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ADAPTATION TO THE HOST-PLANT, AND THE EVOLUTION OF HOST  
SPECIALIZATION, IN 'CYCAD WEEVILS' (COLEOPTERA: BRENTIDAE)

by

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A beetle may or may not be inferior to a man - the matter awaits demonstration; but if he were inferior to a man by 10 000 fathoms, the fact remains that there is probably a beetle view of things of which man is entirely ignorant.

G.K. Chesterton, "On Humility", in *"The Defendant"*, 1901. Quoted by  
R.A. Crowson in *"The Biology of the Coleoptera"*, 1981.



**FRONTISPIECE.** Top left. A group of mature female *Encephalartos altensteinii* plants in which the tallest plant is bearing three megasporangiate cones. Top right. An *Encephalartos longifolius* plant with a single megasporangiate cone. A 30 cm ruler has been placed alongside the cone. Bottom left. A transverse section through a mature megasporangiate cone of *Encephalartos caffer*. Bottom right. An *Encephalartos altensteinii* seed that has been opened to show the large numbers of *Antliarhinus zamiae* individuals that may emerge from a single seed.

## RÉSUMÉ

This thesis deals with host relationships in an enigmatic and seemingly primitive group of weevils belonging to the genus *Antliarhinus* (Coleoptera: Brentidae). These beetles occur only on species of the cycad genus *Encephalartos* and appear to retain an ancient association with cycads, a group of plants that were widespread in the Mesozoic era (ca. 200 MYA) before the rise of the angiosperms and which are now represented by 11 genera with relict distributions in the tropics and sub-tropics.

The primary aim of this research was to determine the possible causes of narrow host specialization in *Antliarhinus zamiae* (Thunberg) and *A. signatus* Gyllenhal, two species which develop exclusively on the ovules of their cycad hosts. The causes of host specialization in phytophagous insects are currently a subject of considerable debate with conflicting viewpoints on the relative importance of evolutionary driving forces, genetic constraints, and present ecological factors, for establishing and maintaining narrow host ranges (Chapter 1). Studies on the causes of host specialization in *A. zamiae* and *A. signatus* provide insights from host relationships that have evolved over a considerable period of time and which may have been influenced by the unique biochemical and morphological attributes of their unusual host-plants.

Chapter 2 concerns host records for *A. zamiae* and *A. signatus*, as well as two other species of *Antliarhinus*. Special emphasis is placed on the effects of plant distribution, plant rarity, and plant taxonomy on the recorded host relationships.

In Chapter 3, the possibility is examined that host specialization in *A. zamiae* and *A. signatus* is a consequence of larval adaptation to the high concentrations of toxic and unusual chemical compounds that occur in the tissues of the cycad ovule. The toxicity of ovule tissues in general, as well as one of the known chemical compounds from ovule tissues, is tested on various insects associated with cycads is examined. In addition, survival and performance of *A. zamiae* and *A. signatus* larvae are compared on different cycad hosts to determine whether differences at this level can explain narrow host ranges in these insects.

Ovipositional traits are examined in Chapter 4 to determine how *A. zamiae* and *A. signatus* oviposit into ovules that are concealed within a compact cone, and the evolutionary history of ovipositional traits is discussed. This provides a basis for establishing the possible effects of ovipositional traits on host specialization in *A. zamiae* and *A. signatus*.

Chapter 5 deals with the effects of ovipositional traits in *A. signatus* and *A. zamiae* on their distribution within cones of *Encephalartos altensteinii*. Particular emphasis is placed on the effects of sporophyll thickness, cone compaction and thickness of the ovule integument in the host-plant on successful oviposition by *A. signatus* and *A. zamiae*.

In Chapter 6, cone and ovule structure, and their effects on oviposition by *A. signatus* and *A. zamiae*, are compared for nine species of *Encephalartos*. The aim of this comparison was to establish whether host specialization by *A. signatus* and *A. zamiae* is affected by the ability of the adult female to oviposit into the ovules of different cycad species.

Chapter 7 deals with variability in snout length in *A. zamiae* females, and its relevance for the ability of *A. zamiae* females to oviposit into the ovules of a broad range of *Encephalartos* species. Attention is also paid to differences in snout length between females from different host populations and the significance of these differences for speciation in *A. zamiae*.

Chapter 8 focuses on the need for synchrony between ovipositional activity and the appropriate stages of cone and ovule development in the host-plant. The significance of synchrony with the host-plant for host specialization is discussed.

Finally, in Chapter 9, the results and ideas presented throughout this thesis are discussed in a short essay on host specialization in *A. zamiae* and *A. signatus*. The importance of larval adaptation to the ovule tissues, adaptation for oviposition into concealed ovules and behavioural synchrony with the host-plant are evaluated. In addition, the importance of an understanding of adaptations to the host-plant, for interpreting host specialization in insect herbivores, is discussed.

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## CHAPTER 1

### INTRODUCTION

Almost all plants are fed upon to some extent by insect herbivores (Thorsteinson, 1960; Strong *et al.*, 1984). Yet the vast majority of insect herbivores feed on only a limited number of the available plant species. Most phytophagous insects feed on a few taxonomically closely related host species, usually within the same plant genus or family (Jermy, 1976; 1984), and many feed on a single plant species. Less than 10% of insect herbivores feed on plants in more than three families (Bernays & Graham, 1988). Thus, although all plants appear to provide the essence for survival of at least some insect herbivores, narrow host specializations predominate amongst insect herbivores.

The predominance of host specialization in phytophagous insects has been interpreted as indicating that, as a rule, selection favours specialization over generalization in almost all insect/plant interactions (see review by Jaenike, 1990). Several attempts have been made to identify a dominant selective force favouring host specialization (see reviews by Brues, 1924; Fraenkel, 1959; Thorsteinson, 1960; Jermy, 1984; Bernays & Graham, 1988; Jaenike, 1990) but it appears that exceptions have always been found to disprove the rule (Barbosa, 1988; Thompson, 1988a). It is worthwhile summarizing the progression of recent ideas in this regard to provide a background for the introduction of alternative theories of host specialization.

Until recently, the most widely accepted theory for the cause of host specialization by insect herbivores has been the coevolutionary theory expounded by Ehrlich & Raven (1964) and subsequently developed by many others (e.g. Breedlove & Ehrlich, 1972; Feeny, 1975; Janzen, 1980a; Spencer, 1988a and other contributions in this volume). In its simplest and strictest form (Janzen, 1980a), the coevolution theory can be summarized as follows. Feeding by insect herbivores is expected to result in reduced vigour, and reduced reproductive potential, of the host-plant. As a result, the plant evolves defences against insect herbivores. Defence genes are expected to spread in populations as a result of the reproductive advantage of "defended" over "undefended" plants. Adaptation by insect herbivores to the plant's defence mechanisms result in an adaptive advantage for these insects, principally due to the absence of other competing insect herbivores. The reciprocal actions of plant defence and counter adaptations in insect herbivores are expected to result in an evolutionary refinement of the plant/insect interaction and increasing host specialization among insect herbivores.

So called "secondary plant compounds", which are phytochemicals with no apparent primary metabolic function (Fraenkel, 1959), have been singled out as the most significant defence mechanisms against insect herbivores (Fraenkel, 1959;

Ehrlich & Raven, 1964; Dethier, 1970). Consequently, biochemical coevolution between insect herbivores and secondary plant compounds has been regarded as the dominant force in the evolution of host specialization in insect herbivores (Ehrlich & Raven, 1964; Dethier, 1970; Labeyrie, 1976; Swain, 1978; Spencer, 1988b).

Secondary plant chemistry is generally acknowledged as the single most important proximal cue for the acceptance or rejection of potential host-plants by insect herbivores (Jermy, 1984; Bernays & Graham, 1988; Jaenike, 1990). However, the adaptive significance of behavioural or physiological sensitivity to secondary plant compounds in insect herbivores, and specifically any coevolutionary interpretations of such sensitivity, have been questioned (e.g. Jermy, 1976; 1984; Bernays & Chapman, 1978; Bernays & Graham, 1988). Important inconsistencies between the chemical coevolution theory and available data are as follows.

1. Chemical coevolution theory accounts mainly for one type of host specialization among insect herbivores, i.e. the existence of taxonomically closely related insects on taxonomically closely related plants. Other major categories of host specialization such as the dispersion of taxonomically similar insects on different families of host-plants are not adequately accounted for (Jermy, 1984).

2. Similarly, reciprocal coevolution requires that genetic lineages of interacting species continue to coexist (Futuyma & Slatkin, 1983). As a result, the phylogenies of insect herbivore taxa and their host-plants may be expected to be congruent. Such congruence has been found in some phylogenetic studies of butterfly/plant relationships (e.g. Benson *et al.*, 1976; Spencer, 1988b) but contrary findings have also been found in butterfly/plant phylogenies (Vane-Wright, 1978) as well as in other insect/plant relationships (Craig *et al.*, 1988). These latter studies show that insect herbivores have shifted between different plant lineages during the course of their evolution.

3. Plants may be subject to various selection pressures over time, of which herbivory by a single insect species will form only a part. Reciprocal coevolution between insect herbivores and their host-plants may, therefore, occur only under specific conditions (Fox & Morrow, 1986) such as in short lived annual plant species where feeding by a single herbivore species may impose significant selection on the host-plant (Strong *et al.*, 1984). In more complex systems, changes in one component species are predicted to affect all other species in the system. For instance, all herbivores feeding on a plant would be expected to respond to the defensive properties of the host-plant even if feeding by only one herbivore species was responsible for the evolution of a particular defensive trait (Fox, 1988). This "diffuse" coevolution may be widespread (Strong *et al.*, 1984)

but is probably difficult to prove since no clear predictions can be made about the outcomes of such interactions (Fox, 1988).

4. Deterrent secondary plant compounds are often not toxic when fed to insect herbivores nor do they necessarily indicate the presence of other toxic compounds in the plant (Bernays & Chapman, 1978; Bernays & Graham, 1988). This means that behavioural responses by insect herbivores to secondary plant compounds may not represent avoidance of plant chemical defences. Responses to chemical cues may have arisen for other reasons.

5. Host relationships may be ecologically labile (Bernays & Graham, 1988). Host shifts by insect herbivores, even between taxonomically distant plant species, have occurred within this century (Brues, 1924; Strong *et al.*, 1984; Bernays & Graham, 1988). Colonization of crop plants, and other introduced plants, by native species provide good examples of such host shifts (Strong *et al.*, 1984). These examples indicate that host shifts by insect herbivores may occur over relatively short periods and are not always limited to plant species with which they share a coevolutionary history.

Some reservations have been expressed about the extent to which each of the above points contradicts the expectations of the coevolution theory (e.g. Rausher, 1988; Ehrlich & Murphy, 1988). However, notwithstanding these reservations, it is widely acknowledged that the role of chemical coevolution as the predominant driving force in the evolution of host specificity in phytophagous insects has been over-emphasized (e.g. Feeny, 1975; Jermy, 1976; 1984; Barbosa, 1988; Bernays & Graham, 1988; Janzen, 1985a,b, 1988; Thompson, 1988a).

The rejection of the coevolution theory as a general explanation for host specificity in phytophagous insects has been accompanied by further attempts to identify a dominant selective force in plant/insect interactions. A notable example is the conclusion of Bernays & Graham (1988) that only predation, specifically by generalist predators, is sufficiently universal to explain host specialization by insect herbivores in general. In their view, differential predation of insect herbivores on different host-plants would, theoretically, select for narrow host specialization on those plants associated with the lowest levels of predation. Host switching is expected to occur as a result of increased levels of predation on established host-plants and the existence of "enemy-free space" (Jeffries & Lawton, 1984) on novel host-plants (Bernays & Graham, 1988).

Several publications have confirmed that differential predation of insect herbivores takes place on different host-plants (Bernays, 1989; Denno *et al.*, 1990). It also seems logical that both cryptic and aposematic insects may be bound to their host-plants as part of their defence against predators. However, the generality of this explanation for the evolution of host specialization in insect herbivores, and the extent to which host switches occur in these insects, still need

to be established. Only a few examples exist of host switches to entirely new hosts (Jermy, 1988). Other host switches may represent induced preferences within an established host range (Jermy *et al.*, 1968) or reversion to a previously broader host range (Brues, 1924). In these instances, predation may offer only an explanation for host shifts within an already narrow host range and would therefore not explain all aspects of host specialization. Put differently, predator avoidance may represent only one level of adaptation to the host-plant. The range of possible hosts may be established at a different level.

Further, evolutionary conservatism of host range (i.e. the retention of similar host ranges in phylogenetically related insects) appears to be common in insect herbivores (Zwölfer, 1982; Jermy, 1984; Jaenike, 1990) and it is doubtful that predation alone could sustain this. Many generalist predators show frequency-dependent preferences for different prey types (Krebs, 1978) with mostly the commonest prey being eaten (Murdoch & Oaten, 1975). Generalist predators, both vertebrates (Murdoch & Oaten, 1975) and insects (Lawton *et al.*, 1974), may learn to feed on common prey types and may establish search images for these prey. They may also focus their feeding activity on areas of the highest prey density (Hassell & May, 1974). These feeding patterns suggest that predation is likely to vary in space and time. Consequently, if predation by generalist predators is the dominant driving force for host specialization among insect herbivores, then temporal and spatial variation in host range should be more common than it apparently is.

Thus although the ecological and evolutionary effects of predation on host specialization in phytophagous insects still have to be widely tested, current evidence does not support predation as the dominant driving force in plant/insect interactions (Fox, 1988; Jermy, 1988; Jaenike, 1990).

A unifying theory for host specialization in insect herbivores that is based upon a dominant selection mechanism, e.g. chemical coevolution or predation, appears to be elusive. The common habit of feeding on plant tissues suggests that, to some extent, insect herbivores must be subject to similar selection pressures. However, the vast array of plant and insect species involved in these interactions, and the diversity of feeding habits, indicate that the causes of host specialization in insect herbivores may be diverse (Craig *et al.*, 1988; Thompson, 1988a). In addition to feeding, various other life history characteristics of insect herbivores may be associated with the host-plant, including mate location (Huignard, 1976; Labeyrie, 1976, 1978), predator avoidance (Jeffries & Lawton, 1984; Bernays & Graham, 1988; Bernays, 1989), oviposition (Masaki, 1986) and tolerance of desiccation (Southwood, 1973). Adaptation to the host-plant at these levels may mean that narrow feeding specialization is simply an evolutionary effect (*sensu* Williams, 1966) of specialization at another level. Genetic constraints on adaptation to the

host-plant at one or more levels (Mitter & Futuyma, 1983; Jermy, 1984, 1988), including phylogenetic constraints on adaptation to the host-plant (Zwölfer, 1982) and pleiotropic effects of one level of adaptation on another, may limit the extent to which insect herbivores can utilize different host-plants and therefore be important for the evolution of host specialization. As a corollary, release from these constraints may facilitate colonization of new host-plants or new parts of plants. Consequently, determining the level(s) of adaptation to the host-plant and understanding host relationships at that level may be crucial to understanding host specialization in insect herbivores.

Such possibly diverse causes of host specialization in phytophagous insects have been rejected by Bernays & Graham (1988) because they do not explain the general predominance of host specialization among insect herbivores. In other words, they may only explain host specialization in specific instances. However, this criticism is only valid if there is indeed universal selection for host specialization. This may not be so since the predominance of host specialization could result simply from increased speciation rates in lineages that have a consistent history of specialization. In other words, specialist species give rise to new specialist species (Jermy, 1988) at a faster rate than generalist species give rise to new generalist (or new specialist?) species. The clustering of host-plant specialists in the most species rich families of insect herbivores (Price, 1980), originally regarded as support for reciprocal coevolution, lends support to this conclusion.

Jermy (1984; 1988) has argued that only changes in the insect nervous system, and concomitant changes in the ability of the insect to recognize and utilize host-plants, are important for host specialization and speciation. In Jermy's view, host specialization arises from limitations on the nervous system to recognize a variety of plant species. Similarly, relatively small changes in the insect nervous system may preadapt insect receptor systems for novel hosts and result in new associations with these hosts. These new associations may lead to a reduction of gene flow between populations on different host-plants and, consequently, to speciation. This theory is intuitively appealing, but there are two assumptions that must be accounted for. Firstly, it is assumed (Jermy, 1988) that changes to the nervous system alone can facilitate colonization of new plants or plant parts. This means that other life history characteristics must be sufficiently plastic to incorporate the new host-plant. However, if this is not so, then selection may be expected to act against genes that facilitate changes to the nervous system and ultimately to host recognition systems. It is, therefore, still necessary to determine at what level of interaction with the host-plant, restriction to only a few host-plants is likely to be established. Secondly, this argument presupposes that speciation of specialist insect herbivores is mostly associated with new host-

plants. However, Mitter & Futuyma (1983) have pointed out that speciation is often not associated with host transfers and they quote an example of leafhoppers from Ross (1962) in which about 70% of speciation events involved no host shifts. In these instances, speciation events may be facilitated by different interactions with the same host-plant. It is therefore important to determine whether different levels of adaptation to the host-plant do affect host specialization and whether they influence the propensity for speciation.

The study of adaptation to the host-plant was applied here to an investigation of host relationships in cycad weevils belonging to the genus *Antliarhinus* (Coleoptera: Brentidae) [the nomenclature and classification used here follow that used by the taxonomists currently revising the genus, i.e. R.G. Oberprieler (in litt., 1989) and G. Kuschel (in litt., 1989) and therefore differs from previous classifications]. In this thesis, special emphasis has been placed on host relationships in *Antliarhinus zamiae* (Thunberg) and *Antliarhinus signatus* Gyllenhal, both species that develop within the ovules of cycads belonging to the genus *Encephalartos* (Zamiaceae) (Annæcke & Moran, 1982; Giddy, 1984). An examination of host specialization in these beetles is appropriate for three reasons.

1. Many species of *Encephalartos* are threatened with extinction (Goode, 1989; Osborne, 1989) and high seed mortality caused by *A. zamiae* and *A. signatus* (Giddy, 1984) may further endanger their survival (Donaldson, In press-a, Appendix 1). Concern has been expressed particularly about the possible spread of these insects to cycads that have not yet been recorded as hosts (Goode, 1989; Donaldson, In press-a). Studying the causes of host specialization in these insects forms part of determining this possibility.

2. Insect herbivores, in which the larvae feed on seeds, are often specific to a single host-plant (Janzen, 1971, 1978; Smith, 1975). For instance, in a study of Costa Rican deciduous forest containing more than 975 species of dicotyledonous plants, 110 species of seed-feeding insects were found (Janzen, 1980b); 83 of these species were reared from only one plant species, 14 species had two host-plants and nine species had three hosts. Only four insect species with seed-feeding larvae were reared from more than three host-plants. Similar specificity has been recorded in other studies of seed-feeding insects (Janzen, 1971; Center & Johnson, 1974; Smith, 1975) and host specialization has usually been attributed to interactions between the insect herbivore and plant defence mechanisms. Seeds are amongst the most nutritious plant tissues (Murray, 1984), illustrated by the high assimilation rates of insects feeding on them (Wightman, 1978), and it is therefore expected that they would need to be protected from herbivores (Janzen, 1969, 1971; Center & Johnson, 1974; Janzen *et al.*, 1977). An array of plant characteristics have been identified as defensive traits against seed-feeding insects in legumes (Janzen, 1969) and a similar array of counteradaptations have been

identified in bruchid beetles (Janzen, 1969; Center & Johnson, 1974). If similarly diverse interactions occur between seeds and the insects that feed on them in other systems, e.g. on cycads, then cycad weevils would be an appropriate group to examine the relationship between adaptation to the host-plant and host specialization.

Seeds and ovules often contain powerful toxins (Janzen *et al.*, 1977; Bell, 1978; Janzen, 1978) that have been considered as potential defence mechanisms against insect herbivores (Bell, 1978; Janzen, 1978). Cycad seeds and ovules are no exception; the persistent megagametophyte, which is the functional equivalent of the endosperm in angiosperm seeds (Bewley & Black, 1978), contains potent mutagens (Bell, 1978; Hoffmann, 1990). The study of host relationships in cycad weevils therefore provides an opportunity to examine the relationship between seed chemistry and host specialization in these insects.

3. Cycads are amongst the oldest plant groups still in existence and have been popularly referred to as "living fossils". Their evolution can be traced back to the Palaeozoic era (Thomas & Spicer, 1987; Sabato, 1990) and they may have predated the angiosperms by more than 100 million years (Table 1.1). Cycads were abundant during the Mesozoic (Thomas & Spicer, 1987) and were probably colonized by insect herbivores during this period (Crowson, 1981). For example, the weevil genus, *Archeorrhynchus* (Curculionoidea), has been recovered from Upper Jurassic fossil beds in which cycads, and the morphologically similar Bennettitales, dominate the plant remains (Crowson, 1981). It therefore seems probable that *Archeorrhynchus* fed either on cycads, or Bennettitales, or both (Crowson, 1981).

Of special significance for this study of current insect/cycad interactions is the retention of apparently ancient cycad host associations amongst some extant insect groups (Crowson, 1981). Most particularly, Crowson (1981) considers that the genus *Antliarhinus* originated from a lineage in which an association with cycads may have been preserved since the Mesozoic. Host relationships in the genus *Antliarhinus* therefore provide a rare opportunity to study a plant/insect interaction that has probably been conserved over a considerable time span (perhaps for as long as 150 million years).

In addition, insect herbivory during the Jurassic and Cretaceous is considered to have had a significant influence on the evolution of plant structures, particularly those associated with the reproductive organs. Protective functions have been proposed for, amongst others, the angiosperm carpel (Stewart, 1983) and the megasporangiate cone of cycads (Crepet, 1979; Stewart, 1983). In many instances it is not possible to test these functions because the causative insects are no longer present. However, in the case of cycads, the condensation of the ovule-bearing structure into a cone protected by sporophylls (modified leaves) is

predicted to have occurred as a result of feeding by beetles (Crepet, 1979) and, more specifically, by primitive long-snouted weevils (Crowson, 1981). *Antliarhinus zamiae* has an extraordinary snout that may be as long as 20mm so that it is possible to test the effects of cone structure in extant cycads on a weevil with an apparently primitive facies.

**TABLE 1.1.** Important events in the evolution of cycads and weevils (Coleoptera: Curculionoidea) relative to major geological time scales. Data summarized here were obtained from Andrews (1961), Crowson (1981), Thomas & Spicer (1987) and Sabato (1990). Estimated ages of time boundaries follow Thomas & Spicer (1987).

Era	Period	Estimated ages of time boundaries (millions of years)	Important events in plant evolution	Important events in insect evolution
Caenozoic	Recent			
	Pleistocene	2		
	Pliocene	5		
	Miocene	25	first records of cycad genus <i>Ceratozamia</i>	
	Oligocene	33		Rise of true weevils (Curculionidae)
	Eocene Palaeocene	55 65	first records of extant cycad genera <i>Dioon</i> & <i>Zamia</i>	
Mesozoic	Upper Cretaceous			first Brentidae
	Lower Cretaceous	144	first record of extant cycad family- Stangeriaceae	
	Upper Jurassic Middle Jurassic		FIRST ANGIOSPERMS	first Curculionoidea
	Lower Jurassic	213	Divergence of <i>Cycas</i> and <i>Zamia</i> lineages	
Palaeozoic	Upper Triassic Middle Triassic Lower Triassic	248	Dominance of Cycadophytes	first Coleoptera
	Permian	286	Extinct cycad genera <i>Archeocycas</i> & <i>Phasmatocycas</i>	FIRST ENDOPTERYGOTE INSECTS
	Upper Pennsylvanian	320	? FIRST CYCADS	

This study was limited to an examination of host specialization in *A. zamiae* and *A. signatus*. Special reference is made to the roles played by larval adaptation to the apparently toxic tissues of the ovule megagametophyte and by adaptation in adult females for oviposition into concealed ovules.

The aims of the study were as follows.

1. To establish the host relationships of species of *Antliarhinus* (Chapter 2). Until the present study, almost nothing was known about these beetles except for some anecdotal reports on *A. zamiae* (Giddy, 1984; Goode, 1989).

2. To determine the role played by larval adaptation to feeding on the ovule gametophyte in the determination of host specialization in *A. zamiae* and *A. signatus* (Chapter 3). Emphasis was placed on these two species because they develop in the same host tissues but have different degrees of host specialization. The comparison of *A. zamiae* and *A. signatus* therefore presented the best chance for resolving the causes of host specialization.

3. To determine adaptations for oviposition into concealed cycad ovules in *A. zamiae* and *A. signatus* (Chapter 4) and to determine the evolutionary development of ovipositional traits by examining additional species of *Antliarhinus*. The evolutionary perspectives gained from this study provided a basis for interpreting the history of host relationships in *Antliarhinus*.

4. To establish whether ovipositional traits in *A. zamiae* and *A. signatus* differentially affect their ability to lay eggs into concealed cycad ovules (Chapter 5) and to assess how such differences may have affected host specialization in each weevil species (Chapter 6).

5. To determine how variability in ovipositional traits may have enabled *A. zamiae* to colonize many morphologically different cycad species (Chapter 7).

6. To establish whether adaptations for oviposition into concealed ovules, or larval adaptation to the gametophyte, necessitates close behavioural synchrony with the phenology of the cycad host by *A. zamiae* and *A. signatus*. Such behavioural synchrony may be expected to influence both host specialization and the possibilities for speciation.

7. Finally, in Chapter 9, a synthesis of host relationships in the genus *Antliarhinus* is presented with particular emphasis on the role of adaptation to the host-plant in the evolution of host specialization in *A. zamiae* and *A. signatus*.

## CHAPTER 2

### HOST RELATIONSHIPS IN THE GENUS *ANTLIARHINUS* WITH SPECIAL REFERENCE TO *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS*

#### ABSTRACT

Collections of cones and other plant parts from 19 species of *Encephalartos* and from the monotypic *Stangeria eriopus* in southern Africa yielded four species of cycad weevils belonging to the genus *Antliarhinus*, namely *A. peglerae*, *A. signatus*, *A. zamiae* and an undescribed species near to *A. verdcourti*. Host records for these weevils show that they were all found only on species of *Encephalartos* but the number of *Encephalartos* species colonized varied between *Antliarhinus* species. *Antliarhinus* sp. nr *verdcourti* was recorded from two host species, *A. peglerae* from five hosts, *A. signatus* from seven hosts, and *A. zamiae* from 13 hosts. Although collections of plant parts were hampered by the rarity of some cycads in the study area, the majority of host records are accurate. Significantly, the host ranges of the more generalist species of *Antliarhinus* always incorporated the host ranges of the more specialized species. This indicates that, to some extent, ancestral host associations have been conserved during the evolution of *Antliarhinus* species. However, speciation events have resulted in either expansion or contraction of host ranges. *Antliarhinus zamiae* appears to be exceptional because its host range has extended to groups of *Encephalartos* that have not been incorporated in the host ranges of other congeneric species.

Any interpretation of host specialization in a phytophagous insect must be based on an understanding of its host relationships in the field (Bernays & Graham, 1988). It is only in this way that host specialization can be understood in the context of the ecological environment in which it may have evolved and in which it is currently maintained (Vane-Wright, 1978; Bernays & Graham, 1988). It is the purpose of the research reported in this chapter to study some aspects of the host relationships of *A. zamiae* and *A. signatus* in the field in order to establish the context in which host specialization should be interpreted.

A first step towards understanding host relationships would be to obtain accurate host records from natural populations so that the extent of host specialization in the "normal" environment of the insect herbivore can be ascertained. This was a priority for this study because so little was known about host relationships in species of *Antliarhinus* except that they were associated with cycads. An association between species of *Antliarhinus* and the cycad genus *Encephalartos* has probably been recognised since Thunberg first described *A. zamiae* in 1784. Since then, *A. zamiae* has become one of the best known insects associated with cycads, principally because it emerges in large numbers from seeds of *Encephalartos* species (Rattray, 1913; Marloth, 1914; Crowson, 1981; Annecke & Moran, 1982; Giddy, 1984) and is regarded as a pest amongst cycad growers (Annecke & Moran, 1982; Giddy, 1984). Possibly because of its notoriety among cycad enthusiasts, *A. zamiae* is generally considered to attack seeds of almost all species of *Encephalartos* (Giddy, 1984; Goode, 1989), but

specific host records were mostly non-existent. With the exception of specimens collected from *Encephalartos altensteinii* (authors of species names are provided in Table 2.2), no *A. zamiae* specimens collected from South Africa, and deposited in major taxonomic collections, had host data attached (G. Kuschel, in litt. 1988). Published host records were mostly anecdotal and were limited to *E. altensteinii* and *E. villosus* (Ratray, 1913), *E. horridus* (Giddy, 1984), *E. lehmannii* (Goode, 1989) and *E. princeps* (Giddy, 1984). A similar paucity of data existed for *A. signatus* which has been recorded only generally as feeding on the ovules of *Encephalartos* species (Goode, 1989).

Accurate host records for *A. zamiae* and *A. signatus* are particularly important because of the possible existence of sibling species or host races on different host species. Most insect herbivores that develop within seeds have narrow host ranges and many are restricted to a single host species (Janzen, 1971, 1980b; Smith, 1975). Taxonomically related insects that feed on seeds may be associated with taxonomically related plant species (Center & Johnson, 1974; Janzen, 1980b), but broad host ranges are apparently rare. The possibility cannot therefore be excluded that the broad host ranges attributed to *A. zamiae* and *A. signatus* in the past, actually represent species complexes in which sibling species feed on different species of *Encephalartos*. Indeed, several species of *Antliarhinus* which are morphologically similar to *A. zamiae* and *A. signatus*, have been described (R.G. Oberprieler, pers. comm.). However, the genus *Antliarhinus* is currently being revised and these species are considered to be synonyms of *A. zamiae* and *A. signatus* (G. Kuschel, in litt. 1989; R.G. Oberprieler, pers. comm.). The collection of data from the field, in which species of *Antliarhinus* can be linked to their host-plants, are therefore needed to confirm these synonymies.

To understand host relationships in *A. zamiae* and *A. signatus* more fully, host records should also be obtained for other congeneric species. These data would indicate whether *A. zamiae* and *A. signatus* have retained the same host species as their congeners or whether they have colonized other cycad species. At least two other described species of *Antliarhinus* are considered to be valid, namely *A. peglerae* Peringuey and *A. verdcourti* Marshall. No host records were available for *A. peglerae* and records for *A. verdcourti* were limited to a collection label listing the host only as the cone axis of a cycad. The collection locality (in Kenya) indicates that the host-plant for *A. verdcourti* is probably *E. tegulaneus* (R. G. Oberprieler, pers. comm.). Nothing further was known about either the host-plants or the biologies of *Antliarhinus* species.

The collection of host-plant data from the field also made it possible to assess the possible influences of plant distribution and plant rarity on host relationships within the genus *Antliarhinus*. Firstly, host specialization in these beetles could arise, in part, from allopatric distributions of *Antliarhinus* populations and cycad

populations. Cycads are distributed in a variety of different habitats and it is possible that *Antliarhinus* species are restricted to only some of these habitats. Secondly, host relationships in species of *Antliarhinus* may have been influenced by the rarity of some cycad species. Plant abundance has been regarded as a major factor influencing the recruitment of insect herbivores by plant species (Strong *et al.*, 1984). A tenet of this argument is that insects colonize rare plants less often than they colonize common plants (Lawton & Schröder, 1977). As a result, *Antliarhinus* species may be expected to be more often associated with common cycads. As a corollary, the most valuable information on host specialization may be obtained from those relationships which deviate from the expected host-species area relationship.

Thus, the aims of this study were the following.

1. To obtain accurate host records for *Antliarhinus* species occurring naturally on cycads in southern Africa. The study was specifically limited to cycad species because all records for *Antliarhinus*, and for the taxonomically related genus *Platymerus* (Oberprieler, 1989), indicate an obligatory association with cycads.

2. To determine if host relationships were affected by allopatric distributions of plants and insects. This was ascertained from field data and was confirmed by transferring *Antliarhinus* species between cycad species cultivated in a botanic garden.

3. To establish whether host relationships have been influenced by the rarity of cycad species.

## MATERIALS AND METHODS

### *Study area*

This study was limited to Africa south of the Limpopo river (i.e. a northern limit of 22°15'S). However, for logistical reasons, all areas of Mocambique that occurred south of this limit were excluded.

### *Cycads within the study area*

The monotypic genus *Stangeria eriopus* (Kunze) Baillon as well as 33 species of *Encephalartos* have been described from the study area (Stevenson *et al.*, 1990; Vorster, 1990). Additional species of *Encephalartos* may be described within the near future since subspecies, and taxonomically distinct populations, have been identified and may be raised to specific status (P. Vorster, pers. comm.). However,

these taxonomic changes within the genus *Encephalartos* are unlikely to influence the interpretation of host relationships in species of *Antliarhinus* since the relevant populations of these cycads are well-known (e.g. Goode, 1989) and they could therefore be considered separately if necessary. In any event, only a few of these taxonomically distinct populations of *Encephalartos* were sampled during the present study.

Within the study area, all species of *Encephalartos* are generally regarded as scarce (Goode, 1989; Osborne, 1989) and one species, *E. woodii*, is extinct in the wild. At least two more species, *E. cerinus* and *E. latifrons*, occur naturally in such low numbers as to be virtually extinct in nature (Osborne, 1989). Most of the remaining species typically have restricted distributions (Goode, 1989) and only a few species, e.g. *E. villosus*, *E. altensteinii* and *E. longifolius*, are widespread. These patterns of distribution and abundance meant that it was often not possible to obtain material from rare species. Records for these species had to be based on personal communication with botanists and amateur enthusiasts who had collected cones and other plant parts in the past.

Because of their scarcity, cycads are legally protected in most areas of South Africa, and permits were required to collect plant parts, particularly cones. The number of cones that could be collected was usually stipulated in the permit. As a result, the number of cones collected for each cycad species (Table 2.1) was often determined by the conservation status of the plant and was not based on any other criteria.

The availability of cones was further influenced by coning frequency. All cycads are dioecious and each sexually mature plant bears either microsporangiate (pollen bearing) or megasporangiate (ovule bearing) cones (see Frontispiece). In species of *Encephalartos*, each plant typically produces a limited number of cones at most once a year. The number of cones varies between species, from one, or rarely two, megasporangiate cones per plant in *E. longifolius* (see Frontispiece) to between three and seven megasporangiate cones per plant in *E. friderici-guilielmi*. Most individual plants do not, however, cone every year and masting (the synchronous production of cones within a population) is common. Periods of two to three years between coning events are characteristic for some populations (Dyer, 1965; Giddy, 1984; Goode, 1989) but longer periods are not uncommon. Examples include, eight years for megasporangiate cones of *E. dolomiticus* in the Transvaal (Goode, 1989), and nine years for megasporangiate cones of *E. longifolius* in the Groendal State Forest (H. Swanenvelder, pers. comm.). *Encephalartos altensteinii* plants in the Kologha Forest near King William's Town have not coned within the tenure of the current forester, a period of 12 years. Thus, even for relatively common species, cones may not be obtained easily.

### *Sampling Procedure*

Microsporangiate cones, megasporangiate cones, and parts of the stems, leaf bases and leaves, were obtained from as many localities as possible. No comprehensive distribution records were available so the localities were identified from the following sources: the PRECIS herbarium database of the National Botanical Institute; herbarium records in the Albany Museum, Grahamstown, and the East London Museum; nature conservation officers in the Cape Province, Ciskei, Natal and Transvaal; the notes of the late C.G. Smith housed in the East London Museum Library, and numerous contacts with professional and amateur botanists. From these records, 74 localities for 19 species of *Encephalartos* and for *S. eriopus* were identified. Sixty-six of these localities were visited periodically (mainly in April/May and October/November) between November 1988 and November 1990 resulting in a total of 198 site visits (some sites were not visited on every occasion). Additional material was sent to me from these localities in June and July 1990. Three further localities were visited once only in January 1989 and the remaining five localities were not visited personally but cones were sent to me from these localities. The greatest emphasis was placed on collections from the Eastern Cape Province of South Africa (see Table 2.1). This emphasis was mainly for logistical reasons but was appropriate because of the concentration of cycad species in this area (12 species of *Encephalartos*, as well as *S. eriopus*, occur here).

Microsporangiate cones and other parts of the plant were dissected to establish the presence of any species of *Antliarhinus*. Where larvae were still present, the tissues were placed in an emergence box at 27°C until adults emerged.

Megasporangiate cones were also dissected to determine the presence of *Antliarhinus* species in the sporophylls, cone axis and ovule integument or seed coat (depending on when the cone was collected). Seeds and ovules were immersed in water. Those seeds and ovules in which the gametophyte had been entirely or partially consumed floated to the surface. "Floater" seeds and ovules were dissected to determine the identity of the insect which had consumed the gametophyte. Samples of "sinker" seeds were routinely dissected but no species of *Antliarhinus* were ever recovered from them.

### *Data analysis*

Host species area relationships were calculated using the number of quarter degree squares in which a plant occurred as an indication of its distribution. Plots were derived from non-transformed data. Log-log plots are more commonly used

**TABLE 2.1.** Sampling details for cones collected from 19 species of *Encephalartos* and from *Stangeria eriopus* between November 1988 and October 1990. For each cycad species the following details are provided: the number of megasporangiate (female) and microsporangiate (male) cones collected; the number of populations visited and the number of populations from which cones were collected; and the number of quarter degree squares in which the species naturally occurs. \* Denotes species in which populations were not visited personally. ° Denotes species from the eastern Cape Province of South Africa and for which the greatest number of collections were done.

Species	Number of cones collected:		Number of populations		Distribution (no. of quarter degree squares)
	female	male	visited	sampled	
° <i>E. altensteinii</i>	47	35	12	7	14
° <i>E. arenarius</i>	5	5	3	1	1
° <i>E. caffer</i>	10	9	3	2	4
° <i>E. cycadifolius</i>	8	7	2	1	2
* <i>E. dyerianus</i>	3	0	*	1	1
* <i>E. ferox</i>	2	0	*	1	2
° <i>E. friderici-guilielmi</i>	30	24	5	5	7
° <i>E. horridus</i>	9	11	3	2	2
<i>E. laevifolius</i>	16	11	4	3	3
<i>E. lanatus</i>	6	8	1	1	1
* <i>E. lebomboensis</i>	2	0	*	1	4
° <i>E. lehmannii</i>	16	15	4	3	7
° <i>E. longifolius</i>	14	15	7	3	10
<i>E. natalensis</i>	6	8	4	2	8
° <i>E. princeps</i>	7	8	5	2	3
* <i>E. transvenosus</i>	3	2	*	1	3
° <i>E. trispinosus</i>	12	10	8	2	4
* <i>E. umbeluziensis</i>	2	0	*	1	1
° <i>E. villosus</i>	36	21	10	5	16
° <i>Stangeria eriopus</i>	10	5	3	3	7
<b>TOTALS</b>	<b>244</b>	<b>193</b>	<b>74</b>	<b>47</b>	

(Strong *et al.*, 1984) but the small number of insect species involved, and the inclusion of zero values, made such transformations inappropriate.

The coefficients of similarity for the distribution of *Antliarhinus* species between different cycad taxa were based on the Bray & Curtis modification of Sorenson's Coefficient (Southwood, 1978). This calculation takes into account both the presence and abundance of species on particular host-plants. The equation is

$$C_N = 2jN / (aN + bN)$$

where  $jN$  = the sum of the lesser values for the species common to both host-plants;  $aN$  = the total individuals sampled on host 'a';  $bN$  = the total individuals sampled on host 'b' (Southwood, 1978). Because considerable numbers of

*A. zamiae* and *A. signatus* may emerge from seeds, their abundance was determined from the proportion of seeds from which they emerged in each host cone. The abundance of *A. peglerae* was similarly determined from the proportion of sporophylls in which they were found in each cone. The abundance of *A. sp. nr verdcourti* was calculated from the number of sporophyll bases (see Chapter 4) in which adults or larvae were found.

Coefficients of similarity were plotted as a dendrogram (see Southwood, 1978). Although this presentation may result in the loss of data (Southwood, 1978) it was used because it illustrates most clearly the relationship between insect host associations and the taxonomic affinities of the host-plants.

#### *Experimental confirmation of host specificity*

Host preferences recorded from field data were verified mostly on plants in the Kirstenbosch Botanic Garden, Cape Town (33°55'S 18°25'E) but also on cones collected in the field (only *E. cycadifolius*, *E. natalensis* and *E. princeps*). Species of *Antliarhinus* were only exposed to plants on which they did not occur, or on which they rarely occurred, in the field (these species are presented later on in Table 2.5). All four species of *Antliarhinus* were released on to *E. cycadifolius*, *E. friderici-guilielmi* and *E. transvenosus*. *Antliarhinus signatus* was also released on to *E. caffer*, *E. princeps*, and *E. villosus*. *Antliarhinus peglerae* and *A. sp. nr verdcourti* were also released on to these cycads with the further additions of *E. horridus* for both species and *E. lehmannii*, *E. longifolius* and *E. natalensis* for *A. sp. nr verdcourti*. As a control, all four *Antliarhinus* species were placed on *E. altensteinii* plants to ensure that utilization of plants in the garden was not prohibited by factors peculiar to the garden (e.g. climate). It is worth noting that Kirstenbosch Botanic Garden has a naturalized population of *A. zamiae* (pers. obs.) although the nearest natural population is over 300 km away.

For each feeding trial, 20 adult males and 20 females of each species of *Antliarhinus* were confined in a fine-mesh bag on the megasporangiate cones of selected *Encephalartos* species. The beetles were placed on host cones in late April 1989 and 1990 for all species except *E. cycadifolius* and *E. friderici-guilielmi* in which beetles were confined on the cones in November 1989. Beetles used in this experiment were reared from cones of *E. altensteinii* and *E. trispinosus* collected in the vicinity of Grahamstown (33°17'S 26°33'E).

## RESULTS

### *Host relationships in the genus Antliarhinus*

Four species of *Antliarhinus* were recorded during this study (Fig. 2.1-2.6); *A. peglerae*, *A. signatus*, *A. zamiae*, and an undescribed species close to *A. verdcourti* (R.G. Oberprieler, pers. comm.). Host records for the four species of *Antliarhinus* are summarised in Table 2.2.

*Antliarhinus zamiae* was recorded from the greatest number of *Encephalartos* species (13, Table 2.2) and, more specifically, also from the greatest number of *Encephalartos* species groups (as identified by Dyer, 1965; Table 2.2). The larvae developed communally within the ovule and destroyed large numbers of ovules in most of their host species (Table 2.3).

Although there were considerable differences in size between *A. zamiae* individuals from the same population and some consistent differences in size between individuals from different host populations (see Chapter 7), they were all identified as one species (G. Kuschel in litt., 1989; R.G. Oberprieler in litt., 1989).

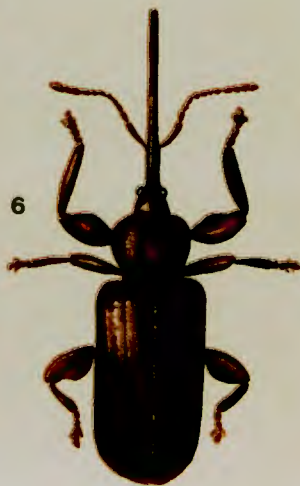
*Antliarhinus signatus* had a far more restricted host range than *A. zamiae* (seven species, Table 2.2) and attacked hosts represented in only three species groups of *Encephalartos* (Table 2.2). Like *A. zamiae*, the larvae of *A. signatus* developed communally only within the megagametophyte of their host ovule. However, *A. signatus* was less common than *A. zamiae* in ovules from almost all host species except *E. longifolius* (Table 2.3).

*Antliarhinus peglerae* was reared from the sporophylls of five host species (Table 2.2). Significantly, *A. peglerae* was the only species associated with the microsporangiate cone of any species of *Encephalartos*; the relevant host species were *E. altensteinii*, *E. longifolius* and *E. trispinosus*. It is remarkable that no parasitoids were ever seen or reared from any of the thousands of individuals of *Antliarhinus* species that were collected, except once when seven presumably generalist parasitoids were reared from *A. peglerae* developing in the microsporangiate cones of *E. altensteinii* (this parasitoid species has been provisionally identified only to family level as Hymenoptera: Pteromalidae).

*Antliarhinus* sp. nr *verdcourti* was bred solely from the megasporangiate cone axes of *E. altensteinii* and *E. natalensis*. Only a few individuals of *A. sp. nr verdcourti* were reared from *E. natalensis* and these emerged from a single cone.

*Antliarhinus verdcourti* was not collected from any cycad species in the study area and no species of *Antliarhinus* were ever found on *S. eriopus*.

**Figs 2.1-2.6.** Adult *Antliarhinus* species in dorsal aspect. 2.1. *A. peglerae* male. 2.2. *A. peglerae* female. 2.3. *A. signatus* female. 2.4. *A. zamiae* female. 2.5. *A. zamiae* male. 2.6. *A. sp. nr verdcourti* female.



5 mm

**TABLE 2.2.** Distribution of *Antliarhinus zamiae*, *A. signatus*, *A. peglerae* and *A. sp. nr verdcourti* on species of *Encephalartos* in southern African. Cycad species are grouped according to their taxonomic affiliations after Dyer (1965). Symbols denote presence (+), absence (-), not collected but reliably confirmed as absent (-<sup>1</sup>), and unknown (?). Cycad species that are extinct in the wild, or virtually so, are identified by an asterisk.

Species of <i>Encephalartos</i>	<i>A.</i> <i>zamiae</i>	<i>A.</i> <i>signatus</i>	<i>A.</i> <i>peglerae</i>	<i>A. sp. nr</i> <i>verdcourti</i>
<i>aemularis</i> Vorster	?	?	?	?
<i>altensteinii</i> Lehmann	+	+	+	+
<i>longifolius</i> (Jacquin) Lehmann	+	+	+	-
<i>natalensis</i> R.A. Dyer & Verdoorn	+	+	+	+
<i>lebomboensis</i> Verdoorn	+	-	-	-
<i>woodii</i> * Sander	?	?	?	?
<i>trispinosus</i> (Hooker) R.A. Dyer	+	+	+	-
<i>horridus</i> (Jacquin) Lehmann	+	+	-	-
<i>princeps</i> R.A. Dyer	+	-	-	-
<i>lehmannii</i> Ecklon & Zheyer ex Lehm.	+	+	+	-
<i>eugene-maraisi</i> Verdoorn	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>dolomiticus</i> Lavranos & Goode	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>dyerianus</i> Lavranos & Goode	-	-	-	-
<i>middelburgensis</i> Vorster <i>et al.</i>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>villosus</i> Lemaire	+	-	-	-
<i>umbeluziensis</i> R.A. Dyer	+	-	-	-
<i>caffer</i> (Thunberg) Lehmann	+	-	-	-
<i>ngoyanus</i> Verdoorn	?	?	?	?
<i>cerinus</i> * Lavranos & Goode	?	?	?	?
<i>arenarius</i> R.A. Dyer	+	-	-	-
<i>latifrons</i> * Lehmann	?	?	?	?
<i>ferox</i> Bertolini f.	+	-	-	-
<i>transvenosus</i> Stapf & Burtt Davy	-	-	-	-
<i>paucidentatus</i> Stapf & Burtt Davy	?	?	?	?
<i>heenanii</i> R.A. Dyer	?	?	?	?
<i>inopinus</i> R.A. Dyer	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>cupidus</i> R.A. Dyer	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>cycadifolius</i> (Jacquin) Lehmann	-	-	-	-
<i>friderici-guilielmi</i> Lehmann	-	-	-	-
<i>ghellinckii</i> Lemaire	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>humilis</i> Verdoorn	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
<i>lanatus</i> Stapf & Burtt Davy	-	-	-	-
<i>laevifolius</i> Stapf & Burtt Davy	-	-	-	-

**TABLE 2.3.** The percentage of ovules colonized by *Antliarhinus zamiae* and *Antliarhinus signatus* in 10 species of *Encephalartos* occurring in South Africa. The total number of ovules sampled for each host species is also provided.

Host species	Percentage ovules colonized		Total number of ovules
	<i>A. zamiae</i>	<i>A. signatus</i>	
<i>E. altensteinii</i>	63	12	18000
<i>E. arenarius</i>	74	8	2000
<i>E. caffer</i>	2	0	2100
<i>E. horridus</i>	87	9	2700
<i>E. lehmannii</i>	41	13	5900
<i>E. longifolius</i>	4	13	6400
<i>E. natalensis</i>	61	13	2400
<i>E. princeps</i>	82	0	2200
<i>E. trispinosus</i>	53	10	3800
<i>E. villosus</i>	87	0	5700

#### *The authenticity of host records*

The accuracy of the above host records may have been influenced by two aspects of the sampling procedure. Firstly, the limited number of cones collected from some localities may not have provided a representative sample for that population. Secondly, the limited number of localities sampled in some cases may mean that cycads were not sampled in areas where they might have been colonized by *Antliarhinus* species. It is important to verify these possibilities, as far as possible, because subsequent comments on host specialization are, of necessity, based on these data.

The best test of the validity of the sampling undertaken here will probably be provided by an analysis of the most comprehensively collected cycad species. Records for the collection of *Antliarhinus* species from these cycad hosts can be analysed to determine whether the number of cones collected from a single locality, or the number of localities sampled, made any difference to the number of beetle species recorded from that host-plant. The cycad species examined for this purpose were *E. altensteinii*, *E. longifolius* and *E. villosus*.

The number of cones collected from any locality had only a small effect on the number of *Antliarhinus* species collected from *E. altensteinii* and *E. villosus*. In four populations of *E. altensteinii*, at most three megasporangiate cones were required to obtain the full spectrum of *Antliarhinus* species typically found in that population (Fig. 2.7). Similarly, in populations of *E. villosus*, all cones contained specimens of *A. zamiae*, the only species of *Antliarhinus* recorded from this host-plant. These collections indicate that only a few cones from any locality (probably no more than

three) are likely to include all the *Antliarhinus* species present in that cycad population.

For almost all cycad species sampled during this study, several megasporangiate cones were collected from each locality. It is therefore unlikely that any populations were under-sampled. Exceptions to this generalization may be *E. ferox*, *E. lebomboensis* and *E. umbeluziensis*, the only species in which fewer than three cones were collected from any one locality (Table 2.1).

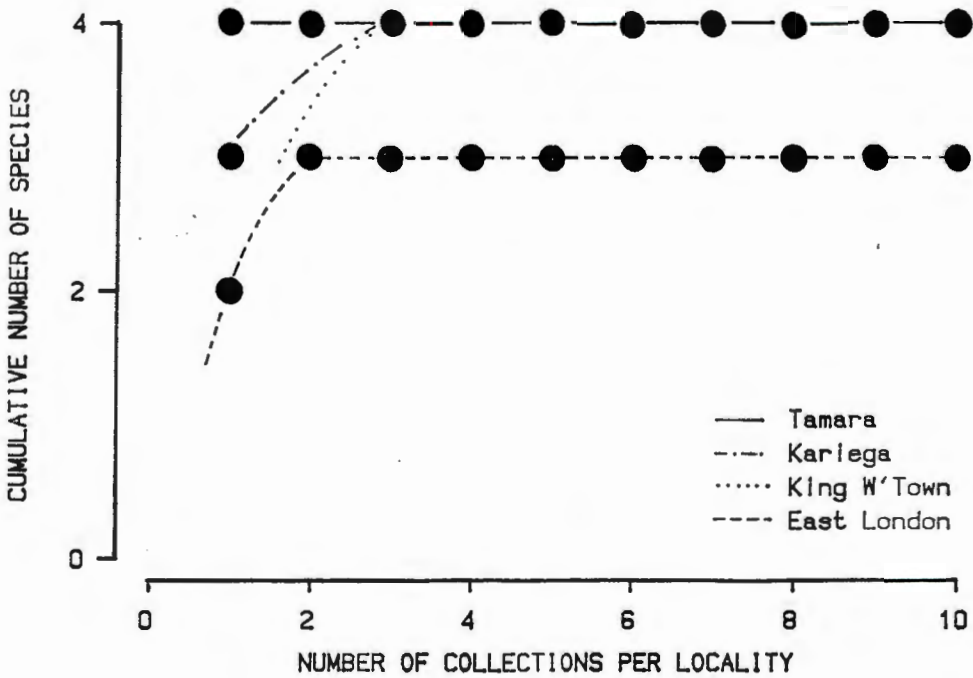


Fig. 2.7. The cumulative number of *Antliarhinus* species obtained from each megasporangiate cone of *Encephalartos altensteinii* collected in four localities. The plot represents collections ranked from the lowest to the highest incidence of weevils in cones and does not reflect the sequence of collecting.

In contrast, the number of localities sampled for any cycad species might have had an affect on the representation of *Antliarhinus* species on some host-plants. An analysis of collections from *E. villosus* showed that all populations had *A. zamiae* present (Fig. 2.8). Similarly, for *E. longifolius*, only one population had

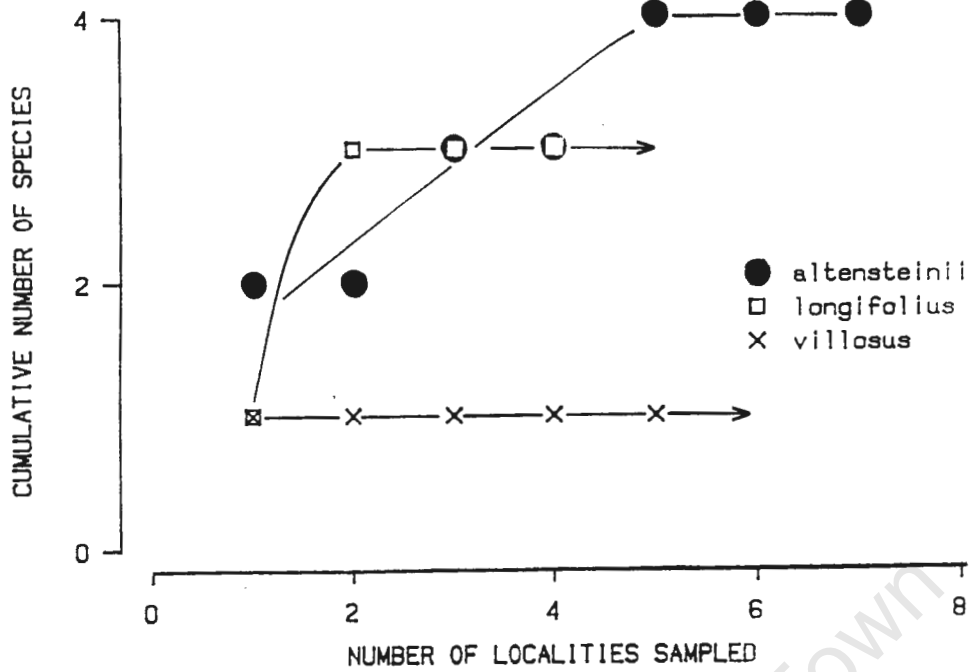


Fig. 2.8. The cumulative number of *Antliarhinus* species collected per locality from megasporangiate cones of *Encephalartos altensteinii*, *E. longifolius* and *E. villosus*. Collections were ranked from the lowest to the highest incidence of *Antliarhinus* species in a population and do not reflect the sequence of collecting.

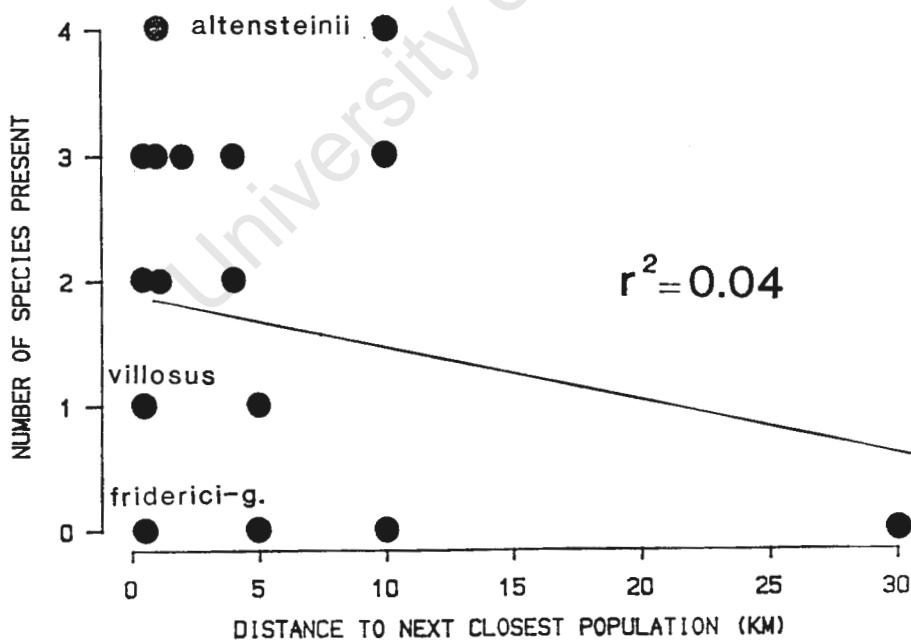


Fig. 2.9. The number of *Antliarhinus* species occurring in 16 populations of eight species of *Encephalartos* relative to the degree of isolation of the host-plant population. The host species with the lowest (*E. villosus* and *E. friderici-guilielmi*) and highest (*E. altensteinii*) representation of *Antliarhinus* species are marked on the graph.

fewer species of *Antliarhinus* than the three species recorded from this host-plant (Fig. 2.8). However, as many as five populations had to be sampled to obtain the full spectrum of *Antliarhinus* species from *E. altensteinii* (Fig. 2.8). This indicates that sampling a greater number of populations may provide a more accurate record of host relationships in some instances.

For several cycad species, specifically *E. arenarius*, *E. caffer*, and *E. horridus*, further sampling was not possible because most of the existing populations had been sampled. Even for more widespread species, such as *E. princeps* and *E. trispinosus*, further collections were impossible due to the low incidence of cones in existing populations (see Table 2.1). The rarity of these species in space or time suggests that samples collected over a longer period might yield additional species of *Antliarhinus* from these cycads.

It is, however, implicit in the above conclusion that species of *Antliarhinus* are only temporarily absent from potential host-plants. In other words, a local extinction event has not been followed by recolonization of that host-plant. The incidence of this effect on host records must be limited. Firstly, there was no correlation between the number of *Antliarhinus* species present in cones and the degree of geographical isolation of the host-plant population (Fig. 2.9). This indicates that isolation alone, and its effects on extinction and recolonization, is not sufficient to explain variation in the number of *Antliarhinus* species present on species of *Encephalartos*. Secondly, collection of cones focused specifically on several sympatric populations of different cycad species (Table 2.4) so that the effect of isolation on host relationships could be distinguished from other effects.

**TABLE 2.4.** Species of *Encephalartos* which occur sympatrically, or allopatrically but close to each other, in at least some areas of their distribution. Only populations which were sampled during the course of this study are listed. \* Signifies populations that are geographically close but remain allopatric.

Species		Locality
<i>E. altensteinii</i>	+	<i>E. arenarius</i> *
<i>E. altensteinii</i>	+	<i>E. caffer</i>
	+	<i>E. trispinosus</i>
<i>E. altensteinii</i>	+	<i>E. princeps</i>
<i>E. altensteinii</i>	+	<i>E. villosus</i>
<i>E. friderici-guilielmi</i>	+	<i>E. natalensis</i> *
<i>E. friderici-g.</i>	+	<i>E. princeps</i>
<i>E. lehmannii</i>	+	<i>E. longifolius</i>

For example, *E. altensteinii* occurred sympatrically with both *E. caffer* and *E. trispinosus* in a locality from which all four species of *Antliarhinus* had been recovered. The absence of all *Antliarhinus* species, except *A. zamiae*, from *E. caffer* is therefore significant. Similarly, the absence of *A. sp. nr verdcourti* from *E. trispinosus* in the same locality must be regarded as more than just an artefact of the collection procedure. The only cycad species in which *Antliarhinus* species may have been underrepresented due to the sampling procedure were *E. horridus*, *E. princeps* and possibly, *E. arenarius*, for the following reasons. (1) Populations were small and were always allopatric with other cycad species even if they occurred within the general distribution of other cycad species (*E. horridus* and *E. arenarius*). (2) Only a limited number of *Antliarhinus* species were present on both hosts in the area of sympatry even though additional species of *Antliarhinus* may have been present in other populations of one of the host species (*E. princeps*).

These results indicate that the restricted collection procedures employed during this study probably had little effect on the comprehensiveness of the host records presented here.

#### *Host-plant distribution and host specialization*

Within the study area, species of *Encephalartos* are distributed along the eastern margin of South Africa from around Willowmore in the south to near the border with Zimbabwe in the north (Fig.2.10). *Stangeria eriopus* has a more restricted distribution within the same general area. Species of *Antliarhinus* were, however, recorded from only some parts of the distribution of *Encephalartos* and were specifically absent from most areas of the Transvaal (Fig. 2.10). Thus the absence of *Antliarhinus* species from some cycad species may be an effect of their allopatric distributions.

The cycad species most likely not to be attacked by *A. signatus* and *A. zamiae* because of their distribution are *E. cupidus*, *E. dolomiticus*, *E. dyerianus*, *E. humilis*, *E. lanatus*, *E. laevifolius*, *E. middelburgensis*, *E. paucidentatus* and *E. transvenosus*. The extent to which the absence of *Antliarhinus* species from these cycads can be attributed to the allopatric distributions of the plants and insects should ideally have been tested by releasing the beetles on to all the affected species in botanic gardens. Unfortunately, of these species, only *E. transvenosus* was available for such a test (see below). The result of this test (see below) supports the conclusion that *E. transvenosus* is not colonized by *Antliarhinus* species only because it occurs outside the distribution range of these beetles.

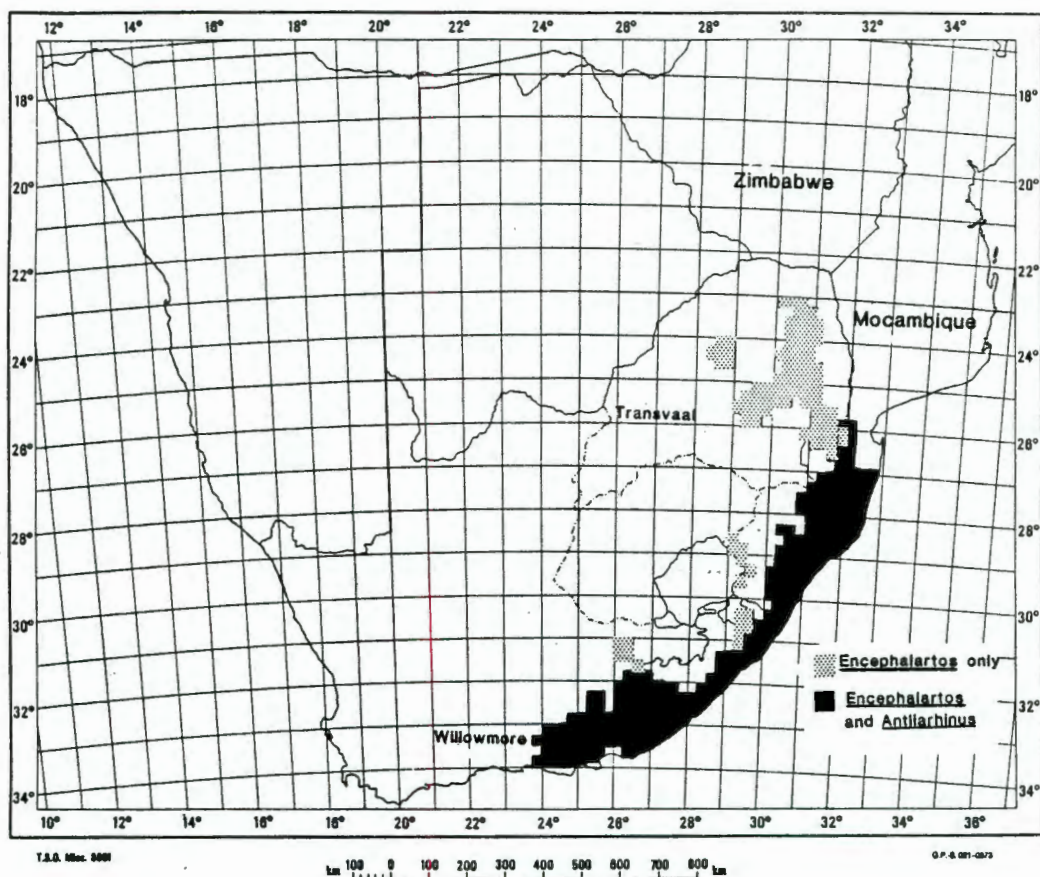


Fig. 2.10. Map of South Africa showing the general distributions of the cycad genus *Encephalartos* and the weevil genus *Antliarhinus*.

The effects of distribution on host relationships in *Antliarhinus* species means that spurious interpretations of relationships could be made if cycad species from the Transvaal are included in analyses. For this reason, all further analyses in this chapter, and in this thesis, are restricted to cycad species that occur within the geographical distribution range of the beetles. Based on the current data, these are the cycad species listed in Table 2.1 with the exception of *E. dyerianus*, *E. laevifolius*, *E. lanatus* and *E. transvenosus*.

### Host species area relationships

The host species area relationship, representing the number of *Antliarhinus* species present on species of *Encephalartos* relative to the distributional area of the host-plant, is presented in Fig. 2.11 for all species of *Encephalartos* from the eastern Cape Province as well as *E. natalensis* from Natal. The initial correlation, as depicted in Fig. 2.11, was weak and statistically insignificant ( $P > 0.05$ ). However, the relationship was almost certainly affected by the absence of all, or most, *Antliarhinus* species from relatively widespread cycad species such as *E. cycadifolius*, *E. friderici-guilielmi* and *E. villosus* (Fig. 2.11). Eliminating these species from the analysis resulted in a significant correlation ( $r^2 = 0.59$ ,  $P < 0.05$ ).

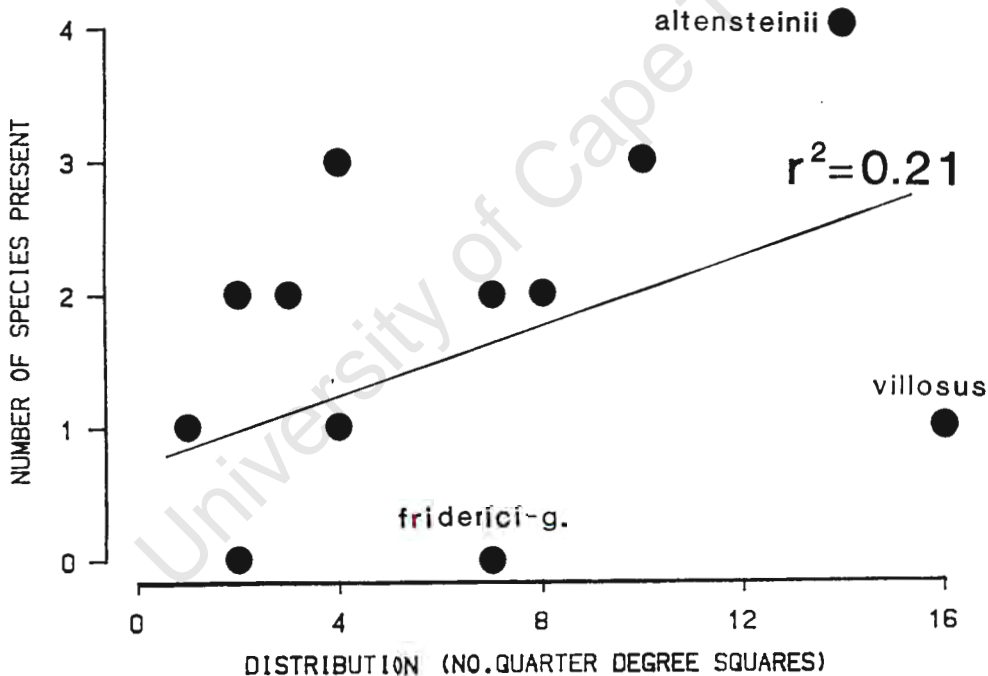


Fig. 2.11. Host species area plot for the number of *Antliarhinus* species present on 12 species of *Encephalartos*. The most widespread cycad species (*E. altensteinii* and *E. villosus*) as well as the species with the lowest representation of *Antliarhinus* species (*E. friderici-guilielmi*) are marked on the graph.

This result indicates that for most species of *Encephalartos* in the eastern Cape Province, a significant proportion of the variation in the distribution of *Antliarhinus* species can be attributed to the rarity of the plant species. Rare plant species have been included in the host range less often than common species. The results from

the host species area relationship also suggest that *E. cycadifolius*, *E. friderici-guilielmi* and *E. villosus* have been incorporated into the host ranges of *Antliarhinus* species less often than would be expected from their distributions.

#### *Host relationships and host-plant taxonomy*

The coefficients of similarity for the distribution of *Antliarhinus* species on their host-plants (only eastern Cape species with the inclusion of *E. natalensis*) are presented as a dendrogram in Fig. 2.12. There is remarkable conformity between this dendrogram and the taxonomic classification of the genus *Encephalartos* presented by Dyer (1965). The most significant deviations from Dyer's (1965) classification are the closeness of *E. horridus* to *E. arenarius* (Fig. 2.12), the distance between *E. altensteinii* and *E. longifolius*, and the closeness between *E. princeps* and *E. villosus* (Fig. 2.12). It is, however, noteworthy that two of these anomalies in the dendrogram (Fig. 2.12) are consistent with a revised classification of the genus *Encephalartos* currently in preparation (R. Osborne, pers. comm.). In Osborne's view, *E. arenarius* and *E. horridus* belong to the same phylogenetic clade and *E. longifolius* and *E. altensteinii* belong to different clades. As a result, the congruence between *Antliarhinus* distribution and host-plant taxonomy may be closer than is evident from Dyer's (1965) classification of *Encephalartos*. The closeness between *E. princeps* and *E. villosus* (Fig. 2.12), for the representation of *Antliarhinus* species, is simply due to *A. zamiae* being the only species recorded from both hosts. In the case of *E. princeps* this may not be a valid representation because of the problems experienced in collecting material from this plant, as explained earlier. The following section on host utilization in the Kirstenbosch Botanic Garden shows that further species of *Antliarhinus* may colonize *E. princeps*. Based on these results, *E. princeps* would be situated near *E. lehmannii* in the dendrogram.

#### *Host utilization in Kirstenbosch Botanic Garden*

When adult *Antliarhinus* spp. were caged on cones in Kirstenbosch Botanic Garden, development was recorded on several hosts from which they had not been recorded in the field (Table 2.5). Most significantly, all four species survived to adulthood on megasporangiate cones of *E. transvenosus*. This confirms the earlier conclusion that some species from the Transvaal may be potential hosts for species of *Antliarhinus*. Similarly, the survival of *A. peglerae* in *E. horridus* and *E. princeps* supports the hypothesis that *A. peglerae* may be absent from these cycad species in the field because of their scarcity in space or time.

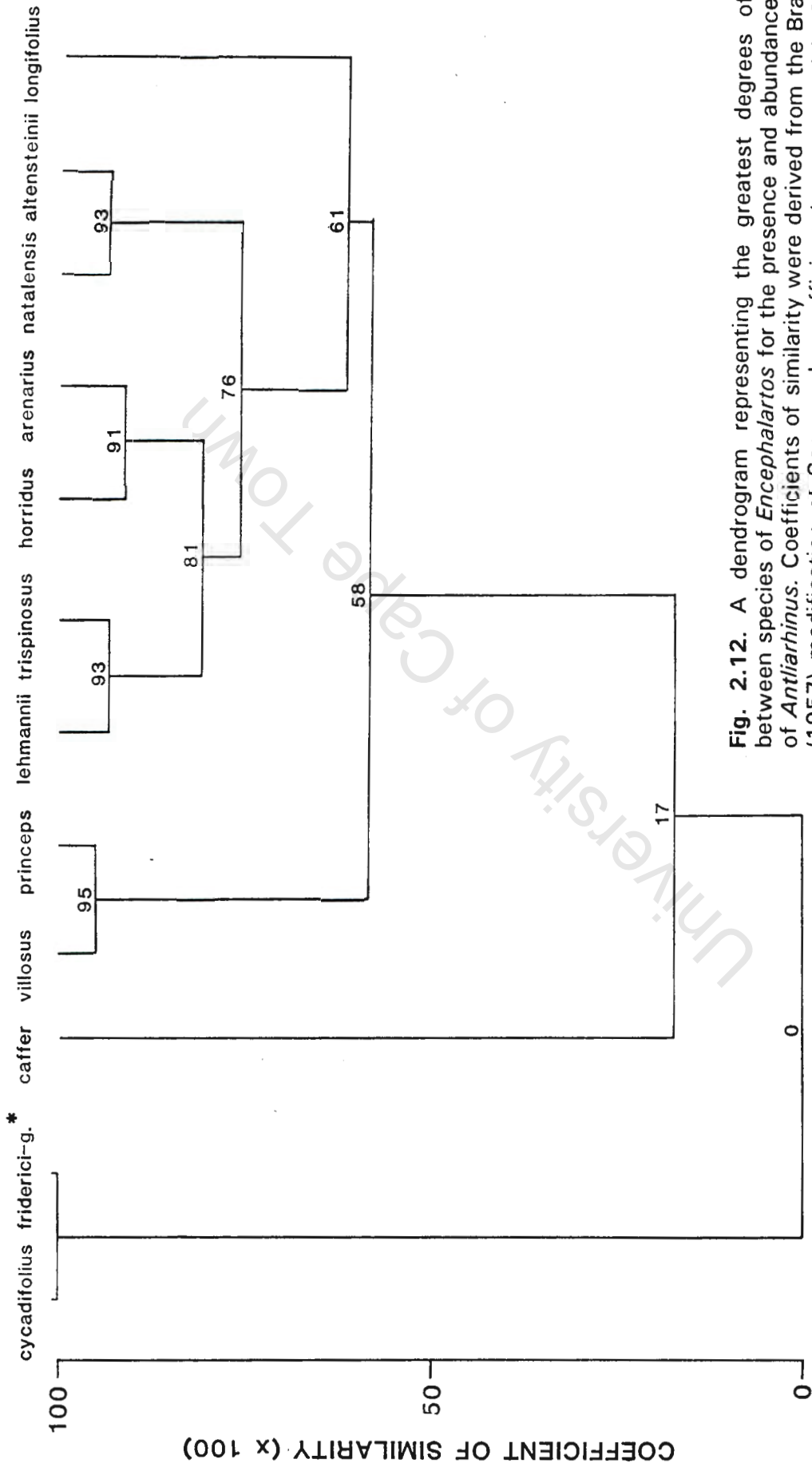


Fig. 2.12. A dendrogram representing the greatest degrees of similarity between species of *Encephalartos* for the presence and abundance of species of *Antliarhinus*. Coefficients of similarity were derived from the Bray & Curtis (1957) modification of Sorenson's coefficient (as presented in Southwood, 1978). *Encephalartos friderici-guiliei* is marked with an asterisk to indicate that its similarity to *E. cycadifolius* is based simply on the absence of any *Antliarhinus* species from both cycad species.

The lack of success for *A. sp. nr verdcourti* on all plants except *E. natalensis* confirms the validity of the field records for this species. The data for *Antliarhinus* species tested on *E. caffer*, *E. cycadifolius*, *E. friderici-guilielmi*, and *E. villosus* also confirm the host relationships recorded in the field.

**TABLE 2.5.** Summary of results for the development of four species of *Antliarhinus* on various species of *Encephalartos* from which they were either not recorded in nature, or were seldom recorded in nature. *Encephalartos altensteinii* was used as a control. A (+) signifies that the weevil species completed development in the host tissues after adult beetles had been confined on the host cone to allow oviposition. A (-) indicates that these plants were rejected and that no oviposition took place.

Host species	Species of <i>Antliarhinus</i>			
	<i>zamia</i>	<i>signatus</i>	<i>peglerae</i>	<i>nr verdcourti</i>
<i>E. altensteinii</i>	+	+	+	+
<i>E. caffer</i>	-	-	-	-
<i>E. cycadifolius</i>	-	-	-	-
<i>E. friderici-g.</i>	-	-	-	-
<i>E. horridus</i>			+	-
<i>E. lehmannii</i>				-
<i>E. longifolius</i>				-
<i>E. natalensis</i>				+
<i>E. princeps</i>		+	+	-
<i>E. transvenosus</i>	+	+	+	+
<i>E. villosus</i>		-	-	-

## DISCUSSION

Host records for *A. zamiae* and *A. signatus* confirm the broad host ranges attributed to these species in previous publications (e.g. Giddy, 1984; Goode, 1989). However, it is wrong to conclude from these host ranges that *A. zamiae* and *A. signatus* attack all species of *Encephalartos*. They were clearly associated with only some species of *Encephalartos*, so that these specific host associations need to be explained.

Host records for the four species of *Antliarhinus* studied in this chapter, namely, *A. peglerae*, *A. signatus*, *A. zamiae* and *A. sp. nr verdcourti*, show that there is a degree of conservatism in host relationships within the genus. All four species of *Antliarhinus* were associated with *E. altensteinii* and, significantly nearly 50% of host relationships in the genus *Antliarhinus* were associated with species in the *E. altensteinii* species group (after Dyer, 1965; Table 2.2). Most of the

remaining host relationships were with species in the *E. trispinosus* species group (Table 2.2). It is therefore clear that differences in host use by different species of *Antliarhinus* represent more than simple omissions or additions from an undifferentiated assemblage of *Encephalartos* species.

It is particularly significant that the host ranges of the more generalist species of *Antliarhinus* always incorporated the host ranges of the more specialized species. In other words, the host range of *A. peglerae* incorporated the host range of *A. sp. nr verdcourtii*, that of *A. signatus* included the hosts attacked by *A. peglerae*, and finally, the host range of *A. zamiae* incorporated all the host-plants colonized by the three other species of *Antliarhinus* examined in this study. This pattern suggests that host range is a species character that has been retained in various speciation events. Probably, the traits involved in host recognition have been retained. However, it is also clear that host ranges have either expanded or contracted as a result of speciation events. Understanding these changes in host range associated with speciation is at the heart of understanding host specialization in these insects.

Differences in life history characteristics are particularly obvious between different species of *Antliarhinus*. *Antliarhinus peglerae* and *A. sp. nr verdcourtii* develop within the sporophyll and cone axis tissues respectively, whereas *A. zamiae* and *A. signatus* develop within the ovules of their host-plants. These different life histories would be expected to be subject to different selection pressures that could affect adaptation to different host-plants. For instance, adaptation for oviposition into ovules would probably vary from adaptation for oviposition into sporophylls. As a result, the change in ovipositional behaviour may result in new selection pressures imposed by the host-plant and, consequently lead to changes in host utilization. Thus, even if the senses associated with host recognition remain unaltered after speciation events, selection may act against indiscriminate host recognition within the ancestral host range if the altered traits resulting from new feeding habits are not adapted to all the host-plants within the ancestral host range. Alternatively, the change in feeding habits may free species from such constraints and allow expansion of host range.

A comprehensive study of altered host ranges accompanying speciation in *Antliarhinus* species would be a major study since so many factors may be involved. However, a comparison of host ranges in *A. zamiae* and *A. signatus* may be more meaningful. Both species have larvae that develop on the ovule gametophyte so that the number of variables in such an analysis would be reduced. Further, Vane-Wright (1978) has pointed out that the most information on the causes of host specialization is likely to be obtained from a comparison of sister species, i.e. species that are separated by a single speciation event. Although the phylogeny of *Antliarhinus* has not been determined, there are good reasons to

believe that *A. zamiae* and *A. signatus* represent such sister species (Chapter 4; Donaldson, in press-b). The remaining chapters of this thesis therefore focus mainly on *A. zamiae* and *A. signatus*.

The most significant differences in host range between *A. zamiae* and *A. signatus*, and therefore those that must be accounted for in an explanation of host specialization, are the absence of *A. signatus* from *E. caffer* and *E. villosus* and the marginal use of *E. longifolius* by *A. zamiae*. In addition, the identification of the causes of the above differences in host range may also account for the absence of both *A. zamiae* and *A. signatus* from all species in the *E. cycadifolius* group of *Encephalartos*. However, it was recognized at this stage that since neither *A. zamiae* nor *A. signatus* feed on cycads in the *E. cycadifolius* group, a comparison of these two beetle species may not explain the omission of cycads in the *E. cycadifolius* group from current host ranges.

Emphasis in the following chapters has been placed on larval adaptation to the ovule gametophyte and adult adaptation for oviposition into concealed ovules as explanations for host specialization in *A. zamiae* and *A. signatus*.

## CHAPTER 3

### HOST SPECIFICITY IN *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS* IN RELATION TO THE BIOCHEMICAL UNIQUENESS AND TOXICITY OF THE CYCAD MEGAGAMETOPHYTE

#### ABSTRACT

The megagametophyte tissues of all extant cycads contain unusual and generally toxic chemical compounds. These compounds, particularly methylazoxymethanol-glycosides such as macrozamin, may act as barriers to colonization by insect herbivores and result in differential performance of herbivore larvae on different cycad taxa. As a result, host preferences in *Antliarhinus zamiae* and *Antliarhinus signatus* may have arisen due to physiological adaptation of their larvae to particular cycad hosts. The apparent barriers to colonization of the megagametophyte by insect herbivores, and differences in performance of *A. zamiae* and *A. signatus* larvae between different cycad taxa were therefore tested in this study. Results presented here show that, in addition to *A. zamiae* and *A. signatus*, four insect herbivores were identified that fed facultatively on megagametophyte tissues. No deleterious effects were observed when two of these insects, *Zerenopsis leopardina* (Lepidoptera: Geometridae) and *Amorphocerus* sp. (Coleoptera: Curculionidae), were reared only on gametophyte tissue. This result indicates that physiological adaptation to megagametophyte tissues occurs in at least four genera and two families and is not unique to *A. zamiae* and *A. signatus*. Larvae of *A. peglerae*, a species considered here to be a model ancestor for *A. zamiae* and *A. signatus*, did not, however, survive on megagametophyte tissues and were killed by the addition of 3% (fresh weight) macrozamin to an artificial diet. Physiological adaptation to the megagametophyte must therefore have been a necessary step in the progression from feeding on cone tissue to feeding on the megagametophyte in species of *Antliarhinus*. However, *A. zamiae* and *A. signatus* larvae performed equally well on both host and non-host species of *Encephalartos* so that differences in host utilization could not have resulted from differential performances of larvae on these plants.

Host relationships in *A. zamiae* and *A. signatus* are characterized by the utilization of only some species of the cycad genus *Encephalartos*, both in the field and in experimental tests of host specificity (Chapter 2). These preferences may have arisen as a result of the differential performance of larvae on different species of *Encephalartos*. In fact, if discrimination between cycad taxa by adult *A. zamiae* and *A. signatus* has evolved solely in response to differential larval performance on different hosts, then the occurrence of these beetles only on species of *Encephalartos* and not on sympatric populations of *Stangeria eriopus* could also be attributed to differences in larval performance between the two cycad genera. The relationship between larval performance on various cycads and host specialization therefore needs to be examined.

Physiological adaptation to the larval food source may be particularly relevant to the understanding of host relationships in *A. zamiae* and *A. signatus* because of the unusual (Moretti *et al.*, 1981; 1983) and generally toxic (Steyn *et al.*, 1948;

Whiting, 1963; Lacquer *et al.*, 1963; Dastur, 1964; Tustin, 1974; 1983) biochemistry of the cycad megagametophyte. Biochemically unusual compounds in plants are thought to act as barriers to colonization by insect herbivores (Strong *et al.*, 1984). Since these compounds would seldom be encountered by insect herbivores, genes that would facilitate physiological adaptation probably occur rarely in insect populations. Similarly, compounds with broad spectrum toxicity may act as barriers to colonization (Bell, 1978; Janzen, 1978). The observation that *A. zamiae* and *A. signatus* are the only known insects that develop obligately on the megagametophyte tissues of any cycad species suggests that the chemistry of the megagametophyte may well act as a barrier to colonization by insect herbivores. Several compounds that are unique to cycads could be implicated in this toxicity.

Methylazoxymethanol (MAM) glycosides, a group of compounds so far found exclusively in cycads (Moretti *et al.*, 1981; 1983), occur in relatively high concentrations in the megagametophyte (De Luca *et al.*, 1980; Moretti *et al.*, 1981; 1983; Yagi & Tadera, 1987). These MAM-glycosides have been identified as powerful carcinogens and mutagens (Matsumoto & Strong, 1963; Kobayashi & Matsumoto, 1965; Druckrey & Lange, 1972; Malevski *et al.*, 1972; Hoffmann, 1990) and as potential neurotoxins (Mettler, 1972). In experimental studies, MAM-glycosides exhibit broad spectrum antibiotic properties (Kobayashi & Matsumoto, 1965) and in non-adapted insect herbivores they cause melanization and death (Kobayashi *et al.*, 1980). It is the de-glycosylated MAM that is toxic through its action as a powerful methylating agent. Liberated MAM causes gene mutations and DNA damage in microorganisms and cultured mammalian cells, mitotic recombination in yeast, sex-linked recessive lethal mutations in *Drosophila*, sister chromatid exchanges in mammalian cell cultures, DNA strand breakage in mammals, and chromosome aberrations in a diversity of organisms (Hoffmann, 1990). The remarkable biocidal activities of MAM suggest that it may be generally toxic to insect herbivores.

In addition to MAM-glycosides, a cycad-specific amino acid,  $\alpha$ -amino- $\beta$ -methylaminopropionic acid (BMAA), has been isolated from the megagametophyte of *Cycas circinalis* L. (Vega & Bell, 1967). BMAA was later found to be widespread in seeds of *Cycas* species (Vega *et al.*, 1968) and may also occur in other cycad genera, e.g. *Zamia* (Norstog & Fawcett, 1989). In experimental studies with chicks (Vega & Bell, 1967; Vega *et al.*, 1968) and macaque monkeys (Spencer *et al.*, 1987), BMAA was shown to be neurotoxic (see also Weiss & Choi, 1988). The presence of BMAA in species of *Encephalartos* is unknown and its effects on insect herbivores have not been established. Nevertheless, it remains a possible barrier to colonization of the megagametophyte.

Chemical barriers to colonization of the cycad gametophyte would not explain host specialization by *A. zamiae* and *A. signatus* within the cycad genus *Encephalartos* unless differences existed between cycad taxa. Studies of cycad chemistry (with the exception of those dealing with BMAA) have often emphasized the ubiquitous nature of cycad compounds (e.g. Moretti *et al.*, 1983), so that the extent of variation in gametophyte chemistry, that could form a basis for discrimination between cycad taxa by *A. zamiae* and *A. signatus*, is not well-known. Nevertheless, some variation in chemistry has been reported.

MAM-glycosides, as a group, have a ubiquitous distribution among cycad species (De Luca *et al.*, 1980; Moretti *et al.*, 1981; 1983). However, because MAM is toxic only in its unconjugated, or aglycone, state (Kobayashi & Matsumoto, 1964; Grab & Zedeck, 1977), the sugar moiety may influence its toxicity. In plant tissues MAM is conjugated with various sugars to form a variety of identifiable glycosides, e.g. macrozamin (MAM-primeverose, Lythgoe & Riggs, 1949), cycasin (MAM-glucose, Nishida *et al.*, 1955), and neocycasin-A (MAM-laminaribiose, Nishida *et al.*, 1959). The toxicity of each glycoside may depend on enzyme activity (specifically glycosidases) in either the host-plant or the herbivore. For instance, in four genera of cycads, glucosidase activity was shown to be several orders of magnitude greater than primeverosidase activity (Yagi & Tadera, 1987). As a result, considerably more MAM was released from the breakdown of cycasin than from the breakdown of macrozamin. Detoxification of MAM by insect herbivores is likely to result from selective enzyme activity to avoid deglycosylation of MAM-glycosides or to reglycosylate MAM with a specific sugar (Rothschild *et al.*, 1986). One MAM-glycoside may therefore be more toxic to a prospective herbivore than another. Since at least some insect herbivores have the ability to distinguish between structurally similar compounds (e.g. Lindroth *et al.*, 1988), and even between different glycosides of the same compound (Ishikawa, 1966; Schoonhoven, 1973), it is conceivable that host relationships are influenced by different glycosides.

The distribution and concentrations of MAM-glycosides have so far been found to be genus-dependent (Moretti *et al.*, 1981; 1983). However, the relative proportions of different glycosides may vary to some extent between congeners (Dossaji & Herbin, 1972; Altenkirk, 1974; Moretti *et al.*, 1981; 1983). For instance, in those species of *Encephalartos* examined so far, macrozamin concentrations varied between 2% and 3% fresh weight (Moretti *et al.*, 1983) and cycasin concentrations ranged from 0.05% to near 0.1% fresh weight (De Luca *et al.*, 1980). Theoretically, the absolute concentrations or relative concentrations of these compounds within the megagametophyte could influence host use by *A. zamiae* and *A. signatus*.

Norstog & Fawcett (1989) have suggested a further possible basis for discrimination among cycad tissues by insect herbivores. Their hypothesis is based on the possible relationship between BMAA and discrimination between male and female sporophyll tissues of *Zamia furfuracea* L. (Cycadales: Zamiaceae) by the weevil *Rhopalotria mollis* Sharp. In the preferred male tissues, vacuolate cells (idioblasts) have been identified that apparently contain BMAA. These idioblasts also occur intact in the faeces of *R. mollis*. In female tissues, not fed upon by *R. mollis*, the idioblasts are largely absent. The inference from these observations is that the weevils can avoid BMAA in male sporophylls by not digesting the idioblasts, but are prevented from feeding on the female tissues by the presence of 'free' BMAA. The 'bound' versus 'free' occurrence of toxins could therefore provide an additional basis for understanding discrimination between different cycad hosts.

Considerable circumstantial evidence therefore exists for the potential role of plant chemistry in the host relationships of *A. zamiae* and *A. signatus*, but this role needs to be tested. To understand the role of physiological adaptation to the cycad gametophyte in the evolution of host specialization by *A. zamiae* and *A. signatus*, three questions need to be answered.

1. Does the chemistry of the megagametophyte pose a significant barrier to colonization of these tissues by insect herbivores? One way in which this could be established is to determine whether other insects feeding on cycads have the ability to develop on megagametophyte tissues. If so, then the rarity of insects feeding on the megagametophyte must be attributable to causes other than the chemistry of these tissues.

2. Was physiological adaptation to the cycad megagametophyte necessary for the evolution of obligate development on the cycad ovule in *A. zamiae* and *A. signatus*? It is conceivable that ancestral species of *Antliarhinus* were exposed to the same chemical compounds in other cycad tissues so that *A. zamiae* and *A. signatus* may have been physiologically preadapted to developing in the megagametophyte. This hypothesis can be tested on *A. peglerae*, here considered to be a model ancestor for *A. zamiae* and *A. signatus* (Chapter 4). If larvae of this species survive on megagametophyte tissues, then it is probable that no specific adaptation to these tissues has taken place in *A. zamiae* and *A. signatus*.

3. Is host specialization in *A. zamiae* and *A. signatus* a result of differential survival of larvae on the megagametophyte tissues of different species of *Encephalartos* and on *Stangeria eriopus*? This hypothesis can be tested by feeding *A. zamiae* and *A. signatus* larvae on the megagametophyte tissues of different cycad species.

## MATERIALS AND METHODS

### *Insect herbivores feeding on the megagametophyte*

A survey of cycad taxa in South Africa was carried out to determine if any insects, other than *A. zamiae* and *A. signatus*, fed, even occasionally, on the cycad megagametophyte. Megasporangiate cones that were collected over a three year period, and which were initially used to determine host relationships of *Antliarhinus* species in Chapter 2 (Table 2.1) were examined in this regard. Cones were dissected to determine the presence of insect herbivore eggs, larvae or adults in the megagametophyte and to determine patterns of feeding damage in these tissues.

### *Toxicity of the megagametophyte for non-obligate herbivores*

Two insect herbivores, *Zerenopsis leopardina* Felder (Lepidoptera: Geometridae) and *Amorphocerus* sp. (Coleoptera: Curculionidae), that fed occasionally on the megagametophyte, were fed continuously from the first-instar onwards on freshly cut blocks (125mm<sup>2</sup>) of gametophyte tissue until they pupated or died. Blocks of megagametophyte tissue were obtained from *E. altensteinii* ovules collected in May 1989. As controls, *Z. leopardina* larvae were fed on young foliage from *E. altensteinii* and *Amorphocerus* sp. larvae were fed on sporophylls from female cones of the same plant. For *Z. leopardina*, feeding took place in 7 mm diameter petri dishes and for *Amorphocerus* sp., feeding trials were carried out in compartmentalized trays in which each compartment measured 20 x 20 x 20 mm. Fresh food was supplied every alternate day and conditions of 25 ± 2°C and 16:8 light:dark were maintained throughout. Each treatment was replicated 10 times.

A similar experiment was carried out with *A. peglerae*. Larvae were fed on megagametophyte blocks under the same conditions as those for *Amorphocerus* sp. (see above). As a control, larvae were fed on blocks of sporophyll tissue from megasporangiate cones.

### *Toxicity of macrozamin for non-specialist herbivores*

*Zerenopsis leopardina*, *Amorphocerus* sp. and *A. peglerae* were reared on artificial, or partly artificial, diets. The former species was reared on a bean diet comprising ground kidney beans (360 g), brewers yeast (48 g), methyl-p-hydroxybenzoate (3 g), ascorbic acid (4.8 g), sorbic acid (2.1 g), 40% aqueous formaldehyde (1 ml) and distilled water (1000 ml). The constituents were finely ground, boiled and then allowed to simmer for 30 mins before the diet was

dispensed into containers. For *Amorphocerus* sp. and *A. peglerae*, 100 g of freeze dried megasporophyll was milled and mixed with methyl-p-hydroxybenzoate (1 g), ascorbic acid (1.5 g), sorbic acid (0.7 g), 40% aqueous formaldehyde (0.3 ml), agar (2 g) and water (300 ml). Again, the diet was first boiled and then dispensed into containers.

Larvae were fed either on the above diets alone (control) or on the same diets with the addition of pure macrozamin in concentrations of 1% and 3% fresh weight (3% fresh weight corresponds to the highest concentrations recorded in *Encephalartos* ovules; Moretti *et al.*, 1983). Larvae were placed individually in capsules containing 3 ml of diet, and fresh diet was supplied every seven days or sooner if larvae had consumed the available food. Each treatment was repeated with 20 larvae.

Pure macrozamin was obtained from the seeds of *E. altensteinii* and *E. longifolius*. The method of extraction was based on that used by Lythgoe & Riggs (1949) and Altenkirk (1974). The megagametophyte was removed from mature seeds and macerated in a kitchen blender. The macerated tissue was then extracted for 3 h with 80% aqueous ethanol (EtOH), in a ratio of 3 l EtOH: 1 kg tissue and the extraction was repeated three times. The solid matter was filtered off and the filtrate was passed through a column packed with a 1:1 mixture of activated charcoal and celite. The column was eluted first with water (1 l), then with 10% EtOH (1 l) and finally with 20% EtOH (1 l). The EtOH fragments were then vacuum distilled to obtain a viscous yellow syrup. Ethanol (95%) was added to the syrup until it became slightly milky. The mixture was then warmed until it became clear and a little more EtOH was added. The solution was then left at room temperature for several days until crystals had formed. Crystals were recrystallized once using 95% EtOH and were then boiled in a Soxhlet extractor with pure methanol for 3 h. The remaining crystals were dissolved in water and recrystallized using 95% EtOH. The resulting crystals were compared to standards obtained from Dr K. Tadera of Kagoshima University, Japan, using melting point, mass spectra and UV spectra.

#### *Differential performance of larvae on different cycad ovules*

To determine differences in the survival and performance of *A. zamiae* and *A. signatus* larvae between different species of *Encephalartos* and *S. eriopus*, first instar larvae were transferred from ovules of *E. altensteinii* to the ovule gametophytes of the appropriate cycad species. *Antliarhinus zamiae* larvae were transferred to *E. caffer*, *E. cycadifolius*, *E. friderici-guilielmi*, *E. lanatus*, *E. longifolius* and *S. eriopus*. *Antliarhinus signatus* larvae were transferred to the same species except that *E. longifolius* was replaced with *E. villosus*. Larvae of

both species were also transferred to the megagametophyte of *E. altensteinii* as a control.

Two methods were used for testing larvae on gametophyte tissue. In the first method, larvae were transferred individually to 125 mm<sup>2</sup> blocks of fresh gametophyte tissue under the same conditions as for *Amorphocerus* sp. (above). This method resulted in high mortality (see Results) of larvae, probably as a result of frequent handling. In the second method, a leather punch was used to remove a small (3 mm diameter) plug of integument and underlying gametophyte tissue from an intact ovule. Five first instar larvae were transferred into the resultant cavity and the integument plug was then replaced. The plug was sealed with melted bees' wax and the entire ovule was dusted with Thiram, a general purpose fungicide. The treated ovule remained untouched until the larvae had developed.

In both experiments, donor and recipient ovules were first washed with 3% sodium hyperchlorite for 10 mins to remove surface contaminants and were then drenched for 10 s in 70% EtOH to complete this process. To further reduce the incidence of contamination, all transfers were carried out in a flow bench and all equipment was sterilised before use. To reduce desiccation, ovules and gametophyte sections were stored in a modified desiccator in which the drying medium had been replaced with a saturated solution of sucrose to obtain a relative humidity of approximately 85% at 25°C.

In all experiments, larvae of *A. zamiae* and *A. signatus* were obtained by allowing adult beetles to oviposit into *E. altensteinii* ovules. The ovules were then dissected to remove the larvae. Eggs were seldom obtained because cones were in short supply, and it usually took between 24 and 48 h to get sufficient females to oviposit into a cone to justify cutting it open. Both *A. zamiae* and *A. signatus* larvae were transferred to recipient ovules using a no. 3 sable-haired brush that had been dipped in distilled and sterilized water.

For each host species, the developmental duration, mortality and adult mass of the insect herbivore were measured.

## RESULTS

### *Insects feeding on the megagametophyte*

In addition to *A. zamiae* and *A. signatus*, four species of insects were found in which larvae fed on the gametophyte of *Encephalartos* species and/or *S. eriopus*. In all cases, feeding on the gametophyte was facultative and was observed only infrequently. The leopard moth, *Z. leopardina*, was recorded feeding on the megagametophyte tissues of *E. laevifolius*, *E. villosus* and *S. eriopus*. The latter record confirms Giddy's (1984) observation that *Z. leopardina* may destroy entire

megasporangiate cones of *S. eriopus*. Larvae of two species of *Amorphocerus*, one in the *A. talpa* Boheman complex from *E. altensteinii* and one unidentified species from *E. friderici-guilielmi* were also found occasionally in the megagametophyte tissues of their host-plants. Usually, the larvae feed on the sporophylls or cone axis. Lastly, larvae of *Platymerus eckloni* Gyllenhal (Coleoptera: Brentidae), belonging to the same tribe as *Antliarhinus*, fed mostly on the ovule integument but sometimes also fed on the periphery of the gametophyte (see also Oberprieler, 1989; Donaldson, in press-a, Appendix 1).

### *Toxicity of the megagametophyte*

Larvae of *Z. leopardina* and *Amorphocerus* sp. (from *E. altensteinii*) showed no significant differences in pupal mass, or larval survival, when fed continuously on megagametophyte tissues (Table 3.1). In fact, developmental duration was significantly shorter for *Z. leopardina* on gametophyte tissue (Table 3.1). The gametophyte does not, therefore appear to be toxic to these herbivores and, consequently, the infrequent feeding on the megagametophyte cannot be ascribed to its toxicity or distastefulness. Effects of larval diet may be manifest only in the adult stage, e.g. by reduced fecundity or longevity, but this possibility was not tested here.

Larvae of *A. peglerae* never survived on megagametophyte tissue.

**TABLE 3.1** The developmental duration, pupal mass and percentage survival to the adult stage of *Zerenopsis leopardina* and *Amorphocerus* sp. larvae reared on blocks of megagametophyte tissue from the ovules of *Encephalartos altensteinii*. Control larvae were fed young leaves or sporophyll tissue. Data represent the mean ( $\pm 1$  S.E.). Initial  $n = 20$ . Significant differences ( $P < 0.05$ , ANOVA) between control treatments and megagametophyte treatments are denoted by an asterisk.

TREATMENT	Developmental duration (days)	Pupal mass (mg)	% survival
<i>Z. leopardina</i> (leaves)	30.2 ( $\pm 1.1$ )*	130 ( $\pm 4$ )	90
<i>Z. leopardina</i> (gametophyte)	26.3 ( $\pm 0.9$ )	132 ( $\pm 5$ )	96
<i>Amorphocerus</i> sp. (sporophyll)	26.4 ( $\pm 0.85$ )	4.9 ( $\pm 0.16$ )	76
<i>Amorphocerus</i> sp. (gametophyte)	25.3 ( $\pm 0.96$ )	5.3 ( $\pm 0.1$ )	68

### Toxicity of macrozamin for non specialist herbivores

Survival of *Z. leopardina* and *Amorphocerus* sp. larvae on artificial diet was not affected by the addition of macrozamin up to a concentration typical for *Encephalartos* seeds (i.e. 3%, Table 3.2). This result would have been expected since both insects survived on blocks of gametophyte tissue and confirms that both species can cope with significant quantities of the most prevalent toxin in *Encephalartos* seeds.

**TABLE 3.2.** Survival to the adult stage of first-instar larvae of *Zerenopsis leopardina*, *Amorphocerus* sp., and *Antliarhinus peglerae* reared on artificial diet. In two treatments, macrozamin had been added in concentrations of 1% or 3% fresh weight. Data represent the numbers of larvae to survive to the adult stage. Initial  $n = 25$ . \*\* =  $P < 0.01$ , ns = not significant.

Herbivore	Concentration of macrozamin			Chi <sup>2</sup>	Significance
	0%	1%	3%		
<i>Z. leopardina</i>	17	20	18	2.03	ns
<i>Amorphocerus</i> sp.	15	12	14	0.75	ns
<i>A. peglerae</i>	14	10	3	12.4	**

In contrast, *A. peglerae* suffered slight mortality when reared on a diet containing 1% macrozamin, and significant mortality when the diet contained 3% macrozamin (Table 3.2). This result indicates that macrozamin was probably responsible for the mortality of *A. peglerae* larvae that were fed on gametophyte tissue (see above).

### Larval survival of *A. zamiae* and *A. signatus* in different cycad species

Transfers of *A. zamiae* and *A. signatus* larvae between cycad species resulted in significant differences in survival and performance only in those larvae reared on *E. cycadifolius*, *E. friderici-guilielmi* (January) and *E. lanatus* (Tables 3.3 & 3.4). In these species of *Encephalartos*, developmental duration was longer (Table 3.4), adults were smaller (Tables 3.3 & 3.4), and mortality was greater (Tables 3.3 & 3.4) than on any of the other species of *Encephalartos* tested here. No survival was obtained on *S. eriopus*.

**TABLE 3.3.** The developmental duration, adult mass and number to survive to the adult stage, of first instar *Antliarhinus zamiae* larvae transferred from the ovules of *Encephalartos altensteinii* to blocks of gametophyte tissue or to intact ovules of six species of *Encephalartos* including *E. altensteinii* (as a control). Data represent the means ( $\pm 1$  S.E.) and 'n' is provided for each species to account for the effects of mortality on the initial number. \*\* =  $P < 0.01$ ; \* =  $P < 0.05$  (ANOVA). na = not applicable because no larvae survived.

Cycad species	GAMETOPHYTE BLOCKS			ENTIRE OVULES	
	developmental duration (days)	adult mass (mg)	n (initial n = 20)	adult mass (mg)	number surviving per ovule (max = 5)
<i>E. altensteinii</i>	19.5 ( $\pm 0.86$ )	10.2 ( $\pm 0.61$ )	10	9.4 ( $\pm 0.22$ )	3.5 ( $\pm 0.47$ )
<i>E. caffer</i>	20.5 ( $\pm 0.71$ )	10.5 ( $\pm 0.64$ )	7	9.2 ( $\pm 0.44$ )	3.0 ( $\pm 0.44$ )
<i>E. cycadifolius</i>	na	na	0	na**	0**
<i>E. friderici-guilielmi</i> (Jan)	na	na	0	7.1**	0.1 ( $\pm 0.16$ )**
<i>ibid</i> (November)	19.0 ( $\pm 0.78$ )	10.1 ( $\pm 0.5$ )	7	8.9 ( $\pm 0.23$ )	2.7 ( $\pm 0.51$ )
<i>E. lanatus</i>	na	na	0	na**	0**
<i>E. longifolius</i>	19.1 ( $\pm 0.79$ )	11.0 ( $\pm 0.57$ )	9	9.9 ( $\pm 0.37$ )	3.4 ( $\pm 0.43$ )

**TABLE 3.4.** The developmental duration, adult mass and number to survive to the adult stage, of first instar *Antliarhinus signatus* larvae transferred from the ovules of *Encephalartos altensteinii* to blocks of gametophyte tissue or to intact ovules of various species of *Encephalartos*. Data represent the means ( $\pm 1$  S.E.) for the numbers of surviving individuals (n) from each host plant. Host species for which larval performance was significantly different from that on *E. altensteinii* (ANOVA) are designated as: \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ . na = not applicable because no larvae survived.

Cycad species	GAMETOPHYTE BLOCKS			ENTIRE OVULES	
	developmental duration (days)	adult mass (mg)	n (initial n = 20)	adult mass (mg)	number surviving per ovule (max = 5)
<i>E. altensteinii</i>	14.1 ( $\pm 0.65$ )	1.8 ( $\pm 0.09$ )	9	2.0 ( $\pm 0.05$ )	3.9 ( $\pm 0.34$ )
<i>E. caffer</i>	15.2 ( $\pm 0.71$ )	2.1 ( $\pm 0.06$ )	7	2.1 ( $\pm 0.06$ )	3.9 ( $\pm 0.4$ )
<i>E. cycadifolius</i>	20**	1.5**	1	na	0**
<i>E. friderici-guilielmi</i> (Jan)	na	na	0	1.6 ( $\pm 0.05$ )**	0.5 ( $\pm 0.2$ )**
<i>ibid</i> (November)	16.0 ( $\pm 0.65$ )	1.8 ( $\pm 0.05$ )	6	1.9 ( $\pm 0.07$ )	2.9 ( $\pm 0.41$ )
<i>E. lanatus</i>	na	na	0	na	0**
<i>E. villosus</i>	15.1 ( $\pm 0.9$ )	2.0 ( $\pm 0.1$ )	6	1.9 ( $\pm 0.11$ )	3.6 ( $\pm 0.3$ )

The statistically highly significant differences between survival of *A. signatus* and *A. zamiae* larvae on *E. friderici-guilielmi* ovules collected in January and those collected in November (Tables 3.3 & 3.4), show that the time at which ovules were collected influenced the performance of larvae reared on them. This is particularly noteworthy since no differences in larval survival were noted between ovules of *E. friderici-guilielmi* collected in November and the control ovules from *E. altensteinii*. This indicates that the time at which ovules were collected was important for larval survival, and this aspect is dealt with in more detail in Chapter 8.

## DISCUSSION

The habit of feeding, as larvae, on the megagametophyte of cycads has evolved in at least six species of insects in South Africa, belonging to four genera, *Antliarhinus*, *Amorphocerus*, *Platymerus*, and *Zerenopsis*, and two orders (Coleoptera and Lepidoptera). The taxonomy of these groups indicates that the habit must have evolved independently a minimum of three times and possibly four since the history of oviposition into cycad ovules in *Antliarhinus* (see chapter 4) and in the related genus *Platymerus* suggests that larval development on ovule tissues evolved independently in each genus. The physiological capacity to cope to some extent with the chemistry of the megagametophyte is, therefore, not uniquely restricted to a peculiar group of weevils that feed obligately on these tissues.

There are, however, distinct differences between the feeding patterns of larvae that feed obligately and facultatively on the megagametophyte. *Antliarhinus zamiae* and *A. signatus* feed exclusively on these tissues and the megagametophyte is usually entirely consumed by the larvae that feed communally within a single ovule. They cannot, therefore, avoid toxins by not consuming megagametophyte tissues or by selectively moving between different host tissues. In contrast, patterns made by larvae that feed facultatively on the megagametophyte were characterized by chewing marks mainly on the surface of the megagametophyte and by only partially consumed ovules. In most cases, a feeding path could be discerned that traversed several host tissues including the megagametophyte. It is therefore possible that these larvae were avoiding parts of the gametophyte or were only able to ingest gametophyte tissue in small quantities.

These hypotheses were tested by feeding *Z. leopardina* and *Amorphocerus* sp. solely on gametophyte tissue. These larvae suffered no greater mortality or loss of performance than larvae reared on other cone tissues that were conventionally fed upon (Table 3.1). Moreover, macrozamin, the most prevalent toxin in seeds of *Encephalartos*, had no apparent effect on the survival or performance of

*Z. leopardina* and *Amorphocerus* sp. larvae. The inference from these data is that *A. zamiae* and *A. signatus* are not unique in their ability to survive exclusively on megagametophyte tissue. Consequently, the evolution of obligate utilization of the megagametophyte by *A. zamiae* and *A. signatus* cannot be ascribed only to the ability of their larvae to develop on megagametophyte tissues. Obligate development on ovule tissues must have been facilitated by the evolution of other traits not related directly to megagametophyte chemistry. These traits are dealt with in the following chapters.

Nevertheless, the susceptibility of *A. peglerae* larvae to macrozamin, and their inability to survive on gametophyte tissue, indicate that dealing with the chemistry of the gametophyte was important for the evolution of larval development on these tissues. *Antliarhinus peglerae* is considered to represent the probable primitive facies for species of *Antliarhinus* (see Chapter 4) and is regarded as a model for a hypothetical ancestor for *A. zamiae* and *A. signatus*. The conclusion that *A. peglerae* cannot survive in gametophyte tissue is therefore significant for understanding the evolution of larval development on ovule tissues. In other words, the evolution of larval development on cycad ovules in *A. zamiae* and *A. signatus* must have been accompanied by a degree of physiological adaptation in their larvae to feeding on these tissues.

#### *Host specificity in A. zamiae and A. signatus*

No statistically significant differences in the performance of *A. zamiae* and *A. signatus* larvae were observed when they were reared on species of *Encephalartos* from which they were not recorded in the field, specifically *E. caffer*, *E. longifolius*, *E. villosus* and *E. friderici-guilielmi* (collected in November). This means that the absence of *A. zamiae* or *A. signatus* from these species in the field is probably not due to the poor performance of their larvae on these species. Rausher (1984) and Jaenike (1990) have criticized tests of larval performance that do not assess possible effects on adults such as reduced longevity or fecundity. Such tests were not feasible for *A. signatus* or *A. zamiae* due to the complex life histories of these insects. Nevertheless, the survival of *A. zamiae* and *A. signatus* larvae on ovules from a range of *Encephalartos* species is consistent with the known chemistry of these plants in which similar profiles for various chemical compounds have been obtained (Moretti *et al.*, 1983; Siniscalco Gigliano, 1990).

Even the poor performance of *A. zamiae* and *A. signatus* larvae reared on *E. cycadifolius*, *E. friderici-guilielmi* (collected in January) and *E. lanatus* does not mean that the absence of *A. zamiae* and *A. signatus* from these species in the field can be attributed simply to this effect. These cycad species belong to a recognized taxonomic group within *Encephalartos* (Dyer, 1965; Goode, 1989) which have certain character traits in common. One such trait is the timing of cone production. Species from the *E. cycadifolius* group typically first produce megasporangiate cones in September and these cones reach their full size, and are receptive for pollination, in about November. They reach maturity in about March. In contrast, most species of *Encephalartos* that are colonized by *A. zamiae* and *A. signatus* first produce cones in January, are receptive for pollination in April and mature in about November. In the latter group, ovules are usually colonized between April and July (Chapter 8). It is therefore possible that development of the ovule influences the stage at which larvae can survive in the gametophyte tissues (Chapter 8) so that the transfer of larvae to ovules from the wrong stage of development would give a falsely negative result for survival in ovules of that species. The differences in survival between larvae transferred to *E. friderici-guilielmi* ovules in January and those transferred in November support this conclusion (Tables 3.3 & 3.4). This result shows that even within the *E. cycadifolius* group of *Encephalartos*, the ovules are suitable for the development of *A. zamiae* and *A. signatus* larvae for at least some stage during maturation. The significance of the synchrony between larval development and ovule maturation for host specialization is examined in greater detail in Chapter 8.

The exceptional mortality of larvae reared on *S. eriopus* may be attributable to various factors but these were not investigated further. It is, however, worth noting that *Encephalartos* and *Stangeria* belong to different families within the order Cycadales (Stevenson, 1990). Moreover, the lineages from which *Encephalartos* and *Stangeria* have evolved may have separated in the Cretaceous (Sabato, 1990) and it is not surprising that the two taxa are biochemically distinct (Dossaji *et al.*, Herbin, 1975; Moretti *et al.*, 1983). The early divergence of the lineages giving rise to *Encephalartos* and *Stangeria* probably means that *Antliarhinus* species have never utilized species of *Stangeria* and the poor performance of *A. zamiae* and *A. signatus* larvae on *S. eriopus* shows that they are not adapted to feeding on this cycad.

In summary, physiological adaptation to the chemistry of the megagametophyte appears to have little to do with differences in host-specificity between *A. zamiae* and *A. signatus*. Physiological adaptation to unusual compounds found in the megagametophyte tissues, e.g. macrozamin, was probably important for the evolution of larval feeding on these tissues but, once the ability to utilize these tissues had evolved, *A. zamiae* and *A. signatus* were probably

preadapted to feed on megagametophyte tissues of any species of *Encephalartos*. Clearly, utilization of different *Encephalartos* species by *A. zamiae* and *A. signatus* must be caused by adaptation to the host-plant at another level, or must be caused by ecological factors such as avoidance of predators. Host specialization as a result of adaptation for oviposition into host ovules is examined in the chapters that follow.

## CHAPTER 4

### ADAPTATION FOR OVIPOSITION INTO CONCEALED CYCAD OVULES IN *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS*

#### ABSTRACT

In the cycad genus, *Encephalartos*, ovules are surrounded by a three-layered integument and, in most species, by substantial sporophyll tissues. Adaptation for oviposition into concealed ovules by *Antliarhinus zamiae* and *Antliarhinus signatus* females may therefore have been crucial for the evolution of obligate development on cycad ovules. Moreover, the evolution of ovipositional traits in each species may have influenced their ability to lay eggs into ovules of different cycad species. Ovipositional behaviour, and the evolution of ovipositional traits, were examined in this study. *Antliarhinus zamiae* females normally used their remarkably long rostrums to bore between adjacent sporophylls and into the ovule. Subsequently, eggs were laid into the ovule via a telescopically extendable ovipositor. In contrast, *A. signatus* females squeezed between adjacent sporophylls and used their relatively short rostrums to bore only through the ovule integument. Eggs were laid into the ovule using a correspondingly short ovipositor. Comparisons of ovipositional behaviour and ovipositor structure within the genus *Antliarhinus* indicate that traits associated with oviposition into cycad ovules evolved from an ancestor that oviposited into somatic cone tissues from within the megasporangiate cone. The consequences of this evolutionary progression for current ovipositional traits and for the interpretation of host relationships in *A. zamiae* and *A. signatus* is discussed.

Data presented in this chapter have been included in a publication currently in press (Donaldson, in press-b).

Results presented in the previous chapter showed that, within the cycad genus *Encephalartos*, host specialization by *A. zamiae* and *A. signatus* was not caused by differential developmental performances of larvae on different host species. While there may be several reasons for the poor correlation between larval performance on different host species and actual host range in insect herbivores in general (Thompson, 1988b), one possible reason for these differences in *A. zamiae* and *A. signatus* may be that adult females are not capable of depositing eggs into the ovules of all the cycad species on which their larvae can survive.

The megagametophyte of *Encephalartos* species, like all other cycads, is surrounded by a three-layered integument comprising two fleshy layers and a third, stony layer, or sclerotesta (Thomas & Spicer, 1987) (see Fig. 4.1). Together, these layers may be several millimeters thick so that the integument alone may provide protection for the megagametophyte from insect herbivores (Stewart, 1983). The ovules are further enclosed within a compact cone comprised of sporophylls radiating from a central cone axis and arranged in spirals around that axis (Plate 4.1). Adjacent sporophylls abut on to one another, thus enclosing the ovules in an almost continuous layer of sporophyll tissue (Plate 4.1). In most species of

**Plate 4.1.**

**Top.** A partially dissected megasporangiate cone of *Encephalartos altensteinii* showing the spiral arrangement of ovules and sporophylls around the central axis.

**Bottom.** A close up of sporophylls and ovules in a megasporangiate cone of *Encephalartos longifolius*.



*Encephalartos*, the ovules are entirely concealed except for a brief period when the sporophylls separate to allow pollen entry (Dyer, 1965; Giddy, 1984). Insect herbivores, in which the larvae feed on the ovule megagametophyte, must, therefore, be able to penetrate these surrounding tissues in order to oviposit into the gametophyte. Determining how eggs are deposited within the megagametophyte, and understanding adaptation at this level may, therefore, be crucial to any interpretation of the determinants of host range in *A. zamiae* and *A. signatus*.

The snout of *A. zamiae* females (Fig. 2.4), which can attain a spectacular length of 20 mm, has been regarded as an adaptation for oviposition into cycad ovules (Crowson, 1981; Howden, MS). However, the mechanism of oviposition has never been identified until the present study. The remains of broken snouts are often seen protruding into the junctions between sporophylls (Howden, MS; pers. obs.) suggesting that the elongate snout is an adaptation for oviposition from outside the cone; either to bore an oviposition hole for subsequent insertion of the ovipositor, or, as occurs in some other weevils (Crowson, 1981), to push eggs down to the larval host tissues. In contrast, Rattray (1913) and J. Bodenstein (pers. comm.) assumed, from the position of oviposition scars, that oviposition occurred from within the cone, presumably after the adult had entered the cone at the time of pollination. If so, the adult female only has to penetrate the ovule integument in order to deposit her eggs and an alternative explanation for the elongate snout is required. An investigation of the ovipositional traits in these weevils was needed to distinguish between these alternatives. A clear understanding of oviposition in *A. zamiae* was also essential because different modes of oviposition may have different consequences for host relationships. Until this study, nothing was known of the ovipositional habits of *A. signatus* or any other species of *Antliarhinus*.

The aim of this study was to examine morphological and behavioural adaptations for oviposition in *A. zamiae* and *A. signatus* in relation to the evolution of larval development on cycad ovules. The evolution of oviposition into the cycad ovule in *A. zamiae* and *A. signatus* may be the result of both specific adaptation for this function, or the result of the fortuitous occurrence of suitable traits due to other evolutionary events (see Williams, 1966; Gould & Lewontin, 1979), including possibly non-adaptive changes such as allometric scaling of ovipositional structures relative to changes in body size (see Gould, 1966; Gould & Lewontin, 1979). The hierarchy of adaptation for oviposition into the host ovules (see Williams, 1966) needs to be established to distinguish between these possibilities. A comparison of ovipositional traits with related taxa is one way in which this can be achieved (Williams, 1966; Clutton-Brock & Harvey, 1979). The study of ovipositional traits was therefore extended to include the only other species of *Antliarhinus* known

from South Africa, namely, *A. peglerae* and *A. sp. nr verdcourti*. The study included an analysis of allometric scaling of body size relative to snout length for all four species of *Antliarhinus*. In addition, observations on ovipositional traits in the related genus *Platymerus* (Brentidae: Antliarhinini), are included, for comparative purposes, in the discussion.

## MATERIALS AND METHODS

Due to the scarcity of megasporangiate cones for many species of *Encephalartos* (see Chapter 2), experiments were restricted to *E. altensteinii*. Adult beetles used in these experiments were collected in April 1989 from *E. altensteinii* at a single locality near King William's Town (32°59'S 27°16'E). Since some individuals of *A. zamiae* and *A. signatus* may differ in several respects from conspecifics on other host-plants, or from other localities (see Chapters 2 & 7), voucher specimens from this collection were sent to the National Collection of Insects in Pretoria and to Dr G. Kuschel, DSIR, Auckland, New Zealand (accession numbers: *A. zamiae* = NBG80; *A. signatus* = NBG81; *A. peglerae* = NBG82; *A. sp. nr verdcourti* = NBG83).

At the time that adults of the four *Antliarhinus* species were collected, they were active on the outside of the cones and their larvae were present within the host tissues. It is therefore probable that some adult females had already oviposited. However, for the present experiments, prior oviposition in the field was not considered to be a problem. Females reared from larvae in the laboratory were not used because elaborate procedures had to be followed to induce them to oviposit (see Chapter 5).

### *Ovipositional behaviour*

In separate experiments for each species of *Antliarhinus*, 10 adult females and 10 males were individually marked and released onto a cone (details below) in the laboratory (ambient light at  $27 \pm 2^\circ\text{C}$ , RH not recorded). The cone was covered with a black cloth to simulate darkness. Beetles were observed for 5 mins every 15 mins during an 8 h period of exposure on the cones, beginning at 08h00. The experiment was repeated three times with a different set of beetles for each replicate. If a beetle entered the cone during the observation period, and was no longer visible, then the cone was partially dissected until the beetle could be clearly seen. Oviposition sites were marked and, at the end of each 8 h period of

observation, the cone was dissected to confirm that deposition of eggs had taken place.

Cones of *E. altensteinii*, collected in Kirstenbosch Botanic Garden (33°55'S 18°25'E) in late April, and in which the sporophylls were beginning to separate to allow pollen entry, were used in all experiments. This stage of cone development corresponded to periods of beetle activity in the field (Rattray, 1913; Chapter 8). Cones were stored in darkness at 5°C between the time they were collected and the time they were used for experiments (a maximum of 10 days).

Finally, to verify data collected in the laboratory, cones of six species of *Encephalartos* were collected in the field and dissected to determine the position of oviposition scars. In each case, the ovules from two complete spirals of sporophylls from the apex to the base of the cone (about 40 ovules per spiral) were taken as representative of the entire cone. The following number of cones were dissected for each species. *E. altensteinii*, 10; *E. ferox*, 2; *E. horridus*, 4; *E. longifolius*, 6; *E. trispinosus*, 4; *E. villosus*, 5.

#### *Ovipositor structure*

Adult females of *A. zamiae*, *A. signatus*, *A. peglerae* and *A. sp. nr verdcourti* were collected from the field and killed with ethyl acetate. Pressure on the abdomen was used to extend the ovipositor which was then severed from the abdomen, cleared in 10% aqueous KOH, and permanently mounted on a microscope slide.

#### *Allometric analyses*

The following measurements were made. The snout, from the tip to the antennal sockets. The body, from the antennal sockets to the tip of the elytra. The extended ovipositor, from its tip to the posterior margin of the elytra.

In allometric studies, head capsule width has often been used as a more accurate measurement of body size than the measurement of body length (e.g. Masaki, 1986). Head capsule width was, however, not appropriate for this analysis because the relationship between body size and head capsule width was not consistent between species of *Antliarhinus*. Thus there would be no way of comparing measurements between different species of *Antliarhinus*.

Allometric regressions were obtained using Log values of all measurements and were derived from the allometric equation  $Y = bx^a$  (Gould, 1966).

## RESULTS

### *Ovipositional behaviour*

When released on to a megasporangiate cone, the initial response of both sexes of all four species of *Antliarhinus* tested here was to seek shelter in the grooves between adjacent sporophylls. Only under cover of darkness did they attempt to oviposit, but once ovipositional behaviour had been initiated they would continue to oviposit even in daylight. Oviposition sites, and the duration of boring through the sporophyll tissues, as well as oviposition, are presented in Fig. 4.1. Additional details follow.

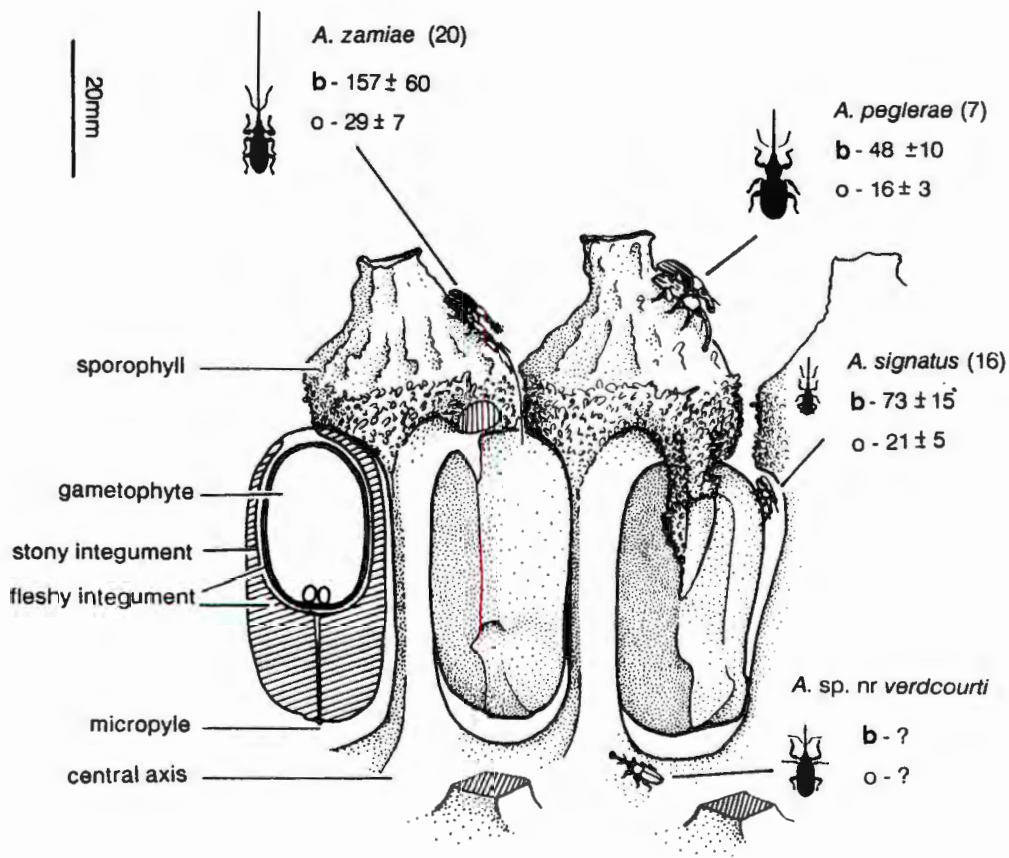


Fig. 4.1. A generalized section of an *Encephalartos* cone showing the oviposition sites of four species of *Antliarhinus*. Hatched lines indicate sections that have been cut away. Statistics represent the mean  $\pm$  1S.E. for the duration, in minutes, of boring (b) and oviposition (o); for each species, *n* is provided in parentheses.

*Antliarhinus zamiae*. Twenty-one of the 30 females tested, used the snout to bore a hole between adjacent sporophylls and through the ovule integument into the gametophyte (Fig. 4.1). Usually, this behaviour resulted in the snout being embedded up to the level of the antennal sockets. The female then withdrew her snout, and immediately inserted her ovipositor. After depositing her eggs (duration recorded in Fig. 4.1), the ovipositor was retracted and the female moved away from the oviposition site. For 17 females, the entire routine took place while coupled with a single male. Dissection of the cone revealed that the female's rostrum penetrated the sporophyll tissue (Fig. 4.2), the integument of the ovule, and approximately 2 mm into the gametophyte. The small diameter of the snout ( $0.105 \pm 0.02$  mm) resulted in only a small entrance hole in the integument but a larger cavity, approximately 1 mm in diameter, was excavated, by the mouthparts, in the gametophyte. Between eight and 28 eggs (mean  $\pm$  1S.E. =  $14.4 \pm 3.7$ ,  $n = 50$  ovipositions) were laid in each cavity.

Although *A. zamiae* adults are dorsoventrally flattened (height =  $0.8 \pm 0.07$  mm), and are therefore capable of entering the cone, only five females entered the cone, all via separated sporophylls in the apical section of the cone. These females were significantly smaller than females that oviposited from outside the cone (ANOVA:  $F = 44.8$ ,  $Df = 1$ ,  $P < 0.01$ ). Once inside the cone, these females moved down to the central axis (Fig. 4.1) where they bored into the locally thickened fleshy integument of the ovule near the micropyle. Boring into the integument lasted between 23 and 65 mins (mean  $\pm$  1 S.E. =  $47 \pm 14.7$ ,  $n = 9$  since some females attempted to bore through the integument more than once) but none of the females inserted their ovipositors. Upon dissection, it was evident that none of the drilling scars (Fig. 4.3) reached the ovule. An analysis of drilling scars in field collected material showed that only 18% ( $n = 200$ ) of scars on the micropylar side of the ovule actually penetrated the integument compared to 95% of scars on the attachment side. In addition, drilling scars were far less frequent on the micropylar side than on the attachment side. In six host species there were significantly more penetration scars on the attachment side of the ovule than on the micropylar side (Table 1).

Four females released on to *E. altensteinii* never attempted to drill into the cone or to enter the cone in any other way.

*Antliarhinus signatus*. The small, flattened, individuals (height =  $0.69 \pm 0.7$  mm) of both sexes squeezed between separated sporophylls to gain access to the ovules. Seven males and four females entered between sporophylls in the lower 1/5th of the cone whereas the remaining 49 adults entered the upper 1/3rd of the cone. When the cone was dissected, the beetles usually moved away from the exposed area, even when observed in darkness using a red light. However, some

**Fig. 4.2-4.9.** 4.2-4.4. Drilling and oviposition scars (arrowed). 4.2. By *Antliarhinus zamiae* on the female sporophyll of *Encephalartos altensteinii*. 4.3. By *A. zamiae* on the micropylar side of the ovule of *E. altensteinii*. 4.4. By *A. peglerae* on the male sporophyll of *E. longifolius*. 4.5-4.8. Extruded ovipositors. 4.5. *A. peglerae*. 4.6. *A. sp. nr verdcourti*. 4.7. *A. signatus*. 4.8. *A. zamiae*. f = foretube, co = cone, r = retractable membrane, c = coxites. 4.9. Rostrum of *A. zamiae*. All scale bars = 1 mm.

females were found with their snouts embedded in the integument about one third of the way down the longitudinal axis of the ovule. The ovule integument is thinnest in this section (see Fig. 4.1). Sixteen females were later observed ovipositing in this portion of the ovule and their position indicated that they may use the adjacent ovule or sporophyll for support when boring into their host ovule. This behaviour may explain why they move away from exposed areas. Dissection of ovules with oviposition scars revealed a cavity in the gametophyte approximately 1mm in diameter into which between 18 and 86 eggs had been laid (mean  $\pm$  1S.E. =  $36.8 \pm 13.7$ ,  $n = 20$ ). The bore of the entrance hole in the integument was approximately equal to the diameter of the snout ( $0.09 \pm 0.019$  mm).

**TABLE 4.1.** Summary of the number of scars caused by *Antliarhinus zamiae* females boring into the micropyle and attachment poles of ovules from six species of *Encephalartos*. Sampling details are given in the text. In all cases the differences between the two poles are highly significant ( $\text{Chi}^2: P < 0.01$ ).

Host species	micropyle pole	attachment pole
<i>E. altensteinii</i>	180	390
<i>E. ferox</i>	5	31
<i>E. horridus</i>	2	70
<i>E. longifolius</i>	1	107
<i>E. trispinosus</i>	133	297
<i>E. villosus</i>	36	156

*Antliarhinus peglerae*. Only seven females of this species attempted to oviposit. The robust adults (height =  $1.74 \pm 0.26$  mm) remained outside the cone, positioned themselves along the vertical axis of a sporophyll, and embedded the relatively short snout either into the adjacent sporophyll or into the sporophyll on which they were standing (Fig. 4.1). Five females inserted their ovipositors, each laying one egg approximately 3 mm below the surface of the sporophyll. In cones collected from the field, oviposition scars were found in similar locations (Fig. 4.4) but in some cases up to three eggs were found within a single cavity.

*Antliarhinus* sp. nr *verdcourti*. The flattened adults (height =  $1 \pm 0.1$  mm) entered the cone through separated sporophylls near the apex of the cone. When the cone was dissected several females were in the process of boring at the base of the sporophyll (Fig. 4.1), or ovipositing there, but could not be induced to repeat this behaviour on an exposed cone axis. Males were not seen in copula with females during boring or oviposition. Only one egg was found in each oviposition site ( $n = 50$  ovipositions) although dissection of 100 oviposition sites from field-

collected material yielded three cavities with two eggs each and one cavity with three eggs.

### *Ovipositor structure*

Howden (MS) has dealt in some detail with the structure of the retracted ovipositor in *A. zamiae* and *A. signatus*. Data presented here focus only on the extruded ovipositor. In all four species of *Antliarhinus* examined here, the ovipositor could be telescopically extruded from the abdomen and comprised three sections (Figs 4.5-4.8). Firstly, as determined by Howden (MS), tergite-8 and sternite-8 are fused to form a cone which is connected to the abdomen by a foretube comprising the membranes joining tergite-7 to tergite-8 and sternite-7 to sternite-8. In unmounted specimens of *A. peglerae* and, to a lesser extent *A. sp. nr verdcourti*, there was a clear evagination where tergite-8 and sternite-8 joined, but the join was almost contiguous in *A. zamiae* and *A. signatus*. The second section is a retractable membrane which in *A. signatus* and *A. zamiae* was clearly muscular throughout its length. When fully extruded the retractable membrane accounted for approximately one third of the ovipositor length in *A. peglerae*, *A. sp. nr verdcourti* and *A. signatus*, and for about half the length in *A. zamiae*. The final section comprised the coxites and styli. In *A. peglerae* and *A. sp. nr verdcourti* the coxites were relatively small, being only two to three times as long as the styli, and were preceded by a membranous section that was clearly not part of the retractable membrane. In *A. zamiae* and *A. signatus* the coxites were elongate and formed one longitudinal half of a tube, the other half consisting of membranous tissue (Figs 4.6 & 4.7).

Although extrusion of the ovipositor did not always follow the same pattern, the following sequence was most common. The cone and foretube emerged first followed by the retractable membrane ensheathing the coxites. When the membrane had extended to the full length of the coxites, the coxites slid out by an unfolding of the membrane along its distal margin.

### *Allometric relationships.*

Extruded ovipositor lengths in *A. zamiae* were strongly correlated with snout length ( $r^2 = 0.98$ ,  $P < 0.001$ ,  $n = 20$ ) and there was a proportional change in all segments of the ovipositor with change in size. When fully extended, the ovipositor was between 0.81 and 0.93 times the length of the snout (mean  $\pm$  1 S.E. =  $0.87 \pm 0.03$ ,  $n = 20$ ) (Figs 4.8 & 4.9). Similar relationships were found for the other species.

The  $\alpha$ -value from the allometric regression,  $Y = bx^\alpha$ , provides an indication of changes in snout length relative to body length for different species of *Antliarhinus*. When  $\alpha = 1$ , snout length changes proportionately with body length. A value  $> 1$  means that larger beetles have disproportionately long snouts, and  $\alpha < 1$  means that larger beetles have disproportionately short snouts. For *A. signatus*, *A. peglerae* and *A. sp. nr verdcourti* there was an almost isometric relationship between snout length and body length ( $\alpha = 0.97$ ) (Fig. 4.10) indicating that there is no selection for snout length variation in beetles of different size. For *A. zamiae*, snout length was proportionately longer than in any of the other species (Fig. 10), irrespective of body size, an indication of selection for longer snouts in this species. Further, there was a positive differential relationship ( $\alpha = 1.4$ ) between snout length and body length in *A. zamiae*. Larger beetles have longer snouts indicating that variation in body size is coupled to selection for snout length.

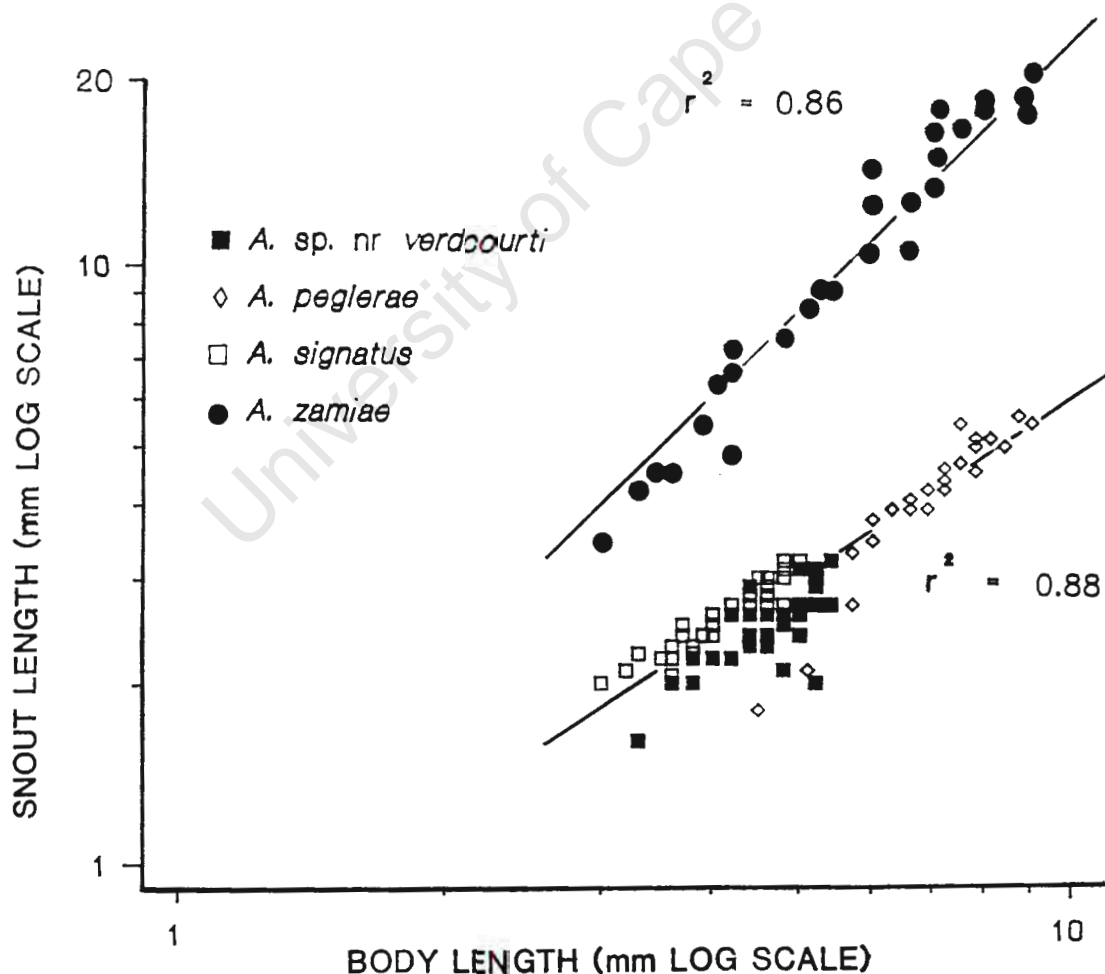


Fig. 4.10. Allometric regressions for snout length against body length for four species of *Antliarhinus*. Details are provided in the text.

## DISCUSSION

Mechanisms for oviposition into the cycad gametophyte are different for adult females of *A. zamiae* and *A. signatus*. The small, dorsoventrally flattened, *A. signatus* rely upon openings between the sporophylls to gain access to the ovule. The female uses her short snout, and equally short ovipositor, to penetrate only through the integument of the ovule. In contrast, *A. zamiae* females most commonly use their extremely long snouts to bore through the sporophyll and, subsequently, the integument of the ovule. Eggs are deposited via an ovipositor of almost equivalent length (the slight discrepancy in length between the rostrum and ovipositor may be attributable to the fact that the rostrum needs to be fractionally longer in order to excavate a cavity in the gametophyte). Although some *A. zamiae* females bore into their host ovule from within the cone, as stated by Rattray (1913), this is exceptional (see Table 4.1) and does not usually result in the deposition of eggs. Dorsoventral flattening enables adult *A. zamiae* to enter the cone, but manoeuvring the long snout within the confines of the cone probably complicates successful penetration of the integument. It is probably equally awkward to manipulate the elongate ovipositor from within the cone.

In both *A. zamiae* and *A. signatus*, infiltration of the barrier posed by the sporophyll tissue could be interpreted specifically as adaptations for reaching the concealed ovules. The adaptive significance of the different modes of oviposition would then be interpreted accordingly. However, this interpretation depends on the evolutionary history of ovipositional traits. Data on oviposition in *A. peglerae* and *A. sp. nr. verdcourti* provide a basis for assessing evolutionary trends leading to oviposition into cycad ovules. Limited data are also available for the related genus, *Platymerus*, so that an outgroup is available for the assessment of characters. The hypotheses presented here, based only on a limited suite of characters, can be tested on a broader character set when the phylogeny of *Antliarhinus* is better known.

### *Evolution of ovipositional traits*

*Antliarhinus peglerae* probably represents the primitive condition within the genus *Antliarhinus* (R.G. Oberprieler, pers. comm.). It is structurally the least modified and is closest in both adult structure and larval host relationships to species of *Platymerus*. In this genus, at least two species (*P. winthemi* Gyllenhal and *P. zeyheri* Gyllenhal) lay eggs singly into the sporophyll tissue and the larvae feed only in this tissue (Oberprieler, 1989; pers. obs.). Similarities between

*A. peglerae* and species of *Platymerus* indicate that species of *Antliarhinus* evolved from an ancestor in which the larvae fed specifically on sporophyll tissue and the adults laid eggs directly into these tissues. Based on this assumption, there are at least three possible pathways for the evolution of oviposition into cycad ovules (pathways A, B & C, Fig. 4.11).

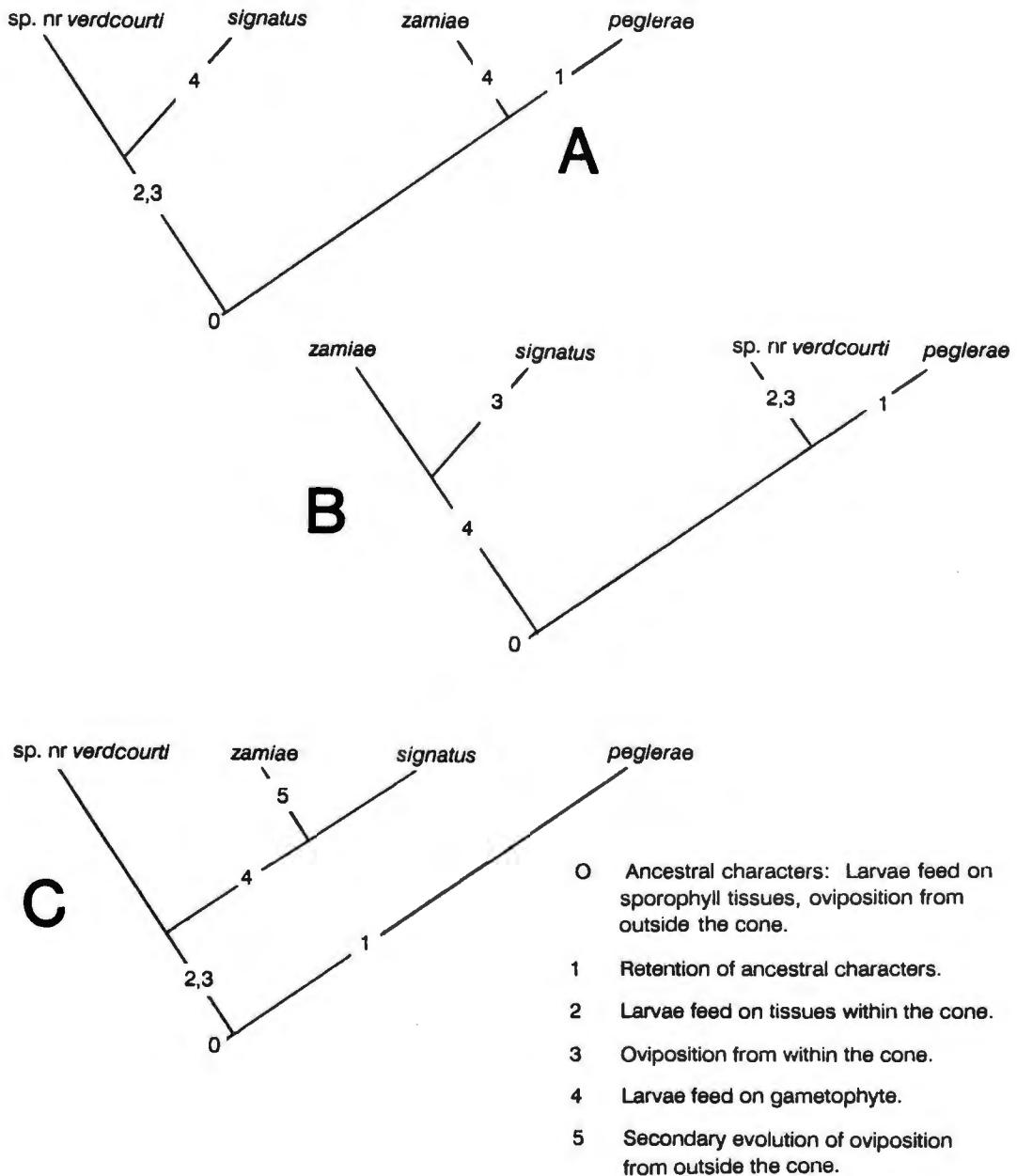


Fig. 4.11. Three possible pathways for the evolution of ovipositional traits in the genus *Antliarhinus*. The analysis was based solely on ovipositional characters. Details are given in the text.

In pathway A, a dichotomy at the level of infiltration into the cone is envisaged as a first step, and the evolution of larval development on cycad ovules as a subsequent step occurring once in each branch. The similarities in oviposition between *A. peglerae* and *A. zamiae*, and between *A. signatus* and *A. sp. nr verdcourti*, appear to support this interpretation. The unique development of obligate feeding on the gametophyte in *A. zamiae* and *A. signatus* (although it may also occur in an undescribed insect found in the ovules of a cycad belonging to the genus *Dioon*, D.W. Stevenson, pers. comm.) suggest, however, that an uncommon set of circumstances existed when this habit evolved. A single evolutionary event leading to larval feeding on the gametophyte is, therefore, more likely. A common origin for *A. zamiae* and *A. signatus* is also supported by the structural similarities in their ovipositors and by the unusual communal development of larvae in both species.

In pathway B, a similar dichotomy is envisaged in which the habit of entering the cone prior to oviposition is considered to have evolved twice, firstly, in *A. sp. nr verdcourti* and secondly in *A. signatus*. The latter species would have evolved from an *A. zamiae*-like ancestor in which oviposition into the ovule occurred from outside the cone. Entry into the host cone requires close synchrony with sporophyll separation at the time of pollination as well as dorsoventral flattening in adult insects. While this combination of characters may have evolved twice, a single event may provide a more parsimonious explanation of the available data. In addition, an acceptable hierarchy of evolutionary events should account for the dorsoventral flattening in adult *A. zamiae* which oviposit from outside the cone.

Pathway C satisfies these criteria. The evolution of dorsoventral flattening and infiltration of the cone, as well as the evolution of larval development on the ovule gametophyte, are predicted to occur only once. In addition, this hypothesis projects that *A. zamiae* evolved from an *A. signatus*-like ancestor. Consequently, the dorsoventral flattening of adult *A. zamiae* would already have existed in the hypothetical ancestor. The character may have been retained because it allows the adults to squeeze between sporophylls and under bark and thereby to avoid detection by predators.

If pathway C is indeed correct, then adaptations for entering the cone (i.e. dorsoventral flattening and behavioural synchrony with sporophyll separation) preceded the evolution of larval development on ovule tissues and did not evolve in *A. signatus* as a means of gaining access to the ovules. Similarly, in this interpretation, the evolution of behaviours and structures in *A. zamiae*, for oviposition from outside the cone, evolved only after the evolution of larval development on the cycad ovule. Thus, in both species, current ovipositional traits may not represent adaptations that led specifically to the evolution of development on the cycad ovule. Nevertheless, ovipositional traits in *Antliarhinus* may have

provided the right conditions for the evolution of obligate development on the cycad ovule.

Current ovipositional traits in *A. zamiae* probably represent new adaptations for oviposition into cycad ovules that accompanied speciation. Speciation is considered to occur mainly in small, isolated populations in which selection pressures differ from those operating on the parent population (Paterson, 1985, 1986; Vrba, 1985). Moreover, the conditions that favour speciation may also result in genetic rearrangements that result in novel traits (Vrba, 1985). It is therefore conceivable that the conditions that were present when *A. zamiae* arose, initially selected for oviposition from outside the cone by *A. zamiae* females and subsequently selected for longer snouts and ovipositors in this species. Moreover, oviposition from outside the cone would have freed *A. zamiae* from the constraints on body size, and probably snout length, that apply to species ovipositing from within the cone. Changes in allometric scaling of snout length relative to body length would then have been possible, thereby facilitating the evolution of new ovipositional functions (see Gould, 1966; Masaki, 1986).

Oviposition by *A. zamiae* and *A. signatus* on various species of *Encephalartos* needs to be examined to determine whether the different ovipositional traits in *A. signatus* and *A. zamiae* affects their ability to utilize particular host species. In Chapter 5, oviposition into a single host species, *E. altensteinii*, is examined to determine which cone and ovule structures are likely to affect oviposition by *A. zamiae* and *A. signatus*. In Chapter 6, cone and ovule structure are examined in nine species of *Encephalartos* to determine whether differences in structure affects the ability of *A. zamiae* and *A. signatus* females to oviposit into the ovules of different cycad species.

## CHAPTER 5

### EFFECTS OF VARIATION IN CONE AND OVULE STRUCTURE WITHIN MEGASPORANGIATE CONES OF *ENCEPHALARTOS ALTENSTEINII* ON OVIPOSITION BY *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS*

#### ABSTRACT

*Encephalartos altensteinii* ovules that are colonized by *Antliarhinus zamiae* and *Antliarhinus signatus* are not distributed randomly throughout the megasporangiate cone. Ovules near the apex and base of the cone are preferred by *A. signatus* whereas ovules situated nearer to the middle of the cone are seldom attacked by this species. A similar, but less significant pattern was observed for ovules colonized by *A. zamiae*. The greater incidence of *A. zamiae* and *A. signatus* in ovules from the apex and base of the cone corresponds with changes in the compaction and thickness of sporophylls in these regions of the cone indicating that these structures affect successful oviposition by *A. signatus* and *A. zamiae*. Forced separation of sporophylls in other parts of the cone results in increased oviposition by *A. zamiae* and *A. signatus* into ovules in these sections of the cone. Compaction between sporophylls prevents *A. signatus* females from entering between the sporophylls and consequently prevents oviposition. In a similar way, thick sporophylls near to the middle of the cone prevent oviposition by *A. zamiae*. Integument thickness of ovules also affects oviposition by *A. signatus*. These results suggest that differences in cone structure between species of *Encephalartos* may affect host specialization in *A. zamiae* and *A. signatus*.

Structures surrounding seeds and ovules, or their ecological equivalents, are expected to have a significant impact on animals that feed on seeds (e.g. Crepet, 1972, 1979; Janzen, 1978; Stewart, 1983; Crepet & Friis, 1987). Protective functions have been postulated for, amongst others, the "massive" ovule integuments in cycads and extinct seed ferns (Stewart, 1983), the cupules of seed ferns (Crepet, 1979; Scott & Taylor, 1983), the interseminal scales of extinct Cycadeioids (Crepet, 1974; Crepet & Friis, 1987), the closed carpel of the angiosperms (Janzen, 1978; Crepet, 1979; Stewart, 1983), the cone scales of conifers (Tripp, 1954; Stewart, 1983), and the megasporangiate cones of cycads (Tang, 1989).

Protection from herbivorous insects is believed to have been particularly important for the evolution of these structures (Crepet, 1979; Stewart, 1983). Insect remains from the Carboniferous contain spores in their guts (Scott & Taylor, 1983) suggesting that, at an early stage of plant evolution, insects fed either on intact reproductive structures or on shed pollen that would have preadapted them for feeding on intact "fruiting bodies" (Malyshev, 1968, in Strong *et al.*, 1984). Further, fossil reproductive bodies of plants from the Upper Carboniferous (Scott & Taylor, 1983) and Mesozoic (Crepet, 1972) show signs of damage, possibly by insects, and the larva of at least one insect from the Upper Carboniferous is believed to have developed in the reproductive organs of gymnosperms (Zherikhin, 1980, as reported by Strong *et al.*, 1984). Seed-feeding weevils may already have

existed in the Upper Jurassic (Crowson, 1981) when cycads were most abundant but before the rise of the angiosperms. In general, it has been proposed that feeding on plant reproductive organs was one of the earliest developments in insect herbivory (Zherikhin, 1980, as reported by Strong *et al.*, 1984) and that feeding by insects on these organs has provided a sustained selection pressure over evolutionary time (Swain, 1978).

These observations have been interpreted as convincing circumstantial evidence that reproductive structures have evolved to protect the seed from insect herbivores (Stewart, 1983). As a result, these structures are expected to have a significant influence on the successful utilization of seeds by insect herbivores (Janzen, 1969, 1971). Host specialization by seed-feeding insects may therefore be influenced by the structures associated with the seeds or ovules of their host-plants.

Data on oviposition in *A. zamiae* and *A. signatus* presented in Chapter 4, suggest that cone and ovule structure in species of *Encephalartos* may have a substantial effect on the ability of these insects to lay eggs into the ovules of their cycad hosts. Female *A. signatus* must first enter the cone between adjacent sporophylls before they can use their short snouts (up to 3.5 mm) to drill through the ovule integument. As a result, they may be excluded from ovules which are surrounded by closely compacted sporophylls, or from ovules which have substantial integuments. Female *A. zamiae* bore between adjacent sporophylls with their elongate snouts and are, therefore, probably not affected by the compaction of adjacent sporophylls. However, sporophyll thickness would be expected to influence the ability of *A. zamiae* females to reach ovules with their long snouts and, consequently, also to oviposit into them. Sporophyll compaction, and thickness of the ovule integument, may therefore be expected to influence oviposition by *A. signatus*, and sporophyll thickness would be expected to influence oviposition by *A. zamiae*.

It is important for understanding host specialization in *A. zamiae* and *A. signatus* to determine whether the respective ovipositional traits of the two species influence their ability to colonize different cycad hosts. Ultimately, this can only be determined by examining the ovipositional responses of *A. zamiae* and *A. signatus* on a variety of *Encephalartos* species and such an analysis is presented in Chapter 6. However, it is first necessary to determine whether oviposition by *A. zamiae* and *A. signatus* is indeed influenced by cone structure, even in a single host species. These results can then form a basis for comparing cone and ovule structure in different species of *Encephalartos* and for interpreting the possible significance of differences in cone and ovule structure for oviposition, and host specialization, in *A. zamiae* and *A. signatus*.

Dissection of *E. altensteinii* cones collected from plants in the field showed that ovules in some parts of the cone, particularly near the apex and base, were more often colonized by *A. signatus* and *A. zamiae* than ovules in other parts of the cone. This observation showed that the distribution of *A. zamiae* and *A. signatus* within cones of *E. altensteinii* might be affected by the ovipositional behaviour of the female. As a result, *E. altensteinii* would be an ideal host-plant on which to test the effects of cone and ovule structure on oviposition by *A. zamiae* and *A. signatus*.

Thus, the aim of this study was to determine whether variation in cone and ovule structure within megasporangiate cones of *E. altensteinii* had any effect on oviposition by *A. zamiae* and *A. signatus*. Two specific hypotheses were tested.

1. That oviposition by *A. signatus* would be influenced mostly by variation in cone compaction and integument thickness.

2. That oviposition by *A. zamiae* would be affected predominantly by variation in sporophyll thickness.

## MATERIALS AND METHODS

### *General procedures*

Sampling and experimental procedures were influenced by three characteristics of the megasporangiate cones of *E. altensteinii*.

1. Mature female plants produce between one and five megasporangiate cones at a time. Generally, the cones first appear in January and reach their full size in late April when the ovules are receptive for pollination (Chapter 8). The cone remains intact for several months after this to allow for the slow process of fertilization and embryo development, and the cone usually disintegrates during November. Some cones may be retained until as late as the following March (pers. obs.). Negligible changes in cone dimensions between the time the cone reaches its full size (May) and the time the cone disintegrates means that comparable measurements can be made anytime over a six month period after May.

2. Ovules, too, reach their full size about the time of pollination. Unfertilized ovules are not aborted, but to all outward appearances continue to develop in the same way as fertilized ovules. This means that estimates of the percentage ovules colonized by *A. zamiae* and *A. signatus* are not compromised by unknown numbers of aborted ovules (Janzen, 1971).

3. The cone is comprised of adjacent rows of sporophylls arranged in spirals around a central axis (see Fig. 5.1B). Each sporophyll covers two ovules. Most megasporangiate cones of *E. altensteinii* had eight spirals of sporophylls and

between 19 and 24 sporophylls in each spiral. As a result, there are between 300 and 500 ovules within a single cone. An analysis of cone and ovule dimensions showed that there were no significant differences between spirals of the same cone for any of the structures tested in this study. For this reason, both field collected data, and experiments, could be restricted to only a few spirals on each cone as a subsample of the entire cone.

#### *Distribution of A. zamiae and A. signatus within cones*

The distribution of *A. zamiae* and *A. signatus* within megasporangiate cones was determined by dissection of 20 mature, but intact, *E. altensteinii* cones collected in November 1988 and 1989 from four localities in South Africa; East London (33°07'S 27°46'E), Grahamstown (33°17'S 26°33'E), Kenton on Sea (33°37'S 26°43'E), and King William's Town (32°59'S 27°16'E). For each cone, the seeds from three spirals of sporophylls (i.e. six spirals of seeds) were opened to determine the presence of either *A. zamiae* or *A. signatus*. At this stage, most *A. zamiae* and *A. signatus* were present as adults, but they could also be distinguished from one another as pupae.

#### *Cone structure*

For each cone collected in the field, the thickness of the sporophylls, measured at the junction between adjacent sporophylls, was assessed for two complete spirals. In addition, the following measurements were made on cones collected in May 1989 from *E. altensteinii* plants in Kirstenbosch Botanic Garden (May corresponds to the month in which oviposition by *A. zamiae* and *A. signatus* usually takes place in the field, Chapter 8).

1. The thickness of the sporophylls at the intersporophyll junction where *A. zamiae* females would insert their rostrums.

2. The thickness of the ovule integument.

3. The compaction of adjacent sporophylls. Only a crude measure of compaction was obtained by equating it to the mass required to force the blade of a 0.7 x 7 mm wide spatula down to the level of ovule. Mass was determined by placing a 10 l beaker on a stand attached to the top of the spatula and filling it with water. A zero value meant that there was no resistance to the spatula reaching the ovule. The maximum possible value was slightly more than 10 kg.

The number of sporophylls, and therefore ovules, in a spiral ranged from 19 to 24. To allow pooling of data from cones with different numbers of sporophylls, each cone spiral was divided into five sections (Fig. 5.1B). The apical and basal

sections each comprised three sporophylls. Sections 2 and 4 (Fig. 5.1B) each contained five sporophylls and the middle of the cone, i.e. section 3, comprised the remaining sporophylls. Only three sporophylls were grouped together in the apical and basal sections of the cone because the greatest variation from the cone average occurred at these points (see Fig. 5.2) whereas the remaining sections were relatively uniform.

#### *Effects of cone and ovule structure on oviposition*

To determine the possible effects of cone and ovule structure on oviposition by *A. signatus* and *A. zamiae*, experiments were carried out on megasporangiate cones collected in May 1990 from the Kirstenbosch Botanic Garden and the Arderne Garden in Cape Town. On each cone, two adjacent spirals of sporophylls (i.e. four spirals of ovules) were separated from the remaining sporophylls in the cone by removing one spiral of sporophylls on each side. The isolated double row of sporophylls could then be sealed off with a fine-mesh gauze and used as an experimental arena.

Beetles used in experiments were collected as adults from mature cones of the previous season (November 1989). These beetles were stored in cardboard boxes in total darkness at  $25 \pm 2^\circ\text{C}$  and 85% RH for the following five months. In April 1990 they were released on to an *E. altensteinii* cone for 48 h and then stored in boxes again until used. Although the factors that initiate oviposition in *A. zamiae* and *A. signatus* are not properly understood, this elaborate procedure was necessary to stimulate oviposition. Since mating takes place only on the host cone (Chapter 4; Donaldson, in press-b), males and females were stored together.

There were 10 replicates for each of the treatments outlined below. To account for intercone differences, treatment and control experiments were done in pairs on the same cone.

*Treatment 1.* Twenty males and 20 females of *A. signatus* were released on to a cone in which the sporophylls had been forcibly separated to expose the ovules underneath. As a control, the same number of adults were released onto an intact cone in which sporophylls abutted tightly on to one another. Since *A. signatus* females cannot oviposit from outside the cone (Chapter 4), this treatment tests the effectiveness with which sporophyll compaction prevents *A. signatus* females from entering the cone. Further, in the opened spiral, the only remaining barrier to penetration of the ovule is the integument, so this treatment also tests the effect of integument thickness on oviposition by *A. signatus*.

*Treatment 2.* The same conditions were applied as in treatment 1, but with adult *A. zamiae*. Since *A. zamiae* females usually oviposit from outside the cone, even when the sporophylls are closely compacted (Chapter 4), the separation of

adjacent sporophylls effectively reduces the thickness of the sporophyll enclosing the ovule. This treatment therefore tests the effect of sporophyll thickness on oviposition by *A. zamiae*. To reduce variability in results that may have arisen as a result of oviposition by *A. zamiae* females with different snout lengths, only females with snouts between 10 and 14 mm long (from the antenna to the tip) were used.

## RESULTS

### *Within-cone distribution of A. zamiae and A. signatus*

In *E. altensteinii* cones collected in the field, there was a distinct distributional pattern of ovules colonized by both *A. zamiae* and *A. signatus* (Fig. 1A). Ovules in the apical and basal sections of the cone were significantly preferred (ANOVA:  $F = 14$ ,  $df = 4$ ,  $P < 0.01$ ) and relatively few of these ovules were not colonized by either *A. signatus* or *A. zamiae*. This pattern was particularly marked for *A. signatus* which was almost never found in ovules from the middle sections of the cone (Fig. 5.1A). Ovules colonized by *A. zamiae* were more dispersed throughout the cone (Fig. 5.1A), but *A. zamiae* occurred significantly less often in ovules from section 4 (Fig. 5.1B) of the cone (based on a Scheffe multiple range test of ANOVA:  $F = 5.4$ ,  $df = 4$ ,  $P < 0.01$ ).

The greater colonization of ovules from the apex and base of the cone by *A. signatus* and *A. zamiae* corresponded to several differences in cone morphology between these sections of the cone and the middle sections of the cone. Firstly, at the time of pollination, sporophylls in the apical and, to a lesser extent, basal section of the cone became separated from each other. The degree of sporophyll compaction in these sections was therefore different from that in the middle of the cone (Fig. 5.2A). Before and after pollination, there was little or no difference in compaction between the sections (Fig. 5.2A). Secondly, at all times, the sporophylls in the middle sections of the cone were thicker than those at the apex and base (Fig. 5.2B). Thickness of the ovule integument also varied between ovules, but the differences were not associated with specific positions within the cone (as determined by an ANOVA comparison of the integument thickness of ovules from the five sections of the cone:  $F = 0.93$ ,  $df = 4$ ,  $P > 0.05$ ).

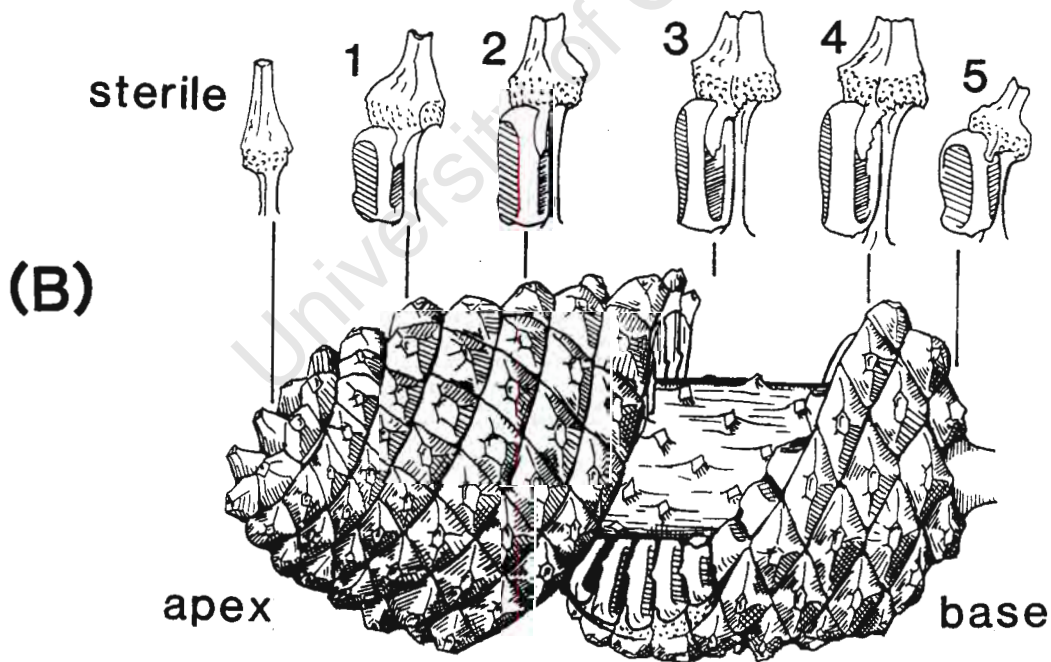
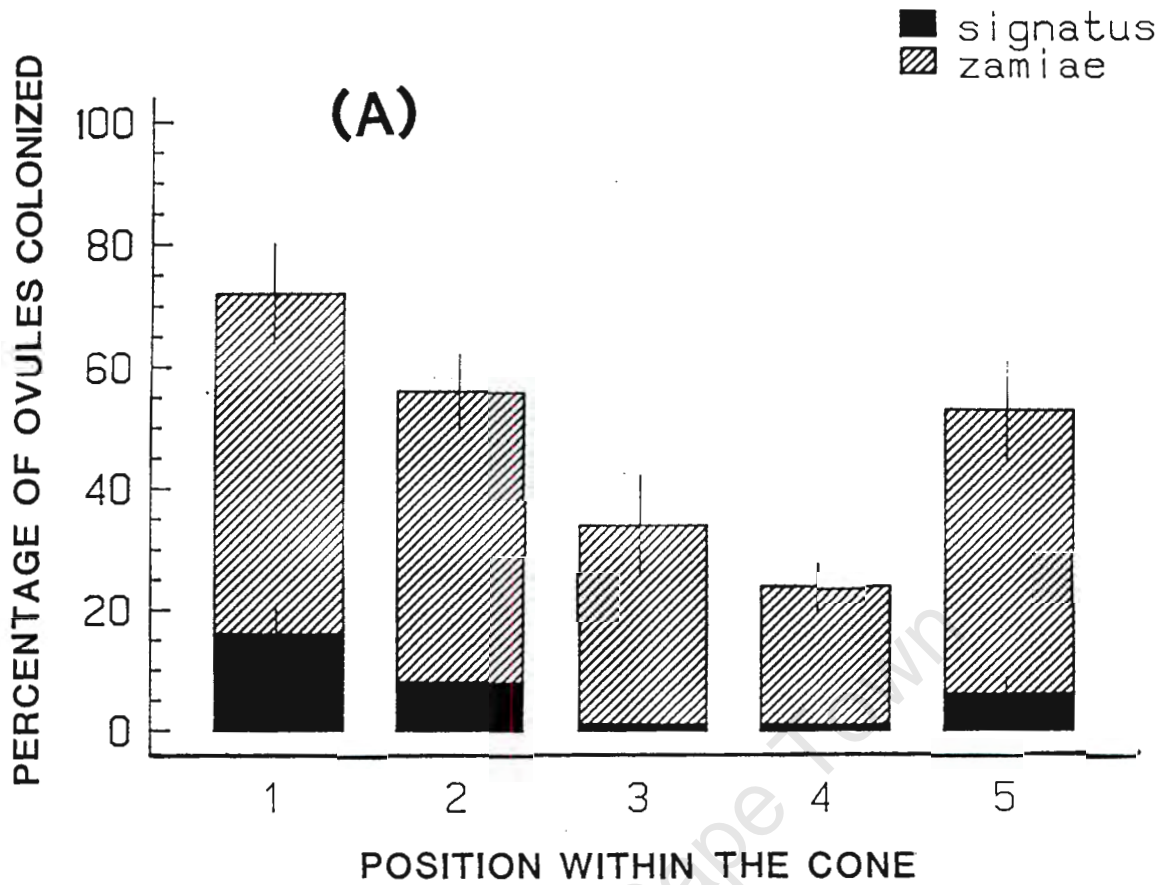


Fig. 5.1 A-B. A. The percentage of *Encephalartos altensteinii* ovules colonized by *Antliarhinus zamiae* and *A. signatus* in each of five sections of the megasporangiate cone (see Fig. 5.1B). Data represent the means  $\pm$  1 S.E. (vertical lines in each bar) for 20 cones. B. A partly dissected megasporangiate cone showing the approximate position of the five cone sections. The shape of the sporophylls and ovules are shown in lateral aspect. Sporophylls at the cone apex are always sterile.

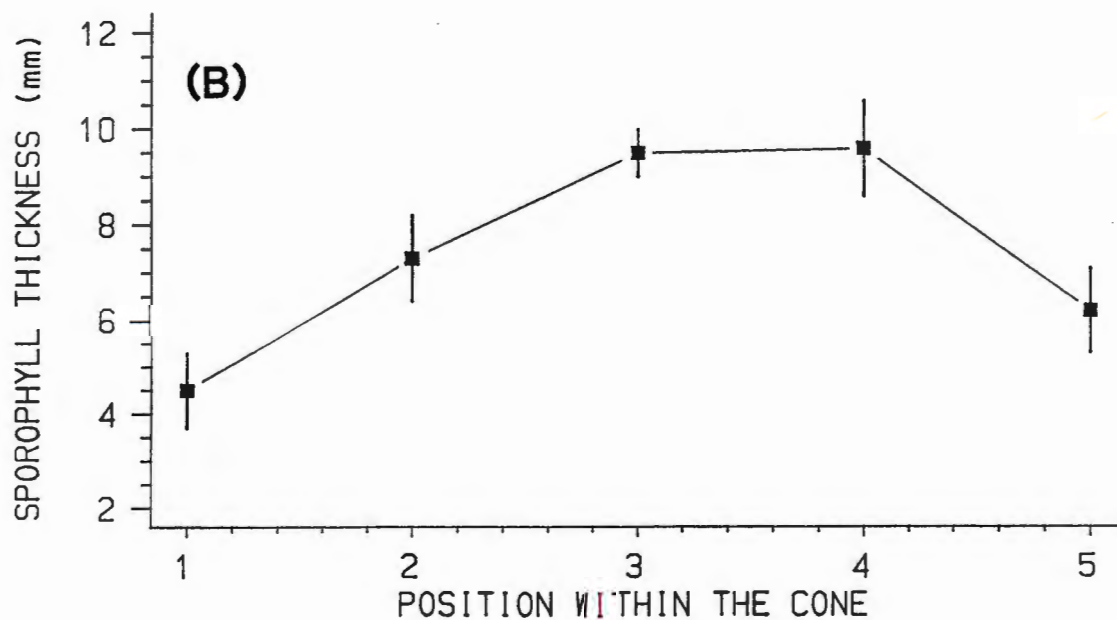
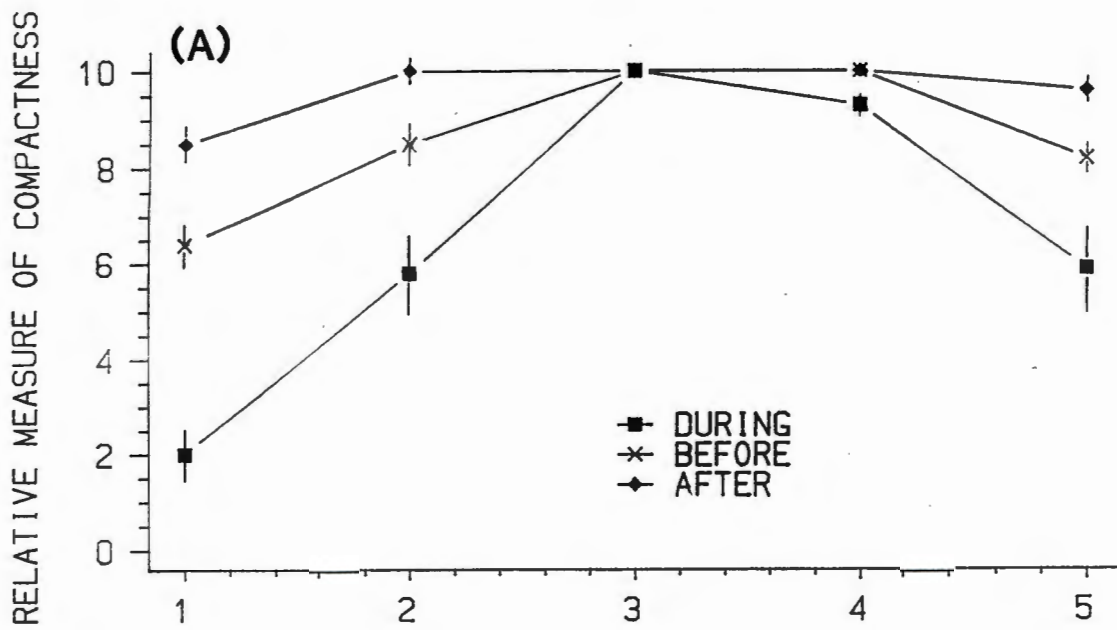


Fig. 5.2 A-B. Differences in the megasporangiate cone structure of *Encephalartos altensteinii* relative to the position within the cone (the five cone sections are illustrated in Fig. 5.1B). A. Compaction between adjacent sporophylls before, during and after the cone opens to allow pollen entry. B. Sporophyll thickness at the junction between adjacent sporophylls.

### Effects of cone and ovule structure on oviposition

In treatment 1, oviposition by *A. signatus* was significantly greater in spirals in which sporophylls had been forcibly separated (ANOVA:  $F = 61.5$ ,  $df = 1$ ,  $P < 0.01$ ). Compaction between sporophylls must, therefore, prevent *A. signatus* females from entering the cone and, consequently, has a substantial effect on oviposition by *A. signatus*.

In opened spirals of treatment 1, there were no significant differences in oviposition by *A. signatus* females between the different sections of the cone (ANOVA:  $F = 0.93$ ,  $df = 4$ ,  $P > 0.1$ ). This indicates that either thickness (or hardness) of the ovule integument has no effect on oviposition by *A. signatus* or that ovules with different integument thicknesses are distributed throughout the cone in an irregular way (i.e. not corresponding to the five recognized divisions of the cone). An analysis of oviposition by *A. signatus* females into ovules with the same integument thickness (i.e. when data for all the ovules with the same integument thickness were pooled) was carried out to distinguish between these alternatives. This analysis showed that there was a significant linear correlation between integument thickness and successful oviposition by *A. signatus* (Fig. 5.3).

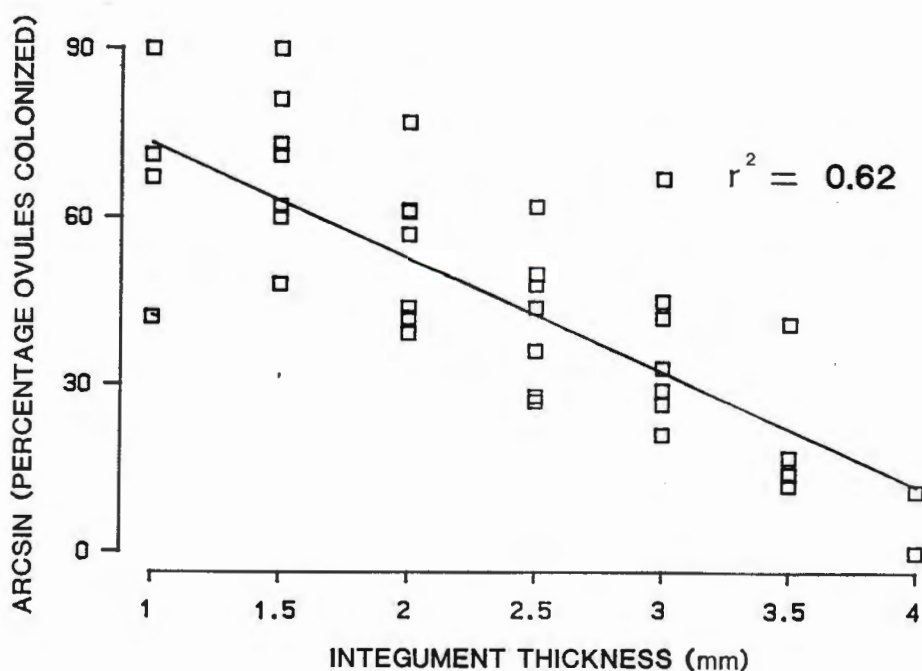


Fig. 5.3. Regression of the percentage of *Encephalartos altensteinii* ovules colonized by *A. signatus* relative to the thickness of the ovule integument.  $n = 41$  points,  $P < 0.01$  (ANOVA).

A gradual decline in successful ovipositions by *A. signatus* females was observed in ovules with thicker integuments (Fig. 5.3) and this reduced ovipositional success was probably caused by females probing ovules with their snouts and ovipositing preferentially into those ovules with the thinnest integuments. In support of this conclusion, is the observation that ovules frequently had scars caused by *A. signatus* females boring into the ovule integument but in which no larvae were found. Ovules with an integument thicker than 3.5 mm were hardly ever colonized by *A. signatus*. This threshold value corresponds to the upper limit for snout length in *A. signatus* (Chapter 4) and it is probable that *A. signatus* cannot penetrate these integuments.

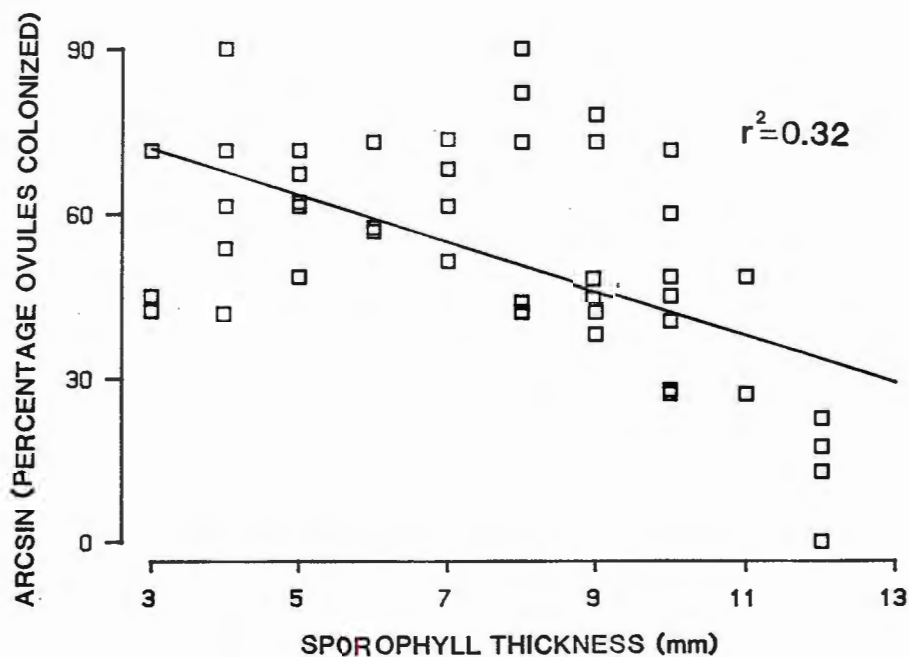


Fig. 5.4. Regression of the percentage of *Encephalartos altensteinii* ovules colonized by *Antliarhinus zamiae* in relation to the thickness of the sporophyll directly above the ovule.  $n = 47$ ,  $P < 0.01$  (ANOVA).

In treatment 2, there was again a significant increase in successful ovipositions by *A. zamiae* females in rows of forcibly separated sporophylls (ANOVA:  $F = 14.08$ ,  $df = 1$ ,  $P < 0.01$ ). Thick sporophylls must, therefore, prevent *A. zamiae* females from boring into ovules in certain sections of the cone.

A weak linear correlation was found between sporophyll thickness in the cone and successful oviposition by *A. zamiae* females in treatment 2 (Fig. 5.4). In this

instance, increase in sporophyll thickness accounted for only 32% of the variability in successful ovipositions. This means that female *A. zamiae* do not always reject ovules if they are covered by substantial sporophylls. However, for sporophylls thicker than 12 mm there was a significant decline in oviposition (ANOVA:  $F = 5.8$ ,  $df = 9$ ,  $P < 0.01$ ) indicating that large sporophylls do indeed prevent oviposition by *A. zamiae*. Although the reduction in oviposition associated with sporophylls thicker than 12 mm (Fig. 5.4) does not correspond to the maximum snout length for *A. zamiae* which may reach 20 mm (Chapters 4 & 7), the beetles used in this experiment all had snouts in the range of 10-14 mm.

The effect of sporophyll thickness on oviposition by *A. zamiae* was also evident in cones collected in the field. Differences in mean sporophyll thickness between cones accounted for 87% of variation in the proportion of ovules colonized by *A. zamiae* in different cones (Fig. 5.5).

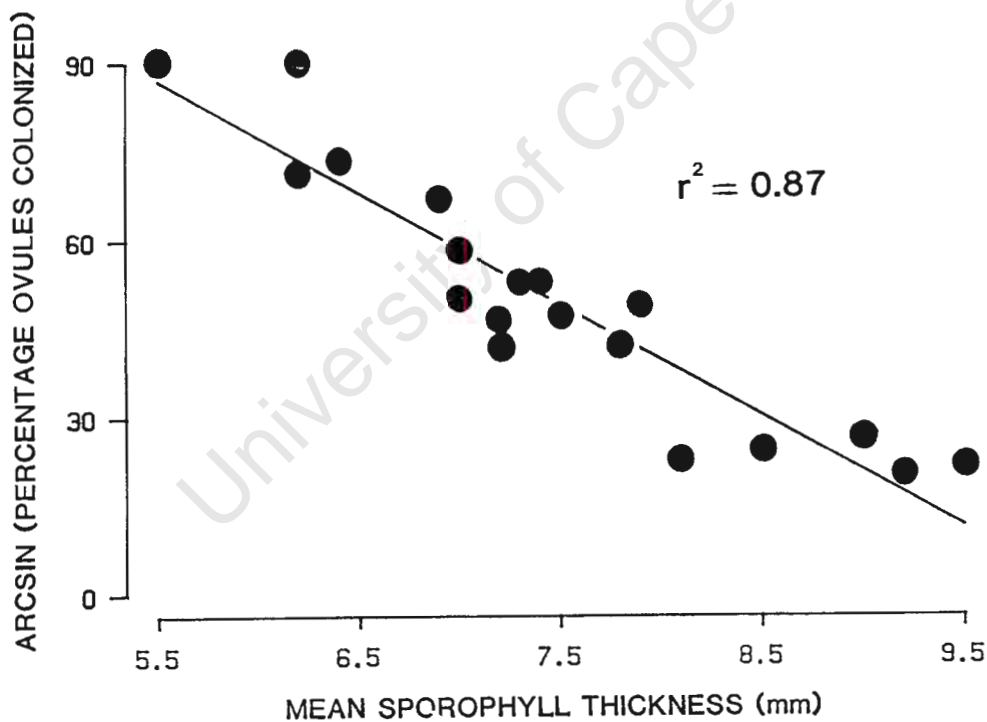


Fig. 5.5. Regression of the percentage of *Encephalartos altensteinii* ovules colonized by *Antliarhinus zamiae* relative to the mean sporophyll thickness for the entire cone in 19 megasporangiate cones collected in the field.

## DISCUSSION

Data presented here support the hypothesis that variation in cone and ovule structure within megasporangiate cones of *E. altensteinii* would have a noticeable impact on successful oviposition by *A. zamiae* and *A. signatus* females. Differences in cone compaction and sporophyll thickness along the axis of the cone (Fig. 5.2A-B) result in a consistent pattern of oviposition by *A. signatus* and *A. zamiae* in which the ovules at the apex and base of the cone are preferred (Fig. 5.1A). *Antliarhinus signatus* oviposits most successfully into ovules with relatively thin integuments (Fig. 5.3) and *A. zamiae* oviposits most successfully into ovules that are covered by relatively thin sporophyll tissues.

The extent to which cone and ovule structure will influence oviposition by *A. zamiae* and *A. signatus* must depend, ultimately, on the extent to which selection imposed by these structures can modify ovipositional traits. In the case of *A. signatus*, successful oviposition depends on the female's ability to enter the cone between adjacent sporophylls. This behaviour appears to be a fixed response and probably shows little variation. As a result, closely compacted sporophylls simply exclude *A. signatus* females from certain parts of the cone. Thickness of the ovule integument may also prevent oviposition by *A. signatus* into the megagametophyte but the extent to which this factor will affect the distribution of *A. signatus* will depend on variability in snout length. Snout length in *A. signatus* varies only slightly between females (from 1-3.5 mm, Chapter 4) and may be constrained by the need to enter the cone and to manoeuvre within the cone (Chapter 4). Consequently, variability in snout length in *A. signatus* females may not be sufficient to allow some females to oviposit into ovules with integuments greater than 3.5 mm.

In contrast, snout length in *A. zamiae* is extremely variable, ranging from 4-20 mm (Chapters 4 & 7). Thick sporophylls may therefore be expected to select for longer snouts, but only exceptionally thick sporophylls (greater than 20 mm) should totally prevent oviposition by all *A. zamiae* females. In host species such as *E. altensteinii*, in which sporophyll thickness ranges from 3 to 18 mm, successful oviposition by *A. zamiae* females may depend on the range of snout lengths in that particular population. In host species with more substantial sporophylls, e.g. *E. longifolius*, it is possible that only females of *A. zamiae* with the longest snouts will be able to oviposit successfully. This possibility is examined in greater detail in chapter 7.

The results presented here provide a basis for further studies. Firstly, it is clear that cone and ovule structure can have a significant effect on oviposition by *A. zamiae* and *A. signatus*. It is therefore necessary to establish to what extent cone and ovule structure varies between different species of *Encephalartos* and to

what extent this variation may affect oviposition and, consequently, host utilization by *A. zamiae* and *A. signatus* (Chapter 6). Secondly, there is a need to understand the extent of variation in snout length in *A. zamiae*. It is proposed here that variation in snout length may enable *A. zamiae* to colonize a greater number of host species because selection imposed by greater sporophyll thickness will simply select for individuals with longer snouts and will not result in exclusion of *A. zamiae* from the host-plant. This suggestion is dealt with in Chapter 7.

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## CHAPTER 6

### VARIATION IN CONE AND OVULE STRUCTURE BETWEEN SPECIES OF *ENCEPHALARTOS*, AND CONSEQUENCES FOR OVIPOSITION BY *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS*

#### ABSTRACT

Cone and ovule structure varies between different species of *Encephalartos*. Sporophyll thickness ranges from around 5 mm in *E. villosus* to an exceptional 21 mm in *E. longifolius*. Similarly, the thickness of the ovule integument varies from less than 2 mm in *E. princeps* to nearly 5 mm in *E. villosus*. In some species of *Encephalartos*, specifically those in the *E. cycadifolius* group (Dyer, 1965), the sporophylls are covered by a woolly tomentum that may interfere with oviposition. *Antliarhinus zamiae* occurs rarely in species with substantial sporophylls, but if the sporophylls are forcibly separated then successful oviposition increases significantly. *Antliarhinus signatus* does not colonize species with thick ovule integuments (generally > 4 mm). *Antliarhinus signatus* females confined on cycad species with thick integuments, such as *E. caffer* and *E. villosus*, did not attempt to oviposit into these species. The inference from this result is that cycad species with thick integuments are not recognized as hosts by *A. signatus* females. Finally, no species of *Antliarhinus* were recorded from cycads in which the cone was covered with a thick woolly tomentum, but the tomentum had no noticeable effect on the ability of the beetles to oviposit. It is concluded that effects of cone structure in different species of *Encephalartos* on oviposition by *A. signatus* and *A. zamiae* can account to some extent for host specialization in these insects.

Results presented in Chapter 5 show that cone and ovule structure have a significant influence on oviposition by *A. zamiae* and *A. signatus* within cones of *E. altensteinii*. Specifically, a thick ovule integument (> 3.5 mm), and closely compacted sporophylls, prevent oviposition by *A. signatus* into many ovules within the cone. Similarly, thick sporophylls, that abut onto adjacent sporophylls, effectively prevent *A. zamiae* females from laying eggs into the underlying ovules. The effectiveness of these barriers against oviposition within one cycad species suggests that host use in general may be determined by the ability of *A. zamiae* and *A. signatus* to oviposit into the ovules of different cycad species.

Successful oviposition by *A. zamiae* and *A. signatus* may not depend only on the host-plant structures mentioned above. In addition, some cycad species, notably *E. cycadifolius* and its close taxonomic relatives (e.g. *E. friderici-guilielmi*, *E. ghellinkii*, and *E. lanatus*) have sporophylls that are covered by a thick woolly layer (Plate 6.1). This tomentum has been implicated in explanations for the absence of *A. zamiae* and *A. signatus* from these cycads (Oberprieler, 1989). The woolly strands are reputed to hinder movement by *A. zamiae* and *A. signatus* and thereby to prevent them from ovipositing into the ovules of these cycads (Oberprieler, 1989). This claim has yet to be substantiated, but indicates a further

**Plate 6.1.**

**Top.** A megasporangiate cone of *Encephalartos lanatus*.

**Bottom.** A close up of a partially dissected megasporangiate cone of *Encephalartos friderici-guilielmi* showing the tomentum (T) on the surface of the sporophylls.



way in which cone structure could influence host specialization in *A. zamiae* and *A. signatus*.

The effects of cone and ovule structure on host relationships in *A. zamiae* and *A. signatus* were examined here. The following hypotheses were tested.

1. That *A. zamiae* would be rare in cycad species with large sporophylls and would not be able to colonize species in which sporophyll thickness at the junction between sporophylls was greater than 20 mm.

2. That *A. signatus* would be less abundant in species of *Encephalartos* with thicker integuments and would be totally excluded from species in which the integument is thicker than 3.5 mm.

3. That a tomentum on the sporophyll surface would prevent oviposition by *A. zamiae* and *A. signatus*.

## MATERIALS AND METHODS

### *Variation in cone and ovule structure*

Cone and ovule structure was examined in nine species of *Encephalartos* from the eastern Cape Province of South Africa, and which occur within the general distribution of *A. zamiae* and *A. signatus* (see Chapter 2). These species were *E. altensteinii*, *E. caffer*, *E. friderici-guilielmi*, *E. horridus*, *E. lehmannii*, *E. longifolius*, *E. princeps*, *E. trispinosus* and *E. villosus*. Each cone was dissected and the following structures were measured. 1. The thickness of the sporophyll immediately above the ovule (Fig. 6.1). 2. The thickness of the integument at its most exposed section, i.e. immediately below the junction between two sporophylls. This measurement was obtained from longitudinally bisected ovules (Fig. 6.1).

The cones measured here were collected from natural habitats in November 1989 for *E. friderici-guilielmi* and in May 1989 and 1990 for the remaining species. Five cones were examined for each species except for *E. caffer* and *E. horridus* for which only four cones were obtained. For each cone, every fourth ovule in two spirals was measured (about 10 samples per cone).

### *Effects of cone and ovule structure on oviposition*

1. *Sporophyll thickness*. To determine the effects of sporophyll thickness on oviposition by *A. zamiae*, 20 males and 20 females were released on to cones of *E. altensteinii*, *E. caffer*, *E. lehmannii*, and *E. longifolius*. In each cone, two spirals

of ovules had been sealed with a net bag; in one spiral, the sporophylls had been forcibly separated and, in the other, the sporophylls remained compacted. Forcible separation of the sporophylls effectively removed the barrier to oviposition imposed by thick sporophylls and was therefore equivalent to a reduction in sporophyll thickness. After 48 h the ovules were dissected to ascertain the number of successful ovipositions into ovules beneath separated or compacted sporophylls. There were five replicates for each cycad species.

2. *Integument thickness and sporophyll compaction.* The effects of these factors on oviposition by *A. signatus* were determined by repeating the above experiment with *A. signatus*. However, *E. longifolius* was replaced by *E. villosus*.

3. *Tomentum on the sporophyll.* Adults (20 of each sex) of *A. zamiae* and *A. signatus* were released on to megasporangiate cones of *E. friderici-guilielmi* collected in November 1989. Cones were presented either as they were collected, or with the tomentum scraped off the sporophylls (five replicates of each). The behaviour of beetles on 'woolly' and 'scraped' cones was observed with special reference to the ability of females to reach the junction between adjacent sporophylls. The ability of *A. zamiae* or *A. signatus* adults to reach the intersporophyll junction was interpreted to mean that the adult female could overcome any barrier to oviposition imposed by the tomentum. Any attempt to oviposit into ovules on 'woolly' or 'scraped' cones was recorded.

## RESULTS

### *Variation in cone and ovule structure*

There was considerable variation in cone and ovule structure between the species of *Encephalartos* examined here (Fig. 6.1). Most noticeable was the substantial variation in sporophyll thickness between species (ANOVA comparison:  $F = 75.19$ ,  $df = 8$ ,  $P < 0.01$  - all differences referred to below are based on a Scheffe multiple range test of the same data). *Encephalartos longifolius* was exceptional with a mean sporophyll thickness of more than 21 mm (Fig. 6.1) which was substantially greater ( $P < 0.01$ ) than any other species. Even *E. lehmannii* with relatively thinner sporophyll tissues of about 14 mm was significantly different to any of the other species examined. At the opposite extreme, mean sporophyll thickness in *E. villosus* was hardly more than 5 mm (Fig. 6.1). Sporophyll thickness in *E. horridus* was also significantly smaller than the other species of *Encephalartos*. The remaining species, *E. altensteinii*, *E. trispinosus*, *E. princeps*, *E. friderici-guilielmi* and *E. caffer* were not significantly different from each other.

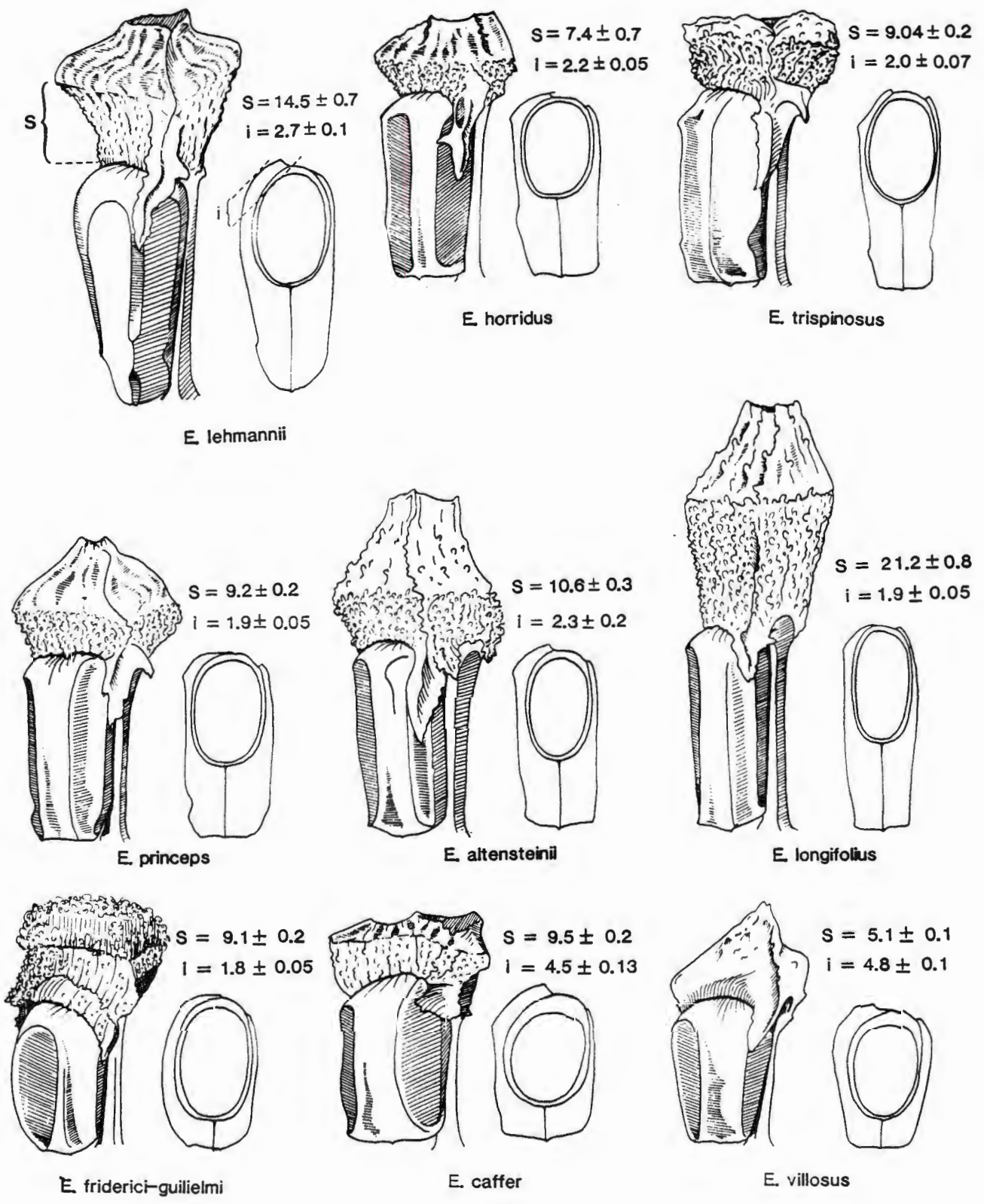


Fig. 6.1. Sporophylls and ovules, seen in lateral aspect, for nine species of *Encephalartos* from the eastern Cape Province of South Africa. For each species, a sporophyll and one of its associated ovules have been drawn. Alongside each of these diagrams is a diagrammatic longitudinal section through the ovule seen from the same perspective. The mean ( $\pm 1$  S.E.,  $n = 50$ ) for the thickness of the sporophyll at its junction with an adjacent sporophyll (s), and the thickness of the integument at its most exposed position (i), are provided for each species. The positions of 's' and 'i' are shown for *E. lehmannii*.

Integument thickness, measured at the most exposed section of the ovule (Fig. 6.1), also varied significantly between species. Exceptionally thick integuments were found in *E. caffer* and *E. villosus* which were not statistically different from each other ( $P > 0.05$ ). However, in both species, the integument was considerably thicker ( $P < 0.01$ ) than in any of the remaining species. The thickness of the integument in *E. lehmannii* was also significantly greater ( $P < 0.05$ ) than in the remaining species.

No measure was obtained of differences in sporophyll compaction between species of *Encephalartos*, but the effects of compaction were tested experimentally (below).

### *Consequences of cone structure for oviposition*

1. *Sporophyll thickness.* Sporophyll thickness was shown to influence, significantly, oviposition by *A. zamiae* in cones of *E. altensteinii* (Chapter 5). A similar effect may therefore be expected in other species. However, *A. zamiae* must penetrate both the sporophyll and integument in order to oviposit within the gametophyte. Therefore, since there is variation in both structures between species of *Encephalartos*, the effects of sporophyll and integument thickness were considered in combination.

The plot of the percentage ovules successfully colonized by *A. zamiae* relative to the combined thickness of the sporophyll and integument in nine species of *Encephalartos* (Fig. 6.2) shows a strong, and statistically significant, correlation ( $r^2 = 0.88$ ,  $P < 0.01$ ) between these variables. This result indicates that the combined thickness of the sporophyll and integument does indeed affect oviposition by *A. zamiae*. The furthest outlier in this regression was *E. caffer* (Fig. 6.2), which deviated substantially from the regression line. Oviposition by *A. zamiae* into *E. caffer* may therefore be influenced by other factors.

The correlation between sporophyll thickness and oviposition success was confirmed by the results obtained from the exposure of *A. zamiae* adults to cones of *E. altensteinii*, *E. caffer*, *E. longifolius* and *E. lehmannii* (Table 6.1). With the exception of *E. caffer*, the number of ovules into which *A. zamiae* females successfully oviposited was significantly higher in those spirals in which sporophylls had been forcibly separated (Table 6.1). The thickness of the sporophyll in these species therefore appears to have a significant impact on oviposition by *A. zamiae*.

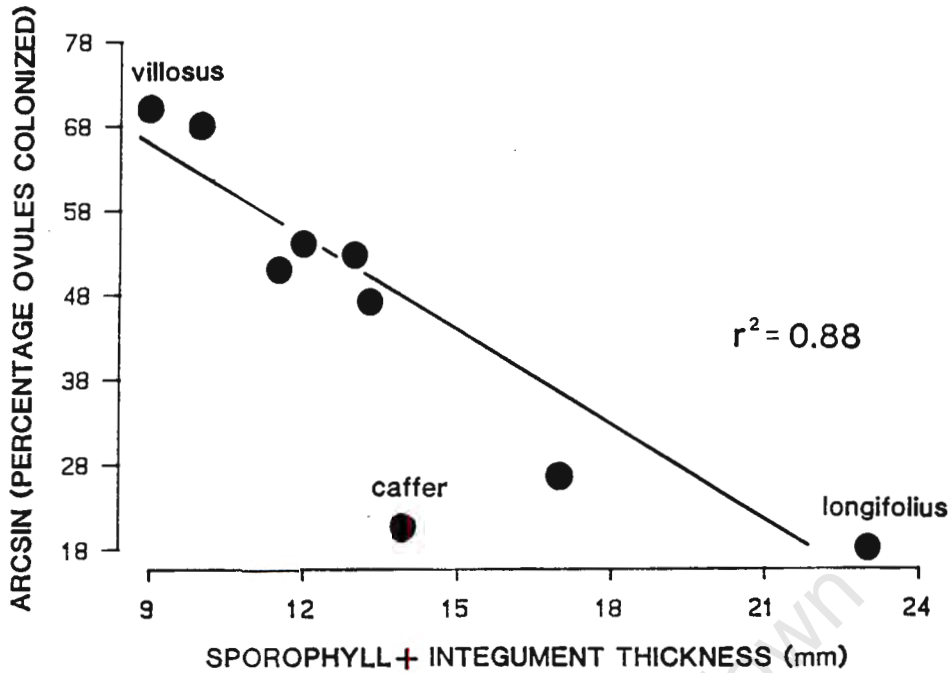


Fig. 6.2. A regression analysis of the percentage of ovules colonized by *Antliarhinus zamiae* (arcsin transformed) relative to the combined sporophyll and integument thickness of host cone in eight species of *Encephalartos*. A value for *E. caffer* is plotted on the graph but was not included in the analysis. Sporophyll and integument thickness represent the mean values depicted in Fig. 6.1. The host species with the lowest and highest levels of colonization by *A. zamiae* are marked on the graph.

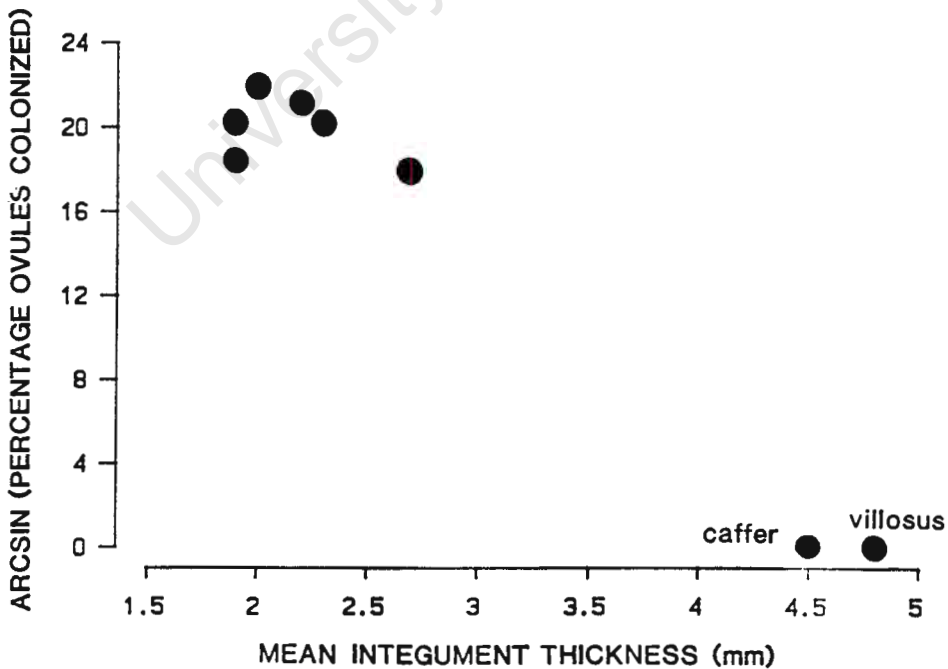


Fig. 6.3. A plot of colonization of ovules by *Antliarhinus signatus* relative to the mean integument thickness (Fig. 6.1) of the host ovule in eight species of *Encephalartos*. Values for *E. caffer* and *E. villosus*, which were never colonized by *A. signatus* are marked on the graph.

**TABLE 6.1.** Mean number ( $\pm 1$  S.E.) of ovules into which *Antliarhinus zamiae* females oviposited in cones of four species of *Encephalartos* in which the sporophylls were either left intact or forcibly separated (open). The results of a one way ANOVA are designated as: ns- no significant difference; \*-  $P < 0.05$ ; \*\*-  $P < 0.01$ .

Host species	Position of the sporophylls		Significance
	Intact	Open	
<i>E. altensteinii</i>	46.2 ( $\pm 3.7$ )	61.4 ( $\pm 3.9$ )	*
<i>E. caffer</i>	1.4 ( $\pm 0.2$ )	2.2 ( $\pm 0.3$ )	ns
<i>E. lehmannii</i>	26.7 ( $\pm 1.2$ )	59.1 ( $\pm 2.9$ )	**
<i>E. longifolius</i>	3.5 ( $\pm 0.4$ )	46.2 ( $\pm 4.3$ )	**

2. *Sporophyll compaction.* Oviposition into ovules in closed and open cones by *A. signatus* (Table 6.2) shows that, for *E. altensteinii*, *E. lehmannii* and *E. longifolius*, successful oviposition increased substantially in opened spirals. This result confirms that cone compaction has a notable effect on oviposition by *A. signatus*. However, in *E. caffer* and *E. villosus* there was no oviposition in either open or closed cone spirals, and some other barrier must therefore be responsible for the exclusion of *A. signatus* from these cycad species.

**TABLE 6.2.** Mean number ( $\pm 1$  S.E.) of ovules into which *Antliarhinus signatus* females oviposited in five species of *Encephalartos* in which the sporophylls were either left intact or were forcibly separated (open). The results of a one way ANOVA are designated as: ns- no significant difference; \*-  $P < 0.05$ ; \*\*-  $P < 0.01$ .

Host species	Position of the sporophylls		Significance
	Intact	Open	
<i>E. altensteinii</i>	10.4 ( $\pm 1.3$ )	47.2 ( $\pm 4.9$ )	**
<i>E. caffer</i>	no oviposition	no oviposition	
<i>E. lehmannii</i>	9.6 ( $\pm 0.8$ )	62.5 ( $\pm 2.7$ )	**
<i>E. longifolius</i>	8.3 ( $\pm 1.0$ )	49.3 ( $\pm 2.9$ )	**
<i>E. villosus</i>	no oviposition	no oviposition	

3. *Integument thickness.* When all species of *Encephalartos* from the eastern Cape were considered, a strong correlation ( $r^2 = 0.95$ ) was observed between integument thickness and oviposition by *A. signatus* (Fig. 6.3). However, the correlation was undoubtedly influenced by the zero oviposition in *E. caffer* and *E. villosus* (Fig. 6.3). Excluding these values results in a weak and statistically

insignificant correlation ( $r^2 = 0.12$ ,  $P > 0.05$ ). The inclusion of *E. caffer* and *E. villosus* in this analysis therefore needs to be justified by examining the effects of integument thickness on oviposition by *A. signatus*.

An analysis of oviposition by *A. signatus* into ovules within cones with open and closed sporophylls in different species of *Encephalartos* showed that integument thickness is a significant barrier to oviposition. In opened spirals of *E. lehmannii*, there was a substantial reduction in oviposition into ovules with an integument thickness greater than 3 mm. This corroborates the earlier result of the effects of integument thickness in *E. altensteinii* ovules on oviposition by *A. signatus* (Chapter 5). Substantial integuments do therefore prevent oviposition by *A. signatus* and therefore provide compelling circumstantial evidence, that integument thickness in *E. caffer* and *E. villosus* prevents colonization of these species by *A. signatus*. However, no oviposition was recorded into ovules of *E. caffer* and *E. villosus*. This was true even in ovules with unusually thin integuments of less than 3 mm. Observations of adult behaviour, and dissection of *E. caffer* and *E. villosus* cones, showed that females had not attempted to oviposit into these ovules. Rejection of *E. caffer* and *E. villosus* as hosts occurred before oviposition took place and did not, therefore, result directly from the female being unable to penetrate the integument.

4. *Tomentum*. The tomentum on sporophylls of *E. friderici-guilielmi* did not appear to impede movement by *A. zamiae* and *A. signatus*. Comparable numbers of beetles were found between sporophylls in 'woolly' cones and cones in which the tomentum had been removed ( $6.2 \pm 1.3$  and  $5.7 \pm 1$  for 'woolly' and 'scraped' cones respectively). Similarly, there were no significant differences between the times taken to find refuge in 'woolly' and 'scraped' cones (ANOVA:  $F = 0.13$ ,  $df = 1$ ,  $P > 0.05$ ). The main reasons why the tomentum did not greatly influence movement of adult beetles appears to have been the compaction of the tomentum and separation of the sporophylls to expose the underlying ovules. Rain and gum exudates compact the tomentum so that it is no longer loose and woolly. Consequently, the beetles are able to walk over the compacted surface and do not have to plough between the tomental strands. Further, in *E. friderici-guilielmi* and other 'woolly coned' species such as *E. lanatus*, the cone structure is such that the sporophylls in some sections of the cone are separated for a considerable period during cone development so that *A. zamiae* and *A. signatus* have no difficulty in reaching the ovule.

Neither *A. signatus* or *A. zamiae* occurred in ovules of *E. friderici-guilielmi*, regardless of whether the tomentum was removed or not.

## DISCUSSION

Considerable differences in cone and ovule structure exist between different species of *Encephalartos*. The purpose of this study was to determine whether these differences can account for the distribution of *A. zamiae* and *A. signatus* among different species of *Encephalartos*.

Mean sporophyll thickness was probably the most variable character between species of *Encephalartos* and it varied from about 3 mm in *E. villosus* to 21 mm in *E. longifolius*. The greater sporophyll thickness in *E. longifolius* certainly affected oviposition by *A. zamiae*. When sporophylls were forcibly separated in this species to simulate smaller sporophylls, oviposition by *A. zamiae* increased greatly. However, sporophyll thickness must have a limited effect on host specialization in *A. zamiae* because, even though *E. longifolius* had the largest recorded sporophylls, it was still recorded as a natural host for *A. zamiae* (Chapter 2). Probably, the exceptional variability in snout length in *A. zamiae* means that even the substantial sporophylls of *E. longifolius* can be penetrated by some females. The extent to which this is possible will depend on snout length variation in *A. zamiae* females, and this is examined in the following chapter.

Results presented in this chapter also confirm that cone compaction influences the distribution of *A. signatus* within cones of host species (Table 6.2). However, differences in cone compaction between cycad species appeared to have no direct effect on host specialization by *A. signatus*. Several non-host species (e.g. *E. villosus*) had ovules that were almost permanently exposed, yet they were not colonized by *A. signatus*.

Integument thickness may exclude *A. signatus* from *E. caffer* and *E. villosus*. These species have exceptionally thick ovule integuments and such large integuments were shown to prevent oviposition by *A. signatus* into ovules in *E. altensteinii* (Chapter 5) and *E. lehmannii* (this chapter). Even though *A. signatus* females did not attempt to oviposit into either *E. caffer* or *E. villosus* this would be expected if they had a poor chance for successful oviposition into these species. Selection would be expected to act rigorously against females that selected hosts into which they could not oviposit.

The woolly covering on the cones of *E. friderici-guilielmi* also does not appear to influence the ability of *A. zamiae* and *A. signatus* to oviposit into the ovules. The only way that the tomentum could be responsible for the exclusion of *A. signatus* and *A. zamiae* would be by preventing them from reaching their usual site for oviposition or by increasing their exposure to predators. These possibilities were tested here and were rejected since the tomentum neither prevented beetles from reaching the junction between sporophylls, nor increased the time they took to find refuge between sporophylls. The tomentum may be associated with

temperature control in megasporangiate cones (Goode, 1989) but it has no apparent effect on host use by *A. signatus* or *A. zamiae*.

In summary, differences in cone structure between species of *Encephalartos* apparently account for the low incidence of *A. zamiae* in cones of *E. longifolius* and probably explain the absence of *A. signatus* from *E. villosus*. The limited effect of increased sporophyll thickness in cycad species, on the host range of *A. zamiae* indicates that snout length is sufficiently variable to accommodate relatively large changes in cone structure. This aspect is dealt with in the following chapter.

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## CHAPTER 7

### VARIABILITY IN SNOUT LENGTH, AND ADAPTATION TO THE HOST-PLANT, IN *ANTLIARHINUS ZAMIAE* FEMALES ASSOCIATED WITH DIFFERENT SPECIES OF *ENCEPHALARTOS*

#### ABSTRACT

Snout length in *Antliarhinus zamiae* females varies between 4 mm and 20 mm. In females reared from *Encephalartos longifolius* and *E. lehmannii*, snout length was generally skewed in favour of longer snouts. This would be expected from the thick sporophylls (Chapter 6) that are characteristic for these host species. In *A. zamiae* females reared from five other host species, bimodal distributions of snout length were observed. This indicates that, on these host-plants, either females with long snouts are favoured or females with short snouts are favoured. Alternatives between these two extremes occur less often and may be selected against. The distributions in snout length may result from different strategies for oviposition in female *A. zamiae*. Some females may oviposit from outside the cone and would be subject to selection for longer snouts. Others may wait for the sporophylls to separate at the time of pollination. In these females there would be no selection for longer snouts. In populations of *A. zamiae* from *E. villosus*, snout length was consistently shorter than in populations from other host species. This consistency was maintained even in areas of sympatry with other host species. The consistency of snout length differences in *A. zamiae* females reared from *E. villosus* and those females reared from other host-plants indicates that sibling species or host races may be involved.

*Antliarhinus zamiae* females oviposit into the ovules of their host-plants by first using their long snouts to bore between adjacent sporophylls to reach the underlying ovule and then inserting their elongated ovipositors to lay eggs (Chapter 4). As a result of this behaviour, successful oviposition depends on the ability of the female to penetrate between adjacent sporophylls with her snout. The results of the previous two chapters have shown that the thickness of the sporophylls surrounding the ovule has a significant influence on oviposition by *A. zamiae* because thick sporophylls prevent the female from reaching the ovule with her snout. Consequently, host specialization in *A. zamiae* may depend on the level of variability in snout length and the extent to which snout length may be altered through selection imposed by the thickness of host sporophylls.

The extent to which selection can modify snout length in populations of *A. zamiae* may have important consequences for host relationships in *A. zamiae*. For instance, the marginal use of *E. longifolius* as a host-plant has been attributed to the exceptionally large sporophylls of this cycad (Chapter 6). However, it is implicit in this interpretation that snout length is subject to selection and that host utilization depends on the constraints on increased snout length in *A. zamiae*. Even host use in cycad species with smaller sporophylls may be affected by the

distribution of snout lengths within *A. zamiae* populations. If all individuals have snouts longer than the maximum sporophyll thickness for any cycad species, then sporophyll thickness presumably will have no effect on oviposition by *A. zamiae*. However, there is probably an upper limit to snout length since the functioning of the snout must be affected if it gets too long. Moreover, selection may favour shorter snouts for other reasons, so that long snouts may only be maintained under exceptional circumstances. Variability in snout length, and snout length adaptation in relation to the host-plant, need to be investigated.

It was noted in Chapter 2 that *A. zamiae* females from different host species had different snout lengths. These differences were regarded as phenotypic variability with probably no significance for the interpretation of species limitations in *A. zamiae* (G. Kuschel, in litt., 1989; R. G. Oberprieler, in litt., 1989). However, results presented in Chapters 5 and 6 show that snout length has important consequences for oviposition so that variability in snout length may represent more than simple phenotypic variation. Based on the data presented in Chapters 5 and 6, it would be expected that snout length in *A. zamiae* would vary in response to selection imposed by sporophyll thickness in the host-plant. As a result, in host populations with small sporophylls, short snouts would be expected and in host populations with large sporophylls, long snouts would be expected. In allopatric populations of *Encephalartos* species with sporophylls of different thicknesses, *A. zamiae* females with different snout lengths may be expected without any need to question the identity of *A. zamiae* as a single variable species. But, if snout length variability differs between *A. zamiae* females from sympatric populations of *Encephalartos* species, then species limitations in *A. zamiae* need to be examined, since sibling species or host races may be involved.

The need to examine the possible existence of sibling species or host races may be particularly true for *A. zamiae* females reared from *E. villosus*. Preliminary data from Chapter 2 indicate that snout length to body length ratios for these females always differed from those from other host species. One aim of this study was, therefore, to determine the extent of these differences and their possible significance for species limitations in *A. zamiae*.

Although it was not the purpose of this study to establish beyond doubt that host races, or sibling species, exist in populations of *A. zamiae*, some of the data needed to determine this possibility were gathered here.

Jaenike (1981) established several criteria for identifying host races in phytophagous insects. Four of these criteria were. 1. The populations must be sympatric and gene flow between populations of the phytophagous insect species must be restricted only by the host-plant on which they occur. 2. Significant genetic differences (e.g. differences in morphology) must exist between the populations on different host-plants. 3. If mating takes place on or near the host-

plant, then assortative mating must occur between insect populations from different host-plants. 4. Differences between host race populations should disappear if individuals from different host races are forced to breed on a single host-plant. If the differences are retained, or if individuals from the two populations do not breed, then the different populations may represent two distinct species and not two distinct host races.

As part of this study, data were collected which would answer some aspects of the questions posed above. More specifically, the aims of this chapter were the following.

1. To determine whether snout length in *A. zamiae* females corresponded to sporophyll thickness in host-plant populations.

2. To establish whether snout length was variable only within certain limits and to establish what those limits were.

3. To determine whether snout length variability differed between *A. zamiae* females from sympatric host populations and to establish whether these differences between *A. zamiae* females were accompanied by assortative mating on particular host-plants. This latter experiment was mainly for females reared from *E. villosus*.

## MATERIALS AND METHODS

### *Measurement of snout length variation between host populations*

Snout length (measured from the snout tip to the antennal sockets) and body length (measured from the antennal sockets to the tip of the elytra) were measured for samples of *A. zamiae* from eight host species. The host species were *E. altensteinii*, *E. horridus*, *E. lehmannii*, *E. longifolius*, *E. natalensis*, *E. princeps*, *E. trispinosus* and *E. villosus*. Adult beetles were obtained from a random sample of seeds collected from the cones described in Chapter 2. For each host species, all seeds which were colonized by *A. zamiae* were mixed and 50 seeds were selected at random. Between two and four females were measured from each seed. For allometric measurements, the sample was more selective and sufficient females were sampled to cover the full range of body size for beetles reared from any particular host species.

More detailed studies of snout length and body length were done for beetles collected from *E. villosus*. For this cycad, cones were collected from six localities; Northern Natal (exact locality unknown), Pietermaritzburg Botanic Garden (29°45'S 30°30'E), Vernon Crookes Nature Reserve (30°20'S 30°45'E), Gonubie (33°00'S

27°57'E), East London (33°05'S 27°47'E) and the Botanic Garden, Grahamstown (33°20'S 26°30'E). At least 50 adult females were measured from cones in each locality.

### *Statistical analyses*

Allometric regressions for snout length relative to body length were obtained from the allometric equation,  $Y = bx^a$  (Gould, 1966) and were based on Log values.

A discriminant function analysis to distinguish between discrete groups of characters in different populations was done using STATGRAPHICS statistical software package (Version 3.1, STSC inc., USA). This was also used for all other statistical analyses.

### *Assortative mating*

Adult beetles were obtained from six host species, namely, *E. altensteinii*, *E. horridus*, *E. lehmannii*, *E. longifolius*, *E. trispinosus*, and *E. villosus* in November 1989 and were stored at 20°C, 80% RH, until the following April. Twenty males and 20 females from each host species were then marked to indicate their host-plant. They were then released separately (10 of each sex) on to either an *E. altensteinii* cone or an *E. villosus* cone for a period of 72 h so that they became accustomed to the host cone. After this period, males and females were released together on to the same species of cone as before (i.e. *E. altensteinii* or *E. villosus*) at the time that the cone was beginning to open for pollination. Pairing of males and females was observed and the original host for each partner was recorded.

## RESULTS

### *Selection for snout length*

Frequency distributions for snout length in *A. zamiae* females from eight species of *Encephalartos* are presented in Fig. 7.1. A normal distribution of snout lengths, with a median value of about 5 mm, was obtained for females reared from *E. villosus*. The mean snout length (Table 7.1) was significantly shorter than that for *A. zamiae* reared from any of the other seven host species (Scheffe multiple range test based on ANOVA:  $F = 132$ ,  $df = 7$ ,  $P < 0.01$ ). Normal distributions

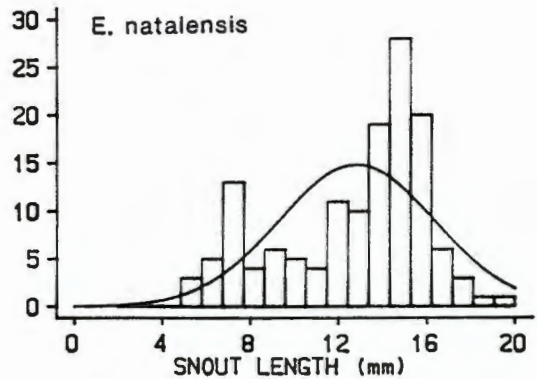
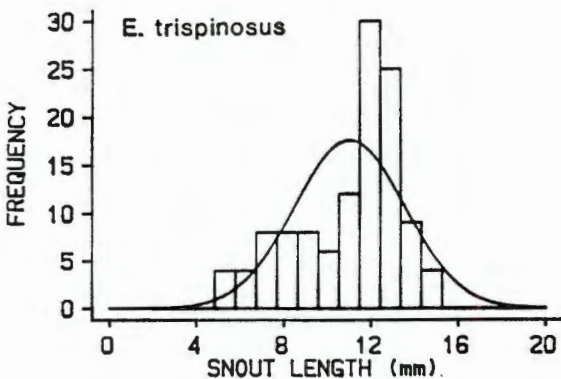
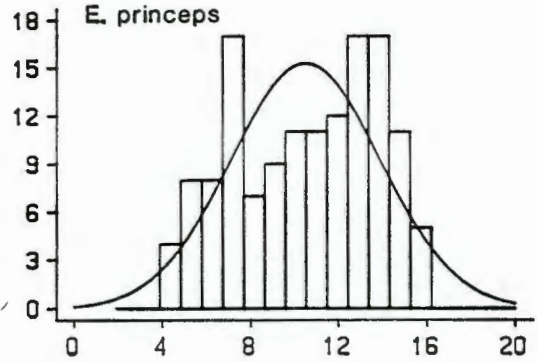
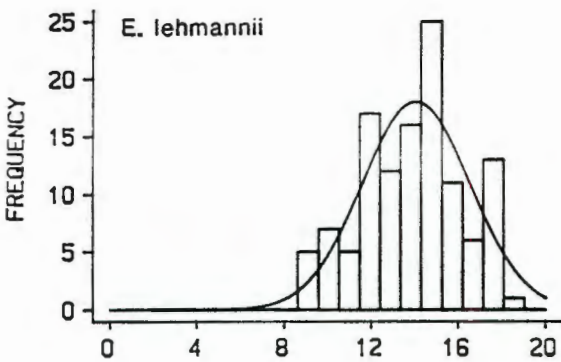
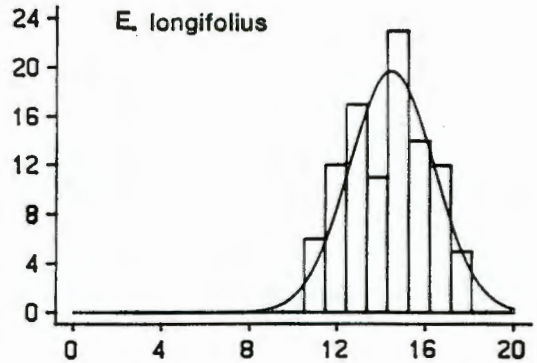
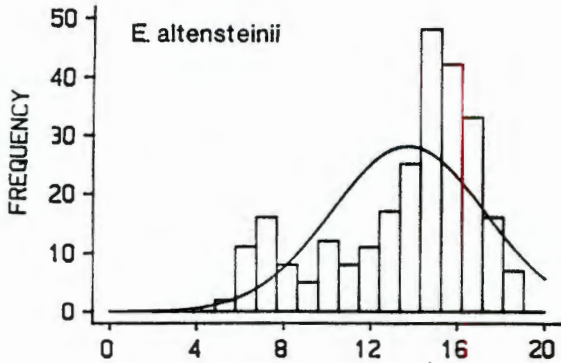
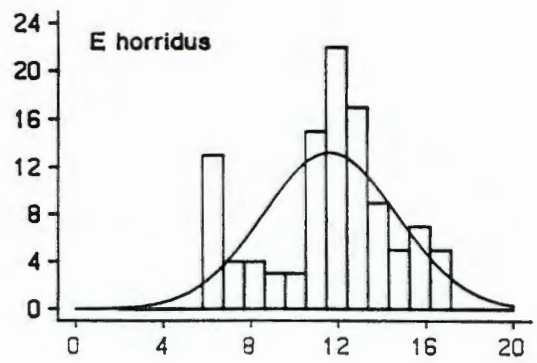
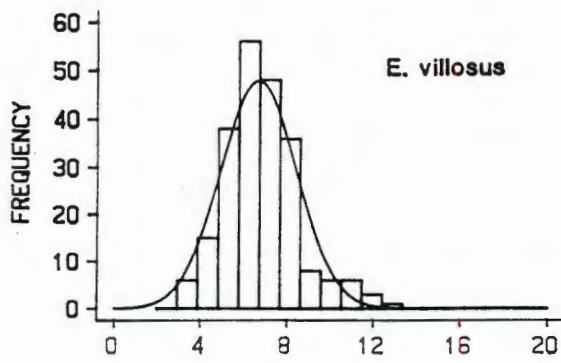


Fig. 7.1. Frequency distributions for snout length in *Antliarhinus zamiae* females reared from seeds in eight species of *Encephalartos*. The solid lines represent the expected normal distribution for the same number of data points and therefore show how the observed frequency of snout lengths differs from a normal distribution. Statistics for deviation from normality are given in Table 7.1.

were also obtained for snout length in female *A. zamiae* reared from *E. lehmannii* and *E. longifolius* (Fig. 7.1). However, in these females snout length was significantly longer (Table 7.1) than for *A. zamiae* females from *E. villosus*. In the remaining five species, snout length distributions were significantly skewed towards longer snouts (Fig. 7.1, Table 7.1) and there was a close correlation ( $r^2 = 0.78$ ) between mean sporophyll thickness in the host-plant and mean snout length in female *A. zamiae* (means provided in Table 7.1). However, for *A. zamiae* females from all host-plants except *E. villosus*, *E. lehmannii* and *E. longifolius*, a distinct bimodal distribution of snout lengths was recorded (Fig. 7.1). This suggests that selection for snout length is not always important or that selection favours two extremes, short snouts and long snouts.

**TABLE 7.1.** Summary of statistics for snout length in *Antliarhinus zamiae* females reared from eight species of *Encephalartos*, as plotted in Fig. 7.1. Mean snout length and its standard deviation, as well as the  $\text{Chi}^2$  analysis for deviation from normal distribution are provided. \*\* Denotes  $P < 0.01$ , ns- denotes no significant difference. In addition, the mean sporophyll thickness for the host plant is provided in the right hand column.

Species of <i>Encephalartos</i>	mean	s.d.	$\text{Chi}^2$	df	significance	n	Sporophyll thickness (mm)
<i>altensteinii</i>	13.7	3.5	115	11	**	261	12.9
<i>horridus</i>	11.6	3.1	33	8	**	107	9.6
<i>lehmannii</i>	14.01	2.4	12.6	6	ns	118	17.3
<i>longifolius</i>	14.5	1.9	6.9	5	ns	100	23.1
<i>natalensis</i>	12.7	3.5	53.1	10	**	139	11.9
<i>princeps</i>	10.5	3.3	67	11	**	137	11.1
<i>trispinosus</i>	11.01	2.5	38	6	**	118	9.4
<i>villosus</i>	6.7	1.7	10.9	5	ns	223	4.8

Allometric analyses of snout length relative to body length showed that short snouts were mostly associated with small beetles. In *A. zamiae* females from two host species, *E. altensteinii* (Fig. 4.11, Chapter 4) and *E. horridus* (Fig. 7.2), a positive differential (i.e.  $\alpha > 1$ ) was obtained for snout length relative to body length. This means that the bimodal distributions of snout lengths observed above were associated with changes in body size, i.e. two extremes were found, small beetles with short snouts or large beetles with proportionately much longer snouts. For *A. zamiae* females from *E. lehmannii*, a less positive differential was obtained (i.e. the  $\alpha$ -value was nearer to 1) and for females reared from *E. longifolius* and *E. villosus* an almost isometric relationship between snout length and body length was obtained (i.e.  $\alpha = 1$ ). In these species, then, increase in body length was not associated with an increase in snout length. For *A. zamiae* females reared from *E. lehmannii* and *E. longifolius* such a result may have been expected if there was a

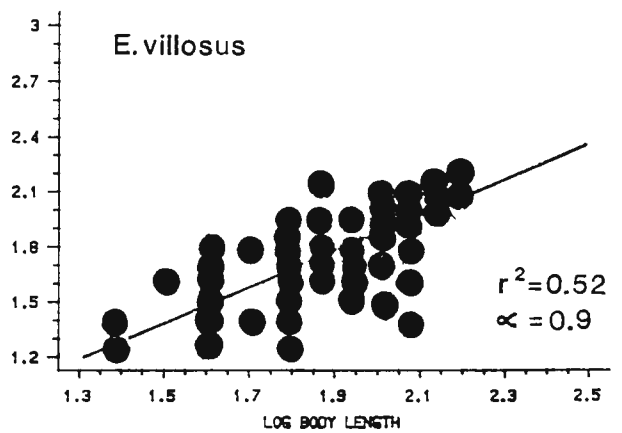
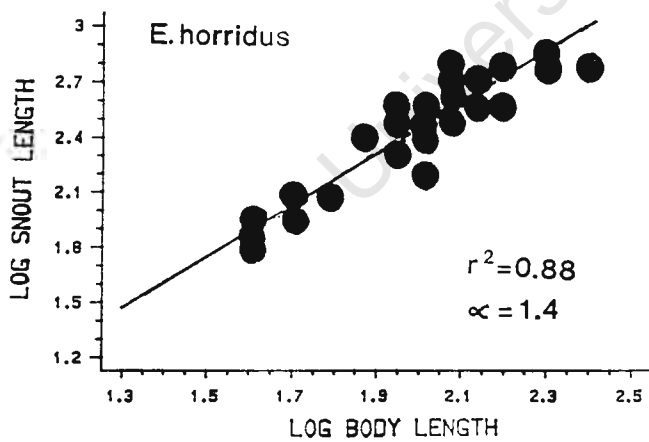
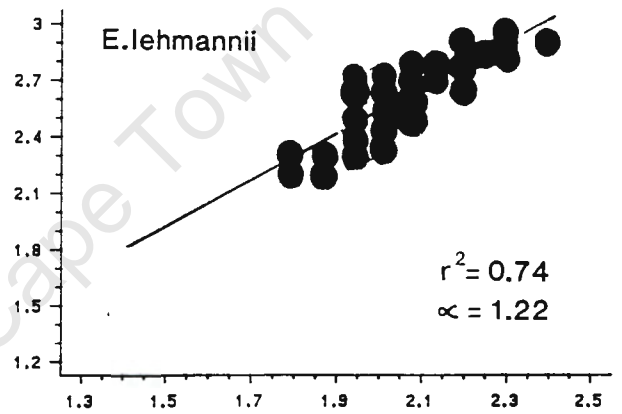
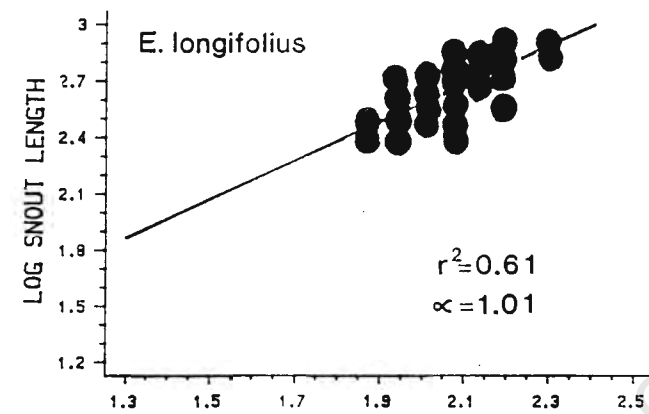


Fig. 7.2. Allometric regressions for snout length relative to body length (Log values) for *Antliarhinus zamiae* females reared from seeds of four species of *Encephalartos*.

maximum limit for snout length. In other words, the population sampled from these two host species may simply represent one extreme of the spectrum of snout length to body length ratios recorded for *A. zamiae* females from other host species such as *E. altensteinii*. However, for *A. zamiae* from *E. villosus*, the isometric relationship was recorded over a much wider range of body size so that in both small and large beetles there was no significant change in the relative length of the snout. *Antliarhinus zamiae* from *E. villosus* appears, therefore, to be subject to different selection for snout length compared to *A. zamiae* from other host species. This would be expected from the much smaller sporophylls of *E. villosus* relative to other species to *Encephalartos*.

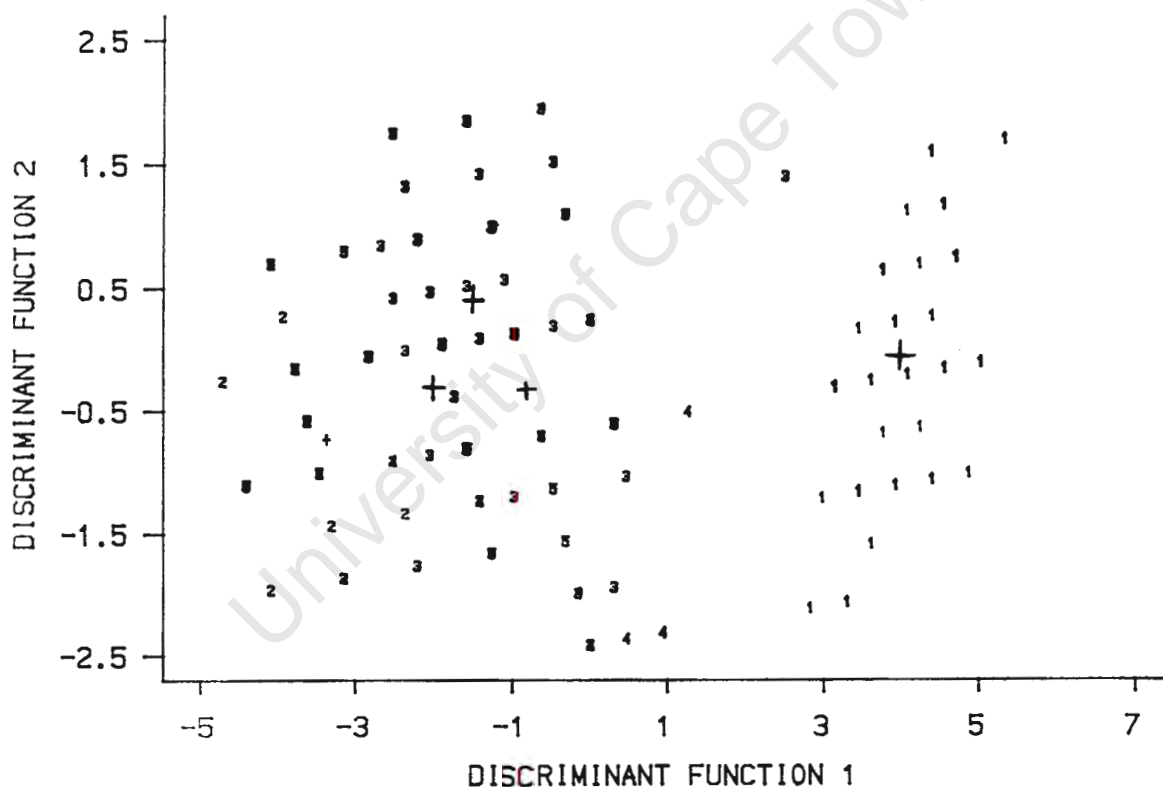


Fig. 7.3. Plot of a discriminant function analysis for snout length (discriminant function 2) relative to body length (discriminant function 1) for *Antliarhinus zamiae* females reared from five species of *Encephalartos*. Numbers represent the host species: 1- *E. villosus*, 2- *E. longifolius*, 3- *E. altensteinii*, 4- *E. trispinosus*, 5- *E. lehmannii*. Crosses mark the centroids for each group.

### *Snout length in females from E. villosus*

The conclusion that *A. zamiae* females from *E. villosus* populations are morphologically different from *A. zamiae* females from other host species is illustrated in a discriminant function analysis of snout length to body length in females from five host species (Fig. 7.3). Individuals from *E. villosus* clearly form a distinct group. This is particularly significant since *E. villosus* occurs sympatrically with *E. altensteinii* and *E. natalensis* in parts of its range so that the integrity of *A. zamiae* from *E. villosus* is apparently maintained even when other host species may influence selection for snout length. Moreover, the distinction between snout length and body length is consistent over a wide area. Samples from four field populations and two gardens (Fig. 7.4) show that in all instances except one collection from the Grahamstown Botanic Garden, snout length to body length ratios in individuals from *E. villosus* differed substantially from the ratios typical for *A. zamiae* females from *E. altensteinii* or *E. natalensis* (Fig. 7.4; only regressions for *E. altensteinii* are provided because they are almost identical to *E. natalensis*). This indicates that the populations on *E. villosus* must somehow be distinct from populations on other host-plants. The anomalous result from the Grahamstown Botanic Garden appears to represent the presence of two different *A. zamiae* populations, one from *E. villosus*, as expected, and a second possibly from *E. altensteinii*. It is possible that, within a garden situation, *A. zamiae* females from *E. altensteinii* may also oviposit into ovules of *E. villosus*.

### *Assortative mating*

In mating experiments, panmictic mating was observed amongst males and females from *E. longifolius*, *E. lehmannii*, *E. trispinosus* and *E. altensteinii* that were released on to cones of *E. altensteinii*. There appeared to be no preference amongst either males or females for mates that had emerged from the same host species ( $\text{Chi}^2 = 0.87$ ,  $\text{df} = 3$ ,  $P > 0.05$ ). In addition, mating on *E. altensteinii* did not appear to influence mating by adults reared from other species.

In contrast, male and female *A. zamiae* reared from *E. villosus* failed to mate at all on cones of *E. altensteinii*. On *E. villosus* cones they paired only with other individuals reared from *E. villosus*. Similarly, males and females from other host species mated only with each other on cones of *E. villosus*. Significantly fewer matings occurred amongst this group on *E. villosus* than on *E. altensteinii* ( $\text{Chi}^2 = 18.6$ ,  $\text{df} = 1$ ,  $P < 0.01$ ).

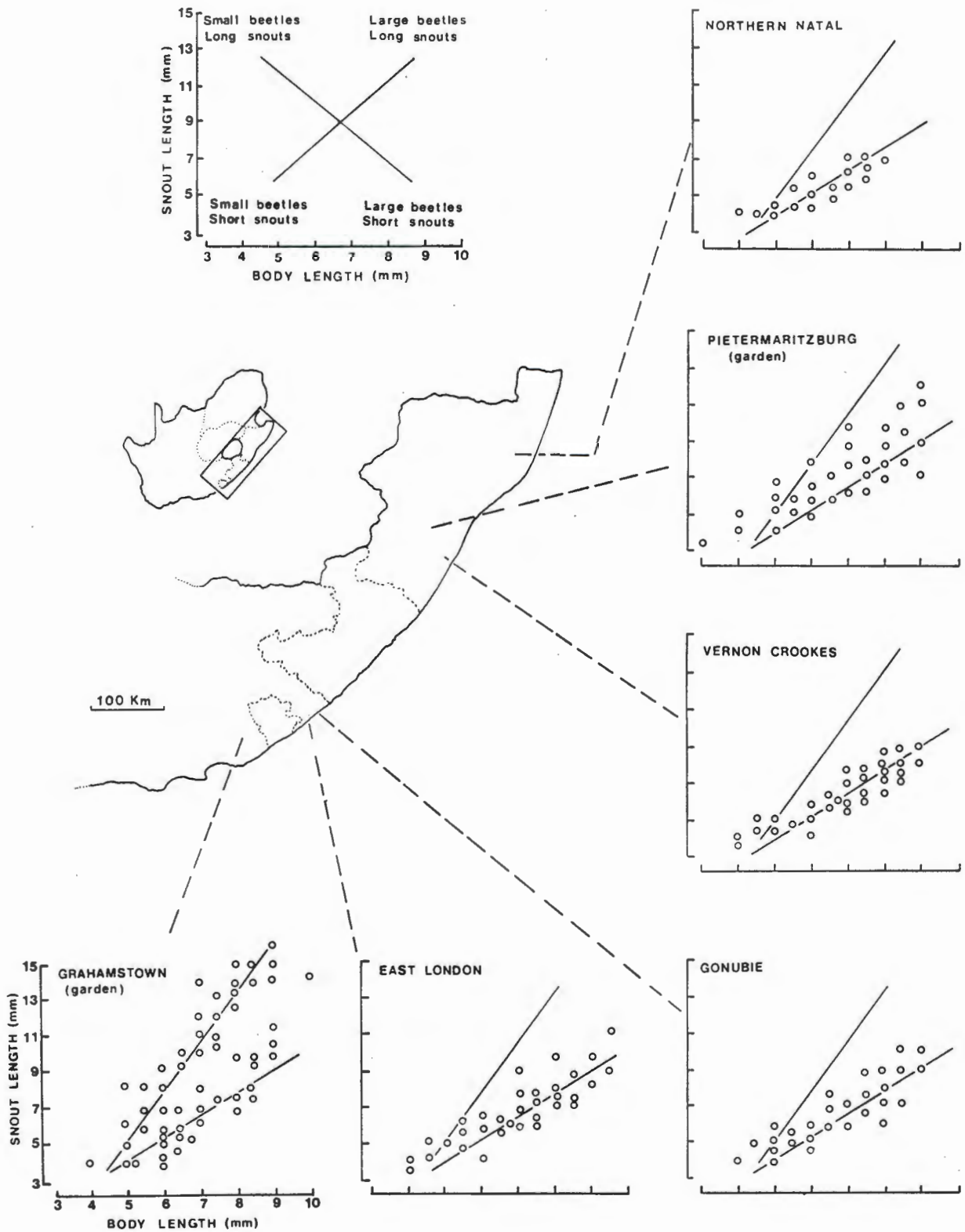


Fig. 7.4. Snout length relative to body length for *Antliarhinus zamiae* females reared from *Encephalartos villosus* seeds from six localities in South Africa. In each graph, two regression lines are provided for reference purposes only and are not derived only from the data presented in the graph. The upper line represents the regression of snout length against body length for all *A. zamiae* reared from *Encephalartos altensteinii* in this study and the lower line represents an equivalent regression for all females reared from *Encephalartos villosus*.

## DISCUSSION

Results presented in this chapter confirm the hypothesis that female snout length in populations of *A. zamiae* would reflect the sporophyll thickness of their host-plants. In all the host species examined here, there was a close correlation between sporophyll thickness and snout length so that sporophyll thickness in the host-plant must select for snout length in *A. zamiae*. However, this conclusion does not explain why, in *A. zamiae* females from at least five host species, there was a bimodal distribution of snout length. The only apparent explanation for this phenomena is the existence of two alternative strategies for ovipositing into the ovule of the host-plant. 1. To bore between the compacted sporophylls of the cone and then to oviposit via the resultant hole. This behaviour was reported in detail in Chapter 4 and would be expected to result in selection for long snouts. 2. To wait until the cone opened for pollination and then to penetrate between the sporophylls. This behaviour was envisaged as the ancestral condition for oviposition in *A. zamiae* (Chapter 4) and would select for short snouts, but also for small adult females that can move between adjacent sporophylls. The bimodal distribution of snout lengths suggests that alternatives between the two extremes are selected against. It is also significant for this argument that, for host species in which *A. zamiae* females have a bimodal distribution of snout length (*E. altensteinii*, *E. horridus*, *E. natalensis*, *E. princeps*, *E. trispinosus*), snout length is strongly skewed towards longer snouts (Fig. 7.1), indicating that oviposition from outside the cone is the most successful oviposition behaviour. This result confirms the results obtained in Chapter 4.

The correlation between snout length and sporophyll thickness, even in cycad hosts with thin sporophylls, indicates that long snouts are only maintained by continuous selection. This again supports the hypothesis that *A. zamiae* originated from an ancestor with a relatively short snout (see Chapter 4). It is probable that, in the evolution of snout length in *A. zamiae*, there has been a trade-off between a short, manoeuvrable snout with the appropriate ovipositional behaviour, and a long unwieldy snout with the concomitant ability to penetrate almost any cycad cone. Due to its unwieldiness, there is likely to be a maximum limit for any extension of snout length and, in *A. zamiae*, this limit appears to be about 20 mm. For this reason, cones with very substantial sporophylls such as those of *E. longifolius* were seldom colonized by *A. zamiae* (see Chapter 2).

Results presented in this chapter confirm that *A. zamiae* females reared from *E. villosus* are distinct from apparently conspecific females reared from other host-plants. *Antliarhinus zamiae* females from *E. villosus* had a consistently different snout length to body length ratio from *A. zamiae* females associated with any other species of *Encephalartos*. These differences were retained in sympatric populations

of *E. altensteinii* and *E. villosus*, as well as *E. natalensis* and *E. villosus*. In other words, even in association with host-plants (*E. altensteinii* and *E. natalensis*) in which selection for long snouts was typically found, *A. zamiae* females associated with *E. villosus* had short snouts.

Snout length variation between *A. zamiae* females from *E. villosus* and other species of *Encephalartos*, indicate that either separate host races must exist on *E. villosus* and *E. altensteinii*/*E. natalensis*, or that populations of *A. zamiae* from *E. villosus* represent a sibling species.

The formation of host races has been reported for several phytophagous insects in which genetically distinct populations occur on different host-plants (Bush, 1975; Singer *et al.*, 1988; Feder & Bush, 1989; Feder *et al.*, 1990a & b; Waldvögel & Gould, 1990). These genetic differences associated with different host-plants have been regarded as a first step towards speciation in phytophagous insects (Bush, 1975; Jaenike, 1990). However, the distinction between sibling species and host races on different host-plants lies in the ability of the host races to mate when forced onto one host-plant (Jaenike, 1981; Katakura *et al.*, 1989). *Antliarhinus zamiae* females from *E. villosus* would not mate with *A. zamiae* females from any other host-plant, even on cones of *E. villosus*, and this indicates that the *A. zamiae* populations from *E. villosus* represent a distinct species. This conclusion needs to be confirmed from mating studies done in the field.

Why populations of *A. zamiae* on *E. villosus* should form a distinct gene pool is not clear. It is possible that females associated with *E. villosus* were not subject to selection for snout length and therefore short snouts spread through these populations. As a result, females from *E. villosus* would be reproductively disadvantaged if they tried to colonize alternative hosts, such as *E. altensteinii*, which have substantially thicker sporophylls than *E. villosus*. Selection against such attempted host shifts may have resulted in a species-specific association with *E. villosus*. What this supposition does not explain is why *A. zamiae* associated with *E. altensteinii* do not colonize *E. villosus* in the field. The longer snouts associated with *A. zamiae* females from *E. altensteinii* should enable them to penetrate the ovules of *E. villosus*. This conclusion is confirmed by the ability of *A. zamiae* females to colonize *E. villosus* ovules both in the laboratory (Chapter 4) and in botanic gardens (Grahamstown Botanic Garden, Fig. 7.4).

A possible explanation for the absence of long-snouted *A. zamiae* from *E. villosus* in the field is that mating between long-snouted *A. zamiae* associated with *E. altensteinii* and short-snouted *A. zamiae* associated with *E. villosus*, disadvantages females from both populations. Such a mating could result both in a long-snouted female that searches for *E. villosus* and a short-snouted female that searches for *E. altensteinii*. The first result would have little consequence for host relationships, but the short-snouted females would, presumably, not be able to

oviposit into *E. altensteinii* ovules. The "unwanted heterozygote" (Vane-Wright, 1978) may therefore select for two distinct populations, one on *E. altensteinii* (or *E. natalensis*) and one on *E. villosus*.

The above sequence of events is consistent with sympatric speciation through host race formation (Bush, 1975). Although such speciation is possible in theory, it is regarded as unlikely in practice (Mitter & Futuyma, 1983) because it requires linkage disequilibrium between the various genetic factors involved (Mitter & Futuyma, 1983). For instance, in the case of *A. zamiae*, it would require linkage disequilibrium between the alleles responsible for host recognition, mate recognition and ovipositional traits, specifically snout length. The conditions that would promote such disequilibrium are apparently rare (Mitter & Futuyma, 1983). Further, selection for assortative mating under the resultant disruptive selection is apparently neither common nor effective in maintaining gene pool integrity (Futuyma & Mayer, 1980). For these reasons, the genetic aspects of host choice and performance on different host-plants would have to be investigated before any conclusions can be drawn on the origins of distinct gene pools of *A. zamiae* on *E. villosus* and on other host-plants.

In summary, variation in snout length is a characteristic of most populations of *A. zamiae* in which the mean snout length is correlated with the sporophyll thickness of the host-plant. Host-use is facilitated by variability in snout length. For *A. zamiae* from most host species, this variability does not necessarily indicate separate gene pools. However, for *A. zamiae* from *E. villosus*, the very different ratios between snout length and body length in adult females indicate distinct gene pools and this was confirmed by the occurrence of assortative mating.

The results presented in this chapter emphasize the need to understand variability in morphological characters for interpreting host specialization in species of *Antliarhinus*. In the following chapter, behavioural characters, specifically the timing of ovipositional activity with appropriate developmental stages of the host-plant, are examined.

## CHAPTER 8

### OVIPOSITION IN *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS* IN RELATION TO THE CONING PHENOLOGY OF THEIR HOST-PLANTS

#### ABSTRACT

Oviposition by *Antliarhinus signatus* is limited to a short period of ovule and cone development when the sporophylls separate to allow pollen entry. This period for individual plants lasts between nine and 14 days and for a population in general may last for approximately six weeks. Oviposition by *A. zamiae* is similarly limited by the hardening of the ovule integument to a period of about two months. The restricted periods of oviposition in both species would be expected to select for behavioural synchrony with the host-plant. As a result, neither *A. signatus* nor *A. zamiae* may be able to colonize cycad species with coning periods that differ from those of their current host-plants. This phenomenon may explain why *A. signatus* and *A. zamiae* are not found on species of *Encephalartos* belonging to the *E. cycadifolius* group.

Results presented in Chapters 5 and 6 showed that differences in ovipositional traits between *A. zamiae* and *A. signatus* can account, largely, for the differences in host ranges between these two species. In other words, regardless of the factors that limit both *A. zamiae* and *A. signatus* to host species within the cycad genus *Encephalartos*, the degree of host specialization by these weevils on species of *Encephalartos* is determined by their ovipositional traits.

In Chapter 2, the possibility was raised that understanding the causes of different host ranges in *A. zamiae* and *A. signatus* could provide an explanation for the absence of both weevil species from cycads in the *E. cycadifolius* group (after Dyer, 1965) of *Encephalartos*. If this is true, then ovipositional traits and, specifically, their adaptation to cone and ovule structure should explain the absence of *A. zamiae* and *A. signatus* from *E. cycadifolius*, *E. friderici-guilielmi* and other species of the *E. cycadifolius* group of species. This hypothesis has been tested throughout this thesis by the incorporation of species from the *E. cycadifolius* group (mostly *E. friderici-guilielmi*) in as many experiments as possible. However, the results of these experiments have shown that cone and ovule structures in species of the *E. cycadifolius* group have no apparent influence on oviposition by *A. zamiae* and *A. signatus* (Chapter 6). Thus, since larvae of *A. zamiae* and *A. signatus* can also develop on the megagametophyte tissues of *E. friderici-guilielmi* (Chapter 3), there is no apparent physical or chemical barrier to colonization of cycads in the *E. cycadifolius* group of cycads by *A. zamiae* or *A. signatus*. It is therefore possible that the absence of *A. zamiae* and *A. signatus* from these cycads is caused by other factors and is therefore beyond the scope of the present work. Such an explanation initially appears likely because no species of *Antliarhinus* feed on cycads within the *E. cycadifolius* group (Chapter 2). The omission of these cycads from the host ranges of all *Antliarhinus* species may,

therefore, have an historical basis that cannot be explained by examining host specialization only in *A. zamiae* and *A. signatus*.

However, an observation in the laboratory suggested that the absence of *A. zamiae* and *A. signatus* from cycads in the *E. cycadifolius* group of *Encephalartos* may be related to the need for *A. zamiae* and *A. signatus* females to synchronize oviposition with specific stages of ovule and cone development in the host-plant. A megasporangiate cone of *E. friderici-guilielmi* had been collected in November 1989 and had been stored at 5°C for five months. It had then been taken out of the store and left on a laboratory bench so that measurements of various cone structures could be taken. At the time, adult *A. zamiae* were present in the laboratory for use in oviposition experiments. When the *E. friderici-guilielmi* cone was later dissected, some of the ovules were found to contain larvae of *A. zamiae*. Female *A. zamiae* must have oviposited into the cone while it was lying on the bench. Unfortunately, there has been no opportunity to repeat the above observation under more rigorous conditions, but this single event indicates that *A. zamiae*, and possibly *A. signatus*, may not colonize *E. friderici-guilielmi* because this cycad species cones at a different time of year to other *Encephalartos* species that are colonized.

No published data are available on coning phenology in species of *Encephalartos*, but personal observations provide some idea of the availability of cones within the distributional range of *A. zamiae* and *A. signatus*. Ten of the 12 species of *Encephalartos* present in the eastern Cape Province of South Africa produce cones at approximately the same time of year. In these 10 species, megasporangiate cones are usually first produced in January and mature between September of the same year and March of the following year. These species of *Encephalartos* are all colonized by *A. zamiae*. In contrast, the remaining two species, *E. cycadifolius* and *E. friderici-guilielmi*, first produce megasporangiate cones in September and these cones mature between March and May of the following year. As already mentioned, these species of *Encephalartos* are not utilized by *A. zamiae* and *A. signatus*.

Although the coning periods of *E. cycadifolius* and *E. friderici-guilielmi* overlap to some extent with those of other cycads in the eastern Cape Province, this does not necessarily mean that *A. zamiae* and *A. signatus* can colonize either *E. cycadifolius* or *E. friderici-guilielmi*. Oviposition by *A. zamiae* and *A. signatus* may be restricted to a specific period of cone development so that behavioural synchrony with this stage of cone development may prevent a host shift to *E. cycadifolius* or *E. friderici-guilielmi*.

Plant structures, particularly non-perennial structures such as ovules and seeds, are not always available for insect herbivores. Consequently, oviposition may be timed to coincide with the appearance of the appropriate plant part in the

environment (Strong *et al.*, 1984; Straw, 1989a). Moreover, in the case of seeds or ovules, the maturation process is associated with the laying down of storage reserves in the endosperm, or in its equivalent, and with changes in water content (Bewley & Black, 1978; Murray, 1984). Oviposition by *A. zamiae* and *A. signatus* may therefore be timed to coincide with a specific stage of ovule/seed development that is best suited to larval survival. Further, maturation of cycad seeds, like other gymnosperms, is accompanied by the organization of the ovule integument into a seed coat, including a hardened sclerotesta (Murray, 1984). In cycads, ovule maturation is also accompanied by the development of the surrounding cone tissues. Consequently, oviposition by *A. zamiae* and *A. signatus* may be timed to coincide with an appropriate stage of ovule or cone development that is best suited to the insect's ovipositional traits.

The aim of the research reported in this chapter was to determine whether oviposition by *A. zamiae* and *A. signatus* was restricted to a limited period of ovule or cone development. Since all species of *Encephalartos* colonized by *A. zamiae* and *A. signatus* cone at approximately the same time of year, this study was restricted to the most available host species, namely *E. altensteinii*.

## MATERIALS AND METHODS

### *The timing of oviposition by A. zamiae and A. signatus*

The period in which oviposition by *A. zamiae* and *A. signatus* usually took place, was established both in the field and in the Kirstenbosch Botanic Garden in the following way. Field data were obtained from cones of *E. altensteinii* collected from natural populations in the vicinities of King William's Town and East London. Cones were collected on or near the first day of each month from February to July in either 1989 or 1990 (a total of 25 cones). Mature cones were obtained from the same localities in October or November of the same year (a total of 20 cones). Each cone was dissected and the number of ovules in which either *A. zamiae* or *A. signatus* were present, was determined. The number of ovules attacked by *A. signatus* and *A. zamiae* in the months of February to July could then be compared with the total number attacked, as measured in October or November. In this way, the peak period of attack by *A. signatus* and *A. zamiae* and by extension, the peak period of oviposition, could be established.

In the Kirstenbosch Botanic Garden, cones were collected at the same time as those collected in the field (see above). Each cone was dissected and the presence of eggs, first instar larvae, second to final instar larvae, pupae or adults of *A. zamiae* or *A. signatus* in ovules was established.

### *Causes of oviposition phenology*

To determine whether the timing of oviposition by *A. zamiae* and *A. signatus* is synchronized with the presence of ovule tissues in a suitable stage of development for larval survival, larvae of both weevil species were reared on ovules collected from February to July and again in November. Five larvae were transferred into each ovule using the methods described in Chapter 3. There were 10 replicates for each treatment.

To determine whether the timing of oviposition was synchronized with stages of cone and ovule development that provided the best conditions for oviposition, the following experiments were carried out. 1. Adult *A. signatus* were released onto cones collected on or near the first day of each month from February to July. Half the sporophylls were left intact and the remainder were forcibly separated. The effects of cone compaction (in closed cones) and integument hardness or thickness (in open cones) on oviposition at different stages of cone development could therefore be ascertained. 2. The same experiment was repeated with *A. zamiae* females to determine if oviposition was synchronized with periods before the integument hardened to form the seed testa or before the sporophylls reached their full size. In both of these treatments, methods were similar to those used in earlier chapters. However, the emphasis here was not just on the structures involved, but on the relationship between oviposition and the ontogeny of these structures.

Penetrability of the ovule integument was measured using a crude penetrometer. A flat platform was attached to a sharpened spike and placed in a vertical position. A 10 l beaker was then placed on top of the platform. The spike was then placed on the ovule integument and water was added to the beaker. The mass required to push the spike through the integument was then used as an indication of integument hardness. The maximum reading was 10 kg.

Separation of sporophylls was measured using the same technique as that used in Chapter 5. However, a second technique was used to determine the duration of sporophyll separation in individual cones, which could only be measured on cones still attached to the host-plant. For this purpose, a 7 x 0.7 mm wide spatula was attached to a kitchen scale with a maximum reading of 5 kg. By inserting the spatula between adjacent sporophylls, the amount of pressure required to push the spatula down to the level of the ovules could be ascertained. In this way, a comparative measurement of sporophyll separation could be obtained with a maximum reading of 5 kg. The duration of sporophyll separation was taken as the number of days for which a reading of less than 5 kg was measured. The duration of sporophyll separation was measured in this way for eight cones.

Dry mass of developing ovules was measured by placing ovules in an oven at 105°C for 24 h and then weighing them.

## RESULTS

### *Timing of ovipositional activity*

In the field, oviposition by both *A. signatus* and *A. zamiae* occurred predominantly in the months of April and May (Table 8.1). No significant increase in the number of ovules attacked by *A. signatus* was recorded in cones collected beyond May. For *A. zamiae* the period of oviposition was longer with significant numbers of ovules still being attacked in June (Table 8.1). Almost identical patterns of ovipositional activity by *A. signatus* and *A. zamiae* were obtained from the more detailed observations in the Kirstenbosch Botanic Garden. Almost no first instar larvae were recorded beyond May for *A. signatus* (Fig. 8.1) whereas large numbers were recorded in June for *A. zamiae* (Fig. 8.2). *Antliarhinus zamiae* therefore appears to be able to oviposit over a longer period than *A. signatus*.

**TABLE 8.1.** Percentage of ovules colonized by *Antliarhinus zamiae* or *Antliarhinus signatus* in cones of *Encephalartos altensteinii* collected from February to July of 1989 and 1990. The percentage colonized was calculated from the number of seeds in which the beetles were found at any stage between February and July relative to the total number of seeds colonized in cones collected in November of the same year. The number of cones collected for each month were, three each for February and March, five each for April to July and 20 for October/November. The mean percentage ( $\pm 1$  S.E.) is provided for each month. \*\* Denotes a significant deviation from total number attacked ( $P < 0.01$ , ANOVA) based on arcsin transformed percentages; \* denotes  $P < 0.05$ .

Species of <i>Antliarhinus</i>	MONTH						
	Feb	Mar	Apr	May	Jun	Jul	O/Nov
<i>A. signatus</i>	0**	0**	7.6** ( $\pm 1.2$ )	14.2 ( $\pm 0.8$ )	14.6 ( $\pm 1.1$ )	13.2 ( $\pm 0.9$ )	13.4 ( $\pm 1.0$ )
<i>A. zamiae</i>	0**	0**	40.6** ( $\pm 4.2$ )	61.8* ( $\pm 5.3$ )	70.8 ( $\pm 5.0$ )	69.6 ( $\pm 4.8$ )	73.8 ( $\pm 4.7$ )

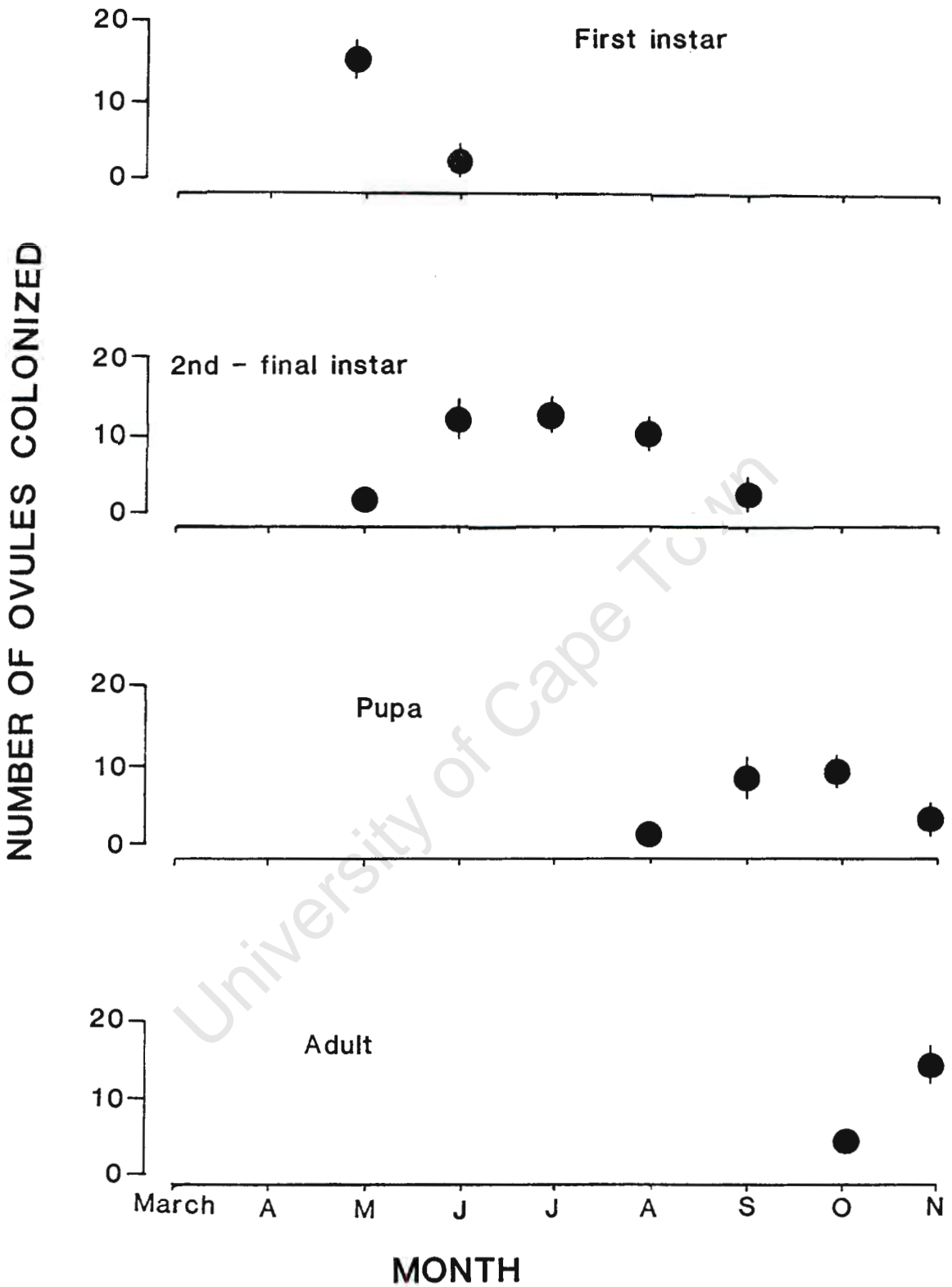


Fig. 8.1. The number of *Encephalartos altensteinii* ovules colonized by *Antliarhinus signatus* in megasporangiate cones collected between March and November 1989 from Kirstenbosch Botanic Garden. The numbers of ovules in which first-instar larvae, second- to final-instar larvae, pupae and adults were found are presented separately. Points represent the means ( $\pm 1$  S.E.) for two cones for March and for July to September, four cones for April and May, three for June and October and seven for November. One hundred ovules/seeds were examined from each cone.

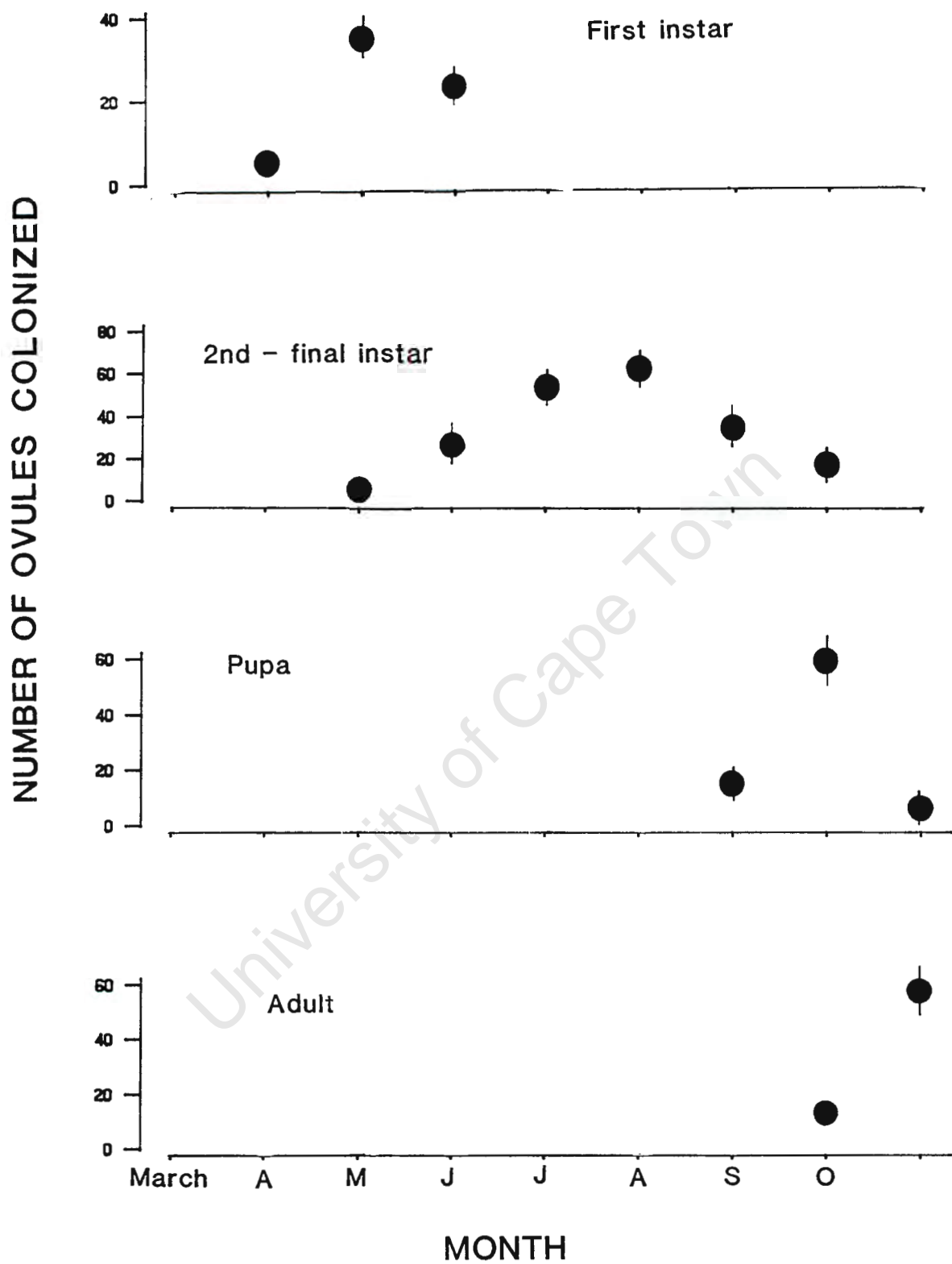


Fig. 8.2. The number of *Encephalartos altensteinii* ovules colonized by *Antliarhinus zamiae* in megasporangiate cones collected from Kirstensboch Botanic Garden between March and November 1989. Other details are provided in Fig. 8.1.

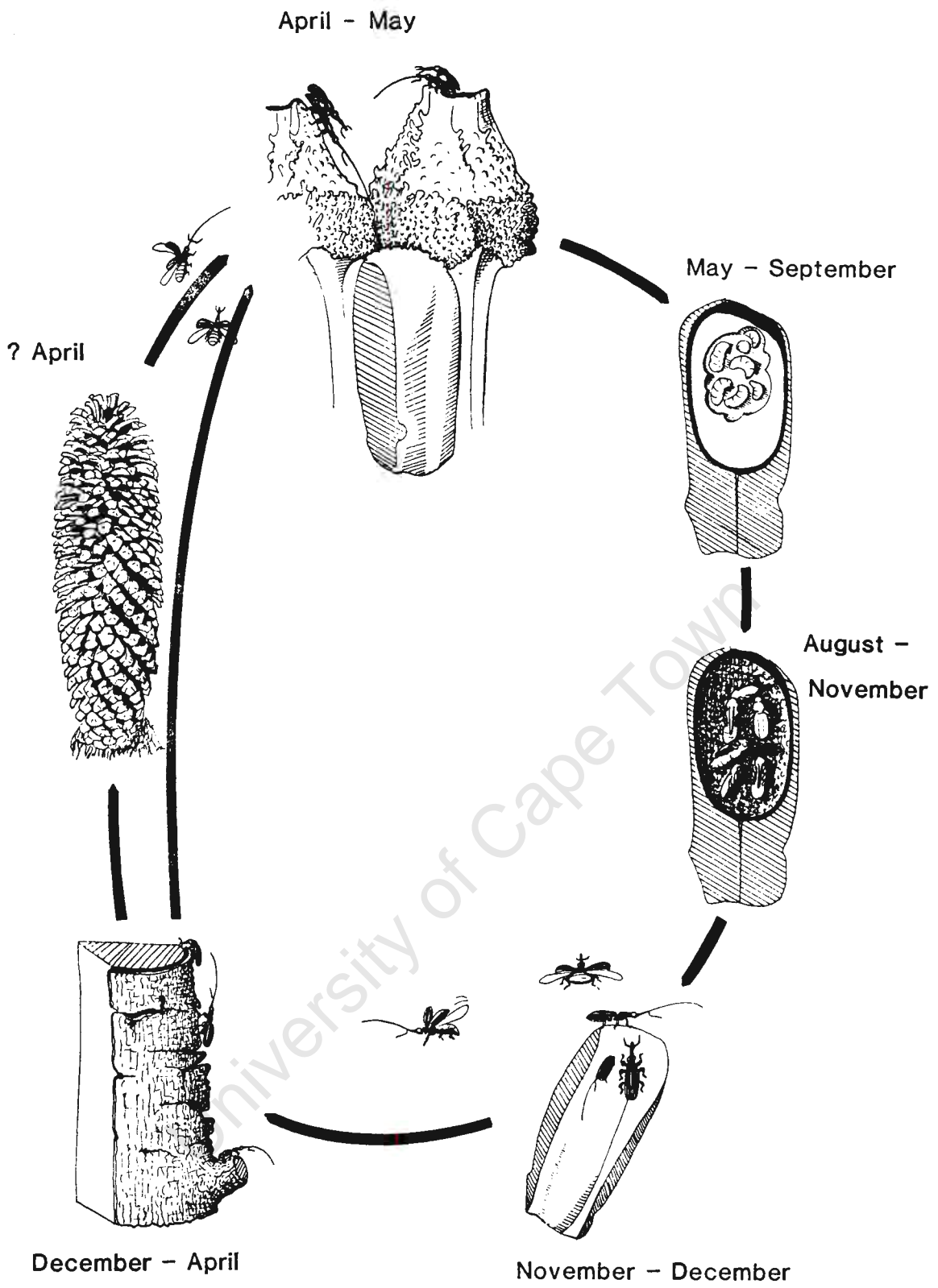


Fig. 8.3. An approximation of the life cycle of the cycad weevil, *Antliarhinus zamiae* based on the data presented in Fig. 8.2. After emerging from the cycad seeds, the adult beetles are reputed to seek shelter under the bark of trees. In April of the following year, they may visit male cones before proceeding to the female cones.

In all other aspects of their development, *A. signatus* and *A. zamiae* were almost identical. In both species the larvae developed within about 6 weeks and remained in their final instar for several weeks before pupating (Figs 8.1 & 8.2). Adults only emerged from the seeds when the cone disintegrated for seed dispersal about November (Fig. 8.3).

#### *The causes of oviposition phenology*

Larvae of both *A. zamiae* and *A. signatus* transferred into ovules collected in March and July showed significantly greater mortality than larvae reared in ovules collected from cones in April and May (ANOVA:  $F = 12.3$ ,  $df = 3$ ,  $P < 0.01$ ). Based on a Scheffe multiple range test of these data, no significant differences in survival ( $P > 0.05$ ) were recorded between larvae reared in ovules from April or May. There were also no significant differences between *A. signatus* and *A. zamiae* for the numbers of larvae surviving in ovules from different stages of development (highest  $\text{Chi}^2 = 0.87$ ,  $P > 0.05$ ).

Higher mortality of larvae in ovules collected in March is almost certainly due to the small size of the gametophyte at this stage (Fig. 8.4). During March and April the megagametophyte rapidly increases in size and attains a greater dry mass, thereby providing more substance for the larvae to feed on.

The reasons for greater larval mortality in ovules collected after July were not specifically tested and it would be groundless to speculate on possible causes. The most important observation from these data is that oviposition by *A. signatus* and *A. zamiae* occurs well within the period of larval survival in host tissues. Further, there is no significant difference in survival between *A. signatus* and *A. zamiae* larvae. The limited period of oviposition by *A. signatus* does not, therefore, result from a narrower period of tolerance of host tissues.

Two significant changes in cone structure occurred within the period of oviposition by *A. signatus* and *A. zamiae*. Firstly, the sporophylls separated to allow pollen entry. This occurred predominantly in late April and early May (Fig. 8.5) but there was some variation between cones (see Fig. 8.5). For individual cones, the period in which sporophyll separation was measured as less than 5 kg lasted between nine and 14 days (mean  $\pm 1\text{SE} = 11 \pm 1.3$   $n = 8$ ). Secondly, the integument became increasingly harder to penetrate (Fig. 8.5) as it attained the dead, stony nature typical of the mature sclerotesta. The influence of these factors on oviposition were investigated further.

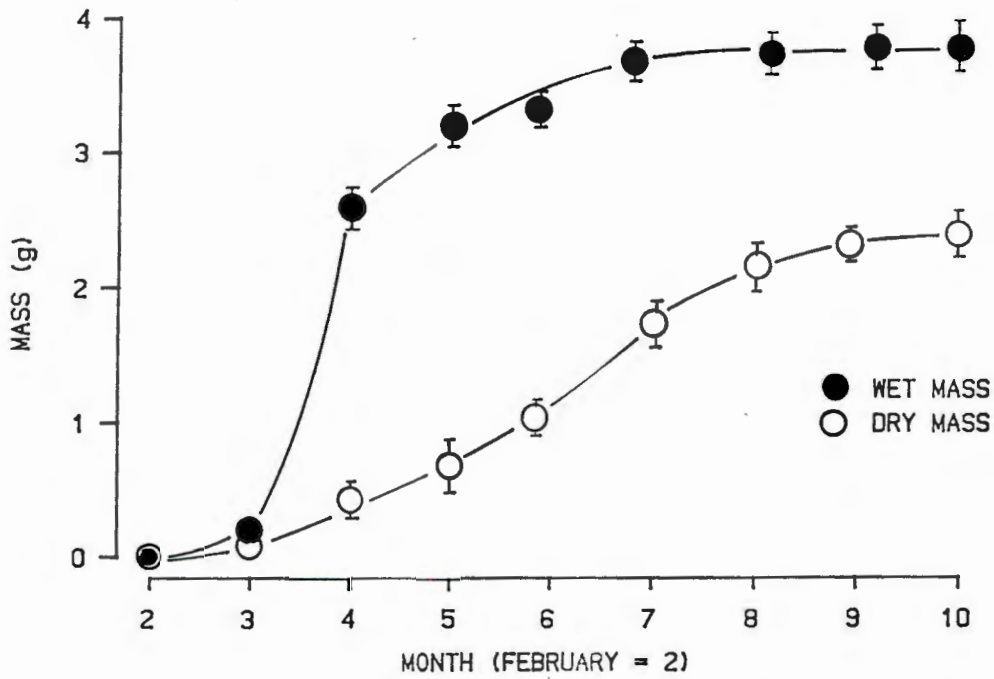


Fig. 8.4. The wet and dry mass of the megagametophyte from the ovules of *Encephalartos altensteinii* collected from Kirstenbosch Botanic Garden between February and November 1989. Each point represents the mean ( $\pm 1$  S.E.) for 10 samples.

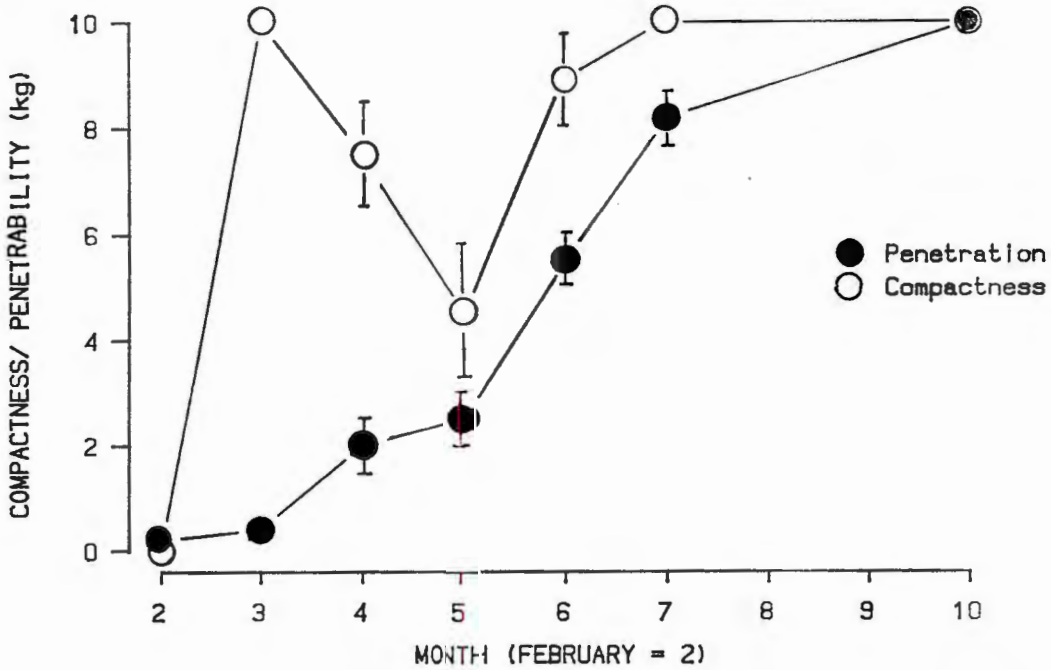


Fig. 8.5. Relative measures of cone compactness in megasporangiate cones, and integument penetrability in ovules of *Encephalartos altensteinii* collected from Kirstenbosch Botanic Garden between February and October 1989. Details of the measurements are given in the text. Each point represents the mean ( $\pm 1$  S.E.) for 10 samples.

A comparison of oviposition by *A. signatus* and *A. zamiae* in compact and open cones showed that successful oviposition by *A. signatus* was significantly higher in open cones. ( $\text{Chi}^2 = 7.4$ ,  $\text{df} = 1$ ,  $P < 0.01$ ). Almost no oviposition occurred in compact cones. In contrast, oviposition by *A. zamiae* was greater in open cones, but not significantly so ( $\text{Chi}^2 = 3.56$ ,  $\text{df} = 1$ ,  $P > 0.05$ ). *Antliarhinus zamiae* females still oviposited into a substantial number of ovules in compact cones.

Oviposition by *A. zamiae* may be influenced by integument hardness, but this could not be conclusively proved. A significantly greater number of successful ovipositions by *A. zamiae* were recorded in May and June than in July ( $\text{Chi}^2 = 8.5$ ,  $\text{df} = 1$ ,  $P < 0.01$ ). Observations of oviposition into ovules collected in July showed that females proceeded to insert their snouts between adjacent sporophylls as normal (see Chapter 4) but that they retracted their snouts without inserting their ovipositors. Dissection of the relevant cones showed that females had penetrated only the outer fleshy integument of the ovule. The stony sclerotesta of the ovule had not been penetrated and was probably too hard for the female to bore through. However, the possibility that the female was responding to a deterrent compound in the outer integument that acts as an indicator of the reduced suitability of the gametophyte for larval survival cannot be excluded.

## DISCUSSION

Results presented here show that *E. altensteinii* ovules are attacked over a relatively short period in about April and May by *A. signatus* and over a longer period from April to June by *A. zamiae*. These findings confirm earlier observations (Chapter 3) that adults of *A. zamiae* and *A. signatus* are active on cones in the field during April and May. They also substantiate the anecdotal references of Rattray (1913) that oviposition into *E. altensteinii* ovules by *A. zamiae* occurs between April and June.

The causes of restricted periods of oviposition apparently differ between *A. zamiae* and *A. signatus*. *Antliarhinus signatus* females must first enter the megasporangiate cone before they can oviposit into the host ovule. Consequently, ovipositional activity by *A. signatus* is limited to a relatively short period (9-14 days) during the development and maturation of the megasporangiate cone when the sporophylls separate to allow pollen entry. In contrast, *A. zamiae* is able to penetrate with its long snout between adjacent, closely compacted sporophylls so that oviposition is not restricted by cone compaction. However, the hardening of the ovule integument, associated with the formation of a seed coat, restricts oviposition into host ovules to a period of about two months. The period in which

oviposition by *A. zamiae* can occur may also be restricted by the greater mortality of larvae in more mature ovules. Oviposition may, therefore, be timed to coincide with the stage of ovule development most suited to larval survival. Mortality in older ovules is almost certainly caused by physiological changes in the ovule gametophyte associated with maturation, but these factors were not investigated further.

The most important observation from the results presented here is that oviposition by both *A. zamiae* and, more particularly, *A. signatus* is restricted to a limited period of ovule and cone maturation. Thus although the host cones may remain on the plant for a considerable period (about 12 months), the ovules are only suitable for oviposition by *A. signatus* and *A. zamiae* for a relatively short period.

Because both *A. zamiae* and *A. signatus* have restricted periods in which they can oviposit into their host-plants, they would be expected to have evolved mechanisms for behavioural synchrony with the host-plant. These mechanisms were not investigated here, but it is possible that *A. zamiae* and *A. signatus* use volatile chemicals emitted by the host-plant to attract insect pollinators. Insect pollination has been proven in some cycad taxa (Norstog *et al.*, 1986; Norstog & Fawcett, 1989; Tang, 1987) and it is suspected that pollinators are attracted by host odours. At least some species of *Encephalartos* emit strong odours at the time of pollination (Ratray, 1913), and it is therefore possible that these odours are used as cues by *A. zamiae* and *A. signatus* to synchronize their ovipositional activities with the right stage of cone and ovule development in their host-plants.

The behavioural mechanisms required to synchronize oviposition by *A. zamiae* and *A. signatus* with the appropriate stage of host development could conceivably restrict these weevils to host species with similar coning periods. As a result, they may not have colonized *E. cycadifolius* and *E. friderici-guilielmi*. It must be pointed out, however, that no species of *Antliarhinus* develop on either *E. cycadifolius* or *E. friderici-guilielmi*. The absence of other species of *Antliarhinus* from these cycads may, therefore, be attributable to similar causes. *Antliarhinus sp. nr verdcourti*, like *A. signatus*, enters the host cone at the time of sporophyll separation (Chapter 4) and, consequently, would be subject to the same limitations as *A. signatus*, i.e. oviposition must be synchronized with a period of sporophyll separation. *Antliarhinus peglerae* oviposits into sporophyll tissues at the time that sporophylls open for pollination (Chapter 4) and this may have arisen because they also oviposit into sporophylls of male cones (Chapter 2) which are only present for a short period and are obviously present when pollination takes place. It therefore seems likely that oviposition in all four species of *Antliarhinus* studied here, may be synchronized with a short period of cone and ovule development that coincides with the pollination period of the host species.

Behavioural synchrony between insect herbivores and their host-plants may have important consequences for host specialization (Straw, 1989a & b; Wood *et al.*, 1990). Synchrony with host-plant phenology may result in a restricted host range for an insect herbivore, or may even promote speciation if different populations are adapted to host-plants with different phenologies (Straw, 1989b; Wood *et al.*, 1990). In the case of *Antliarhinus*, it is possible that the two genera, *Antliarhinus* and *Platymerus*, diverged as a result of behavioural specialization with the coning period of their host-plants. *Antliarhinus* has become associated with *Encephalartos* species, all having a common coning period from January to December, and *Platymerus* has become associated with *E. friderici-guilielmi* that cones from September to April.

In summary, the absence of *A. zamiae* and *A. signatus* from species in the *E. cycadifolius* group of *Encephalartos* cannot be explained as a direct consequence of ovipositional traits in these weevils. However, the need to synchronize oviposition with specific periods of host development has probably prevented *A. zamiae* and *A. signatus* from colonizing *E. cycadifolius*, *E. friderici-guilielmi* and other species in the *cycadifolius* group of *Encephalartos*.

In the following chapter, a synthesis of the results presented so far in this thesis, is presented.

## CHAPTER 9

### ADAPTATION TO THE HOST-PLANT, AND THE EVOLUTION OF HOST SPECIALIZATION, IN *ANTLIARHINUS ZAMIAE* AND *ANTLIARHINUS SIGNATUS*

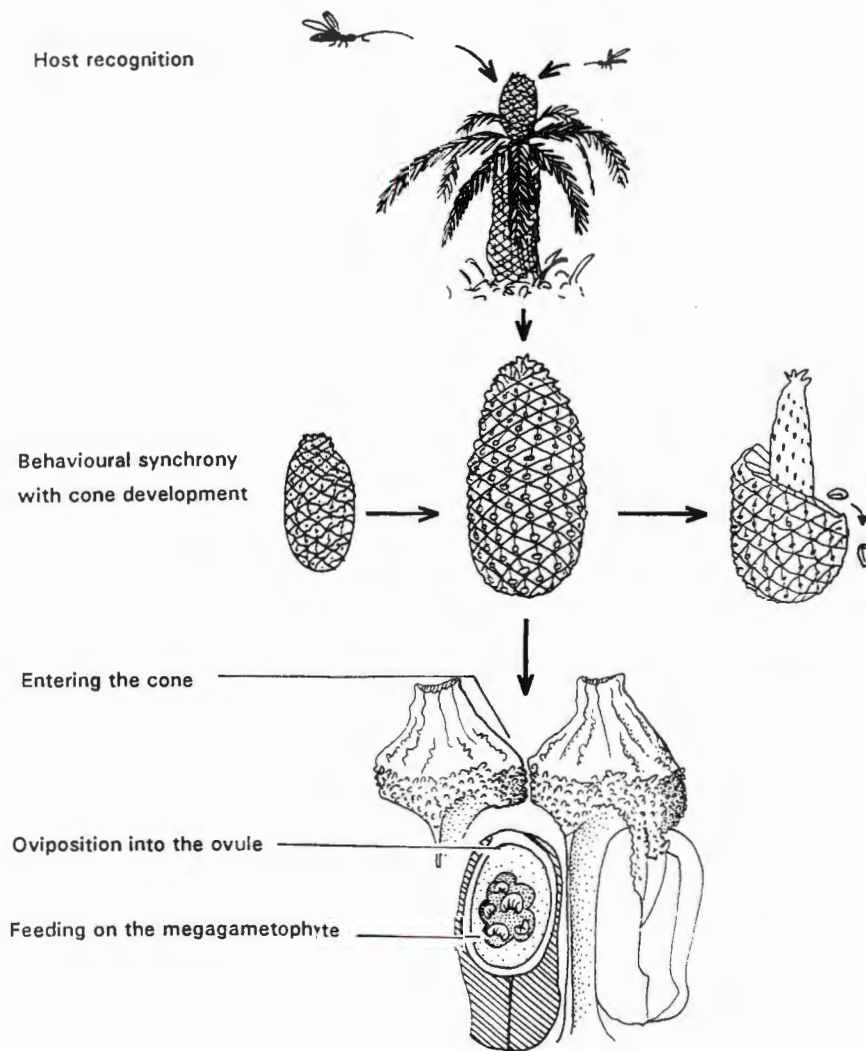
#### ABSTRACT

This chapter is essentially a synthesis of the results and ideas presented in the previous sections of this thesis. The central focus of this work has been the evolutionary consequences of adaptation to the host-plant for host specialization by the cycad weevils *Antliarhinus zamiae* and *A. signatus*. Differences in ovipositional traits between the two species can account for most of the differences in host utilization between *A. zamiae* and *A. signatus*. Oviposition in *A. signatus* is constrained by the need to enter the cycad cone before oviposition because movement within the cone requires a small body size and short snout. As a result, snout length cannot respond to selection imposed by thick ovule integuments in potential host plants and, consequently, these structures act as barriers to colonization. *Antliarhinus zamiae* oviposits from outside the cone and has been freed from the constraints that limit snout length. As a result, *A. zamiae* has been able to colonize more species of the cycad genus *Encephalartos*. However, host specialization may also be affected to some extent by behavioural synchrony with appropriate stages of host development, by physiological adaptation to host tissues, and by the ability to recognize suitable plant species as hosts. Adaptation to the host plant may therefore occur at different levels with different consequences for host specialization in insect herbivores.

This thesis reports on host relationships in cycad weevils belonging to the genus *Antliarhinus*, with particular emphasis on the causes of host specialization in *A. signatus* and *A. zamiae*. Evidence has been presented to indicate how different adaptations to the host-plant influence host specialization by these weevils.

Amongst the most striking differences between *A. zamiae* and *A. signatus* is their ovipositional traits, and these differences clearly show how adaptation to the host-plant can influence host specialization in insect herbivores. *Antliarhinus signatus* females oviposit from within the cone of their host-plants and are morphologically adapted for squeezing between the sporophylls of the host cone. In other words, they are small, dorsoventrally flattened beetles with relatively short snouts (usually < 3.5 mm). As a consequence of their short snouts, *A. signatus* females are unable to penetrate ovules that have thick integuments (Chapters 5 & 6) and may, therefore, be unable to colonize cycad species which typically have thick integuments, e.g. *E. caffer* and *E. villosus*. In contrast, *A. zamiae* females are adapted for oviposition from outside the cone, allowing greater variation in body size and, more importantly, greater variation in snout length. As a result of the greater variability in these characters, *A. zamiae* females can oviposit into a broad range of *Encephalartos* species and *A. zamiae* has, therefore, colonized a greater number of *Encephalartos* species than has *A. signatus*. This comparison emphasizes the need to understand how insect herbivores are adapted to their

host-plants, and how limited variability in a particular trait may influence host specialization.



**Fig. 9.1.** A diagrammatic representation of the various interactions between *Antliarhinus zamiae* and *A. signatus* and their cycad host.

It would be naïve to assume that ovipositional traits alone are responsible for host specialization in *A. signatus* and *A. zamiae*. An array of other factors may also be involved (Fig. 9.1). The evidence shows that adult females of both *A. signatus* and *A. zamiae* do not recognize some *Encephalartos* species as hosts (Chapters 2 & 6). Host recognition systems may, therefore, be dedicated, specifically, to only a few plant species and it is clear that colonization of new host species must be accompanied by some change to the nervous system. Host specialization in

*A. signatus* and *A. zamiae* may also be affected by behavioural synchrony with particular stages of cone development. One consequence of this behavioural synchrony with the host-plant, is that host use may be restricted to plants that cone at a particular time of year (Chapter 8). Finally, data on larval development on different cycad species suggest that host specialization may be influenced by physiological adaptation to larval host tissues. Although *A. signatus* and *A. zamiae* larvae can apparently develop on all species of *Encephalartos*, they do not survive on *S. eriopus* (Chapter 3), the only other cycad taxon occurring in the same environment. The most likely first step in any expansion of host range for *A. zamiae* and *A. signatus* would be to colonize other cycad taxa, because these plants have at least some features in common with their present host-plants (Jermy, 1984; Berenbaum, 1990). This step would almost certainly require physiological adaptation to the new host's tissues.

The restrictions on host range expansion in *A. signatus* and *A. zamiae*, as detailed above, probably did not arise independently of each other, but were influenced by existing restrictions. For instance, it would be expected that the evolution of the host recognition system would be influenced by the ability of the female to oviposit into the plants that are recognized as hosts. Alternatively, ovipositional traits may have evolved that are specifically adapted only to those plants that the insect initially recognized as hosts. Similarly, physiological adaptation to host tissues may have occurred only on those plants that the adults recognized as hosts (although this is not necessarily the case, e.g. Thompson, 1988b). In other words, these traits may have evolved under conditions in which there was no selective advantage for utility on other host-plants.

The evolution of character traits that now restrict host range may also have been influenced by the ecological conditions that existed in the environment in which they evolved. For instance, the lineage from which *Antliarhinus* species evolved may have become restricted to cycads when these plants were the dominant vegetation in their environment (i.e. in the late Jurassic, Crowson, 1981). Subsequent adaptation to these host-plants may have resulted in increasing specialization on cycads even though the plants are now relatively scarce. In other words, the accumulation of cycad-specific character traits in the ancestors of *Antliarhinus*, at a time when ecological conditions favoured colonization of cycads, may have initiated a process of specialization on cycads that still has implications for host specialization in *A. zamiae* and *A. signatus*.

It must be stressed that, regardless of the sequence of evolution for characters that affect host utilization by *A. zamiae* and *A. signatus*, each trait that limits the ability of the insect to colonize new host species contributes to host specialization by making it less likely that a change in one character will allow colonization of a new host-plant. This reasoning is similar to Fisher's (1958) conclusion that, when

there are many characters under consideration, the chances of a mutation occurring that moves all characters to an optimum (in this case for the colonization of new hosts) is vanishingly small (see also Hastings & Hom, 1990). However, this conclusion assumes that there is limited variability in each character that affects host utilization, and that it is this limitation that promotes host specialization. The factors that limit adaptation to a broad array of host-plants therefore need to be discussed.

Ultimately, the extent to which a particular trait will limit host use by an insect herbivore depends on two factors. Firstly, the heterogeneity of the plants in the insect's environment (Feeny, 1975; Fox & Morrow, 1981; Denno & McClure, 1983; Strong *et al.*, 1984) and, secondly, the variability in the insect character trait (Fry, 1989; Via, 1990). The discussion is, therefore, divided into two parts. Firstly, the role of coevolutionary forces is evaluated since coevolutionary forces may contribute to the heterogeneity of the plants on which the insects feed. Secondly, genetic and epigenetic (i.e. the expression of genetic characters in the phenotype) factors that may limit variability in insect character traits are discussed.

#### *The role of coevolution*

Superficially, host specialization by both *A. signatus* and *A. zamiae* could be equated with the predictions of a coevolutionary relationship. The development of cone and ovule structures, of particular coning phenologies, and of toxic chemical compounds in the megagametophyte, in species of *Encephalartos* could, theoretically, be viewed as defensive responses to feeding by *A. signatus* and *A. zamiae*. Ovipositional traits, behavioural synchrony with the host-plant and larval adaptation to the chemistry of the megagametophyte in *A. zamiae* and *A. signatus* could then be viewed as coevolutionary responses to the plant's defence mechanisms.

While these hypothetical scenarios are feasible, they are not consistent with data on the host relationships of *A. signatus* and *A. zamiae*. Development on cycad seeds by larvae of *A. signatus* and *A. zamiae* apparently evolved only after *Antliarhinus* had become isolated on the genus *Encephalartos* (Chapter 4) and after the divergence of the lineages giving rise to *Antliarhinus* and *Platymerus* (Chapter 4). As a result, any characteristics in species of *Encephalartos* that have evolved as defenses against *A. signatus* or *A. zamiae*, should only be present in species that have an historical relationship with these beetles. However, within the genus *Encephalartos*, species that have probably never been colonized by any species of *Antliarhinus*, e.g. species in the *E. cycadifolius* group, have substantial sporophylls, compact cones, infrequent coning cycles, and similar chemistry to species that are

colonized by *A. zamiae* and *A. signatus*. In fact, other cycad genera such as *Macrozamia* and *Lepidozamia* have similar characteristics (Tang, 1989; Siniscalco Gigliano, 1990; Stevenson, 1990) so that these features may represent plesiomorphic characters in the cycad lineage from which all three of these genera originate (see Stevenson, 1990). By all accounts, ovipositional, behavioural and physiological adaptations for feeding on ovule tissues by *A. signatus* and *A. zamiae* have evolved in response to plant characters that were already in existence. There is also no evidence to suggest that cone structure or coning phenology in species of *Encephalartos* have changed in response to feeding by *A. signatus* or *A. zamiae*. Host relationships in *A. signatus* and *A. zamiae* are more consistent with a sequential series of events (Jermy, 1984) in which the evolution of plant characters was followed by the evolution of appropriate traits in *A. signatus* and *A. zamiae*, without any reciprocal interaction. Coevolution can, therefore, be ruled out as a contributing factor in the evolution of host specialization in *A. zamiae* and *A. signatus*.

#### *Adaptation to the host-plant and host specialization*

Essentially, host specialization in phytophagous insects may have two alternative causes. Firstly, if an insect herbivore is potentially able to recognize, oviposit, and feed on any plant species, then host specialization must be caused by selective forces, acting in the insect's present environment, that favour utilization of one plant species, or a group of plant species, over all other plants within the that environment. In this instance, host use may vary from one environment to another and may change within an ecological time scale. Theories in which host specialization is considered to result from predator avoidance (Bernays & Graham, 1988) or from optimal foraging decisions (e.g. Levins & MacArthur, 1969) are based on this premise. Alternatively, host specialization may result from inherent qualities of the insect such as the ability (or inability) to recognize and/or utilize different plant species due to constraints imposed by genetic or epigenetic factors. In other words, host range may be a species character that is subject to the same genetic and epigenetic constraints as other species characters such as wing venation. This view is embodied in the contention that host specialization in insect herbivores arises from constraints on the insect nervous system to recognize more than a limited number of host-plants (Jermy, 1988).

These two alternative explanations for host specialization in insect herbivores are not necessarily mutually exclusive. For instance, genetic constraints may restrict host utilization to a particular group of host-plants, but selective processes may further restrict host utilization within a particular ecological environment

(e.g. Rausher, 1984; Horton *et al.*, 1988). Nevertheless, the distinction between the two alternatives is crucial to an understanding of host specialization in insect herbivores. If host specialization can be explained as a consequence of genetic and epigenetic constraints accompanying the process of adaptation to the host-plant, then there is no need to seek adaptive explanations for host specialization above this level (see Williams, 1966).

Jermy (1984, 1988) has emphasized the importance, for host specialization, of constraints on the insect nervous system for recognising chemical cues from different host-plants. Changes in host-plant chemistry, for whatever reason, may affect the ability of an insect herbivore to recognize or utilize that plant if the chemical changes occur in compounds used as host recognition cues. Thus, unless corresponding changes alter the insect's host recognition system, such changes in host chemistry must lead inevitably to narrower host ranges. Jermy (1984, 1988) has placed particular emphasis on the fact that changes in plant chemistry may fortuitously affect insect host range without the need for specific adaptation to the host-plant.

If constraints on the nervous system alone are responsible for host specialization in insect herbivores, then it should be expected that phylogenetically related insects should be subject to similar constraints. However, comparison of species pairs in monophyletic lineages have shown that phylogenetically related species may differ substantially in the breadth of their host ranges (Vane-Wright, 1978; Craig *et al.*, 1988). These differences could, theoretically, arise from incidental changes to the host recognition system in only one species in a monophyletic lineage (Jermy, 1984). However, mutation rates for these sorts of changes should be equivalent in phylogenetically related species, indicating that other factors contribute to narrow host ranges in insect herbivores.

For many insect herbivores, successful utilization of a plant species may require more than just recognition of the plant as a suitable host. Depending on the life history characteristics of the insect herbivore, successful utilization may depend on the insect's ability to digest the plant tissues, to lay eggs into the appropriate tissues (e.g. Straw, 1989b; *Antliarhinus* spp. in this study), to synchronize its activities with plant tissues at the appropriate stage of development (Evans *et al.*, 1989; Straw, 1989b; Wood *et al.*, 1990) and to link up with mates attracted to the same host-plant (Jaenike, 1990). These activities may require specific adaptation to the host-plant so that utilization of many different plant species will depend on the inherent variability in these traits and on the factors that may limit the expression of variability.

Variability in a particular trait may be influenced by the evolutionary history of the insect. The evolution of new adaptations in organisms in general, takes place within a phylogenetic context (Williams, 1966; Gould & Lewontin, 1979; Eldredge

& Salthe, 1984) so that the options available for new adaptations may be determined to some extent by past events. However, as mentioned above, it is unlikely that phylogenetic constraints alone can account for host specialization (Jaenike, 1990). Differences in host range within monophyletic groups (see Vane-Wright, 1978; Craig *et al.*, 1988) provide the clearest example that phylogenetic constraints on new adaptations are not sufficient to explain narrow host ranges. For example, *A. signatus* and *A. zamiae* have similar evolutionary histories, yet *A. zamiae* has evolved new adaptations for oviposition that allow an expansion of host range. Similarly, Bernays & Janzen (1988) showed that phylogenetic constraints on head size and mouth part morphology did not account for host specialization in sphingid and saturniid moths.

Variability in traits associated with the host-plant may also be affected by interactions between different traits. For example, the constraints limiting oviposition into *Encephalartos* ovules by *A. signatus* females do not necessarily imply that genetic variability for longer snouts does not exist in *A. signatus* females. It does mean that any possible variability is not expressed in the phenotype. Eldredge & Salthe (1984) pointed out that there is a genealogical hierarchy involved in the evolution of character traits. Population structure depends on organismic properties such as life history characteristics, which depend on nervous and anatomical characters, which depend in turn on gene action. Constraints may occur at one or several of these levels with varying effects on the remaining levels of organization (Eldredge & Salthe, 1984). For instance, Dover (1982) showed that there can be considerable variation at the molecular level of the genome without any noticeable change in the phenotype. The expression of alternative genetic characters may, therefore, be affected by higher levels of organization. In *A. signatus*, it is possible that genetic variability in snout length exists but cannot be expressed because of the limitation on body size imposed by the need to enter the cone prior to oviposition.

Such a latent variability in snout length may account for the changes in ovipositional function accompanying the speciation event that gave rise to *A. zamiae*. Disruptive factors that promote speciation (see Paterson, 1985; 1986) may also incidentally result in genetic rearrangements (Vrba, 1985). Thus, during the process of speciation, the behaviour associated with oviposition may have changed sufficiently to allow oviposition from outside the cone and this would have been accompanied by greater expression of snout length variability. As a result of this change in ovipositional behaviour, *A. zamiae* was incidentally endowed with the potential to expand its host range beyond that which is possible in *A. signatus*.

It is clear from this example, that the evolution of new adaptations to the host plant may not always result in greater host specialization. Williams (1966) asserts

that speciation has been accompanied mainly by the substitution of one adaptation for another. If these substitutions result in release from constraints on host utilization, then expansion of host range may occur. For instance, lepidopterous caterpillars may be prevented by physiological constraints from feeding on plant species that contain light-activated toxins. However, the evolution of leaf-tying behaviour in these larvae, resulting in protection from sunlight, may allow expansion of host range to include plants containing phototoxic chemicals (Sandberg & Berenbaum, 1989).

The observation that new adaptations involving a single trait can result in range expansion raises the question as to why adaptation to the host-plant should result in host specialization among insect herbivores. It seems that, at least to some extent, adaptation to the host-plant involving more than one character may promote host specialization. Braker (1989) observed that, in acridoid grasshoppers, the evolution of oviposition on the host-plant (as opposed to in the soil) was almost invariably accompanied by a narrower host range. Whereas 59% of these grasshoppers were broadly polyphagous, all acridoid species ovipositing on their host plants were either monophagous or oligophagous. The combination of adaptation for oviposition on the host-plant and feeding on the host-plant therefore appeared to have a more significant effect on host specialization than feeding alone. Adaptation to the host-plant at a second level may, therefore, add additional constraints to the utilization of more than one host-plant. Some feeding habits may require specific adaptation to the host-plant at several levels and this may explain the high degree of host specificity in insects with seed-feeding larvae (Janzen, 1969; Center & Johnson, 1974) as well the low recruitment of gall formers and leaf miners by introduced plants such as European thistles introduced into North America (Strong *et al.*, 1984; Zwölfer, 1988).

Population genetic studies indicate that, where several genetic characters are involved in adaptation to the environment (e.g. to the host-plant), the maintenance of variability is more difficult to explain than its absence (Via, 1984, 1990; Futuyma & Peterson, 1985; Hastings & Hom, 1990). Models show that in these situations, genotype-environment interactions often lead to fixation of only one genotype (Hastings & Hom, 1990). As a result, limited variability in insect responses to their host-plants should, perhaps, be expected. Variability may, however, be maintained by polymorphisms (Vane-Wright, 1978; Mitter & Futuyma, 1983) or by pleiotropic interactions between characters that are adapted to the same environment (Hastings & Hom, 1990). These genetic factors may result in 'multiple niche polymorphisms' (Mitter & Futuyma, 1983) or multiple adaptive peaks (Hastings & Hom, 1990) in which different insect phenotypes could be specifically associated with either different host-plants, different plant phenotypes

within the same host species (Thompson, 1988c,d; Fry, 1989, Karban, 1989) or even to different parts of the same plant (Whitham & Slobodchikof, 1981).

The limited ability of insect herbivores to be adapted to a wide range of host-plants means that they may often be associated with particular plant phenotypes or particular plant species in any one environment. This situation may increase the likelihood of speciation events among insect herbivores. Firstly, in these 'specialist' species, the chances of populations being isolated in environments that are different from those of the parent population are greater, and therefore increase the chances for allopatric speciation (Paterson, 1985; Wade & McCauley, 1988). Secondly, at least in theory (see Mitter & Futuyma, 1983), the association of different insect genotypes with different host-plants increases the likelihood that speciation can occur in sympatry (Bush, 1975; Mitter & Futuyma, 1983; Scriber, 1983; Crego *et al.*, 1990; Karowe, 1990; Wood & Keese, 1990). Increased speciation rates in insect herbivores with a high degree of host specialization may then offer an explanation for the predominance of host-plant specialists among insect herbivores.

In conclusion, research presented in this thesis has shown that adaptation to the host-plant can have important consequences for host specialization in insect herbivores. Moreover, it has shown that host specialization may be influenced by interactions with the host-plant at more than one level. The most important conclusion that can be drawn from this work is the need to have a broad perspective of an insect herbivores relationship with its host-plant when assessing the causes of host specialization.

## SUMMARY

1. This study is about host relationships in the cycad weevils *Antliarhinus zamiae* and *Antliarhinus signatus* and how host specialization in these weevils may be affected by adaptation for larval feeding on their cycad hosts and adaptation for oviposition into the larval food source.

2. *Antliarhinus zamiae* and *A. signatus*, as with all known species of *Antliarhinus*, develop exclusively on species of the cycad genus *Encephalartos*. Both species develop, as larvae, on the megagametophyte tissues of the host ovule. *Antliarhinus zamiae* had the broadest host range for any species of *Antliarhinus* (13 host species) whereas *A. signatus* had a narrower host range comprising seven species of *Encephalartos*. The host range of *A. zamiae* incorporated all the host species attacked by *A. signatus*.

3. *Antliarhinus peglerae*, which usually feeds on the sporophyll tissues of *Encephalartos* species, and which is considered to be a model ancestor for *A. zamiae* and *A. signatus*, does not survive on megagametophyte tissues. Larvae of *A. peglerae* also die when fed an artificial diet containing 3% macrozamin, a generally biocidal compound found in megagametophyte tissues. This result suggests that adaptation to feeding on megagametophyte tissues was a prerequisite for the evolution of larval development on cycad ovules in *A. zamiae* and *A. signatus*.

4. Larvae of both *A. signatus* and *A. zamiae* developed equally well on ovule tissues from a range of *Encephalartos* species, including species from which they were not recorded in the field. This result indicates that differences in host utilization between *A. signatus* and *A. zamiae* did not arise from physiological constraints on larval adaptation to feeding on megagametophyte tissues from different species of *Encephalartos*.

5. Substantial differences in ovipositional behaviour were observed between *A. signatus* and *A. zamiae*. *Antliarhinus zamiae* females usually used their extraordinarily long snouts to bore between the compacted sporophylls of their host-plants in order to reach the ovules underneath. Eggs were deposited via an almost equally long ovipositor that was inserted into the hole bored between the sporophylls. Unlike *A. zamiae*, *A. signatus* females first had to enter the cone between separated sporophylls before they could bore through the integument of the host ovule. *Antliarhinus signatus* females had a small body size and a short snout, probably to facilitate movement within the cone of their host-plant.

6. Within cones of *E. altensteinii*, *A. signatus* was only found in ovules situated near to the apex and base of the cone. The sporophylls in these areas of the cone separate to allow pollen entry and therefore allow *A. signatus* females to enter the cone to oviposit. Ovules attacked by *A. zamiae* were more evenly distributed throughout the cone but ovules that were not attacked were usually associated with exceptionally thick sporophylls.

7. Oviposition by *A. signatus* into ovules of *E. altensteinii* was also affected by the thickness of the ovule integument. Ovules with integuments thicker than 3.5 mm were seldom attacked because snout length in *A. signatus* females has a maximum limit of about this value. Oviposition by *A. zamiae* into ovules of *E. altensteinii* was affected by the thickness of sporophylls covering the ovules.

8. *Antliarhinus signatus* was absent from species of *Encephalartos* in which the mean thickness of the ovule integument was greater than 4 mm. This observation suggests that *A. signatus* is absent from these cycad species because *A. signatus* females cannot penetrate the ovule integument. *Antliarhinus zamiae* was less common in species of *Encephalartos* in which mean sporophyll thickness exceeded 15 mm indicating that *A. zamiae* females cannot penetrate the thick sporophylls in order to oviposit.

9. Snout length in females of *A. zamiae* ranged from 4-20 mm. Generally, in any population of *A. zamiae*, female snout length corresponded to the sporophyll thickness of the host-plant, but a bimodal distribution of snout lengths was obtained for females reared from four host species. Snout length either corresponded to the thickness of the host sporophyll or was significantly shorter than the thickness of the host sporophyll. This result suggests that some females may oviposit only when the sporophylls separate to allow pollen entry and are therefore not affected by sporophyll thickness. Snout length never exceeded 20 mm even if the sporophyll thickness of the host-plant was greater than 20 mm. This result indicates that host utilization by *A. zamiae* may be affected by the maximum length of the female's snout.

10. Oviposition by both *A. signatus* and *A. zamiae* was synchronized with specific periods of cone and ovule development in the host-plant. Oviposition by *A. signatus* was timed to correspond to a brief period of sporophyll separation at the time of pollen entry. Oviposition by *A. zamiae* took place over a longer period and was timed to occur before the ovule integument hardened to form the seed testA. Close behavioural synchrony between ovipositional activity in *A. signatus*

and *A. zamiae* and host-plant development probably prevents host shifts to species of *Encephalartos* with different coning phenologies.

12. This study suggests that adaptations for oviposition into the ovules of their host-plants has had a significant effect on host specialization by *A. signatus* and *A. zamiae*. Understanding adaptation to the host-plant, and specifically constraints on adaptation to a broad array of plant species, is therefore important for understanding host specialization in insect herbivores.

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**INSECT PREDATION OF OVULES IN THE SOUTH AFRICAN SPECIES OF  
*ENCEPHALARTOS* (CYCADALES: ZAMIACEAE)**

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**ABSTRACT**

South African species of *Encephalartos* reputedly suffer considerable ovule mortality as a result of insect predation. In some species, such as *E. lehmannii*, 100% of the ovules in a single cone may be preyed upon (Giddy, 1974). The resultant reduction in potential seed set could have serious implications for the conservation of cycads, particularly for species which are already threatened. However, the extent of ovule predation in different species of *Encephalartos* and the potential for ovule predators to colonize new cycad hosts are unknown. In this study, ovule and seed predation were examined in 19 species of *Encephalartos*. Six ovule predators were identified. Four of these insect species preyed facultatively on the megagametophyte of their host species and seldom caused substantial mortality. In contrast, two specialist ovule predators, *Antliarhinus zamiae* and *Antliarhinus signatus* together destroyed between 2 and 96% (mean values) of their host's ovules and may therefore have a considerable influence on seed production. Both species were absent from the Transvaal region of South Africa but may yet colonize species of *Encephalartos* that grow here. However, successful colonization may depend on the attributes of the potential host plant since some species of *Encephalartos*, notably *E. cycadifolius* and *E. friderici-guilielmi* were never preyed upon by *A. zamiae* or *A. signatus* even when they occurred within the distributional range of these beetles.

**INTRODUCTION**

South African species of the cycad genus *Encephalartos* (Zamiaceae) are perhaps unique among cycads worldwide because their seeds and ovules are apparently heavily preyed upon by insects (Rattray, 1913; Giddy, 1974; Goode, 1989). In some instances, 100% predation of seeds collected in the field has been reported (Giddy, 1974). Such seemingly extraordinary levels of predation are of concern for the conservation of these cycads since many populations of *Encephalartos* are already vulnerable (Giddy, 1974; Goode, 1989). More specifically, insect predation is perceived to pose two potential threats to cycad survival. 1. That seed and ovule predators may colonize new hosts thereby placing additional pressures on species of *Encephalartos* that may already have poor recruitment. 2. That predation could have an increased impact on recruitment in populations that are declining due to other factors such as habitat destruction and the removal of mature plants by collectors. In other words, as population numbers decline,

insect predators may destroy more significant proportions of the potential seed crop. This may be particularly true of specialist seed predators that are restricted to cycad species.

The aims of this study were to determine the following. 1. The identity of insect ovule and seed predators and their distribution on South African species of *Encephalartos*. 2. To establish the potential for colonization of new hosts by the most damaging ovule predators. 3. To determine whether ovule and seed predation was higher in small populations.

## METHODS

To determine the extent of ovule and seed predation in species of *Encephalartos*, megasporangiate cones from 19 species (see Table 1) were collected between November 1988 and November 1990. A total of 232 cones from 71 localities were examined. Each cone was dissected and all seeds and ovules were placed in a basin of water. "Floater" seeds and ovules were dissected to confirm insect predation and to identify the insect predator. A sample of "sinker" seeds and ovules was opened to confirm the absence of seed predators.

Populations of *E. altensteinii* and *E. villosus* were most extensively sampled to determine the effects of population size on ovule and seed predation. Cones were collected from small populations (less than approximately 200 mature plants) and larger populations. In total, 47 megasporangiate cones of *E. altensteinii* and 36 cones of *E. villosus* were examined.

To establish host specificity in the most damaging ovule predators, *Antliarhinus zamiae* (Thunberg) and *Antliarhinus signatus* Gyllenhal (Coleoptera: Curculionoidea), 50 adults of each sex were confined in mesh bags on the cones of non host species in Kirstenbosch Botanic Garden, Cape Town, or on to isolated cones collected from natural populations. *Antliarhinus zamiae* adults were released on to *E. cycadifolius*, *E. friderici-guilielmi*, *E. laevifolius*, *E. lanatus* and *E. transvenosus*. *Antliarhinus signatus* adults were released on to the same plants but with the addition of *E. caffer*, *E. princeps* and *E. villosus*, species from which they were not collected in nature. In addition both species were released on to cones of *E. altensteinii* as a control. Only complete development of the insect predators on the host plant was interpreted as successful colonization.

## RESULTS AND DISCUSSION

**Extent of insect predation of ovules-** Six species of insects were found that killed ovules by feeding on them (see Table 1). In all cases, penetration of the integument and at

least partial consumption of the megagametophyte appeared to be necessary to kill the ovule. Four of the insects, *Zerenopsis leopardina* Felder (Lepidoptera: Geometridae), *Platymerus eckloni* Gyllenhal (Coleoptera: Curculionoidea), and two species of *Amorphocerus* (Coleoptera: Curculionidae) fed facultatively, and only occasionally, on the megagametophyte. They fed more commonly on the megasporophylls (*Z. leopardina* and *Amorphocerus* spp.) or the fleshy integument of the ovule (*Amorphocerus* spp. and *P. eckloni*). As a result, these facultative ovule predators accounted for relatively little direct mortality of ovules (Table 1). In some instances, feeding by these insects appeared to facilitate pathogenic infection of the ovules so that they may be indirectly responsible for far greater mortality of ovules. However, without detailed studies of the sequence of infection, it is groundless to speculate on the role of predation in the onset of disease.

The most devastating ovule predators were *Antliarhinus zamiae* and *A. signatus* (Table 1), two weevil species that probably have an ancient association with species of *Encephalartos* (Crowson, 1981). *Antliarhinus zamiae*, alone, accounted for more than 60% of ovule mortality in eight host species. In combination with *A. signatus*, mean ovule mortality was often around 80% (Table 1) and it was not uncommon for all the ovules in a single cone to be predated. The exceptionally high mean mortality for *E. horridus* ovules (96%) is testament to the potential effects of predation by *A. zamiae* and *A. signatus* on seed production.

Thus, in general, these data support previous claims that *A. zamiae* (Giddy, 1974; Annecke & Moran, 1982; Goode, 1989) and to a lesser extent *A. signatus* (Goode, 1989) are highly destructive of *Encephalartos* ovules.

The actual effects of high ovule mortality on recruitment are unknown. If significant, density dependent, mortality of seeds occurred during or after dispersal, then it is possible that predispersal mortality of ovules would have a negligible effect on reproduction (Harper, 1977). However, in the absence of such data it may be prudent to assume that very high ovule mortality has some effect on reproduction.

**Distribution of *A. zamiae* and *A. signatus***- Although *A. signatus* and, more particularly, *A. zamiae* have relatively broad host ranges, neither species was recorded on all species of *Encephalartos* (Table 1). They were notably absent from almost all cycad species in the Transvaal province of South Africa (Fig. 1) despite close taxonomic affinities between some Transvaal species of *Encephalartos* and species occurring elsewhere that were preyed upon by *A. zamiae* or *A. signatus* (e.g. *E. dolomiticus* from the Transvaal and *E. lehmannii* from the eastern Cape Province). It is possible that the beetles have not colonized cycads in these areas simply due to an unsuitable climate or due to geographical barriers. However, unconfirmed reports from collectors in the Transvaal claim that *A. zamiae* and *A. signatus* have become naturalized in gardens in the region presumably through the importation of infested ovules into the area. Although garden situations cannot be equated with natural habitats, it remains a possibility that these predators could spread to

Transvaal cycads unless they are prevented from doing so by attributes of the potential host plant.

*A. zamiae* and *A. signatus* were also never recovered from species groups that included taxa with particularly woolly cones, i.e. *E. cycadifolius*, *E. friderici-guilielmi*, *E. ghellinckii*, *E. laevifolius*, and *E. lanatus*. Cones of the related species, *E. humilis*, were not examined but I have not come across any records of ovule predation in this species. At least some of these cycads, e.g. *E. cycadifolius* and *E. friderici-guilielmi*, are distributed within the general range of *A. zamiae* and *A. signatus* so that they are not excluded as hosts by geographical or climatic barriers. The most plausible explanation for their omission from the host range of *A. zamiae* and *A. signatus* appears to be incompatibility between the insects and these cycads. Similarly, the absence of *A. signatus* from *E. caffer* and *E. villosus* (Table 1) may be caused by inherent qualities of these plants. This conclusion was confirmed by the host specificity tests carried out in the Kirstenbosch Botanic Garden.

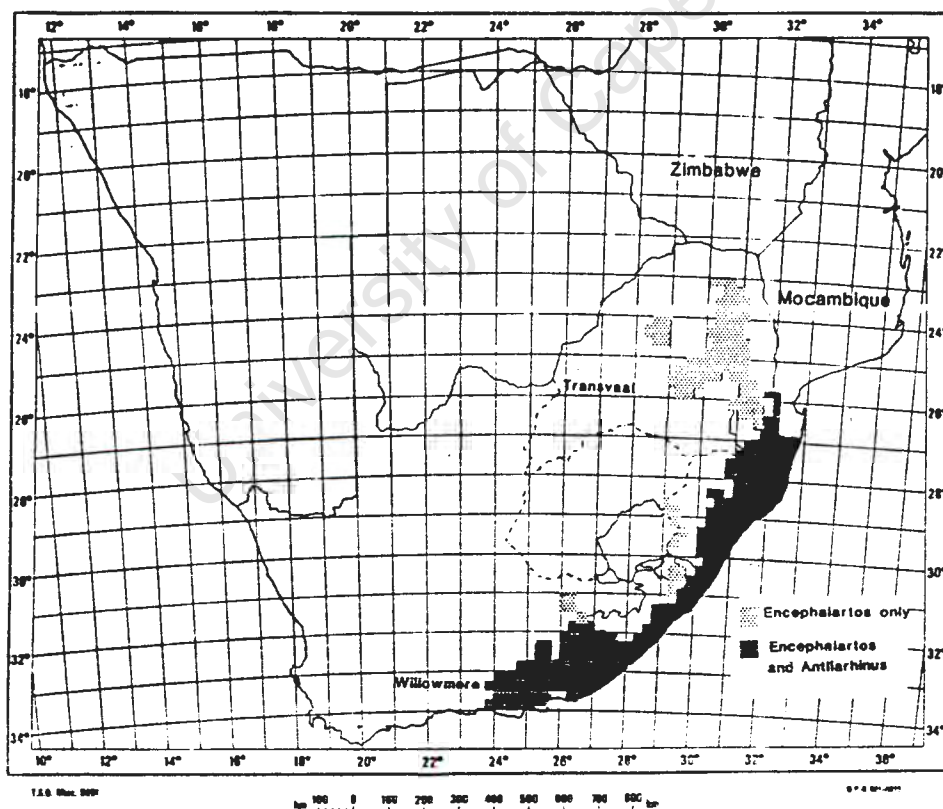


Fig. 1. Map of South Africa showing the general distributions of the cycad genus *Encephalartos* and the cycad weevils *Antliarhinus zamiae* and *Antliarhinus signatus*.

Host specificity in *A. zamiae* and *A. signatus*- *Antliarhinus zamiae* and *A. signatus* developed normally on at least one species of *Encephalartos* from the Transvaal when confined to plants in Kirstenbosch Botanic Garden. Their release on to cones of *E. transvenosus* resulted in more than 60% predation of ovules by *A. zamiae* and about 15% mortality as a result of predation by *A. signatus*. The number of ovules preyed upon was not significantly different from predation in *E. altensteinii* ( $P > 0.05$ ). This result confirms the prediction that *A. zamiae* and *A. signatus* have the potential to colonize some Transvaal species. Cones from *E. dolomiticus* or its near relatives were not available for testing, but a similar result would be expected.

*Antliarhinus signatus* also developed successfully on *E. princeps*. Since *E. princeps* is distributed within the range of *A. signatus*, the absence of this weevil from *E. princeps* may reflect a temporary absence from the populations sampled in this study. In this case *A. signatus* may be recovered from *E. princeps* populations in future.

Neither *A. zamiae* or *A. signatus* developed successfully on *E. cycadifolius*, *E. friderici-guilielmi*, *E. laevifolius*, or *E. lanatus*. Dissection of these cones showed that adults had made no attempt to oviposit into the ovules and that they did not recognize these cycad species as host plants. Oberprieler (1989) has attributed the absence of *A. zamiae* and *A. signatus* from ovules of *E. friderici-guilielmi* to the woolly covering present on the megasporophylls. However, the tomentum has little effect on the movement of beetles on the cone (Donaldson, unpublished data) and is therefore unlikely to hinder oviposition. It is more likely that major differences in coning phenology between these cycads and other species of *Encephalartos* have resulted in the absence of predation by species of *Antliarhinus* (Donaldson, unpublished data).

*Antliarhinus signatus* also failed to colonize *E. caffer* and *E. villosus* in the botanic garden thus confirming the field results in which this seed predator was always absent from these cycads. The most plausible explanation for this phenomena is that *A. signatus* females are not able to oviposit into the ovules of either *E. caffer* or *E. villosus*. *Antliarhinus signatus* females have a relatively short rostrum (ca. 3mm) which they use first to penetrate the ovule integument near to the attachment to the megasporophyll and then to excavate a hole in the gametophyte into which they lay their eggs (Donaldson, in press). Due to the limitations of snout length, female *A. signatus* are only able to penetrate integuments that are approximately 3mm thick in the regions near the attachment to the megasporophyll. As a result they could be excluded from cycads such as *E. caffer* and *E. villosus* which often have integuments greatly in excess of 3mm thick in this region (Donaldson, unpublished data).

In general, these host specificity data show that *E. friderici-guilielmi* and related plants are unlikely to be colonized by *A. zamiae* or *A. signatus* regardless of where they occur and the same could be said for colonization of *E. caffer* and *E. villosus* by *A. signatus*. However, other species of *Encephalartos* that are currently not predated by *A. zamiae* or

*A. signatus* should be considered as potential host plants, particularly *E. transvenosus* and probably *E. eugene-maraisii*, *E. dolomiticus*, and *E. dyerianus*

**Population size and ovule predation-** Significantly greater predation of ovules was recorded in small populations of *E. altensteinii* (mean predation = 75%) and *E. villosus* (mean predation = 93%) than in large populations (means of 61% and 84% for *E. altensteinii* and *E. villosus* respectively;  $\chi^2$  on raw data,  $P < 0.01$ ). The reasons for this difference were not investigated but could be due to predator satiation in large populations or to density dependent mortality of adult beetles in large populations. Adult *A. zamiae* and *A. signatus* seek shelter under bark between coning cycles (Ratray, 1913) and probably suffer their greatest mortality at this stage (Donaldson, unpublished data). If shelter sites are limited, then density dependent mortality of adult beetles would be expected. Since large populations of cycads are likely to host greater numbers of beetles, mortality of adult beetles may be higher in these populations. As a result, predation in the following season would be lower than in a smaller cycad population in which the adult beetles may have suffered relatively less mortality due to their lower initial density.

These results indicate that predispersal predation of ovules may increase as populations decrease in size. However, it should be stressed that this conclusion is based on a relatively small sample. Collecting over a longer period and from more populations is needed to confirm this result. In addition, the effects of predispersal ovule mortality on recruitment needs to be studied to determine whether increased predation in small populations has any real effect on reproduction.

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TABLE 1. Percentage of ovules or seeds destroyed by insect predators in 19 species of *Encephalartos*. For each cycad species, the mean percentage of ovules preyed upon by *Zerenopsis leopardina* (Lepidoptera), *Amorphocerus* sp. 1 and sp. 2, *Platymerus eckloni*, *Antliarhinus zamiae* and *Antliarhinus signatus* (all Coleoptera) is given. In addition, the total percentage of ovules preyed upon and the number of megasporangiate cones sampled are provided. Percentages were rounded off to the nearest integer except where the value was less than 1.

Species of <i>Encephalartos</i>	<i>Zerenopsis leopardina</i>	<i>Amorphocerus</i> sp.1	<i>Amorphocerus</i> sp.2	<i>Platymerus eckloni</i>	<i>Antliarhinus zamiae signatus</i>	<i>Antliarhinus signatus</i>	Total predation (%)	Number of cones
<i>altensteinii</i>	0	0.02	0	0	63	12	75.02	47
<i>arenarius</i>	0	0	0	0	74	8	82	5
<i>caffer</i>	0	0	0	0	2	0	2	10
<i>cycadifolius</i>	0	0	0	0	0	0	0	8
<i>dolomiticus</i>	0	0	0	0	0	0	0	3
<i>ferox</i>	0	0	0	0	62	0	62	2
<i>friderici-</i>								
<i>guilielmi</i>	0	0	5	7	0	0	12	30
<i>horridus</i>	0	0	0	0	87	9	96	9
<i>laevifolius</i>	0.2	0	0	0	0	0	0.2	16
<i>lanatus</i>	0	0	0	0	0	0	0	6
<i>lebomboensis</i>	0	0	0	0	51	0	51	2
<i>lehmannii</i>	0	0.4	0	0	41	13	54.4	16
<i>longifolius</i>	0	0.3	0	0	4	13	17.3	14
<i>natalensis</i>	0	0	0	0	61	13	74	6
<i>princeps</i>	0	6	0	0	82	0	88	5
<i>transvenosus</i>	0	0	0	0	0	0	0	3
<i>trispinosus</i>	0	0	0	0	53	10	63	12
<i>umbeluziensis</i>	0	0	0	0	81	0	81	2
<i>villosus</i>	0.6	0	0	0	87	0	87.6	36