POLLINATION ECOLOGY OF THE
EXTANT CYCADOPHYTA

Submitted in partial fulfilment of the degree
BSc. (Hons.) Plant Science
October 2009

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ABSTRACT

Cycads are ancient grouping of gymnosperms of great significance in terms of all botanical disciplines (e.g. Ecology, Systematics, and Physiology). They are living fossils providing unprecedented access to the past as they have undergone little change. They are however also highly endangered. Their pollination is then one of the chief areas of concern for these reasons. Cycad pollination has been a source of controversy for almost half a century. It was first considered to follow the coniferous anemophilous (wind pollination) pattern for nearly a half century, despite indications of entomophilous (insect pollination). In this paper I investigate whether the application of pollination syndrome, to a thoroughly described reproductive event (including structural morphology) from literature, could settle the question. I find that the reproductive ecology and morphology of cycads are inconsistent with the anemophilous pollination syndrome and adhere much more closely to cantharophily (beetle pollination). These indirect conclusions are then supplemented with a review of the literature on direct experimental evidence, the findings of which mostly discredit wind pollination and affirm insect pollination in all cycad genera investigated. This work provides support for pollination syndromes as a tool to infer pollinators, not only in angiosperms but also in other seed plants. It also settles the debate surrounding the pollination ecology of cycads by describing the obligate mutualism that occurs between the insects and their host.

Keyword: Cycads; pollination; syndrome; entomophily; anemophily.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction</strong></td>
<td>4</td>
</tr>
<tr>
<td>Taxonomy and distribution</td>
<td>7</td>
</tr>
<tr>
<td>Conservation status</td>
<td>12</td>
</tr>
<tr>
<td>Pollination syndromes</td>
<td>14</td>
</tr>
<tr>
<td><strong>Reproductive event</strong></td>
<td>16</td>
</tr>
<tr>
<td>Morphology of reproductive structures</td>
<td>16</td>
</tr>
<tr>
<td>Male strobili</td>
<td>16</td>
</tr>
<tr>
<td>Female strobili</td>
<td>17</td>
</tr>
<tr>
<td>Reproductive event ecology</td>
<td>21</td>
</tr>
<tr>
<td><strong>Indirect evidence of pollinating agent</strong></td>
<td>24</td>
</tr>
<tr>
<td>Wind</td>
<td>24</td>
</tr>
<tr>
<td>Insect</td>
<td>27</td>
</tr>
<tr>
<td><strong>Direct evidence of pollinator</strong></td>
<td>29</td>
</tr>
<tr>
<td>Host specificity</td>
<td>29</td>
</tr>
<tr>
<td>Pollination ecology</td>
<td>33</td>
</tr>
<tr>
<td>Pollinator attraction</td>
<td>38</td>
</tr>
<tr>
<td>Cycad toxicity</td>
<td>39</td>
</tr>
<tr>
<td>Rewards</td>
<td>40</td>
</tr>
<tr>
<td><strong>Conclusion</strong></td>
<td>42</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>43</td>
</tr>
</tbody>
</table>
INTRODUCTION

Cycads are a group of higher plants of the Superdivision Spermatophyta that includes all seed bearing plants and are more specifically classed in the non-flowering plants grouping Gymnospermae (Simpson 2006). Cycads have been recognised as a distinct natural grouping for over two hundred years due to their very similar morphological features (Grobbelaar 2002). Cycads are the most basal lineage of the seed bearing plants (Chaw et al. 2000; Simpson 2006) with a fossil record extending back to the Palaeozoic, possibly from the late Carboniferous (~300–325 million years ago) but at least from the early Permian (~280 mya) (Schneider et al. 2002). They were dominant in terms of diversity and distribution throughout the Mesozoic (Schneider et al. 2002) with the Jurassic (144–213 mya) being coined as the age of the cycad (Terry 2001; Hall et al. 2004). Extent taxa, however, are considered to have evolved relatively recently, originating around 144-70 mya (Terry 2001; Schneider et al. 2002; Vovides et al. 2003). Of note is the striking resemblance of the fossil taxa to the extant species (Klavins et al. 2003 cited in Sharon et al. 2005) prompting cycads to be referred to as living fossils. This provides an almost unprecedented situation where inferences about extinct taxa, and the evolutionary history of others, can be made be through the study of extant plants (Terry et al. 2004).

The extant species of cycad share a large number of traits: all cycads are dioecious (sexes on separate individuals) bearing cones as reproductive structures (Hall et al. 2004); they are generally long-lived (Giddy 1974); have slow growth rates; have late reproductive maturity (Grobbelaar 2002; Terry et al. 2008); have various specialist interaction such as nitrogen fixing root-bacteria (Grobbelaar 2002), have strikingly similar vegetative and reproductive forms (Figure 1) (Giddy 1974; Grobbelaar 2002), are late recruiters and produce few offspring (Donaldson 1995 cited in Golding & Hurter 2003); and are poisonous, containing many compounds or compound-groups exclusive to the division (Schneider et al. 2002; Brenner et al. 2003). Their reproductive ecology can be interpreted as K-strategists (focus on survivorship not reproductive output), though some cycads are r-selected (Donaldson 1995 cited in Golding & Hurter 2003). In terms of Grime's (1977) classification they can be classed as stress tolerant (Table 1). They fit the category quite well with their: slow growing, long-lived nature; tough evergreen leaves, which are retained for many years; mostly tree and shrub habit; and comparatively low investment into seed compared to vegetative organs.
Table 1: Characteristics of stress tolerant plants (modified from Grime 1977)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology of shoot</td>
<td>Extremely wide range of growth forms</td>
</tr>
<tr>
<td>Leaf form</td>
<td>Often small or leathery, or needle-like</td>
</tr>
<tr>
<td>Litter</td>
<td>Sparse, sometimes persistent</td>
</tr>
<tr>
<td>Maximum potential relative growth rate</td>
<td>Slow</td>
</tr>
<tr>
<td>Life forms</td>
<td>Lichens, perennial herbs, shrubs, and trees (often very long lived)</td>
</tr>
<tr>
<td>Longevity of leaves</td>
<td>Long</td>
</tr>
<tr>
<td>Phenology of leaf production</td>
<td>Evergreens with various patterns of leaf production</td>
</tr>
<tr>
<td>Phenology of flowering</td>
<td>No general relationship between time of flowering and season</td>
</tr>
<tr>
<td>Proportion of annual production devoted to seeds</td>
<td>Small</td>
</tr>
</tbody>
</table>

Figure 1—Almost universally applicable description of cycad morphology (Giddy 1974).
Cycads are considered extremely important for various reasons: firstly due to their evolutionary history and taxonomic position; secondly as they are considered ‘flagship’ species for conservation due to large-scale endangered status (Donaldson 2003); thirdly because of their unusual life histories and traits; and lastly due to their horticultural appeal (Whitelock 2002 cited in Terry et al. 2008). One important and far reaching ecological consideration is their pollination (Terry et al. 2004). The development of the flower in the Angiospermae (flowering plants) and the associated insect pollination is a source of constant debate due to the poor fossil record in this regard (Endress 1994; Simpson 2006). The study of extant cycad pollination might thus prove invaluable, as inference can be made towards the ancient conditions of the cycads and possibly subsequent lineages (Terry 2001). However in terms of pollination no group has resulted in such controversy as the cycads.

The debate surrounding cycad pollination is on whether they are wind or insect pollinated. Cycads were considered to be wind pollinated; as most other thoroughly studied gymnosperms are (Grobbelaar 2002; Schneider et al. 2002; Kono & Tobe 2007). This was based on Charles Joseph Chamberlain’s work (1919, 1935 cited in Schneider et al. 2002) whom was undoubtedly the authority on the taxa at the time stating: “There have been many reports of insect pollination, but in a rather extensive field study in which all the gene-ra have been examined, nothing has been observed which would indicate anything but wind pollination so characteristic of the whole group of gymnosperms” (Chamberlain 1919: 1965 edition, page 127 cited in Grobbelaar 2002). This view would be the prevailing case for the following half a centaury with even recent publication such as the Dendrological Foundation of southern Africa’s Tree Atlas (Section 1) stating: “Cycads are wind pollinated; the possibility of accidental insect pollination by predators however cannot be excluded” (von Breitenbach & von Breitenbach 1992). This generalisation has persistent even though observation of insect associations with cycad strobili, and their speculated pollination thereof, were mentioned around the same time by authors such as Pearson (1906), Rattray (1913) and Marloth (1914) (all cited in Donaldson 1997). Direct experimentation and observations of pollination however were absent with no work done on cycad reproductive biology (Vovides et al. 1997) and evidence was largely circumstantial (Tang 1987a; Niklas & Norstog 1984). Recently studies into cycad pollination have been conducted, prompted primarily by the various indirect lines of evidence and observations casting doubt on the generalisation made by Chamberlain.
Taxonomy and distribution

The currently around 300 recognised species in 11 genera (Hill et al. 2003) share a distinct classification up to division level and only split at the family level (Table 2):

Division: Cycadophyta;
Class: Cycadopsida;
Order: Cycadales.

Traditionally three families have been recognised: Cycadaceae, Zamiaceae and Stangeriaceae. The genera recognised per family are (Figures from Hill undated): Cycadaceae represented only by Cycas (~90 spp.) (Figure 2D, E); Stangeriaceae compromising two genera, the monotypic genus Stangeria (Figure 2L) and Bowenia (2 species, Figure 2B); Zamiaceae including Ceratozamia (16 spp., Figure 2J), Chigua (2 species, Figure 2F), Dioon (11 spp., Figure 2I), Encephalartos (~62 spp., Figure 2K), Lepidozamia (2 species, Figure 2C), Macrozamia (~38 spp., Figure 2H), Zamia (~56 spp., Figure 2G) and the monotypic genus Microcycas (Figure 2A). The number of recognised cycad species has seen a dramatic increase past 30 years with taxonomic revisions of Australian cycads already been undertaken (Donaldson 2003).

Table 2—Current taxonomic standing of the cycads (modified from Hill undated)

<table>
<thead>
<tr>
<th>Order</th>
<th>Suborder</th>
<th>Family</th>
<th>Subfamily</th>
<th>Tribe</th>
<th>Subtribe</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycadales</td>
<td></td>
<td>Stangeriaceae</td>
<td>Stangerioideae</td>
<td></td>
<td></td>
<td>Stangeria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bowenioideae</td>
<td></td>
<td></td>
<td></td>
<td>Bowenia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zamiaceae</td>
<td>Encephalartoideae</td>
<td>Diooeae</td>
<td>Encephalartae</td>
<td>Encephalartinae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Macrozamiinae</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Lepidozamia</td>
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<td></td>
<td></td>
<td></td>
<td>Ceratozamia</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Microcycas</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Zamia</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Chigua</td>
</tr>
</tbody>
</table>

Figure 2 (Next page)—Examples of extant cycad species of all genera. A, Microcycas calocoma; B, Bowenia spectabilis (Eising undated); C, Lepidozamia peraffskyana; D, Cycas thouarsii (Female); E, C. revoluta (Male); F, Chigua restrepoli (Jones 1993); G, Zamia furfuracea; H, Macrozamia communis; I, Dioon spinulosum; J, Ceratozamia robusta; K, Encephalartos ferox; L, Stangeria eriopus (Severed male strobilus in picture). A–E, G–J: www.flickr.com. K–L: P. Rousseau.
However according to the most current paper on cycad phylogeny by Zgurski et al. (2008) the family Stangeriaceae is not monophyletic (corroborated by Hill et al. 2003; Rai et al. 2003, Bogler & Fancisco-Ortega 2004; Chaw et al. 2005). This family has been recognised morphologically but has found little molecular support (Zgurski et al. 2008). Zgurski et al. (2008) presents a single tree of greatest agreement (Figure 3) between their and the recent molecular work of Hill et al. (2003) and Chaw et al. (2005) and the morphological classification by Hermsen et al. (2006) except where noted. I have modified their tree to incorporate their results, i.e. *Dioon*’s placement in bold. They found that the genera *Bowenia*, *Stangeria* and *Dioon* all have uncertainty in phylogenetic placement (Zgurski et al. 2008). The confidence levels for the various other clades however are high with the Division being considered strongly monophyletic (99% bootstraps—Schneider et al. 2002). *Cycas* is considered basal through molecular (100% bootstrap support Zgurski et al. 2008) and morphological support (Grobbelaar 2002; Donaldson 2003; Hermsen et al. 2006). *Ceratozamia*, *Microcycas*, *Zamia* and *Stangeria* form a clade (89–98% support), as does *Encephalartos*, *Lepidozamia* and *Macrozamia*, with a sub-clade consisting of *Encephalartos* and *Lepidozamia* found (100% bootstrap).

![Figure 3](image-url) — Greatest agreement tree for cycad genera. Outgroup= *Ginkgo biloba*. Symbols corresponding to *Bowenia* and *Stangeria* show possible placements. Double arrows represents departure from Hermsen et al. (2006). Modified from Zgurski et al. (2008).
Distribution-wise cycads are centred around the tropics and subtropics of the formerly united supercontinents Laurasia and Gondwana including four centres of diversity (Figure 4) in: the Americas (new world/north and south America), Africa, Asia and Australia (Donaldson 2003). Species only occur a few degrees above and below the tropics of Cancer and Capricorn (Figure 4) being confined between 40° south and north of the equator (Grobbelaar 2002). A high level of endemism is found in terms of genera: *Ceratozamia* and *Dioon* are endemic to Mexico and bordering countries; *Zamia* has 68.5% of its species endemic to one country (Donaldson 2003); *Microcycas* is endemic to Cuba (Jones 2002); *Chigua* is only known from the type locality in Colombia (Donaldson 2003); *Encephalartos* is endemic to Africa (45 taxa endemic to one country and 18 to two, with South Africa containing more than half of Africa’s cycad diversity—Donaldson 2003); *Stangeria* is endemic to the South African east-coast (Donaldson 2003); *Bowenia, Macrozamia* and *Lepidozamia* are endemic to Australia (representing 25% of the world cycad flora—Donaldson 2003); and *Cycas* has diversity centres in Vietnam (24 species), China (21), Thailand (10) and Indonesia (7) (Donaldson 2003). However ~25% of *Cycas* species have a cosmopolitan distribution—ranging from South-East Asia (including Japan) through India down to Madagascar and the African east coast covering most islands in-between (Jones 2002; Donaldson 2003; Hill et al. 2003).

Figure 4—Worldwide Cycadophyta distribution: (1)—*Ceratozamia, Zamia, Dioon, Microcycas* and *Chigua*. (2)—*Encephalartos, Stangeria, Cycas thouarsii* (3)—*Cycas* (4)—*Macrozamia, Lepidozamia, Bowenia*. (Hill et al. 2003).
**Conservation status**

Cycads are an ancient group no longer in its evolutionary heyday facing a clear extinction crisis facing the division (Donaldson 2003). Cycads are among the most threatened groups of plants worldwide with ~50% of the species included in the IUCN Red List and 82% being listed as threatened or worse (Donaldson, 2003). In terms of the four centres of diversity Donaldson (2003) tallied all known species by their respective status under the IUCN (Figure 5). The Americas and Africa both have almost 20 Critically Endangered species, while Australia is the only region with more Least Concern species than more critical categories. Asia also has concerning statistics with almost twice as many vulnerable taxa than Endangered and Critically Endangered ones—these could very easily become endangered should ineffective conservation be implemented. Of great concern is the fact that three of the four centres of endemism (Australia, Mexico and South Africa) are known to be affected by illegal trade (Gilbert 1984, Osborne 1990 cited in Golding & Hurter 2003).

![Figure 5](image-url) — Diversity and status of the world’s cycad flora in the four main regions of diversity. EW = Extinct in the Wild, CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, LC = Least Concern, DD = Data Deficient. Modified from Donaldson (2003).
Specifically in Africa, two species are Extinct in the Wild, 18 are classified as Critically Endangered (26%), eight are classified as Endangered with a further 18 (26%) classified as Vulnerable (Figure 5). Importantly three species were Data Deficient which, along with speculation that a wealth of species is still to be discovered in tropical Africa (Goode 1989, 2001; Donaldson 2003), could inflate these numbers even more. This has been found as a recent review on Encephalartos’ status yielded five undescribed taxa, all being endemic (Golding & Hurter 2003). Golding & Hurter (2003) thus urge cycad taxonomists to develop consistent infra-generic classification to aid with the Red Listing process. All Encephalartos species are listed under CITES as Appendix 1 (the strictest control group—Grobbelaar 2002; Golding & Hurter 2003) to avoid identification errors (Hutton and Dickson 2001 cited in Golding & Hurter 2003).

Various life history traits also contribute to the threatened status of cycads: cycads are stress tolerators, with a low competitive ability and high vulnerability to disturbance (Donaldson 1995 cited in Terry et al. 2008); they have a long juvenile stage (5–20+ years—Giddy 1974); occur in disjoint populations increasing inbreeding (Terry et al. 2008 with cited literature); have localised seed dispersal (< 100 m) (Terry et al. 2008 with cited literature); and very low seedling recruitment (Goode 2001; Grobbelaar 2002). On behalf of the IUCN’s Cycad Specialist Group Donaldson (2003) compiled the most comprehensive conservation review for all cycad species worldwide. In this “Status survey and conservation action plan” he stresses the importance of ecological consideration for conservation whether in situ or ex situ. Cycads do not exist in isolation, they require a minimum viable population (Golding & Hurter 2003) to not only support themselves, but also their various ecological processes often unique to the division. For example they form symbiotic interaction with nitrogen fixing cyanobacteria, arbuscular mycorrhizae, vertebrate dispersal agents along with predation by weevils and moth larvae (Giddy 1974; Goode 1989, 2001; Grobbelaar 2002). The pollination ecology of these taxa then also forms one of the primary considerations for conservation of both ecological functions and the species themselves. For some taxa it is already too late: *E. woodii* has no female individuals left, as possibly does *E. brevifoliolatus* (Grobbelaar 2002) which, along with *E. relictus* (Hurter & Glen 2001 cited in Golding & Hurter 2003), are all Extinct in the Wild. For others time is of the essence: the Cuban endemic *Microcycas calocoma* has almost no recruitment in situ (Donaldson 2003) and both the *Chigua* species have only been legitimately collected a handful of times, *C. restrepoi* only four times and *C. bernalii* only once with no reproductive material ever seen (Hill undated).
Pollination syndromes

Pollination syndromes are primarily an Angiospermae (flowering plant) concept used to infer pollinators in the absence of direct evidence. By definition it is a suite of floral traits associated with attraction and utilisation of specific pollinators (Fenster et al. 2004). Forseeably this can be generalised to any reproductive structure as the aim is to suit a pollinator’s behaviour, needs and characteristics (e.g. morphology) to facilitate pollination. Sets of traits in combination then select for a specific pollinators (Fenster et al. 2004). Although there are multiple pollination syndromes, the debate focuses on insect (with beetles being the most observed) and wind pollination. The various characters and states associated with these syndromes (in angiosperms) are compiled below (Table 3) from Faegri and Van Der Pijl (1966), Endress (1994) and Proctor et al. (1996).

Table 3: Selected pollination syndromes and the floral characters associated with them

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Cantharophily (Beetles) (Figure 6)</th>
<th>Anemophily (Wind) (Figure 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthecis</td>
<td>Day time</td>
<td>Daily pollen release pulse</td>
</tr>
<tr>
<td>Colour</td>
<td>Colours dull, cream/off-white, greenish, brownish</td>
<td>Perianth reduced or absent. Mostly colourless, green or brown</td>
</tr>
<tr>
<td>Reward</td>
<td>Copious pollen, feeding tissues, nectar or other exudates</td>
<td>None</td>
</tr>
<tr>
<td>Odours</td>
<td>Strong fruity or aminoid</td>
<td>None</td>
</tr>
<tr>
<td>Floral shape and symmetry</td>
<td>Dish/bowl shaped, flat, cylindrical, amorphic, halomorphic</td>
<td>Regular, open</td>
</tr>
<tr>
<td>Arrangement</td>
<td>Solitary when large, in dense inflorescence when small</td>
<td>Unisexual often dioecious plants</td>
</tr>
<tr>
<td>Nectar guides</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Landing pads</td>
<td>Large flowers</td>
<td>None</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust, special feeding tissue in some</td>
<td>Not robust</td>
</tr>
<tr>
<td>Crypticness</td>
<td>Rewards easily accessible. Sexual organs exposed, ovary hidden</td>
<td>Anthers and stigmas well exposed. Flowers borne free from obstructions.</td>
</tr>
<tr>
<td>Relative size</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Thermogenicity</td>
<td>Mostly thermogenic</td>
<td>None</td>
</tr>
<tr>
<td>Sexual organs</td>
<td>Epigynous, other organs exposed</td>
<td>Stigmas (pollination drop) large and divided. Anthers (pollen sacs) pendulous</td>
</tr>
<tr>
<td>Pollen</td>
<td>Pollen main attractant, copious amounts</td>
<td>Pollen copious, dry, powdery, smooth, often in bladders</td>
</tr>
</tbody>
</table>
As an introduction then we have seen the relative importance of cycads in terms of their evolutionary position (being basal to all extant seed plants) and conservation status (being highly endangered worldwide). The implementation of a successful conservation plan will necessitate a thorough understanding of their ecology (Donaldson 2003). None less so than their pollination ecology which in many cases is the pivot point of a taxa’s long-term survival. In this review of the literature then, I will aim to investigate the cycad reproductive event and compare it with the previously mentioned pollination syndromes reaching a conclusion of which it fits best, i.e. indirect evidence (pg 24). I will also present direct experimental evidence (pg 31) on the pollination ecology of the cycads. The final goal will be to conclude which pollination agent the extant cycads make use of—either wind or insect pollination—and settle the debate.
REPRODUCTIVE EVENT

With a few exceptions cycads show a high similarity in their reproductive events, from structural morphology to the accompanying ecology activities, I will thus describe the event in terms of these two categories—morphology and ecology—noting exceptions where applicable.

Morphology of reproductive organs

Male strobili

All male cycads form similar cone or strobili (microstrobili), the size, colour and number of which is highly variable across species (Giddy 1974; Goode 1989; Grobbelaar 2002), though all are slender in comparison to their female counterparts (von Breitenbach & von Breitenbach 1992). The male strobilus has four main constituents: (1) microsporangia (pollen sacs) housed on (2) microsporophylls (male cone scales) connected to (3) a central axis running the length of the strobilus, resting on a (4) peduncle which attaches it to the apex of the trunk. The microsporangia are found abaxially on the microsporophylls (Figure 8A, D) and house the microspores (male gametophytes) released as pollen grains. Microsporophylls are modified leaves consisting of: a narrow stalk (attached to the central axis); beyond which they are widened and flattened (the area carrying the microsporangia) (Figure 8A); ending terminally in the bulla (shield) which faces outward (Figure 8C, F). Some sporophylls however are sterile bearing no microsporangia often found at the top of strobili (Figure 8C). The bullae form a tight mesh sealing the strobilus until maturation with genus-specific sporophylls arrangement patterns found: Bowenia, Chigua, Zamia and Ceratozamia (Figure 8F) have hexagonal bullae arranged alternately in vertical rows; Microcycas and Stangeria (Figure 8G) have diamond shaped bullae alternating in vertical rows; Cycas, Dioon, Macrozamia, Encephalartos (Figure 8C, H) and Lepidozamia (Figure 8E) have diamond shape bullae that are spirally arranged (Grobbelaar 2002). The central axis is subtended by a peduncle (Figure 8F,G) of variable length and is sometimes absent (Figure 8H). When mature the central axis of the strobilus lengthens within a short time period (few days) resulting in gaps forming between neighbouring sporophylls (Grobbelaar 2002—Figure 8H). The size and distribution of these are species-specific and may be: very minute or quite large, even or uneven, spirally/staggered or complete (Figure 8E). Soon after opening the pollen sacs rupture, releasing pollen (Jones 2002).
Figure 8—Microstrobilus morphology. A, Abaxial sporophyll view with burst microsporangia; B, Adaxial view of (A), note attachment to central axis; C, Sterile sporophylls at strobilus top; D, Burst and unripe pollen sacs; E, Lepidozamia peroffskyana opening asymmetrically; F, Ceratozamia sporophyll arrangement; G, Stangeria sporophyll arrangement; H, Multiple Encephalartos ferox strobili at various stages of maturity. A-B: Grobbelaar 2002; C-F: www.flickr.com; G-H: P. Rousseau.
Female strobili

Markedly similar to their male counterpart, only Cycas has a clearly divergent morphological form. Instead of resembling the connate strobili of its male counterpart, Cycas female cones (megastrobili) bear seeds along the lower margins of numerous congregated megasporophylls at the trunk apex (Figure 9) (Simpson 2006). These sporophylls are extensions of the stem forming modified leaves, though less so than other genera’s, still looking markedly leaf-like (Figure 9D). Dioon has similar leaf-like sporophylls (Figure 11B) but these are far more compact and attached to a central axis (Grobbelaar 2002). The structure formed by the compacted yet individual sporophylls is termed a pseudostrobilus (Grobbelaar 2002). Megasporophylls have long stalks, bearing ovules on either side and ending in a sterile lamina-like structure of varying design (lobed, entire, or dissected) (Grobbelaar 2002). During maturing the sporophylls are first folded onto themselves (adaxially) forming a tight ball (Figure 9A), later “opening” to orientate themselves abaxially (Figure 9B) when receptive—exposing the ovules (Grobbelaar 2002). One to six ovules are borne on a single sporophyll (Figure 9C, D) (Grobbelaar 2002).

Figure 9—Cycas female pseudostrobili. A, C. revoluta closed; B, C. revoluta open; C, C. thouarsii pseudostrobilus; D, Multiple ovules and seeds on sporophylls. www.flickr.com.
The typical megastrobilus design is very similar, although broadly ovoid (von Breitenbach & von Breitenbach 1992), to the microstrobili described previously with: (1) megasporophylls which house; (2) ovules (forming seeds); attached to a (3) central axis; resting on a (4) peduncle (Figure 10). Each strobilus may contain several hundred seeds with exact numbers variable both within and between species (Donaldson 1993), number of strobili however is a far more preserved trait with many species prone to single strobilus production (Giddy 1974; Goode 1989; Grobbelaar 2002). Opposed to their male counterparts, all megastrobili are produced simultaneously and reach maturity in unison (Donaldson 1993). Female strobili also have fewer, but larger sporophylls (Check undated) than do their male counter-parts. The shape of both megastrobili and sporophyll stems from the ovules they bear, as these (at maturity) are significantly larger in relation to microsporangia. Megasporophylls are thus made up of a narrow stalk with two (rarely one) ovules to either side nestled in the terminal bullae (Figure 10, 11). The micropylar end (pollen entrance) is arranged distally to the bullae facing the central axis (Figure 12B) (Jones 2002; Simpson 2006). Again Dioon stands out as having ovules each with their own stalk whilst all other genera have sessile ovules (Figure 11) (Grobbelaar 2002). Both terminal and basal sporophylls in most cases are smaller than the rest, often being sterile as well (Figure 13). Percentages of sterile sporophylls are taxonomically correlated with some forming substantial amounts—39% in *E. trispinosus* (Figure 13A), while others have only a few—6% in *E. cycadifolius* (Figure 13B) (Grobbelaar 2002).

**Figure 10—*Encephalartos* spp. megastrobili internal morphology. A, *E. middelburgensis* top view with central axis around which seeds are arranged nestled in the sporophylls. Note two seeds per sporophyll; B, bottom view of (A). Note peduncle and removed seeds showing sporophyll stalks and two seed shaped hollows; C, *E. trispinosus* with removed sporophylls and seeds, note spiral arrangement and marks on central axis where sporophylls were attached. A-C: P. Rousseau.**
Figure 11—*Dioon edule* megastrobilus parts. A, Leaf-like megasporophylls attached to the central axis; B, Stalked ovules. A–B: Grobbelaar (2002).

Figure 12—Megastrobilus morphology. A, Sporophylls with two seeds, one on either side of a narrow stalk attached to the central axis; B, Flipside of (A) with stalk no longer visible, micropylar end indicated by arrow. Modified from Grobbelaar (2002).

Figure 13—*Encephalartos* female strobili. A, *E. trispinosus*, red square showing sterile sporophylls (Rousseau P); B, *E. cycadifolius* with little to no sterile sporophylls (Xaba 2009).
During their receptive period megastrobili open for a few days (to weeks) then close completely for the duration of strobilus maturation (Stevenson et al. 1998). As with the microstrobili a conserved sporophyll pattern is found, while in megastrobili genus-specific opening patterns also occur (Grobbelaar 2002): The taxonomic clade Bowenia, Ceratozamia, Chigua and Zamia develop fissures almost anywhere except in the top sterile sporophylls which are fused (Figure 14D); vertical fissures occur in Microcycas (Figure 14C); in Dioon basal sporophylls curve outwards (abaxially) (Figure 14A); in Stangeria sides of sporophylls curve inwards (adaxially); and the three taxonomically related genera Macrozamia, Encephalartos and Lepidozamia have mostly their top sterile sporophylls separating.

Figure 14—Megastrobili opening patterns. A, Dioon mejiae, red square shows separated sporophylls (Haynes undated); B, Encephalartos ferox, top sporophylls open slightly, lower ones shut (www.flickr.com); C, Curved Stangeria eriopus sporophylls (University of Wroclaw undated). D, Fully open Zamia fischeri except for top fused sporophylls (www.flickr.com); E, Microcycas calocoma with vertical fissures (Hill undated).
**Reproductive event ecology**

Temperature may influence coning, with plants at different localities coning or becoming receptive at different times based on temperature differences (Grobelaar 2002). The number of strobili per plant is heavily dependent on individual age which is related to the size of the plant (Ornduff 1991), time since previous coning and resources available (Donaldson 1993). Maximum numbers of strobili per plant are highly conserved within a species (Donaldson 1993) ranging from between one and six per stem (Grobelaar 2002). Environmental dependency is much more pronounced in female individuals, as the reproductive event is more taxing on them (Cheek undated) due to their higher resource allocation (Ornduff 1991). This has been seen in studies on *Zamia pumila* (Ornduff 1991) with the sexes differing in leaf area, leaf number, stem shape and size at first reproduction. Successive participation in reproductive events is also uncommon, especially for female individuals (Ornduff 1991; Wilson 2002). Coning readily occurs in cycles with males coning every 1-2 years and females every 4-5 years (Giddy 1974; Grobelaar 2002; Jones 2002). Masting may also occur in some cycads, though evidence therefore is not overbearing (Donaldson 1993 with cited literature). The main activities during pollination however occur during the opening of both males and megastrobili, coinciding with pollen maturity and ovule receptiveness respectively. With notable exceptions this period is coupled to thermogenesis, the exudation of the pollination droplet and excretion of volatiles.

Thermogenicity or the production of heat, often exceeding the ambient temperature by several degrees (Terry 2001), is coupled strongly to pollen maturity (Terry *et al.* 2004) (Figure 15A & 17). Tang (1987b) in a study on the thermogenicity in all extant genera found 42 of 43 species were thermogenically active, with only *Stangeria eriopus* not showing thermogenesis. Heat production also followed a circadian rhythm (Figure 15) (Tang 1987b). In terms of the sexes only some species had heat production in female strobili and where this occurred it was always lower than in their male counterparts. Starch content was the characteristic most correlated with heat production as megastrobili consistently had lower concentrations than conspecific males (Tang 1987b).
Figure 15—Daily thermogenic activity of cycad strobili. A, *Zamia furfuracea* (dashed line = ambient temp.) over 9 days indicating decline during post-pollination period, arrows show start and end of pollen release; B, Male *Encephalartos hildebrandtii* temperature during a single day (dashed line = ambient temp.). A-B: Modified from Tang 1987b.

As with all gymnosperms the onset of receptiveness is announced by the ovules excretion of a pollination droplet at the micropylar end (Figure 12B) (Simpson 2006). This liquid substance is chemically similar to angiosperms nectar (Baker & Baker 1975 cited in Tang 1987b) and consists of water, various sugars and trace amino-acids (Simpson 2006). As pollen is introduced into sporophyll fissures it becomes trapped by this sticky exudate.

Lastly both male and megastrobili are strongly odorous at maturity (Tang 1987b), though females are weaker (Tang 1985 cited in Tang 1987b). In his experiment on heat production, Tang (1987b) found that all thermogenically active plants were odorous and delimited three scent types: fruity (*Bowenia, Ceratozamia* and *Zamia*), resinous or fruity (*Encephalartos, Lepidozamia, Macrozamia*) and musty (*Cycas, Dioon* and *Microcycas*) though there were exceptions in almost all cases. He also found that the odours released were identical in the micro- and megastrobili (Terry *et al.* 2004), a finding reiterated by analyses of scent constitutes by Pellmyr *et al.* (1991).
In conclusion, we find that though I have discussed the various processes and structures of cycad reproduction separately, they all form part of an intricate integrated event. For instance the emission of volatiles is strongly correlated with thermogenic activity of strobili (Tang 1987a), which in turn is initiated during sporophyll opening due to strobilus lengthening (Grobbelaar 2002) when pollen/ovules are mature (Figure 17). Also, as the pollination droplet can be excreted and retracted, Donaldson (1997) found that the excretion is strongly correlated with thermogenic activity in *E. villosus* (Figure 16).

![Figure 16](image_url)

**Figure 16**—Correlation between microstrobilus temperature and megastrobilus pollination droplet excretion in *Encephalartos villosus* (Donaldson 1997).

![Figure 17](image_url)

**Figure 17**—Temperatures above ambient of microstrobili before, during and after dehiscence (Tang 1987b).
INDIRECT EVIDENCE

Here I will attempt to use the described reproductive event and structures to determine which of the two pollination syndromes fits cycads the best.

Wind pollination

Anemophily can be inferred for cycads due to the following traits as per Table 2:

I. Pollen is light, dry, and copious (Niklas & Norstog 1984; Wilson 2002).
II. The exposed nature of receptive Cycas ovules (Niklas & Norstog 1984).
III. Some taxa do not produce heat (Tang 1987b).
IV. Some species are not odorous (Tang 1987b).
V. No landing pads are presented.
VI. Masting occurs (Donaldson 1993).

Additional characteristics include: a superficial resemblance with coniferous ovulate cones which are exclusively wind pollinated (Niklas & Norstog 1984; Simpson 2006); taxa such as Stangeria (Procheș & Johnson 2009), Zamia furfuracea (Tang 1987a) and Cycas (Niklas & Norstog 1984) producing fertile seed ex situ; and the absence of insect visitors to one or both sexes’ strobili (Grobbelaar 1999 cited in Grobbelaar 2002; Terry et al. 2004).

Some experimental investigations into wind pollination include Niklas & Norstog (1984) who set out on the basis of aerodynamic theory. Similar studies had been conducted on conifers (Niklas & Paw U 1983) where strobilus morphology, surface texture, and adhesive properties influenced wind currents and preferentially deposited pollen at the ovules. Niklas & Norstog’s (1984) experiment involved megastrobili of Z. furfuracea and Z. pumila, Dioon edule and C. circinalis, all three genera with different opening patterns (Figure 14). Wind tunnel experiments found that each genus produced a distinctive, non-random pollen deposition pattern (Figure 18). Regions of deposition were mostly areas where sporophylls opened: at Dioon’s basal sporophylls; a small crack in Zamia; and on the erect sporophylls of Cycas. Pollen tallies indicated no preferential deposition to the windward side, instead spread evenly around the circumference (Figure 18). Strobili interiors however showed little direct pollen deposition at ovules, even in Cycas. They conclude therefore that wind pollination, in its strictest sense (direct movement of pollen to ovules), only occurs by chance and not design—in sharp contrast to the clearly efficient conifer aerodynamic system (Niklas & Paw U 1983).
Figure 18—Airflow-disturbance patterns of cycad megasporobilli. A, Dioon; B, Zamia spp.; C, Cycas. First two images represent two airflow speeds (100 cm.s⁻¹/200 cm.s⁻¹), third image refers to number of pollen grains per cm². Note non-random wind currents forming with rather uniform pollen deposition. Modified from Niklas & Norstog 1984.
Field studies have also found pollen transfer via wind to be lacking, with pollen numbers decreasing significantly within a few meters of the source being practically absent within ten (Stevenson et al. 1998; Hall et al. 2004; Kono & Tobe 2007). Airborne pollen around receptive females has also been found to be minimal (Hall et al. 2004). For example in C. panzhihuaensis, pollen densities decreased substantially within 2.55 m from the source (Wang et al. 1997 cited in Kono & Tobe 2007), on average only 40 grains of pollen found its way to Z. furfuracea megastrobili (Stevenson et al. 1998) and airborne loads of Lepidozamia peroffskyana pollen trapped in the vicinity of males was often minimal and always sporadic (Hall et al. 2004). This stands in contrast to expectations of large quantities of pollen, not only being released and airborne, but also finding their way to ovules (Proctor et al. 1996).

Additional difficulties and inconsistencies with anemophily (from Faegri and Van Der Pijl 1966; Endress 1994; Proctor et al. 1996) are also found:

I. Stems are not very tall in many species (Grobelaar 2002; Jones 2002).

II. Ovules are not exposed in all other genera (Hall et al. 2004), strobili are often obscured by large persistent leaves (Kono & Tobe 2007).

III. Pollen release and ovule receptiveness are not synchronised within or between populations (Grobelaar 2002; Hall et al. 2004; Kono & Tobe 2007).

IV. Pollination periods are often during the rainy season (Kono & Tobe 2007).

V. Most genera produce both heat and strong odour during receptive periods (Tang 1987b).

VI. Woolly strobili may hamper entrance of wind blown pollen (Grobelaar 2002).

VII. Forest species experience little or no wind, with strobili borne close to ground level often covered in layers of litter (Tang 1987a; Grobelaar 2002; Wilson 2002).

VIII. Some species in fact have large, heavy and/or sticky pollen (Stevenson et al. 1998).

IX. Many species of most genera conserved ex situ do not produce fertile seed at all, despite conditions conducive to wind pollination prevail (Niklas & Norstog 1984; Wilson 2002).

X. In Microcycas calocoma in situ fertilisation does not occur, although plants grow in sufficient numbers and distance (Vovides et al. 1997 with cited references).
Insect

Many of the inconsistencies with wind pollination can either be directly explained or dismissed by insect pollination.

Canarthophily can be inferred to cycad based on the following characteristics (Table 2):

I. Thermogenic activity of both sexes during receptive periods (Tang 1987b)

II. Strong odours produced by both sexes (Tang 1987b), the constituents of which include insect attractants and deterrents (Azuma & Kono 2006 cited in Kono & Tobe 2007). Fruity, musty and resinous smells fit the canarthophily profile (Tang 1987b).

III. Pollen is adhesive and produced in sufficient quantities to be considered as reward.

IV. Beetle pollination of primitive taxa (Kono & Tobe 2007 with cited references).

V. Arrangement in a compacted strobili (Grobbelaar 2002).

VI. Robust nature of strobili with plenty of feeding tissue (Grobbelaar 2002).

VII. Hidden ovules (Grobbelaar 2002).

The other indicator of insect pollination is the observed presence (Figure 19) of the same insect species at both sexes' strobili (especially during maturity) (Grobbelaar 2002). Although these observations do not concretely prove pollination, they do strengthen the case therefore.

Observations include:

I. Pearson (1906 cited in Stevenson et al. 1998) observed beetles on Encephalartos villosus and credited Joseph Hooker as suggesting cycads are entomophilous. He however did not observe actual transport of pollen to female strobili.

II. Rattray (1913 cited in Stevenson et al. 1998) investigated the interactions between weevils and E. villosus and E. altensteinii. He stated "The whole life-history [of the weevil] appears so dependent on Encephalartos that it would be only reasonable to find that the cycad had demanded a *quid pro quo* and had turned the visits to use."

III. Marloth (1914 cited in Donaldson 1997) proposed the main pollinator of E. villosus could be a seed parasite (Figure 19) based on observations of the insect's behaviour and presence on strobili.

IV. Baird (1938 cited in Norstog et al. 1986) hypothesised that the insects observed on Macrozamia riedlei might be secondary pollen carriers of pollen into the megastrobili.

V. More recently various authors report insect visitation (Schneider et al. 2002).
Figure 19—Insect observations on cycads. A, *Antliarhinus zamiae* weevil, the common seed parasite, covered in pollen; B, *Macrozamia lucida* male strobilus with thrips present. A Rousseau P. B: Terry undated.

In conclusion on indirect evidence and predictions based on pollination syndrome, I find that the reproductive event of cycads is unlike that of anemophilous plants and fits biotic insect pollination and more specifically the cantharophily syndrome much better.
DIRECT EVIDENCE

Indirect evidence however has never provided solid scientific arguments as the scientific method rests on direct empirical experimentation. This was provided by Norstog et al. (1986) who found the first unequivocal evidence of insects as the pollination agents in cycads. After them various authors conducted exclusion experiments (excluding wind, insect and both) to determine the contribution of each agent (Table 4). To date only the genera Chigua and Microcycas have no confirmed insect pollinated species (Tang 2004 cited in Vovides 1991): Chigua has not been investigated due to the political climate in Colombia (Hill undated), while there is strong circumstantial evidence that Microcycas is insect pollinated (Vovides et al. 1997; Chaves & Genaro 2005). In terms of insect diversity with the exception of the thrip genus Cycadothrips a basal clade to of Thysanoptera (Mound & Terry 2001), all other known pollinators are beetles (Coleoptera) (Terry et al. 2005; Terry et al. 2007), from the family Curculionidae and the superfamily Cucuoidea (Oberprieler 1995 cited in Suinyuy et al. 2009; Tang 2004 cited in Terry et al. 2005; Vovides 1991) with the subfamily Xenoscelinae (Cucuoidea: Erotylidae) associated with cycad genera on every continent, i.e. Africa (Donaldson 1997), central America (Vovides 1991), south-east Asia (Tang et al. 1999 cited in Hall et al. 2004) and Australia (Forster et al. 1994). However only a quarter of the ~300 cycad species have confirmed insects associates, with even less confirmed as polliants (Terry et al. 2004; Suinyuy et al. 2009). In most papers however the insects associated with cycad strobili are novel species and in some cases genera (Ornduff 1992). These insects also seem to be in obligate, host specific mutualisms and have their life cycle bound to the cycad and its reproductive event (Stevenson et al. 1998; Terry et al. 2007; Terry et al. 2008)

Host specificity

Host specificity in most cycad genera seems to be high with several having their own genus of pollinator with each cycad species making use of a different species of insect (Stevenson et al. 1998). This can most prominently be seen through the sympatric occurrence of species with no hybrids forming though they are hybridisable through artificial pollination (Grobbelaar 2002; Terry et al. 2004). Species-specificity has been found in Lepidozamia where the insect taxonomist Rolf Oberprieler (Hall et al. 2004) considers the Tranes sp. on L. peroffskyana (Figure 19C) to be a different species from: T. insignipes found on L. hopei, T. lyteroides the pollinator of Macrozamia communis and Tranes sp. on M. machinii. Both Bowenia species
have their own Milotranes species pollinating them (Wilson 2002). Zamia is more lax with Rhopalotria mollis (pollinator of Z. furfuracea) living on Z. loddigesii and Z. spartea, but not Z. integrifolia (Stevenson et al. 1998). In Macrozamia Terry (2001) cites previous works’ strobili surveys which suggest that four Macrozamia species are Cycadothrips spp. specialists (Figure 19D), eight are Tranes spp. (Curculionidae) pollinated and three have a co-pollination system. In contrast to the higher species-specificity resulting in non-hybridisation of Tranes pollinated Macrozamia; hybrids are evident among some Cycadothrips-pollinated species (Jones 2002). Strict host specialization has also been postulated for species of Encephalartos in Africa (Oberprieler 1995, 2004, Donaldson, 1997, 1999 cited in Suinyuy et al. 2009), for instance in the E. cycadifolius, E. friderici-guilielmii, E. ghellinckii, E. humilis, E. laevifolius, and E. lanatus clade (Treutlein et al. 2005 cited in Suinyuy et al. 2009) one finds a common suite of Coleopteran pollinators proven in some (E. cycadifolius and E. friderici-guilielmii) while only present and speculated in the others. Suinyuy et al. (2009) speculate that there may be at least two different cycad pollination systems within the southern African Encephalartos: the first involving Cucujoidea (families Boganiidae and Erotylidae) and is less host specific (Donaldson 1997); the second involving both Cucujoidea beetles and Porthetes (Curculionidae), with Porthetes being highly species-specific (Donaldson 1997). In terms of the northern species up to eastern and central Africa this is most probably not the case as Porthetes appears to be absent (Donaldson 1999 cited in Suinyuy et al. 2009). On the other hand genera such as Stangeria (Grobbeelaar 2002) which are known to naturally pollinate in cultivation worldwide, might do so due to a lack of host specificity or cosmopolitan distribution of their pollinators (Kono & Tobe 2007; Proches & Johnson 2009). Between genera then there seems to be conserved taxonomic association between pollinator and plant, while within genera however there is of yet no concrete correlation between morphological or taxonomic alliances and pollinator associations (Terry 2001; Suinyuy et al. 2009). I however hold a strong suspicion that with the help of thorough pollinator mapping, taxonomic revisions and reproductive ecology descriptions, patterns will present themselves.

In terms of the four centres of diversity (Figure 4), and the continents they represent, the insect pollinators are taxonomically unrelated (Leschen 2003) with similarities in the pollination systems being due to convergent evolution into a specialised niche (Oberprieler 1995a, 1995b cited in Hall et al. 2004). Langurid beetles which pollinate American and African cycads are only distantly related (Schneider et al. 2002) which indicates that such beetles could have been linked with cycads before the separation of the continents (R.G. Philip Rousseau 26111889 30/47
Oberprieler pers. com. cited in Schneider et al. 2002) or might indicate that interactions have evolved independently on different continents (Terry et al. 2005; Suinyuy et al. 2009). Host specificity may be induced by two main mechanisms, temporal isolation and recognition isolation (Stevenson et al. 1998; Schneider et al. 2002). In Caribbean Zamia species those that are incompatible to each others’ pollinators often cone in another season providing phenological incompatibility (Stevenson et al. 1998). This could possibly also be the case for various sympatrically (in situ or ex situ) growing species. Behavioural recognition and host finding seems to take the form of strobilus activity timing of thermogenesis and volatile emission (Terry et al. 2004), where these occur simultaneously with different pollinator’s activity and may serve to aid specificity. For example Tranes-pollinated Macrozamia species are clearly different from two Cycadothrips-pollinated species in the timing of these activities (Terry et al. 2004). The Tranes taxonomic grouping pollination systems of Lepidozamia, Macrozamia and Bowenia are speculated to be kept distinct due to weevil behaviour differing between the genera (Hall et al. 2004). This adds ecological support to the taxonomic hypotheses on host specificity (Hall et al. 2004).

Figure 20—Pollinators. A, Rhopalotria mollis (Curculionidae) (Schneider et al. 2002); B, Carpophilus hemipterus (Nitidulidae) (Gross 2007); C, Tranes lyteroides (Curculionidae) (Kennedy undated).
Table 4—Direct experimental evidence of insect pollination (chronologically by species).
Note—Erotylidae has nomenclatural priority over Languriidae used in other works (Leschen 2003)

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Pollinator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zamia furfuracea</td>
<td>Rhopalotria mollis (Figure 20A) (Curculionidae)</td>
<td>Norstog et al. 1986, Figure 20A</td>
</tr>
<tr>
<td>Zamia pumila</td>
<td>Rhopalotria slossoni + Paraxonotha zamiae (Curculoidea: Erotylidae)</td>
<td>Tang et al. 1987a</td>
</tr>
<tr>
<td>Encephalartos villosus</td>
<td>Porthetes sp. (Curculionidae), Metacucujus goodie (Cuculoidea: Boganiidae) + Erotylidae sp. nov.</td>
<td>Donaldson 1997</td>
</tr>
<tr>
<td>Dioon calijano</td>
<td>Rhopalotria bicolor (Curculionidae)</td>
<td>Vovides 1991</td>
</tr>
<tr>
<td>Macrozamia communis</td>
<td>Tranes lyterioides (Figure 20C) + T. sparsus (Curculionidae); Cycadothrips chadwicki (Thysanoptera: Aleothripidae)</td>
<td>Terry 2001</td>
</tr>
<tr>
<td>Macrozamia macdonelli</td>
<td>Cycadothrips albrechtii (Thysanoptera)</td>
<td>Mound &amp; Terry 2001</td>
</tr>
<tr>
<td>Bowenia spectabilis</td>
<td>Milotranes prosternalis (Curculionidae)</td>
<td>Wilson 2002</td>
</tr>
<tr>
<td>Bowenia serrulata</td>
<td>Milotranes subopaca (Curculionidae)</td>
<td>Wilson 2002</td>
</tr>
<tr>
<td>Lepidozamia peroffskiana</td>
<td>Tranes sp. (Curculionidae)</td>
<td>Hall et al. 2004,</td>
</tr>
<tr>
<td>Macrozamia lucida, Macrozamia machinii</td>
<td>Tranes sp. (Curculionidae) + Cycadothrips chadwicki (Figure 19C) (Thysanoptera: Aleothripidae)</td>
<td>Terry et al. 2005</td>
</tr>
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<td>Cycas revoluta</td>
<td>Carpophilus chalybeus (Coleoptera: Nitidulidae)</td>
<td>Kono &amp; Tobe 2007</td>
</tr>
<tr>
<td>Stangeria eriopus</td>
<td>Carpophilus hemipterus (Figure 20B) + C. fumatus + U. picinus (Coleoptera: Nitidulidae)</td>
<td>Procheş &amp; Johnson 2009</td>
</tr>
</tbody>
</table>
Pollination ecology

Of the few directly proven cases of insect pollination in cycads (Table 4), even less have been investigated for the particulars of the interaction. All known ones however are highly specialised dependent mutualisms (Oberprieler 2004, Tang 2004 cited in Schneider et al. 2002; Brenner et al. 2003; Terry et al. 2008) where neither party has long-term survivability without the other (Schneider et al. 2002). These interactions are analogous to the angiosperm fig/wasp, yucca/moth, and senna/moth obligate mutualism (Schneider et al. 2002) where pollinators need the plant’s reproductive event to complete their life cycle (Terry et al. 2008). In cycads the pollinator’s life cycle revolves around the male strobili with visitation to female strobili culminating in specific behaviours that ensures the transfer of pollen to the pollination drop (Suinyuy et al. 2009). Visitation to megastrobili therefore is not a necessity though pollinators are very efficient and strobilus fertility may approach 100% (Stevenson et al. 1998). Comparable pollinator life cycles have been found in Zamia furfuracea, Z. pumila (Figure 24), Macrozamia communis (Stevenson et al. 1998), Dioon edulis, D. califanoi (Vovides 1991), Bowenia spectabilis, B. serrulata (Wilson 2002) Encephalartos villosus (Donaldson 1997) and Lepidozamia peroffskyana (Hall et al. 2004). Life cycles are not identical however, e.g Lepidozamia, Macrozamia and Bowenia share the Tranes weevil taxonomic group as pollinators which are broadly similar, though weevil behaviour is slightly divergent between the cycad genera (Hall et al. 2004).

No pollinators are usually found on pre-dehiscent strobili (Figure 21) (Suinyuy et al. 2009). Insect that start the annual breeding cycle usually emerge from a dormant stage (Norstog et al. 1986) from various parts of the cycad or its immediate environment (e.g. stems or the surrounding soil) (Norstog et al. 1986; Tang 1987a). Once emerged insects enter maturing (L. peroffskyana—Hall et al. 2004) or matured (Vovides 1991; Schneider et al. 2002) microstrobili (Figure 21). In the case of Cycas revoluta however sap beetles have been observed to settle in megastrobili (Kono & Tobe 2007). Microstrobili often become mature slightly before megastrobili, releasing pollen before any megastrobili are receptive (Procheș & Johnson 2009). This might be to allow the pollinator’s population to build up before initiating pollination. In some cases some preference towards specific microstrobili is present as in Z. furfuracea and M. communis (Norstog et al. 1986; Tang 1987a; Pellmyr et al. 1991; Terry 2001).
Most pollinators complete their life cycles exclusively on the microstrobili (Mound & Terry 2001; Wilson 2002) using the strobili as brood sites (Norstog et al. 1986; Tang 1987a; Donaldson 1997; Wilson 2002; Hall et al. 2004; Terry et al. 2005). Pollinators are usually few in number at first (although Tranes sp. on L. peroffskyana number around 700—Hall et al. 2004) and the first breeding cycle is short, increasing the pollinator numbers in succeeding generations (Stevenson et al. 1998). The microstrobili provide shelter (thrips drawn to tight places—Terry 2001) and food for adults and larvae (Vovides 1991; Stevenson et al. 1998). Food is usually in the form of pollen (Porthetes however doesn’t feed on pollen—Donaldson 1997) and strobilus tissue (always the end of the strobilus’ lifetime—Hall et al. 2004). Most early activity (mating, male aggression etc.) occurs on the external bullae (Hall et al. 2004) with insects moving into strobili to feed and oviposit (Vovides 1991; Schneider et al. 2002; Hall et al. 2004). Once larvae hatch they feed on pollen and burrow into sporophyll tissue to moult, later to emerge and continue feeding (Stevenson et al. 1998; Suinyuy et al. 2009). Only early instars feed on pollen, with later instars eating only parenchyma tissue or each other (Stevenson et al. 1998). This ends in the microstrobili being completely hollowed out by the boring and final emergence of mature insects (Norstog et al. 1986; Hall et al. 2004) usually only after microsporangia were fully developed (Breckon & Ortiz 1983; Donaldson 1997; Hall et al. 2004). It is noteworthy that larvae of *Rhopalotria mollis* (on *Zamia*
**furfuracea)** abstain from eating pollen, resorting to cannibalism, and pupate in only 10 days (Stevenson et al. 1998). Because of the staggered male maturation and coning, a constant source of food and broodsites are available allowing many generations of beetles to develop within a reproductive event (Norstog and Fawcett 1989 cited in Schneider et al. 2002).

Numbers of pollinators at the end of this cycle can exceed many hundreds and in the case of thrip pollinators many thousands (estimated 20,000—Mound & Terry 2001). Once insects metamorphosed into adults they chew exit holes becoming covered in pollen in the process (Figure 22). Many insects seem to be specifically (morphologically) adapted for pollen adherence (Schneider et al. 2002; Hall et al. 2004).

**Figure 22—**Cycadothrips albrechtii hatching form microsporangium of Macrozamia macdonnellii with pollen attached (Mound and Terry 2001).

After strobili reach the end of their lifetime, insects move to the outside and fly to other strobili, including nearby female ones (Hall et al. 2004). In their observations Terry (2001) found in early dehiscence microstrobili (10%–20% dehisced sporangia), thrips were equally in leaving and arriving, at 40%–70% dehiscence, most thrips trapped were leaving the strobili, and finally at 190% dehiscence, most thrips were leaving microstrobili. The departure from their brood site is often conducted en mass (Terry 2001; Terry et al. 2004). The timing thereof seems to very species-specific (Breckon & Ortiz 1983), e.g. Tranes activity often occurs around sunset while the Cycadothrips move close to noon (Terry et al. 2004; Hall et al. 2004). The time of departures might be linked to some environmental cue (Terry 2001) as thrips on *Macrozamia macdonnellii* were seen to move during the late afternoon, when direct sun was blocked and a sharp fall in temperature and rise in humidity occurred (Mound & Terry 2001; Terry 2001). When these criteria are met insect move to the outside of strobili and take flight in short succession usually being heavily coated with pollen (Figure 23) (Norstog et al. 1986; Mound & Terry 2001; Hall et al. 2004; Suinyuy et al. 2009). Once in-flight, pollinators are drawn to receptive/dehiscent strobili (Hall et al. 2004). Pollination is
mediated once pollen ladened insects enter megastrobili (Wilson 2002; Terry et al. 2007). Visitation numbers are skewed between the sexes with far less insects visiting megastrobili than microstrobili (Terry et al. 2005). Pollen delivery however is substantial and theoretically more than enough to ensure 100% fertilisation (Terry 2001). Once on megastrobili insects often display very different behaviour than they do on males, with little activity on the surface (e.g. mating) (Hall et al. 2004). Most pollinators move into megastrobili soon after arrival (Mound & Terry 2001; Hall et al. 2004) and have specific behaviours within the strobilus to ensure the successful transfer of pollen, by concentrating their activity around the micropyle (Donaldson 1997; Terry et al. 2005). Insects rarely oviposit in female tissue with larvae almost absent (Breckon & Ortiz 1983; Stevenson et al. 1998) and megastrobili are also not fed upon (Stevenson et al. 1998). Reasons for this are discussed in the section on attraction and toxicity.

Figure 23—Insects leaving microstrobili of Macrozamia spp. before take-off. (a) Tranes sp. weevil dusted in pollen, (b) Cycadothrips chadwicki (Terry et al. 2005).

Once the cycad population nears the end of its reproductive event, insects start to enter their dormant stage (diapause) which may last for 2 years (Norstog et al. 1986), or theoretically longer. Pollinators pupate in casings often filled with toxins obtained from the cycad (Brenner et al. 2003). How and where they pupate differs between taxa and includes vegetative parts of the cycad or in the surrounding soil (Norstog et al. 1986; Vovides 1991; Wilson 2002). This explains why pollinators do not starve and die out during non-coning years (Norstog et al. 1986). Diapause is entered by larvae that do not develop into adults and serve as the starting breeding stock for the next season when they finally emerge as adults (Stevenson et al. 1998).
Figure 24—Life cycle of *Pharaxonotha zamiae* on *Zamia pumila*. A, Emerged adults leave soil and visit dehiscent microstrobili or receptive megastrobili; B, Beetles feed on pollen; C, Oviposition in microsporophylls; D, Eggs hatch, larvae feed on pollen; E, Larvae burrow into sporophylls to moult; F, Later instars feeding on sporophylls; G, Larvae feed on central strobilus axis an peduncle, but not the stem; H, Last larval instar drops to the ground; I, Larvae enter soil to (J) pupate. K, From (A) beetles may visit megastrobili. Tang 1987a.
Pollinator attraction

How insect behaviour is manipulated by the plants has to do with various cues indicating the status of the strobili. At maturity sporophylls open giving access to the interior. This is accompanied by thermogenesis as well as the secretion of odour (Tang 1987b) coupled to pollen dehiscence and pollination drop excretion respectively (Tang 1987a). The specifics thereof such as volatile profiles, release times and timing of thermogenesis seem to mediate pollinator movement between strobili (Terry et al. 2004, Terry et al. 2008). Pollinators are conspicuously absent from strobili that are not mature and are thus not thermogenic or odorous (Suinyuy et al. 2009) with the lower visitation to females being correlated with the lower volatile production and thermogenic activity (Tang 1987b; Terry et al. 2005; Procheş, & Johnson 2009). Activities of pollinators are closely linked to these two activities with movement between strobili often due to an increase thereof (Figure 25) (Mound & Terry 2001; Schneider et al. 2002). Odours of the different sexes have also been found to be similar (Breckon & Ortiz 1983; Mound & Terry 2001; Terry et al. 2004; Procheş, & Johnson 2009). Increases in production at specific times during maturation have been found to increase manyfold (Terry et al. 2004), even a million fold in some species’ microstrobili (Terry et al. 2007). Odour has also been linked to pollinators, e.g. Stangeria has fermented fruit scented compounds that are the known foodstuff of sap beetles (the plant’s pollinator); some compounds found in all Coleopteran pollinated cycads are absent from thrip pollinated Macrozamia species and vice versa (Terry et al. 2004).

![Figure 25—Strobilus thermogenesis and thrip activity in *Macrozamia macleayi*. Note peak in temperature correlated with increase in thrip movement (Terry *et al.* 2004).](image)
In a noteworthy investigation Terry et al. (2007) looked at the effect of *Macrozamia lucida*’s volatiles on its pollinator, *Cycadothrips chadwicki*. They found that in a “two-way choice” between male sporophyll volatiles and air: thrips are attracted/neutral to sporophylls early in the day, repelled at midday, and attracted at later times. This is directly correlated to *in situ* observation on strobili (Figure 25): thrips remain on strobili in the mornings (low volatile emissions); are repelled at midday (high emissions) and attracted again later in the afternoon (low emissions). They conclude that volatile changes sufficiently explain the thrips behaviour, although temperature and light could also modulate their effects (Terry et al. 2007). They conclude the pollination strategy as a “push-pull” interaction where thrips are driven from microstrobili to increase the visitation of the non-essential megastrobili. Whether this interaction is applicable to Coleopteran pollinators remains to be investigated. This might then explain in part why insects have such differing behaviours on megastrobili, as females have a comparable bouquet to males, they too may repel insects by their volatile emissions (Stevenson et al. 1998).

Thus the two main criteria affecting pollinator’s attraction and possibly ultimately pollinator specificity is timing of thermogenesis and volatile emission and volatile ingredients (Terry et al. 2004).

**Cycad toxicity**

Unlike the well-known obligate mutualisms of angiosperms, the interactions in cycads do not revolve around the female reproductive structures and seed parasitism (Terry et al. 2005). Insects are known not to even feed on megastrobili tissue, with the main hypothesis concerning this being the toxicity of cycads (Stevenson et al. 1998). Cycad pollen however is also poisonous, prompting Schneider et al. (2002) to speculate that the interaction must have developed firstly with ancestral beetles evolving to cope with the toxins only later transferring pollen. It does however seem that the megastrobili is not the focal point of the pollination mutualism (discussed further under the section on rewards).

The main difference in terms of toxicity in microsporophyll and megasporophylls tissue centres around a type of storage cell called an idioblast. In microstrobili idioblasts are intact and lack plasmodesmata, whereas in the megastrobili, idioblasts have plasmodesmata and are sometimes ruptured, releasing their poisonous contents (Vovides et al. 1993 cited in Brenner et al. 2003), including neurotoxins (BMAA and Cycasin —Stevenson et al. 1998; Vovides
This rupturing usually occurs just prior to receptiveness and the subsequent pollination event (Stevenson et al. 1998). This has been confirmed by various stains at the different stages of the strobilus’ life cycle where a conspicuous lack of idioblast cells is found just prior to strobilus receptiveness (Stevenson et al. 1998). As seeds develop on the plant for many months after pollination (Grobbelaar 2002) the megasporangium tissue is needed to facilitate nourishment of the developing seed (Norstog et al. 1993, Vovides et al. 1993 cited in Brenner et al. 2003). This is off course of great value to females who guard their developing seeds, and the structure needed therefore, against damage (Terry et al. 2005). Females thus seem to attract insects to pollinate them yet offer no reward for such services, in fact they do the opposite, they guard against any other activity except pollination by the visitors. This has begged the question as to why pollinators visit non-rewarding megastrobili that are not necessary for them to complete their life cycle at all.

**Rewards**

Rewards in terms microstrobilus visitation have been laid out in detail, boiling down to food and broodsite. Thermogenesis has been speculated to also aid in the insects life cycle (Seymour 1997, Seymour & Schultze-Motel 1997, Ervik & Barfod 1999 cited in Terry et al. 2004): enhanced growth and development of immature stages; stimulating mating; warming pre-flight muscles; increasing dispersion of CO2; and coaxing pollinators to leave. The megastrobilus however does not have a clear function in the life cycle nor do pollinators feed on any part thereof (Hall et al. 2004) This begs the question: what rewards do the megastrobili offer pollinators for their services? Tang (1987a) was one of the few early authors to venture any hypotheses with following works (amongst others: Donaldson 1997 Stevenson et al. 1998; Terry 2001; Schneider et al. 2002; Wilson 2002; Hall et al. 2004; Terry et al. 2005; Procheş & Johnson 2009) exploring these to some extent. Hypotheses are as follows:

1) **Mimicry**

No reward is offered but insects are attracted through mimicry of the male reproductive structures. This is supported as some species which have similarly coloured and textured strobili (e.g. *Zamia pumila*—Tang 1987a). Male and females are both thermogenic at the same times during the receptive period (Terry 2001). Odours have been found to be very similar between the sexes (Breckon & Ortiz 1983; Tang
1987b; Procheş, & Johnson 2009). This would tie into the strategy to limit pollinator
time and activity at the ovules thus limiting damage (Baker 1976 cited in Tang 1987a).

2) Pollination droplet
During the receptive period the pollination droplet is excreted to trap pollen. Beetles may
visit the megastrobilus for this nutritious liquid (Breckon & Ortiz 1983; Tang 1987a;
Donaldson 1997; Kono & Tobe 2007). As mentioned the chemical composition is
broadly similar to nectar of many flowering plants (Baker & Baker 1975 cited in Tang
1987a). As drops are secreted and retracted on a daily basis studies have found drop
excretion not to be directly correlated with insect activity (Tang 1987a). Also the
pollination droplet is speculated to be of little use for larger pollinators though there is
evidence of thrips feeding thereon (Terry et al. 2005)

3) Refuge
Here the large strobili sheltering pollinators against the elements or predators might be
visited as refuge sites. This is consistent with observation on insects that seem to
spend most of their time inside strobili (Tang 1987a).

4) Breeding sites
This was proposed as a strategy closely related to other obligate mutualisms in
angiosperms where pollinators feed on seeds (Terry et al. 2004). A study on E. villosus
specifically asking whether there is a floral parasite mutualism found that no such
mutualism occurred (Donaldson 1997). Subsequent studies on the nature of the
pollination mutualisms found in cycads have also failed to present a pollinator that fits
this profile (possible Cycas revoluta—Kono & Tobe 2007).

These hypotheses presented are not all mutually exclusive. At present the attraction of
pollinators seems most likely to be through microstrobilus mimicry (Tang 1987a, 1987b;
Pellmyr et al.1991; Procheş, & Johnson 2009), though other rewards are not exclusive to this
attraction strategy. In the boarder scheme, pollinators of cycads have an ecological niche as
reward (Schneider et al. 2002) where: the cycads toxins are useful and employed (Norstog et
al. 1986; Tang 1987a); relatively few herbivores disturb the broodsit; a specialist diet is
followed (toxic pollen); and the pollinator’s survival is coupled to the cycad’s, which in a
manner of speaking has lasted for millennia.
Conclusion

Norstog & Nicholls (1997 cited in Schneider et al. 2002) compare these recent findings on the pollination ecology of cycads with a “the Rosetta Stone”. They view cycads as unique due to them combining insect pollination, symbiotic nitrogen fixation in root nodules, high toxicity and survivorship, with comparatively little morphological changes through their evolutionary history. A thorough understanding surrounding them would thus provide a multitude of otherwise unattainable insight into most other plant taxa. Due to the poor fossil record in most cases, especially with angiosperm origins (Endress 1994), a study of these living fossils can be used to make inference around ancient conditions and evolutionary paths.

Though cycads have been generalised to be wind pollinated form the early 1900 (Chamberlain 1935 cited in Procheş, & Johnson 2009), it has clearly been shown through direct experimentation that all investigated genera are insect pollinated (Procheş, & Johnson 2009). The fact that the ill informed and unscientifically founded generalisation persisted for half a century must serve as a reminder to what science is all about, empirical observation and experimentation. That being said even without these direct lines of evidence a thorough understanding of a taxon’s biology could have been indicative. Through the application of pollination syndromes to the Cycadophyta’s reproductive event, I have shown that wind pollination was a stretch and that biotic pollination fitted far better. It is commonly now believed that all cycads are insect pollinated (Chaves & Genaro 2005) however studies to confirm this are both essential and lacking (Terry 2001).

It is however paramount to thoroughly understand this interaction in the light of conservation both in situ and ex situ. A great deal of the world cycad flora is conserved in botanical gardens and private collections. If plants are however conserved in isolation, then the only thing we are conserving is a species list for future generations. To truly conserve nature’s diversity we must conserve it an entity (Donaldson 2003). This includes their pollinators which are dependant on the cycads to complete their life cycles (Schneider et al. 2002). In many cases then, because of the species-specificity, the loss of a single cycad species would also directly result in the loss of an insect taxa. A positive sign is the three recent papers (Kono & Tobe 2007; Procheş, & Johnson 2009; Suinyuy et al. 2009) on the subject which show the continued interest and investigation thereon.
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