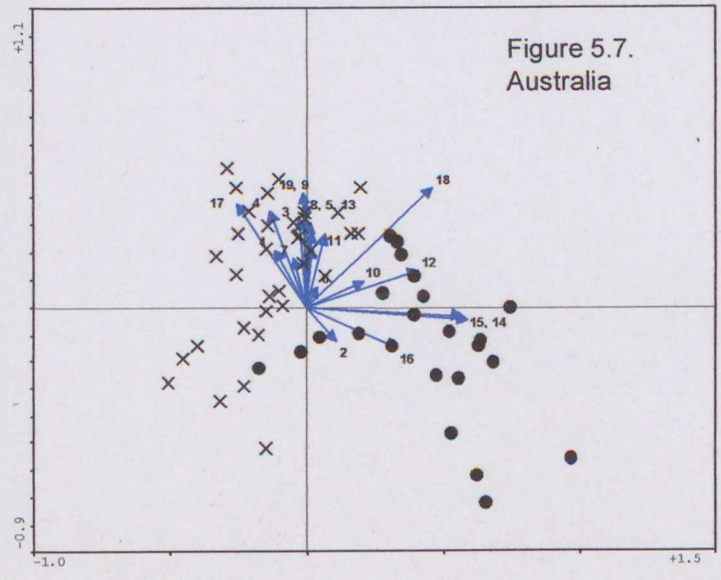


Table 5.2. Two-dimensional morphospace areas and nearest neighbour distances for PCA Axes 1 and 2, and PCA Axes 2 and 3, for the Scarabaeinae faunas of three biogeographic regions. Area values are given for the total assemblage, for each functional and nesting group and for areas of overlap. Mean, maximum and minimum nearest neighbour distances are given for the whole fauna in each case.

	Africa		Neotropics		Australia	
	Axes 1 & 2	*Axes 2 & 3*	*Axes 1 & 2*	*Axes 2 & 3*	*Axes 1 & 2*	*Axes 2 & 3*
Morphospace areas						
All species	4.301	2.593	3.138	2.126	1.514	1.166
Rollers	2.41	1.055	1.60	0.757	0.82	0.603
Tunnellers	2.045	1.207	1.437	1.014	0.55	0.448
Dwellers	0.082	0.058	0.345	0.133	N/A	N/A
Overlap between rollers and tunnellers	0.426	0.131	0.26	0.088	0.125	0.03
Overlap between rollers and dwellers	0	0	0.266	0.039	N/A	N/A
Overlap between tunnellers and dwellers	0.078	0.058	0.031	0	N/A	N/A
Coprophages	4.301		1.498		1.303	
Necrophages	1.628		2.266		N/A	
Generalists	1.365		2.916		0.889	
Overlap between coprophages and necrophages	1.628		1.219		N/A	
Overlap between coprophages and generalists	1.365		1.49		0.799	
Overlap between necrophages and generalists	1.031		2.228		N/A	
Nearest neighbour distances						
Mean nearest neighbour distance	1.084	0.724	0.889	0.689	0.680	0.561
Maximum nearest neighbour distance	2.902	2.430	2.607	2.165	1.815	1.654
Minimum nearest neighbour distance	0.016	0.002	0.005	0.006	0.009	0.011

5.3.2 Functional groups

From table 5.1, and figures 5.5 - 5.7, we see that axis 2 is most closely correlated with measurements of the hind leg: 14 (curved length of tibia), 15 (straight length of tibia), 12 (length of femur) and 16 (degree of tibial curvature). These are clearly the same rolling characteristics identified in the Neotropical analysis of chapter 3. The rollers from all regions are strongly positively correlated with these measurements, whilst the tunnellers have a strong negative correlation with them, so that there is a highly significant difference between the two functional groups ($F_{1,2} = 170.1$, $p < 0.0001$), (Tukey HSD tests $p < 0.0001$), regardless of continent. Rollers and tunnellers were also found to be significantly separated in morphospace by axis 3 ($F_{1,2} = 43.7$, $p < 0.0001$), (Tukey HSD tests $p = 0.001$), although this is not as clearly defined as on axis 2. Axis 3 is most closely correlated with the measurements 3 (maximum pronotal width), 5 (maximum elytral width), 13 (width of femur). The tunnellers are however largely separated from the rollers by a positive correlation with the measurements 17 (width of tibia), and 4 (related to pronotal length). These are by and large the same digging characteristics associated with axis 3 in the Neotropical analysis (figures 3.9 – 3.11).

The distribution of the Australian rollers was significantly different to that of the Neotropical and African rollers along axis 1 ($F_{1,2} = 14.8$, $p < 0.0001$), (Tukey HSD tests $p < 0.001$) although the distribution of the Australian tunnellers was not found to be significantly different to the African and Neotropical tunnellers, indicating a greater size difference between the continents in the rollers. It is evident that the Australian fauna does however lack the larger bodied tunnellers found in the other continents. As regards the shape component of the morphology, the Australian rollers were found to be significantly different to the African rollers on axis 3 ($F_{1,2} = 3.13$, $p = 0.048$), (Tukey HSD test $p = 0.038$), and the Australian tunnellers different to the African tunnellers on axis 2 ($F_{1,2} = 6.77$, $p = 0.002$), (Tukey HSD tests $p = 0.001$). No significant differences were found between the morphospace occupied by the Neotropical rollers or tunnellers with any other region, on any other axis, suggesting that the Neotropical fauna is somehow intermediate in species distribution, between the other two regional faunas.

On the other hand, the distribution of dwellers in the morphospace of the Neotropics and Africa were found to be significantly different to each other along both axes 2 ($F_{1,2} = 27.0$, $p < 0.0001$), and 3 ($F_{1,2} = 23.2$, $p = 0.0002$). From figures 5.2 and 5.3, we can

see that the African dwellers (*Oniticellus*) are positioned in the tunneller morphospace, whilst the Neotropical dwellers (*Eurysternus*) are positioned in the roller morphospace. Australia has no dwellers, and cannot be included in this comparison. There is the hint of a similarity in the morphology of the two dwelling groups on axes 2 and 3, as they both have a negative correlation with measurement 18 (area of tibia). On axis 3 (and axis 4, which is not shown due to the low amount of variation explained), both groups are also seen to be negatively correlated with measurements 7 and 8 (width and length of head), and 9 and 10 (height of and relative length of the elytra), indicating smaller heads and more 'shallow' and parallel-sided abdomens.

5.3.3 Feeding groups

The distribution of the different feeding groups in the morphospace of the three regional pools is displayed in figures 5.8-5.10 (for PCA axes 1 and 2). Although the Australian coprophages were found to be significantly smaller than the Neotropical or African coprophages ($F_{1,2} = 9.73$, $p = 0.0001$), (Tukey HSD tests $p < 0.006$), this merely reflects the overall differences in size distribution of the different regional faunas. No other significant differences were found. Out of interest, in the African fauna, the coprophages occupy an area of morphospace almost 3 times larger than that occupied by the necrophagous or generalist species. In contrast, the Neotropical coprophages occupy a space some 50% smaller than the generalist or necrophagous species (see table 5.2). Australian coprophages and generalists occupy more similar areas of morphospace.

Figures 5.8 to 5.10. PCA biplots illustrating the distribution of feeding behaviour through the morphospace of three different biogeographic regions.
Feeding groups: White = Coprophagous, Blue = Necrophagous, Yellow = Generalist.

Figure 5.8. The African fauna

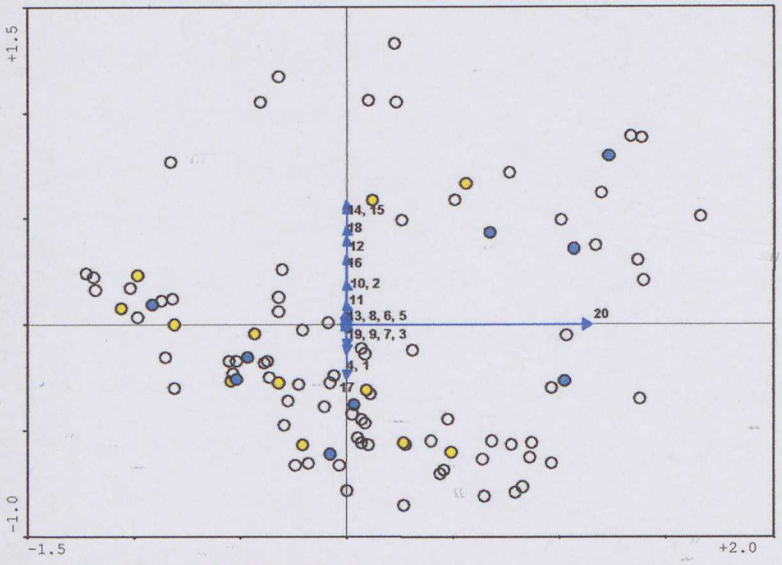


Figure 5.9. The Neotropical fauna

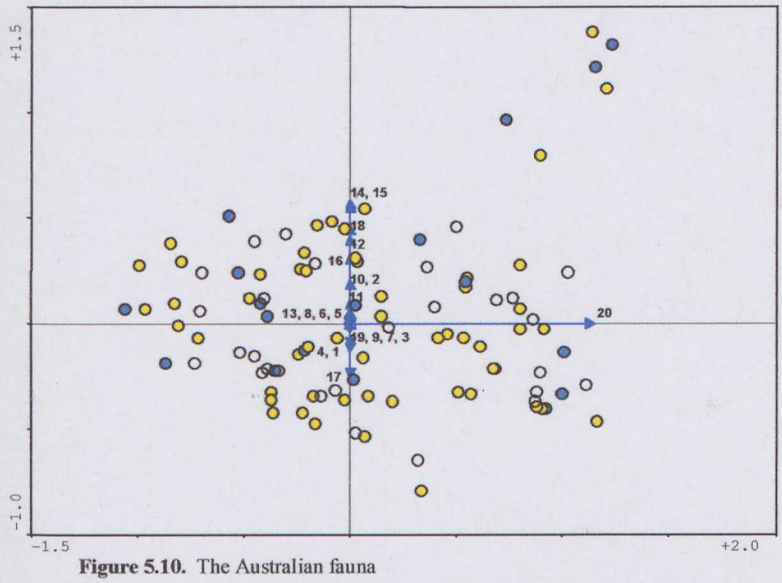
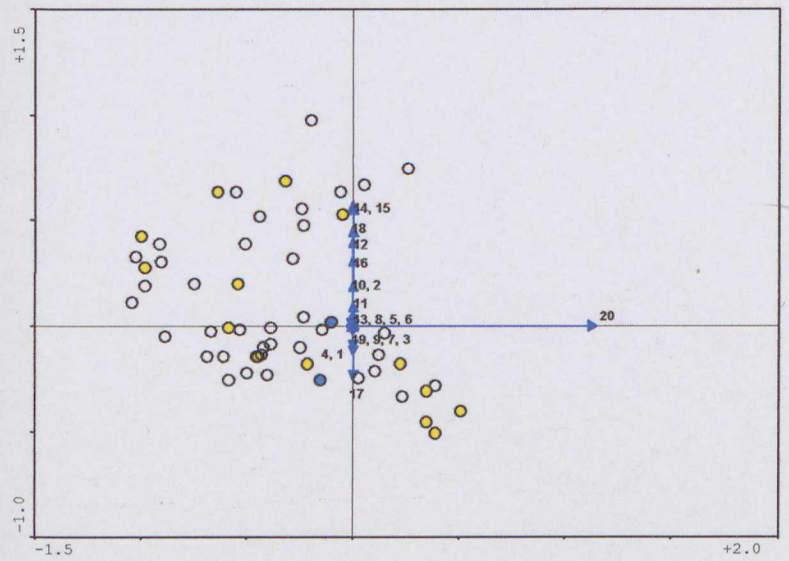


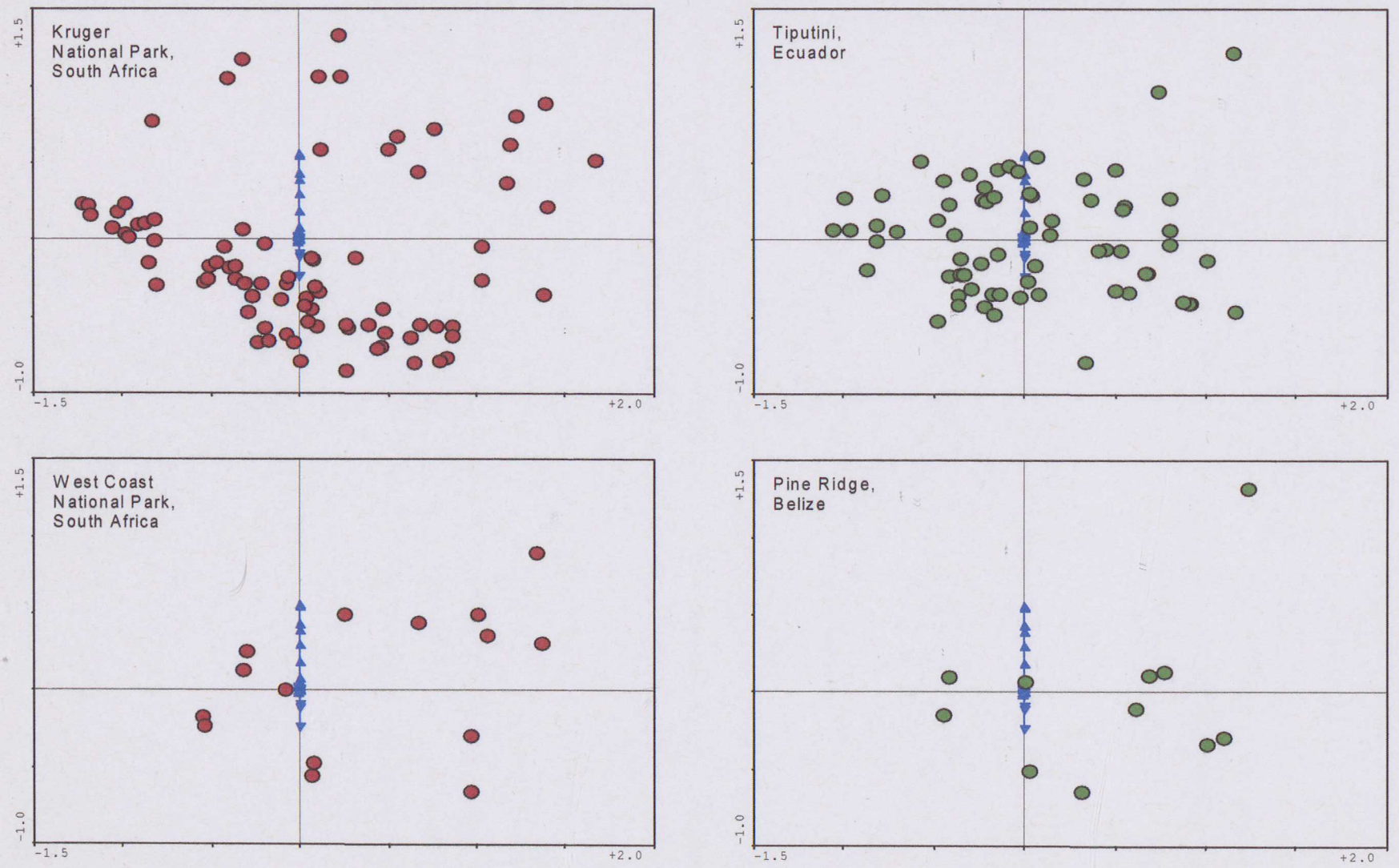
Figure 5.10. The Australian fauna



5.3.4 A comparison of species rich and less rich habitat types between regions

The occupation of morphospace, on PCA axes 1 and 2, by two assemblages in favourable African and Neotropical habitats (Kruger and Tiputini), and two in harsher African and Neotropical habitats (West Coast and Pine Ridge), is illustrated in figure 5.11. The favourable (species rich) habitats by and large reflect the morphospace occupied by their respective regional faunas (figures 5.2 and 5.3). The harsher habitats are much more depauperate, but the species of each are still well distributed throughout the regional morphospace, rather than being concentrated in any particular area. A series of MANOVA tests showed no significant differences, in the species distribution in space, between the species rich and species poor habitats in Africa (Kruger and West Coast) or the Neotropics (Tiputini and Pine Ridge), on any axis. Neither was a significant difference found between the two species poor habitat types (West Coast and Kruger). However, a comparison of the fauna of the two favourable habitat types (Kruger and Tiputini) returned a p value of 0.131 for axis 2 ($F_{1,2} = 2.3$). Although this is statistically non-significant, it suggests that the species distribution in morphospace of the favourable habitats in Africa and South America are still more divergent from one another, than are the species poor habitats, which had a p-value of 0.945 for this axis ($F_{1,2} = 0.004$), when tested against each other. Figure 5.11 tends to suggest that the African fauna is more spread out along axis 2, whilst the Neotropical species tend to be more closely clustered.

Figure 5.11. The assemblage morphospace of species rich and less rich habitats, compared between Africa and the Neotropics. Axes 1 and 2 are presented.



5.3.5 Phylogeny and morphology

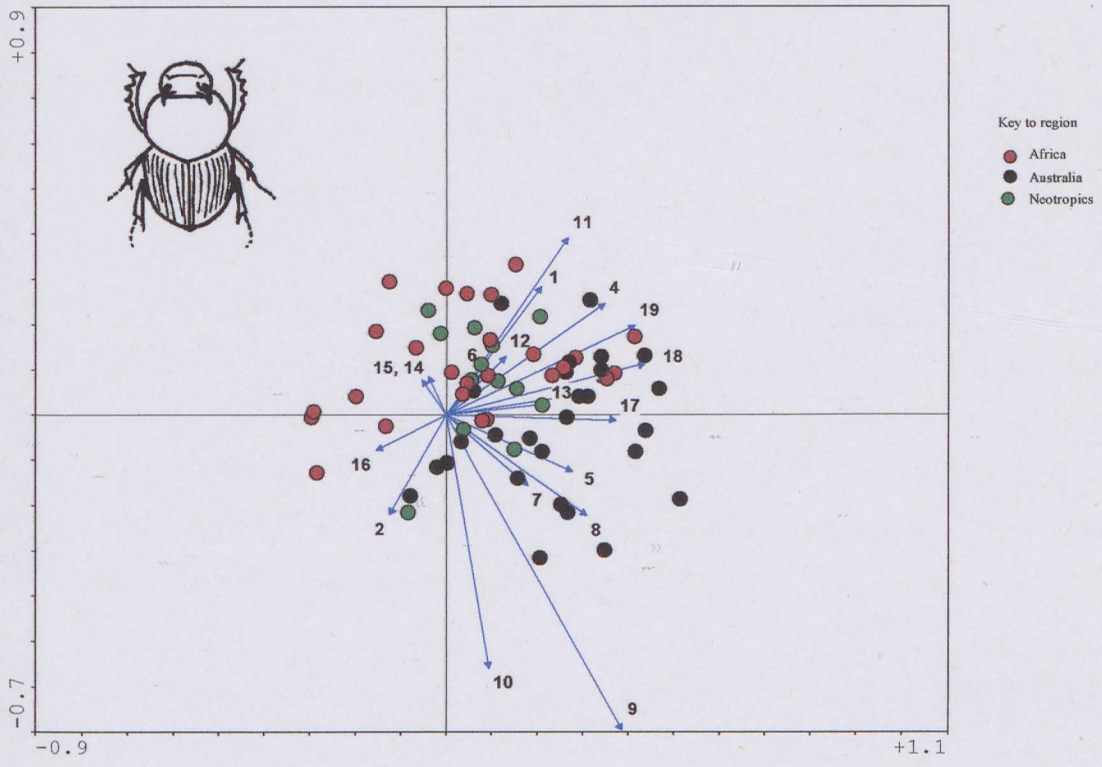
The Mantel test for correlation between the phylogenetic and morphometric distances between species was found to be statistically significant on axis 2, and almost significant on axis 3 (table 5.3). The Mantel statistic (r) is however low for both axes 2 and 3, suggesting that there is a considerable level of noise in the data. Nevertheless, this is an intriguing result, and because axis 2 and 3 most strongly divide rollers and tunnellers, this suggests an association between phylogeny and the gross morphology of rolling or tunnelling.

Table 5.3. Mantel test results for association between morphometric and phylogenetic distances.

Morphospace Axis	Standardized Mantel Statistic (r)	p value
Axis 1	-0.0024	0.441
Axis 2	0.0363	0.047
Axis 3	0.0325	0.067

Upon examination of the distribution of the *Onthophagus* species from the three continents, the Australian species were found to be positioned significantly differently along axis 3, when compared to both African and Neotropical species ($F_{1,2} = 9.59$, $p = 0.0002$), (Tukey HSD tests $p < 0.015$). No significant difference was found between the African and Neotropical *Onthophagus* species on any axis. Figure 5.12 illustrates the distribution of the *Onthophagus* of each region along axes 3 and 4. The morphological measurements positively correlated with this include maximum height of elytra (9), length of elytra (10) and length of head (8), as well as width of elytra (5) and width of head (7). This suggests that the Australian *Onthophagus* have generally larger body proportions.

Figure 5.12. A comparison of the morphospace occupied by species of the genus *Onthophagus* in three biogeographic regions. PCA axes 3 and 4 are presented.



Discussion

5.4.1 Continental differences in ecomorphology

From this global-level analysis, one of the most important findings is that the dung beetle faunas of Africa, Australia and the Neotropics are largely distributed throughout morphospace in the same way. Closely associated with this is the finding that the key functional morphology of the rollers and tunnellers is generally very similar between regions, and the same functional characters which were seen to separate the Neotropical fauna in morphospace are also seen to separate the faunas of Africa and Australia in the same way. Whilst axis 1 is constrained to represent size alone, by virtue of the methodology used, axis 2 was most closely correlated with rolling characteristics and axis 3 with digging characteristics, and these were seen to be equally important to structuring the assemblages of all regions.

Size explains most variation in the morphology and will clearly effect the functional role of an organism in a variety of ways, such as its resource use and competitive abilities. The faunas of Africa and America have a very similar body size distribution to each other, but the Australian fauna almost totally lacks the large-bodied species (rollers or tunnellers) which are found elsewhere. The largest-bodied genus, *Aulacopris*, although not represented here, does reach up to 19 mm in length (Cambefort 1991a), but has only three species, and these are largely restricted to the south-east of the country. Quite why the most species-rich Australian assemblages, in the relictual forests of northern Queensland, do not reach the body sizes seen in Africa or the Neotropics is unclear, although it is possible that they might be subject to some form of phylogenetic constraint. With placental mammals only recently introduced to Australia, this may be related to the small pelleted dung typical of marsupials. Energy use tends to increase with body size in a taxon (Currie and Fritz 1993), and it appears that there might be a metabolic constraint to body size in dung beetle assemblages (Chown and Steenkamp 1996). Thus if larger bodied species need larger dung resources, they may be limited by inter-species competition, particularly in a species-rich habitat such as the Australian rainforests.

This size discrepancy of the Australian fauna almost certainly has an effect on the average nearest neighbour distance, and total area of morphospace occupied, since these

are both considerably smaller than for Africa and the Neotropics. The greater volume of morphospace occupied by the African fauna indicates a greater diversity of body size and shape, and presumably a greater variety of ecological roles. The Australian fauna on the other hand is relatively clustered (on all of the axes examined), and occupies a considerably smaller area of morphospace, suggesting less functional variety. It is also noteworthy that none of the four different rolling tribes in the African fauna (Sisyphini, Scarabaeini, Gymnopleurini and the African canthonines) actually overlap with each other on the first two axes, and instead have a clear tendency to cluster with other members of the same tribe. This is suggestive of a phylogenetic constraint to their morphology, although it could indicate the need for a greater ecological 'distance' between the groups, due to inter-species competition for instance. Non-competitive diversification (natural drift in morphology) is also a potential mechanism leading to different morphologies. However, all of the Australian rollers are canthonines, which seem to be a closely related group (chapter 2). If phylogeny does constrain morphology in some way, this may also explain their tendency to cluster in morphospace, and lack of ecological variety.

5.4.2 Functional groups compared

The rollers and tunnellers of the three different regions can be said to be largely convergent in the position of the morphospace occupied. The rollers all tend to have longer hind femora and tibiae, which also have a greater degree of curvature. As discussed previously, this seems to be a required adaptation for the formation and rolling of dung balls. Conversely, the tunnellers tend to have shorter, straighter legs, with wider tibiae, which presumably gives them more power and surface area to dig with, and a bigger pronotum to house the increased muscle mass necessary for digging burrows. Many distantly related groups, endemic to different regions, occupy very similar areas of the morphospace, indicating similar gross morphology, and by extension, equivalent ecological roles. For instance, in the morphospace describing the 'large tunneller niche', the American Phanaeini and the African Onitini occur very close to each other. Due to similar morphology, these two groups were once thought to be sister taxa (Zunino 1985), but this has been consistently rejected by my molecular data. The rolling morphology has also independently evolved in a convergent manner, on what appears to be a number of occasions, indicating the significance of such morphology to this mode of nesting. Furthermore, such convergence signifies that the

functional morphology of a dung beetle cannot be entirely constrained by phylogenetic history.

5.4.3 Morphological requirements of living in dung

Unrelated dung beetle taxa can however apparently fill the same functional role in independent assemblages, without strongly converging on the same morphology. The two dwelling groups are an interesting example of this. The sister group to *Eurysternus,* the American dwellers, was estimated to be the American canthonines, and Eurysternus evidently still maintains a largely roller-like morphology (albeit slightly more tunneller-like than the canthonines – see figure 5.3). The African dwellers, represented here by *Oniticellus*, were estimated to be nested within the Onthophagini, and evidently still maintain a very tunneller-like morphology. There is some apparent convergence in morphology, identified primarily on axes 3 and 4, which explain relatively little of the morphological variance, but these included having tibiae of low surface area, smaller heads and more shallow and parallel-sided abdomens. This may reflect the requirements of a dwelling lifestyle. For instance, dwellers do not need to dig burrows or bury dung balls in the soil, which might explain the relatively low surface areas of their tibiae, and smaller heads, both of which are normally used to move earth. Given that rolling and tunnelling characteristics dominate the phenology of most other groups, an organism which only digs in dung will not need the same type of morphology. The shallow, parallel sided abdomen of both groups (relative to their sister taxa), is more of a 'streamlined' body shape, reminiscent of that seen in the other scarabaeoid dwellers, the Aphodiinae. This may reflect a switch in the selective pressures upon morphology, from the need to dig or roll, offering least resistance (being 'hydrodynamic') when moving through dung. Incidentally, of the most commonly found non-scarabaeoid Coleoptera groups in the dung insect community, the first are the Staphylinidae, which have a long and very slender morphology, and can be seen to slip through the dung with minimum resistance. The second group are the Hydrophilidae, which being a water beetle group, have a highly streamlined body form. Future work might compare the morphology of the scarabaeine dwellers with the Aphodiinae dung beetles in particular, to test for convergence in body form due to similar functional lifestyles.

5.4.4 Morphological trade-offs and strategies to avoid them

In chapter 3, I suggested that there might be a trade-off in the morphology of rollers, between rolling characteristics and tunnelling characteristics. Thus to be a more efficient roller, it was hypothesised that a beetle would move further away from the tunnelling morphology, and thus become less effective at burying its dung balls. Because of the apparent convergence in body form in independently derived rolling groups, such a hypothesis should apply to any of the rolling groups, from any region. One of the most functionally derived group of rollers, in terms of their morphology, are the Old World Sisyphini, consisting of two genera, *Neosisyphus* and *Sisyphus*. These are the species with the highest axis 2 values in figure 5.2, reflecting their extremely long, slender and seemingly delicate legs (a hind leg is illustrated in figure 5.1, and the position of the *Neosisyphus* species indicated on figure 5.2). This extreme morphology would seem to subject this taxon to the greatest trade-off of any group of rollers, as far as reducing their digging abilities. Rollers often dig relatively shallow nests (Hanski and Cambefort 1991d), presumably because of their relatively poor capacity for tunnelling, but several species of *Neosisyphus*, with the longest legs, actually avoid burrowing altogether, and instead attach their dung balls to the grass stems or simply leave them on the surface (Paschalidis 1974).

I suggest that it is precisely because of this trade-off, that the Argentine Eucraniini, which are classified as rollers (Cambefort 1991a), do not in fact roll dung, but carry it. In this way, they presumably do not lose any of their digging abilities, which are after all, crucial to their way of life, since they live in (and must therefore excavate) permanent burrows. In a similar way, the Scarabaeini genus *Pachysoma*, which also lives in burrows, in the arid areas of south-western Africa, is known to drag pieces of dung forwards, held between the hind legs (Scholtz 1987), unlike the typical 'normal' rolling behaviour of the rest of the tribe Scarabaeini. One of the most striking things about these two groups is their remarkable morphological similarity, illustrated in plates 5.1 and 5.2 below, which has frequently led to assumptions that they might be related. The phylogeny strongly suggests that they are not, and in fact *Pachysoma* and the eucraniines are derived from rolling and tunnelling ancestors respectively. Instead, it seems they have converged on a remarkably similar body form, so that they might

benefit from an ability to translocate dung (by carrying or dragging), whilst still being able to dig deep, permanent burrows. The large pronotum which is common to both presumably houses powerful digging musculature, since both groups are also flightless - probably to conserve water in the arid environments to which both taxa are restricted. An important conclusion from this is that strong environmental pressures can prevail over phylogenetic ancestry to influence the morphology of a scarabaeine. Unfortunately, no eucraniines have been measured, although a *Pachysoma* species was collected as part of the West Coast National Park assemblage, and is indicated on figure 5.2. It apparently still retains some of its ancestral (rolling) morphology, but has the lowest axis 2 values of any of the Scarabaeini.

Plate 5.1. A species of Eucraniini, *Anomiopsoides pereirai*. Taken from Halffter and Matthews (1966). Note the clypeal prongs used in conjunction with the front legs to carry dung.

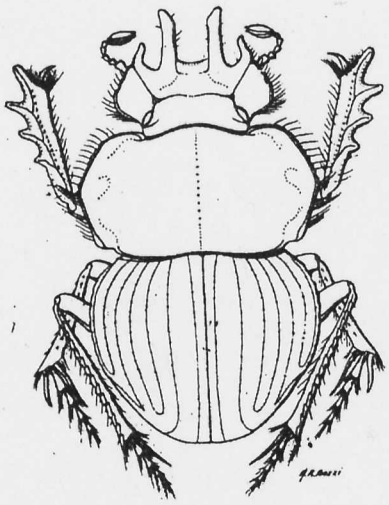
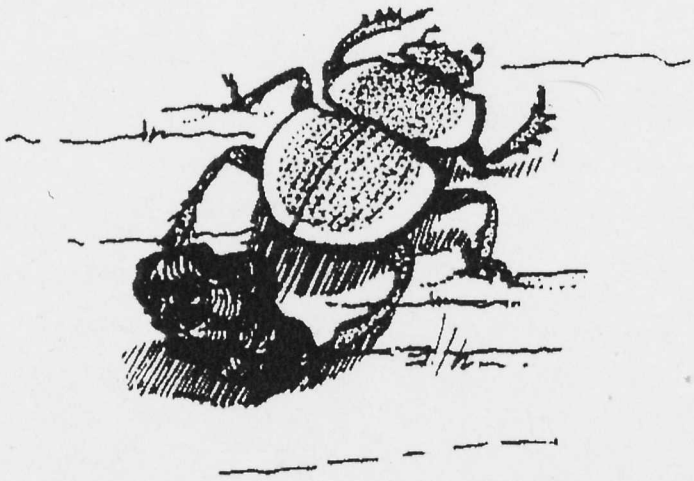


Plate 5.2. *Pachysoma striatum* dragging dung with its hind legs. Taken from Scholtz (1987). Note that *Pachysoma* tends to have a greater degree of curvature to its hind legs than the Eucraniini – evidence of its rolling ancestry.



5.4.5 Quality of the habitat

Although it is not conclusive, there is a suggestion that the species rich habitats are more divergent in their occupation of morphospace than the less species rich habitats. Figure 5.11 illustrates this, by demonstrating how the species of the Kruger assemblage tend to occupy more peripheral positions within its total area of morphospace; in particular they are more widely dispersed along axis 2. In contrast, the species of the Tiputini assemblage are more clustered on axis 2, with less apparent diversification between the rollers and tunnellers. The distribution of species in the more depauperate assemblages was not significantly different from the richer assemblages in their respective regions, and were evidently similar to one another, although this may be a function of the low species richness. The species of both Pine Ridge and the West Coast National Park are quite widespread in morphospace, supporting the idea that this may reflect niche partitioning in harsh environments (chapter3).

Whilst we saw a divergence in functional composition in the species rich habitat types in chapter 4 (figure 4.5), all functional groups were still well represented. In figure 5.11 however, the same functional groups from the two different assemblages are positioned differently along axis 2, so that its appears that the morphologies of the African rollers and tunnellers are more different to one another, whilst the rollers and tunnellers at Tiputini are more similar (even if no statistical significance can be attached to this).

There are several possible reasons why this might be so. Firstly, there may be a phylogenetic component to the morphology, where different origins of rolling have slightly different morphological adaptations, which have persisted in the different lineages. What is interesting is that the potential divergence in morphologies is seen on axis 2, the same axis which the Mantel test found to be correlated with phylogenetic distance. This suggests that there is a phylogenetic component to the main morphological characteristics of rollers and tunnellers, which endures through cladogenesis, just as we have seen in the two independent dwelling lineages. In this case, the relatively wide distribution of the different African rolling groups may reflect phylogenetic divergence between them or independent origins of the behaviour. Unfortunately, the phylogeny does not have the required level of resolution to explore this idea further with the African groups. However, the Neotropical rollers do appear to

consist of a closely related set of taxa, which would support this idea, since they have a tendency to be less divergent in morphospace than the African rollers.

An alternative explanation might be habitat-related. Tiputini and Kruger are certainly good representatives of the most species-rich and diverse assemblages that exist on the two continents, but the different habitat types means that they are likely to be subject to different environmental filters. Therefore, although each fauna has the same broad functional groups, their natural history may be subtly different because of the habitat, so influencing their functional morphology. It might be informative to include an African rainforest assemblage in the analysis, so as to compare more similar habitats. However, as discussed previously, the taxa of the African forests are to a large degree, of the same genera as those occurring in the savannas. Furthermore, I have noted a crucial difference in the species richness of rollers in the African forests compared to the Neotropical ones. In Africa, the rollers only really flourish in the open habitats, whereas the opposite is seen in the Neotropics. In the Ivory Coast for example, Cambefort and Walter (1991) reported the presence of only 5 species of roller, compared to 21 in a nearby savanna. Thus there appears to be a clear difference in the most benevolent habitat type for the rollers of the two continents, which I have suggested may be due to the type of habitat which acted as the primary theatre of evolution in the two regions. For some reason, the African savanna-dwelling rollers may be more competitive if they are more functionally different to the tunnellers. The opposite seems to be true in the Neotropics, where both functional groups are less differentiated from each other. Perhaps there is an optimum morphology for a dung beetle in a rainforest, which largely precludes the African rollers from utilising such habitats because of their more extreme morphologies, and which constrains the Neotropical beetles to be relatively conservative in their form. Such a hypothesis certainly requires more investigation, including data on the African forest assemblages, and perhaps a comparison of the natural history and function of the African and Neotropical rollers, to identify how this might affect their morphology. Of course it is quite likely that environmental differences and phylogenetic history would both play a significant part in this apparent divergence.

5.4.6 Phylogenetic influences on morphology

Species located in similar regions of the morphospace can be interpreted to be influenced by similar relationships between morphology and ecology, but this can also be evidence of shared ancestry (Ricklefs and Miles 1994). From the convergence of the Eucraniini and *Pachysoma* (although unmeasured in morphospace), it appears that the environment or ecology of a dung beetle can select for a particular body form, which may be quite different from the ancestral morphology. Two further examples of this are found in the Australian fauna. The tunnelling genera *Coptodactyla* and *Demarziella* were consistently recovered in the phylogeny within the Australian canthonines, although they were originally considered to be the sole representatives of the tribes Coprini and Dichotomiini in the continent. The evidence that they are nested within the endemic rollers is therefore good. In some cases, the addition of species to a community may only be facilitated by the development of novel ways of exploiting the available resources (Ricklefs and Schluter 1993). In this case, these taxa have undergone a transition from rolling to tunnelling, presumably because the tunnelling niche would have been effectively empty before the colonisation of the continent by *Onthophagus*, and is still relatively unoccupied compared with the fauna of Africa and the Neotropics. This is a particularly good example of adaptive radiation in the Scarabaeinae, seemingly to take advantage of an empty or less-competitive niche space. The position of the two genera is indicated on figure 5.4, and both are found well within the 'tunnelling' morphospace, but each has invaded a different area of it. The species of *Coptodactyla* are seen to be amongst the largest tunnellers in the fauna, with a mean body length of 13.5 mm, whilst the species of *Demarziella* are much smaller, with a mean length of only 4mm (Cambefort 1991a). Because *Demarziella* and *Coptodactyla* do not appear to be closely related, this represents two independent switches in functional behaviour, accompanied by a shift in functional morphology. Not only does this finding refute the suggestion that rolling is always a more derived behaviour than tunnelling, but it also indicates that knowledge of phylogenetic history can not necessarily predict morphology, and neither can morphology always predict phylogeny.

Just as *Demarziella* and *Coptodactyla* have apparently become adapted to a tunnelling way of life to increase the niche separation between them and their sister taxa, it seems that the *Onthophagus* of Australia may have also undergone a morphological change (as depicted in figure 5.12) in order to exploit a less competitive niche. In Australia, they

are seen to have generally larger body proportions, and are found to be in a similar area of morphospace (on axis 3) as groups such as *Catharsius*, *Copris* and *Dichotomius*, which are all large tunnellers in other regions of the world. I suggest that the *Onthophagus* in Australia are not only exploiting the niche of small tunnellers, but that of large tunnellers, which is otherwise unoccupied in Australia by any other group except *Coptodactyla*, which has also apparently undergone morphological adaptation for similar reasons. It seems likely that a change in the morphology of *Onthophagus* (along axis 3) would be less constrained by phylogenetic inertia than the kind of change undergone by *Coptodactyla* and *Demarziella*, which must have undergone much more significant morphological changes in order to switch functional groups (along axis 2). This is probably why phylogeny was found to be correlated with axis 2 in morphospace, but not with axis 3.

In contrast to these examples, the findings of the Mantel test, although rather over-simplistic, suggests a general correlation between the gross morphology of rollers and tunnellers and their phylogenetic history. Such a finding can be supported by a number of examples. One of these is *Heliocopris marshalli*, the only large tunneller of any continent, with a positive axis 2 value (indicated in figure 5.2). It is therefore positioned in morphospace more closely to various members of the Scarabaeini tribe than to other tunnellers. This is unusual, since the rollers and tunnellers tend to diverge more in morphospace as they become larger, but if we consult the phylogeny, it becomes evident that the genus *Heliocopris* is consistently recovered as the sister group to the roller *Anachalcos*, and is the only tunnelling taxon in an otherwise rolling clade (see figure 2.3). Just as the morphology of the African and Neotropical dwellers also appears to be constrained to some degree by their phylogenetic history, so *Heliocopris* appears to still retain some of the key characteristics of its ancestors.

5.4.7 Conclusions

The morphometric study of an assemblage or regional pool is a useful way to characterise the ecology of the organisms in question, and gives us an important mechanism with which to compare and contrast the faunal structure of different regions. However, to fully exploit the descriptive power of this approach we need a good understanding of the phylogenetic and biogeographic history of the organisms, since these are essential elements influencing the ecology and hence the morphology of the

different taxa. Using our estimates of the scarabaeine history, we have addressed questions about how certain traits characterising a particular function have originated, and identified convergent 'trajectories' in the morphospace of independent species pools. Knowledge of the phylogenetic history has also allowed us to identify true convergence in morphology (adaptation), as distinct from shared ancestry, whilst the morphology has also supported earlier phylogenetic estimates of independent ancestry (in the dwellers for example).

The ecology, and hence morphology of a species is apparently influenced by an inextricable combination of phylogenetic and biogeographic history, regional and local environmental conditions, and inter-species interactions. In the Scarabaeinae, it seems that the key aspects of functional morphology (roller or tunneller characteristics) are generally constrained by ancestral history. This means that a taxon will generally not diverge from an ancestral rolling or a tunnelling morphology, unless environmental or ecological pressure is significant enough to 'override' this phylogenetic constraint. It seems that if the taxon can function effectively without altering its functional morphology, these traits will persist (in the dwellers or Heliocopris for instance). However, when subject to strong inter-species competition perhaps (*Demarziella*, *Coptodactyla*), or to a harsh environment (*Pachysoma*, Eucraniini), species will apparently converge on the most effective morphology to suit its function. Although the different continental faunas are seen to diverge in various ways, at the level of the regional pool, ecologically similar scarabaeine groups occupy similar morphological space. Dung beetle assemblages can be said to largely converge on an analogous structure, with the differences seen explained by unique phylogenetic or environmental circumstances.

Chapter 6. Summary and Conclusions

Throughout the preceding chapters, I have attempted to identify the key patterns and processes in the phylogenetic history and structure of local and regional dung beetle assemblages. However, it would be useful in this final section to summarise the key findings of the project and to attempt to bring together the various strands of argument, in order to identify the most important processes which appear to have been central to the evolution of dung beetle assemblages across the world.

6.1 Phylogenetic history

One of the primary aims of this study was to produce an estimate of the Scarabaeinae phylogeny, in order to address a number of unanswered questions as to the origins of dung feeding and the ancestral feeding strategy, whether the main functional groups were indeed monophyletic, and how the group had evolved in different biogeographic regions.

Despite a lack of robust resolution at the deeper nodes of the phylogeny, it appears that dung feeding evolved at a basal node in the Scarabaeinae, from the presumed primitive state of saprophagy, which still persists in extant taxa at the base of the tree. A number of the scarabaeine tribes were found to be polyphyletic or paraphyletic. Most notably, the Canthonini and Dichotomiini appear to be artificial groups, consisting of a variety of distinct lineages. All three nesting strategies (rolling, tunnelling and dwelling) were also found to be polyphyletic, and there appears to be considerable plasticity in the functional roles of the group. Rolling appears to have arisen on several different occasions and, significantly, to have first originated at a very basal node in the Scarabaeinae. Dwellers have originated independently from both rolling and tunnelling ancestors. Another key finding was that the phylogenetic history of the group seems to be closely associated with continental history. This means that the regional species pools of the three main biogeographic regions (Australia, the Old World and the Neotropics) appear to be dominated by a lineage endemic to that region.

6.2 Functional diversity

Another major goal of this project has been to elucidate the factors responsible for the observed patterns of diversity in dung beetle assemblages, at both a local and a regional scale. Assemblages were characterised in terms of their functional diversity and compared by multivariate ordination both within and between biogeographic regions. From a range of environmental variables (acting from a regional to a local level), the variation in diversity across local assemblages within a region was found to be most closely correlated with habitat type, both in the Neotropics and Africa. Compared globally, the most species-rich habitat type was seen to vary between regions. Functional diversity (nesting group) was seen to diverge in the most species-rich assemblages of different regions, but to be convergent in less species-rich habitats, where environmental conditions were harsh. This was suggested to be due to strong environmental filters having a greater influence on functional diversity than phylogenetic ancestry or local species interactions. A regional investigation into assemblage structure, as defined by the species feeding behaviour, showed similar convergence under less favourable conditions, although not so clearly. There was also a correlation between the species richness of necrophagous and generalist Scarabaeinae and the richness of small, omnivorous and carnivorous mammals.

Feeding behaviour in assemblages was found to be extremely variable both within and between regions. The Neotropical taxa clearly have the greatest propensity for necrophagy (or generalism), whilst coprophagy predominates in the African assemblages, regardless of habitat type. Non-coprophagous feeding behaviour is taxonomically widespread, and no apparent phylogenetic or morphological constraint is evident, rather it seems that the unique biogeographic history of each region, and local factors (species-species and species-environment interactions) influence the diet choice to a greater degree. Diet choice seems to be an important mechanism of niche partitioning, as the most morphologically and ecologically similar species were seen to have the greatest variation in feeding behaviour in the Neotropics.

6.3 Morphological variation

The ecological separation of functional groups was reflected by their separation in morphospace, and the role of phylogenetic inertia was determined to be a significant

component in the maintenance of key functional traits within lineages. Evidence was seen of convergent ecology in independent lineages, without accompanying convergence in form (in the dwelling groups), indicating the strength of historical effects upon ecomorphology. However, in some instances, phylogenetic ancestry can apparently be subordinate to strong environmental factors or species interactions driving niche diversification, since convergence of morphology and function in unrelated groups was also identified. This phenomenon may be of key significance in explaining the cause of the numerous switches in functional roles observed in the phylogeny. An ecological cost of specialisation was identified, in terms of a trade-off between rolling and tunnelling morphologies. Overall, morphospace has an approximately similar shape and functional structure between continents, but the subtleties of area and morphospace occupancy are subject to differences attributable to unique regional histories.

6.4 The evolution of dung beetle assemblages

In addition to the key evolutionary hypotheses of scarabaeine evolution, this project has identified a number of major drivers of dung beetle assemblage structure and diversity. I have presented a variety of findings which, considered together, suggest that any assemblage is influenced at a variety of levels, from large-scale regional historical processes to local environmental factors and species-level interactions. By virtue of the scales at which these different drivers are acting, they can be ordered into a hierarchy of importance, although it is of course the amalgamation of all of these effects which ultimately determines the structure of the assemblage. The most influential and pervading of these drivers is phylogenetic history. As we have seen, the phylogeny of the group closely reflects the environmental and geological history of the separating continents, so that the effects of phylogenetic and biogeographic history cannot be clearly separated with the data at hand. As such, 'history' influences each regional species pool, local assemblage and individual species in a different way. This is best explained in terms of a series of processes or filters, operating at different temporal and spatial scales (Angermeier and Winston 1998, Lawton 2000).

Within regions, I have identified habitat type to be the local environmental variable most strongly correlated with functional diversity. This is however, a composite of a range of environmental factors, and will also reflect the vegetational and climatic

history of the continent. The species richness and functional diversity of scarabaeine assemblages are seen to be affected by habitat quality, but also by the unique histories, persistence and absolute age of the habitat types on a given continent.

Only after these regional and local filters have influenced the species pool do the smaller-scale species-level interactions play their role in assemblage structure. Although there is little direct evidence of inter-specific competition in dung beetles, a number of examples of apparent niche partitioning and diversification have been observed in this project, and it seems that interactions at this level do play a critical role in the localised structure of assemblages. Up until now however, these relatively small-scale processes have been the subject of most research. The combination of techniques employed in this project has enabled us to take a broader view of the field, by incorporating information from the level of species interactions right up to continental scale processes. The phylogeny has provided a critically important framework in which to set this study, even if it is not yet fully resolved. The project has also produced a beneficial way of quantifying and comparing local and regional scarabaeine assemblages, using morphospace, so that we can view and interpret them in a totally new way. It will also help to set the local scale studies in a regional, historical context. A key prediction from this study might be that other assemblages or taxa which also appear to be highly influenced by inter-species competition for patchy resources (and hence subject to strong pressures for convergent evolution), should still reveal strong historical differences when considered in a cross-regional context.

6.5 Avenues of future research

A number of possibilities for future study suggest themselves from this project. I include here several of those which might most enhance the research done in this project, and some of the most potentially interesting ones.

The addition of more characters to the phylogenetic data set is of paramount importance to obtaining a more robust phylogeny. To estimate the relationships between the more basal clades requires a number of gene loci which are relatively more conserved than the three genes investigated in this project. 18S has been tried for this purpose, but proved too conservative, and so choice of the gene region is critical. Having better support for the more terminal relationships, only a few taxa from each monophyletic

clade would need to be sequenced. To more fully elucidate the origins of the basal groups (the various 'canthonines' and 'dichotomiines') would be of particular interest, since this would support or refute many of the arguments regarding the biogeographic history of the group presented here. One further group would be particularly interesting to add to the phylogeny - the Madagascan canthonines, which comprise 14 different genera. Based on morphology, these have been suggested to be most closely related to the Australian canthonines (F. Genier pers. comm.). The isolated history of Madagascar since the early Cretaceous (Cox and Moore 1993) means that the island may represent a fourth main biogeographic realm for dung beetles, just as the endemic lemur families of Madagascar suggest a basal division from the other primate lineages.

A Madagascan dung beetle assemblage would also be of great interest to compare with the other regional faunas regarding assemblage structure. The Madagascan fauna is primarily restricted to tropical forest, and the application of the standardised trapping protocol here, as well as in a West African rainforest would enable an interesting comparison of Australian, African, Madagascan and South American rainforest assemblages; four (potentially) phylogenetically independent lineages, evolving in the same habitat type. Such a study would allow us to largely factor out habitat effects, and address purely historical influences on assemblage morphospace and functional diversity.

Quite how the genus *Onthophagus* has been so successful, colonising all major biogeographic regions, and contributing some two-fifths of all scarabaeine species, remains uninvestigated. In particular its presence in Australia takes some explaining, when only one other clade, apparently of ancient, possibly Gondwanan, origin occurs there. Additional sequencing of species from different regions, and the application of a molecular clock to the group might prove more informative as to how old the group is, and how rapidly it is able to speciate. This may tell us whether the group was in fact present in Australia before its division from Africa, or (more likely) whether it is simply successful at colonising new assemblages across barriers impenetrable to other groups. Matthews (1972) has suggested that *Onthophagus* entered Australia from the north on up to 34 different occasions. Phylogenetic data would be able to clarify which are the sister taxa of the Australian species, as well as the number of colonisation events.

The evolution of different feeding behaviours within the Scarabaeinae appears to be an important mechanism of niche differentiation, and in the evolution of different lineages and regional species pools around the world. Although no phylogenetic or morphological constraint has been observed, this may be due to the level of investigation undertaken. For example, the linear measurements of gross functional morphology may not elucidate any more subtle differences in the morphology of the different feeding strategies – in the mouthparts, or gut morphology for example. Gut morphology proved highly informative in the characterisation of various termite functional groups, where gross morphology did not (Donovan *et al.* 2000). Certain scarabaeine lineages may have a greater predisposition to carrion feeding due to a particular functional adaptation which enables a wider range of resources to be utilised. Use of the phylogeny, information on diet from the standardised trapping protocol and more detailed investigation of the morphology associated with feeding may reveal hitherto unrecognised explanations of feeding behaviour, at both a species and a regional level.

In order to investigate further the way in which new species are able to colonise dung beetle assemblages, and how the assemblage evolves, one could attempt to identify how species are added to the morphospace of an assemblage. Are they added to the periphery of morphological (and hence ecological) space, or within the space of the established species (and thus sharing similar niche space with them). Ricklefs and Travis (1980) found that bird species were added to the periphery of ecomorphological space, whilst the interior of the community remained quite conserved. One particular way of addressing this in dung beetle assemblages might be to examine the success or failure of the various species introduced to Australia in the last 30 years to deal with the cattle dung problems. Some of these species have reportedly been more successful than others, spreading from the cattle pastures into the endemic assemblages of the open forests and rainforests (G. Monteith unpublished). Have the successful species joined these assemblages at their periphery, and might the less successful species have been prevented from colonising them because their ecologies would dictate that they be added to the established space? The addition of the introduced species to the Australian morphospace data set might prove highly informative.

Finally, although the combination of the various approaches employed in this project has enabled us to view the evolution of dung beetle assemblages in a new way, it would

benefit from one or two novel analytical techniques with which to more fully explore the data collected. For example, a means to better integrate and test the association between the phylogenetic and morphometric information might reveal the full extent of phylogenetic constraint on morphology and assemblage structure.

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