The Biology and Systematics of *Bowenia* Hook ex. Hook f. (Stangeriaceae: Bowenioideae)

Thesis submitted by

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…….. ……..

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Statement of Systematic intent

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Abstract

The contents of this thesis describe a study of the Biology and Systematics of *Bowenia* Hook. ex Hook. f. (Cycadales, Stangeriaceae, Bowenioideae). The genus contains two recognised extant species, *B. spectabilis* (Hook. ex Hook.) and *B. serrulata* (W. Bull) Chamberlain. They are restricted to small areas of tall moist and closed forests of central and northeast Queensland, Australia, respectively. The genus was named after Sir George Ferguson Bowen (1821-1899), the species epithet *spectabilis* refers to the spectacular leaves with pinnules (1st order leaflets) with entire margins in the first case and *serrulata* to the serrate margins of the pinnules in the second. The species are unique in the extant cycads in having bipinnate foliage.

Surprisingly little is known about the biology of the members of this genus and this study redresses that situation. In addition, the systematics of *Bowenia* currently present difficulties for taxonomists and management authorities, as there is confusion about the number and distribution of species of *Bowenia* and the status of disjunct and morphologically different populations in northeast Queensland. As there is considerable interest in harvesting *Bowenia* leaves for the Australian and international 'cut flower' markets, clarification of the systematics of the genus is necessary for its effective management, and this study addresses this need.

The strategy adopted for the study was to undertake intensive fieldwork in central Queensland, become familiar with the taxon growing there, and then use that knowledge to facilitate studies in north Queensland. Studies in the field were complimented by work in the laboratory and in the greenhouse.

Bowenia contains a suite of toxins, is slow growing, reproductive events occur once a year and immediately prior to the onset of the 'wet' season, and access to study populations at the appropriate times was often difficult. These factors meant that the fieldwork required collecting sufficient data for analysis extended for a decade. The sequence of events in the study was to collect data on the morphology, reproductive biology, insect associations and genetic profiles of the taxa and integrate them in a database that could latter be used to provide characters for a phylogenetic analysis and a subsequent review of the systematics of the genus.

Studies of the morphology of plants in six populations representing the two recognised species and both morphological forms found in north Queensland, found that they could not readily be differentiated on the basis of leaf, pinnae and pinnule number or morphometrics or the size and branching habit of the subterranean stem. As these characteristics had initially been used to distinguish the two species, a search was made for other characters on which to base a phylogenetic analysis.

The pollination of the taxa was found to be obligate entomophilic and mediated by Molytine weevils involved in species-specific 'brood site reward' pollination syndrome. *Miltotranes prosternalis* (Lea) was demonstrated to be the pollination vector of all northern populations and *M. subopacus* (Lea) to be the pollination vector of plants in Central Queensland. In addition, it was discovered that the leaf beetle *Lilioceris nigripes* (Fabricius), whose range includes that of *Bowenia*, distinguished between the populations in central and northeast Queensland but not the northern populations.

Studies of the karyomorphology of representatives of the two currently named species and the disjunct and morphologically variable northern populations indicated the presence of two taxonomic entities. The first was the population in central Queensland corresponding to the currently recognised *B. serrulata* and the second comprised all the populations, irrespective of their pinnule and root morphology, in northeast Queensland.

A phylogenetic analysis using twelve characters across the six populations of *Bowenia* confirmed the presence of just two species, conforming to those previously named. A comparison of the result of the phylogenetic analysis with the distribution of the two taxa show that *Bowenia serrulata* (W. Bull) Chamberlain is restricted to central Queensland and *B. spectabilis* Hook. ex Hook. is restricted to northeast Queensland, and the two are and have been divided for millions of years by the intervening megathermal Burdekin Gap. In addition, the results show that *B. spectabilis* is morphologically variable and that plants with pinnules with serrate margins in northeast Queensland are examples of phenotypic variation within this species.

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Dr Robyn Wilson assisted me in the field, advised on matters statistical, reviewed drafts of this thesis and papers resulting from this study, and good-naturedly tolerated my enthusiasm for all things ‘cycad’. Paul Forster, of the Queensland Herbarium, provided friendship and intellectual support, and was ably assisted in this regard by Lou Randall and Dr Roy Osborne. Dr Gordon Guymer, Director of the Queensland Herbarium, gave permission for access to the collection. I collaborated with Dr Goro Kokubugata of Tsukuba Botanic Gardens on the karyological studies reported here, and it was a pleasure to work with him at JCU and the Queensland Herbarium while he was a visiting fellow in 2000. Dr Elwood Zimmerman, Dr Rolf Oberprieler and Tom Weir of the ANIC, Canberra, and Ross Storey of QDPI, Mareeba, advised on the entomological aspects of this study and I thank them all.

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I particularly wish to express my gratitude to my family, Robyn and Cleo, who provided physical and intellectual support during a study involving a good deal of discomfit and far too many ticks and leaches - I owe them both more than I can ever express.

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_____ (1993b) The relationship between *Cycas ophiolitica* K.D. Hill Cycadaceae), the butterfly *Theclinesthes onycha* (Lycaenidae), the beetle *Lilioceris nigripes* (Coleoptera: Chrysomelidae) and the ant *Iridomyrmex purpureus*. 1991 Symposium Series. UCQPGSA, Rockhampton.

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_____ (2003) A profile of the Queensland rainforest cycad *Bowenia spectabilis* (Stangeriaceae). *Wodyetia* 7(3): 3-8.

Kokubugata, G., Kondo, K., **Wilson, G.W.**, Randall, L.M., Schnas, A and Morris, D.K. (2000) Comparison of karyotype and rDNA-distribution in somatic chromosomes of *Bowenia* species (Stangeriaceae, Cycadales). *Australian Systematic Botany* 13(1): 15-20.

_____, Hill, K.D., **Wilson, G.W.**, Kondo, K. and Randall, L.M. (2001) A comparison of chromosome number and karyotype in somatic chromosomes of Stangeriaceae (Cycadales). *Edinburgh Journal of Botany* 58(3): 475-481.

Poster papers

Wilson, G.W. (2002) Insect Pollination in the rainforest cycad *Bowenia.* Ecology 2002, Annual Conference of The Ecological Society of Australia, Cairns, Australia.

Kokubugata, G., Kondo. K., Wilson, G.W. and Randall, L.M. (1999) Comparison of karyotype and rDNA-Distribution in Somatic Chromosomes of *Bowenia* species (Stangeriaceae, Cycadales). XVI International Botanical Congress, St Louis, Missouri.

Technical Reports

Wilson, G.W. (1995) Invertebrate pollination vectors, herbivores and defenders of the rainforest cycads *Bowenia spectabilis* and *B. 'Tinaroo'.* Report to the Wet Tropics Management Authority, Cairns, Australia.

Chapter 1 Introduction

This study considers the biology and systematics of *Bowenia* Hook. ex Hook. f. in the Subfamily Bowenioideae in the Family Stangeriaceae of the Order Cycadales. Surprisingly little is known about the biology of the members of this genus of cycads and this study redresses that situation. In addition, the systematics of *Bowenia* currently presents difficulties for taxonomists and management authorities, as there is confusion about the number and distribution of species of *Bowenia* and the status of disjunct populations in northeast Queensland. As there is considerable interest in harvesting *Bowenia* leaves for sale in the Australian and international 'cut flower' markets, clarification of the systematics of the genus is necessary for its effective management, and this study also addresses this need.

No previous studies of the infrageneric systematics of *Bowenia* have been conducted and the status of the disjunct northern populations has not been addressed. A biosystematic approach is used in this study; biological, morphological, chromosome, ecological and biogeographical data are integrated to determine the status of the species and forms of the genus.

This study sets out to

- describe aspects of the biology and ecology of *Bowenia,*
- consider evolution and speciation in the genus,
- present a revision of the systematics of *Bowenia*

and whilst doing so, contribute to the debate on

- the role of 'refugia' in the persistence of Wet Tropics flora, and the
- discussion on the use of morphological traits of fossil plants as indicators of palaeoclimate and -ecology.

It should be noted that under Article 29 of the International Code of Botanical Nomenclature, any revision of the systematics of *Bowenia* presented in this thesis would not qualify as an effective and valid publication of it.

This chapter introduces the cycads, and *Bowenia* in particular, and provides details of the origins and affinities, fossil record and palaeoecology, present distribution and status, and systematics and phylogenetics, of them. Data that distinguish *Bowenia* are emphasised and details that might assist in addressing the above listed aims, are discussed.

1.1 Introduction to the Cycadales

Cycads are dioecious gymnosperms characterised by girdling leaf traces, simple ovulate cones, motile gametes, haustorial pollen tubes, apogeotropic roots (Crane 1985; Stevenson 1990), and unique secondary compounds including methylazoxymethanol (MAM) glycosides (Siniscalco Gigliano 1990). The origins of the cycads are in the Palaeozoic (Mamay 1969, 1976; Leary 1990; Stewart and Rothwell 1993) with the medullosan progymnosperms ('seed ferns') the likely ancestors (Stewart 1983; Crane 1985; Doyle and Donoghue 1986). The Order is one of four in the gymnosperms, the others being the Ginkgoales, Coniferales and Gnetales, and is a sister clade to all extant seed plants (Laconte and Stevenson 1990; Nixon et al. 1994) (Figure 1.1).

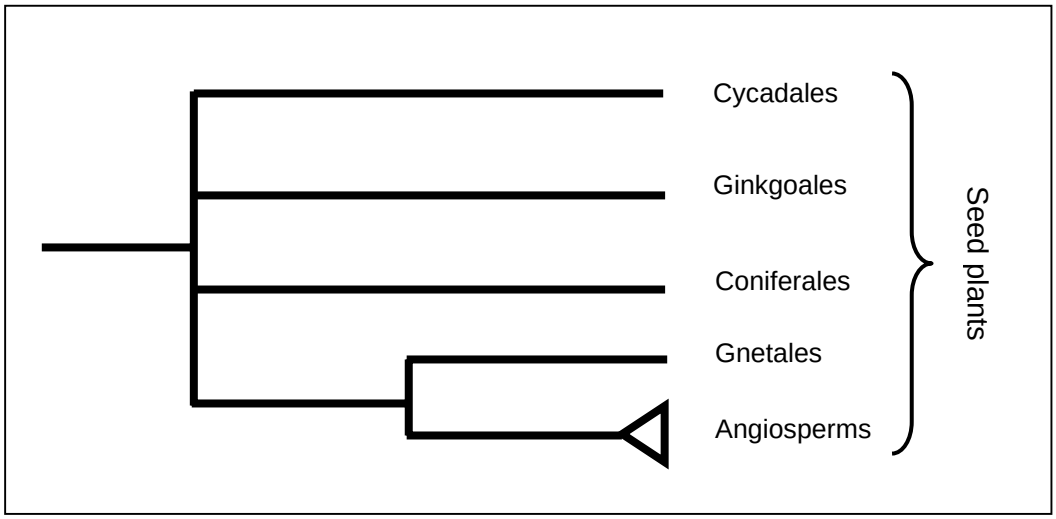


Figure 1.1 Cladogram showing simplified relationships of the seeds plants (adapted from Judd et al. 1999)

Cycads are currently the subject of considerable interest by the general public and scientific communities. The extent of their use in landscape gardening and interest of collectors and the societies and journals that result from that interest are testament to the public popularity of the group. A recent international 'sting' operation involving police and cycad collectors and the subsequent prosecution of some of those collectors highlights the economic value of and illegal trade in this group. The scientific interest in cycads is due to the fact that they are an ancient and phylogenetically discrete order that exhibits features, e.g. motile gametes and haustorial pollen tubes, indicative of early stages of evolutionary trends, and as such, they are of interest to evolutionary biologists and botanists.

The Order Cycadales includes the families Cycadaceae Linnaeus 1753, Zamiaceae Reichenbach 1837 and Stangeriaceae Johnson 1959, containing one, eight and two genera respectively (Stevenson 1992). Contrary to the 'popular' belief that cycads are relics of a bygone age and no longer evolving (Levin and Wilson 1976) there is evidence that they are actively speciating, particularly in *Cycas* in Australia (Jones 1993). In a recent review, Pienaar and van Rensburg (2001) listed 301 species of extant cycads (Table 1.1).

Table 1.1 The families, genera and number of species of extant cycads.

Family	genus	number of species
Cycadaceae	*Cycas* L.	105
Stangeriaceae	*Stangeria* Th. Moore	1
	Bowenia Hook. ex Hook. f.	2
Zamiaceae	*Ceratozamia* Brongn.	16
	Chigua D. Stevenson	2
	Dioon Lindley	12
	Encephalartos Lehmann	64
	Lepidozamia Regel	2
	Macrozamia Miq.	40
	Microcycas A. DC.	1
	Zamia L.	56

1.1.1 Introduction to *Bowenia* Hook. ex Hook. f.

Bowenia is a Queensland endemic and, with *Stangeria*, constitutes the family Stangeriaceae (Johnson 1959; Stevenson 1992). The genus contains two described extant species, *B. spectabilis* Hook. ex W.J. Hook. and *B. serrulata* (W. Bull) Chamberlain (Figure 1.2a,b). *Bowenia* is unique in the extant cycads in all species having bipinnate, sometimes tripinnate (Wilson 1996), foliage.

Figure 1.2 *Bowenia spectabilis* (a) and *B. serrulata* (b) in natural habitat.

Joseph Dalton Hooker described the genus and *B. spectabilis* in 1863 from material collected by Walter Hill at Rockingham Bay, north Queensland in 1862. The genus was named, at Hill's request, after Sir George Ferguson Bowen (1821-1899), first governor of Queensland, and the species epithet refers to the spectacular leaves with pinnules (1st order leaflets) with entire margins (Figure 1.3a). Charles J. Chamberlain described *B. serrulata* in 1912 from material collected at Byfield, central Queensland; the specific epithet refers to the serrate margins of the pinnules (Figure 1.3b).

Figure 1.3 Pinnules of (a) *B. spectabilis* and (b) *B. serrulata.*

1.2 Distribution of the extant cycads

The distribution of the extant genera is pan-tropical with some taxa extending to temperate areas (Figure 1.4). The areas of greatest diversity are Central America with five genera in Zamiaceae, and Australia, with four genera in three families, i.e. Cycadaceae, Zamiaceae and Stangeriaceae.

Figure 1.4 The distribution of the extant cycads (adapted from Jones 1993).

1.2.1 Distribution of *Bowenia*

The extant species of *Bowenia* are restricted to tropical Queensland (Jones 1993). *Bowenia spectabilis* occurs in the Wet Tropics bioregion (Goosem et al. 1999) of northeast Queensland with disjunct populations at McIlwraith Range and Starke on Cape York Peninsula and *B. serrulata* to a small area around Byfield in the Central Queensland Coast bioregion (Young 1999).

The earliest fossils attributed to *Bowenia* date from the Tertiary, with *B. eocenica* R.S. Hill and *B. papillosa* R.S. Hill, described from Eocene deposits at Anglesea, Victoria and Nerriga, New South Wales respectively (Hill 1978). Fossils of *Bowenia* are also known from Early Eocene deposits at Regatta Point, Tasmania (Hill 1998), mid-Eocene deposits at Maslin's Bay, South Australia (Scriven 1993), and Miocene deposits at Baralaba in central Queensland (Christophel pers. comm. 1999) (Figure 1.5).

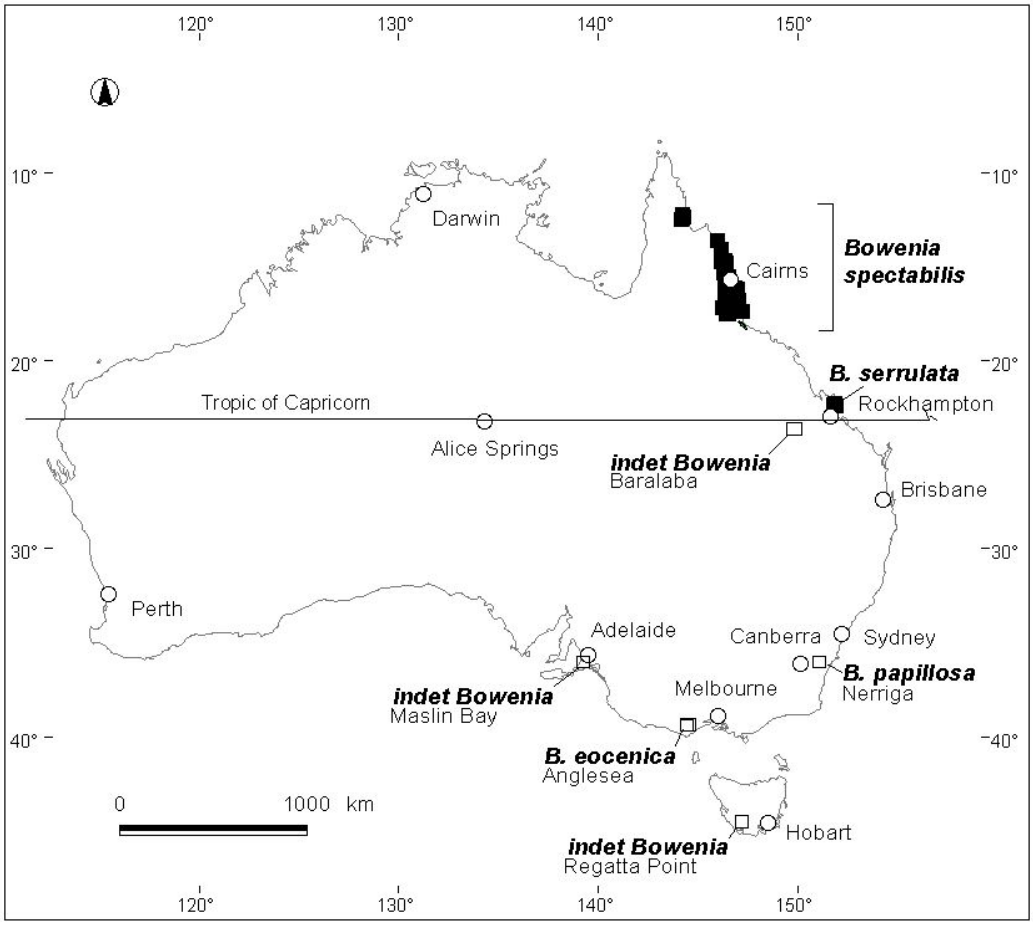


Figure 1.5 The distribution of extinct (□) and extant (■) species of B*owenia.*

Bowenia spectabilis occurs in rainforest and fringing eucalypt forest from Cardwell to Cooktown in the Wet Tropics while the populations at McIlwraith Range and Starke on Cape York Peninsula are restricted to isolated rainforest communities. Palaeoclimate data indicate that the latter populations have been separated by the megatherm (Nix 1991a) Normanby Gap since cooler and wetter mesotherm (Nix 1991a) conditions prevailed in the Holocene or Pleistocene (Kershaw 1994) and the distribution of the species was likely to have been continuous. In the northern portion of the Wet Tropics, *Bowenia* straddles the Black Mountain Corridor (BMC) where a rainforest link breaks and reforms with minor fluctuations in the climate, most recently rejoining in a mesotherm period of the Holocene 7500-6000 years BP (Nix 1991). *Bowenia serrulata* occurs in rainforest and fringing eucalypt forest in Central Queensland and is separated from *B. spectabilis* by 800 kms and the megatherm Marlborough and Burdekin Gaps that have existed since the onset of continental scale drying in the Mid-Late Miocene, 12-6 MY ago (Martin 1977, 1991; Kemp 1978; Sluiter and Kershaw 1982; Adam 1992; Truswell 1993).

These data mean that extant *Bowenia* occur on both sides of Holocene, Pleistocene and Tertiary-aged barriers that have been demonstrated to be the cause of speciation events or the isolation and subsequent genetic variation in vertebrate species (Winters 1988; Joseph and Moritz 1993; Joseph et al. 1995; Moritz et al. 1997; Schneider et al. 1998; Winters 1997; Williams and Hero 1998).

Despite inferences these barriers have had similar affects on plants, few supporting data are available (Webb and Tracey 1981; Nix 1991b). The presence of *Bowenia* in areas of known periods of isolation provides an opportunity to relate any genetic variation in them with the duration of their isolation and assist in describing the evolutionary processes involved in the separation of any taxa recognised. This inquiry is important as *Bowenia* with pinnules with serrate margins also occur in north Queensland, with populations at Tinaroo and Kuranda in the Wet Tropics known in the nursery industry as *B. Tinaroo* and *B. Kuranda* respectively (Gummow pers. comm. 1994). Foliage from these populations cannot currently reliably be distinguished from that of *B. serrulata* using morphological characteristics. The taxonomic status of these populations is uncertain and determining it is the prime reason for this study.

Three alternative views of the status of these populations suggest themselves;

- they are *B. serrulata* and have been separated from the Byfield population for 6 - 12 Ma,
- they are ecotypes of *B. spectabilis*, or
- they are a third species of *Bowenia*.

It should be noted that K.D. Hill is quoted in Jones (1993) as proposing the last but did not formalise it in a later treatment of the genus (Hill 1998). Interestingly, in Jones (2002), *Bowenia* sp. 'Tinaroo' is still listed and the note 'in many respects this species is almost intermediate between *B. serrulata* and *B. spectabilis*, but it has not originated as a hybrid' is included. The hypothesis tested in this study, the second of the above alternatives, that they are an ecotype of *B. spectabilis*, is true.

Hill (1998) noted that the pinnule margins of all fossil material were serrate and considered this a derived condition from an ancestor with pinnules with entire margins, e.g. like *B. spectabilis*. This is an application of the Nearest Living Relative (NLR) hypothesis which states that 'living plants most similar in form to extinct taxa in the same or closely related genera are most likely to occupy similar niches and be subject to similar climatic regimes'.

Environmental parameters are widely used in defining terrestrial biomes (Whittaker 1975) and plant physiognomy, particularly of leaves, in the classification of vegetation types, e.g. of the Australian rainforests (Webb 1959, 1968, 1978). The largest leaves and leaves with entire margins are found in rainforest where water and temperature stress on plants is minimal (Bailey and Sinnott 1915, 1916; Wolfe 1971, 1978; Givnish 1979). A reduction in leaf size and serration of the margins are adaptations to increasing stress. In tropical rainforests, average leaf size declines with the average temperature of the coolest month (Leigh 1990), and many species exhibit phenotypic plasticity in this and related characteristics.

These relationships have been used in association with NLR data sets in reconstructions of the palaeoecology of extinct floral assemblages, (see Wolfe 1979, 1990, 1993, 1995; Davis and Taylor 1980; Wolfe and Upchurch 1987; Gregory and Chase 1992; Wing and Greenwood 1993; Carpenter et al. 1994).

Jordan (1997a) noted that these studies focused on leaves of woody dicotyledonous plants well represented in the fossil record, and that 'the leaves of other groups do not appear to have the same response to climate'. Jordan (1997b) presented details of conflicting evidence of the effects of climate on leaf physiognomy of gymnosperms, including *Bowenia*, in the Early Pleistocene flora of Regatta Point, western Tasmania, in support of his comments. However, studies by Hill and Carpenter (1991) and Hill (1994), of the Palaeogene *Acmopyle* and *Dacrycarpus* (Podocarpaceae) in Tasmania indicated leaf size and stomatal density in these taxa were related to prevailing climate conditions.

In this study to see if the populations and/or species can be distinguished on the basis of morphological characteristics the mean leaflet length, width, surface area and margin morphology and the number of pinnae on the largest leaf of 100 plants of *B. serrulata* and nominate and putative *B. spectabilis* was recorded and compared. In addition, the data were examined to see if pinnule size is correlated with environmental parameters and if a cline from large entire to smaller serrate–margined leaflets occurs in northern populations of *Bowenia*.

1.3 Systematics and phylogenetics of the extant cycads

The classification of the Cycadales by Stevenson (1992) presented in Table 1.2 is currently the most widely accepted and is followed in this study.

Table 1.2 A provisional classification of the Order Cycadales.

Order Cycadales	
Suborder Cycadineae	
Family Cycadaceae	Genus *Cycas*
Suborder Zamiieae	
Family Stangeriaceae	
Subfamily Stangerioideae	Genus *Stangeria*
Subfamily Bowenioideae	Genus *Bowenia*
Family Zamiaceae	
Subfamily Encephalartoideae	
Tribe Diooeae	Genus *Dioon*
Tribe Encephalarteae	
Subtribe Encephalartinae	Genus *Encephalartos*
Subtribe Macrozamiinae	Genus *Macrozamia*
	Lepidozamia
Subfamily Zamioideae	
Tribe Ceratozamieae	Genus *Ceratozamia*
Tribe Zamieae	
Subtribe Microcycadinae	Genus *Microcycas*
Subtribe Zamiinae	Genus *Zamia*
	Chigua

It is widely accepted, e.g. Chase et al. (1993) and Nixon et al. (1994), that the order is monophyletic but the relationship of the families and genera has proven difficult to resolve. This is particularly true for *Stangeria* and *Bowenia,* which are morphologically atypical in the cycads. *Stangeria* was initially identified as *Lomaria coriacea* Schrader with the error only realised when a specimen growing in England, produced a cone. Baillon finally published the current combination of *S. eriopus* in 1892 (Jones 1993). *Bowenia* also has fern-like foliage and *B. serrulata* is sold in markets as the 'Byfield Fern'.

Bowenia was first assigned to Zamiaceae, although problems existed with this placement. Johnson (1959) assigned *Bowenia* and *Stangeria* to Stangeriaceae in the subfamilies Bowenioideae and Stangerioideae respectively. Stevenson (1990), elevated these subfamilies to familial rank but in 1992 reverted to Johnson's classification (Figure 1.6). More recently, on the basis of molecular sequence data, Stevenson has proposed reassigning *Bowenia* to Zamiaceae (Forster, pers comm. 2002). In contrast, in a recent review by Schneider et al. (2002) of the evolution, toxins, herbivores and insect pollination of the cycads, they cite plastid *rbc*L gene sequence data by Treutlein and Wink (2002) as the basis for keeping the genus in a monotypic Boweniaceae.

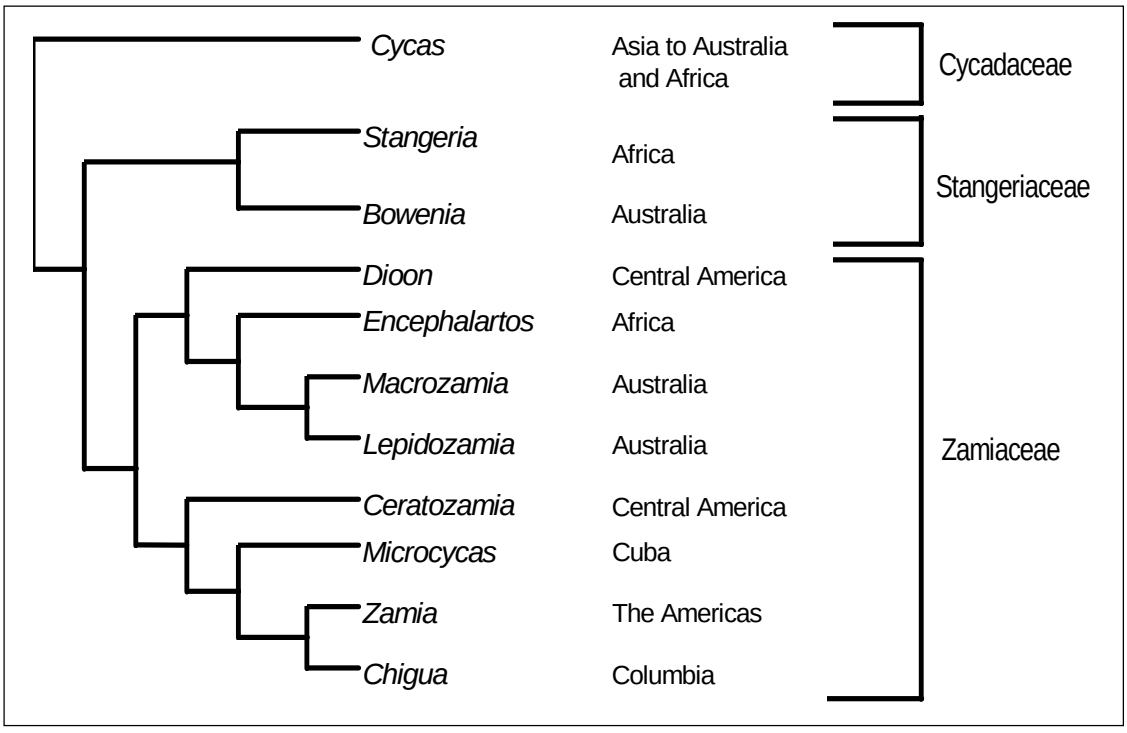


Figure 1.6 A consensus cladogram of the genera and families of extant cycads (adapted from Stevenson 1992).

These changes in the classification of *Bowenia* emphasise the problems experienced, even by professional systematists, in resolving the relationships and phylogenetic placement of the genus.

1.3.1 Taxonomy and infrageneric systematics of *Bowenia*.

Prior to its formal description by Hooker in 1863, *Bowenia* had been known from leaf fragments collected by Cunningham in 1819 from near the Endeavour River, north Queensland. It was first brought to public attention by William Bull in his ***A Retail list of New Beautiful and Rare Plants offered by William Bull (1878) V,4***. Bull listed *B. spectabilis* var. *spectabilis* and *B. spectabilis* var. *serrulata*, with himself as author of both. A type specimen for the latter variety (sic) is not cited and, regrettably, it cannot be determined if it refers to what was later described as *B. serrulata* or to material from north Queensland. Within a year the plants were listed in ***Illustrerte Gärten Zeitung 23 (1879)99, t.15*** with Bull mentioned in the type citation, and in ***L'Illustrations Horticole 26 (Dec. 1879) 184, t. 366*** as *B. spectabilis* var. *serrulata* André, an illegitimate name as Bull had already used it. The comment '*originaires des mêmes contrées'* (sic) in the latter suggests that the description had been copied directly from Bull's publication.

There is some evidence that Walter Hill collected the type of *B*. *spectabilis* var. *serrulata* in north Queensland. In ***A Synopsis of the Queensland Flora,*** F.M. Bailey (1883), described *Bowenia* collected by Thozet at 'Maryvale' near Byfield in Central Queensland, as *B. spectabilis* var. *serrata.* Bailey was Hill's successor as Queensland Government Botanist, was with him when he collected the material sent to Hooker, and would have been aware of the description of *B. spectabilis* var. *serrulata.* It appears likely that he intended to differentiate the two; certainly, his is the first formal record of *Bowenia* from central Queensland.

The specific status of *Bowenia* from central Queensland was recognised in the description of *B. serrulata* by Chamberlain in 1912. He distinguished the species from *B. spectabilis* by differences in the morphology of pinnule margins and the stem and root mass, but this did not clarify the status of north Queensland populations with pinnules with serrate margins. The confusion was recently demonstrated in the Australian Heritage Commission report *Tropical Rainforests of North Queensland: Their Conservation Significance* (Keto and Scott 1986), with both *B. serrulata* and *B. spectabilis* listed as being present.

At the time of the submission of the report, specimens in the Queensland Herbarium of *Bowenia* with pinnules with serrate margins collected at Kuranda, Tinaroo and nearby Mt Haig in north Queensland, were labelled as *B. spectabilis* var. *serrata,* the same as used by Bailey (1883) for material from central Queensland (Figure 1.7). In 1991 the HISPID name *Bowenia* sp. (Mt Haig L.W. Jessup 910) was applied to them, but in July 2000, on the basis of karyological work by Kokubugata et al. (2000), they were reassigned to *B. spectabilis* (Forster, cited in Holland et al. 2001).

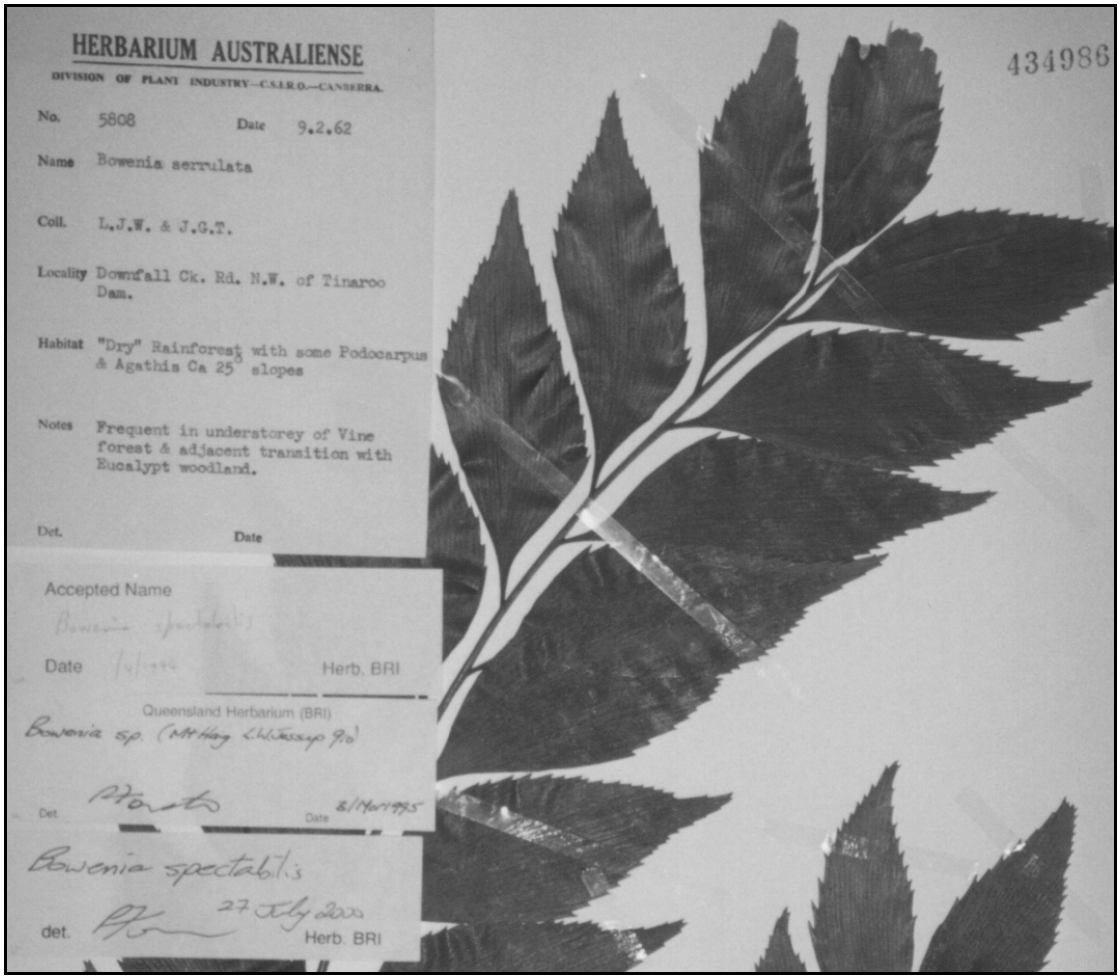


Figure 1.7 Scanned images of a portions of a sheet from the Queensland Herbarium of *Bowenia* with pinnules with serrate margins collected in north Queensland; note the changing labelling of the specimen.

Additional evidence of the ongoing confusion about the number and distribution of species of *Bowenia* is found in the authoritative *Flora of Australia* series. When providing a key to distinguish the species in a treatment of *Bowenia* for Volume 48 of the Flora, Hill (1998) used a combination of pinnule morphology and number of pinnae on the largest leaves, i.e.

1 pinnules entire or few irregularly lacerate
B. spectabilis

1: pinnules regularly serrate

2 stems with 5-30 leaf-bearing branches;
largest leaves with more than 12 pinnae
2. B. serrulata

2: stems with 1-5 leaf bearing branches;
largest leaves with less than 11 pinnae
1. B. spectabilis

Whilst keys are not intended to describe taxonomic affiliations the criteria used in this key indicate that some *B. spectabilis* have pinnules with serrate margins and that *B. serrulata* might occur in north Queensland, i.e. plants with multi-branched stems and >12 pinnae. This differs from the determinations applied in the Queensland Herbarium and does not accommodate plants with sparsely branched stems, leaves with >12 pinnae and pinnules with serrate margins.

Confusion about the taxonomy and infrageneric systematics of *Bowenia* has existed for 125 years (Hill 1978). As this confusion has been on the basis of morphological characteristics, this suggests a resolution of the status of the various populations of *Bowenia* may be found by re-examination of the morphological data and then the consideration of additional characters. The need is to determine the phylogeny of the members of the genus using an expanded data set and then use it to guide and support a taxonomic revision. The results can then be applied to the phylogenetic relations of populations on a geographic scale. In addition, an accurate description of diversity in the genus is a prerequisite to the conservation and management of it.

1.4 The Species concept used in this study

This study is designed to resolve the infrageneric relationships of *Bowenia*; this infers the delineation of operational taxonomic units generally referred to as ‘species’. The concept of the species is much debated and no single definition pleases all or adequately applies to every situation with Levin (2000) observing that ‘species concepts are only tools that are fashioned for characterizing organic diversity’ and that ‘any attempt to neatly fit biological diversity into a single species concept is likely to be futile’.

As a result and before describing the methods used in this study, and to provide a philosophical basis on which they were conducted, I now briefly consider the species concepts available and justify my choice of them.

Charles Darwin (1859, 1871) drew attention to the concept of the 'species' in *On the Origin of the Species by means of Natural Selection* at a time when the role of genetics and the existence and function of the DNA molecule were not known. At that time and for most of the subsequent century, the mechanistic concept, which is based on the 'speciation process' and the idea of cohesion and mechanisms that cause a population of organisms to retain a discrete identity, prevailed.

The best-known example of this species concept is the Biological Species Concept (BSC), espoused by Dobzhansky (1937a, 1941) and Mayr (1942, 1963, 1970, 1982a), i.e. 'a species is a reproductive community of populations (reproductively isolated from others) that occupies a specific niche in nature'. This concept has dominated the zoological literature since the time of its proposal and, until recently, the botanical literature as well (Judd et al. 1999).

Other variations of the mechanistic species concept have been proposed, the Ecological Species Concept (Van Valen 1976), the Recognition Species Concept (Peterson 1985) and the Cohesion Species Concept (Templeton 1989). Van Valen aroused the most debate on the topic and defined a species as 'a lineage (or closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage inside its range, and which evolves separately from all other lineages outside its range'.

A second set of species concepts can be classified as historical and consider the ontogeny of the species, thereby adding a temporal aspect to the concept. They include the Evolutionary Species Concept of Simpson (1961) and Wiley (1981), the Phylogenetic Species Concept of Nixon and Wheeler (1990) and Baum and Shaw's (1995) Genealogical Species Concept.

A third set of species concepts can be classified as phenetic, e.g. Michener (1970) and Sokal and Crovello (1970) and are based on the overall similarities of species, which are separated from other species by a gap in variation, without consideration of evolutionary processes and phylogeny.

Of the concepts presented above, the phenetic concept is rejected specifically because it does not take into consideration evolutionary processes and phylogenetic relationships and as such is likely to contribute little to explaining 'how' taxa arise and what their relationship is. Of the remaining alternatives, the Ecological Species Concept (ESC) of Van Valen (1976) and the Phylogenetic Species Concept (PSC) of Nixon and Wheeler (1990) initially appear to best address the tenets expounded in this study. However, the ESC does not consider a temporal component of the species and therefore is rejected. The PSC is similarly rejected because it does not accommodate the idea of discrete species that can interbreed if the opportunity arises; something that is known to occur in *Bowenia* (pers. obs); the rigorous application of this precept may prejudge what is and is not a species in the *Bowenia*

In this study, I have adopted Levin's (2000) Ecogenetic Species Concept that recognises the role of ecological criteria and unique genetic systems in the formation of a species. The parameters of this species concept include those in this study and the philosophy of it concurs with my own. In addition, I believe this is a more useful species concept in a study considering taxa of great antiquity that occur in disjunct areas of similar habitat and comparisons of phenotypic and genotypic characters and ecological associations are made.

1.5 Taxa recognised in this study

For the purpose of this study the following taxa were recognised; *B. serrulata* in Central Queensland and nominate *B. spectabilis* with pinnules with entire margins and putative *B. spectabilis* with pinnules with serrate margins in north Queensland. *Bowenia* with pinnules with entire margins at Starke and McIlwraith Range on Cape York Peninsula were considered 'putative' *B. spectabilis* so as not to assume their phylogenetic status. The taxa recognised represent the foliage types and the geographic range of the genus.

1.6 Study Populations and site data

The study population of *B*. *serrulata* is located at Byfield in Central Queensland. The population of nominate *B. spectabilis* is located at Tarzali on the Atherton Tableland and the populations of putative *B. spectabilis* are located at Kuranda and Tinaroo on the Atherton Tableland and at Starke and the McIlwraith Range on Cape York Peninsula.

The location of the study populations and intervening megatherm barriers, Normanby Gap, Black Mountain Barrier, Burdekin Gap and Marlborough Gap respectively north to south, are indicated in Figure 1.8 and precise details of location, bioregion and altitude are presented in Table 1.3.

Table 1.3 Site details of the study populations of *Bowenia.*

taxon	**location**	**bioregion**	**latitude** (°S)	**longitude** (° E)	**altitude** (m)
Bowenia serrulata	Byfield	CQC[1]	-22.85	150.65	10
nom. *B. spectabilis*	Tarzali	WT[2]	-17.4168	145.6014	760
put. *B. spectabilis*	Tinaroo	WT	-17.1667	145.55	720
	Kuranda	WT	-16.8167	145.6333	440
	Starke	CYP[3]	-14.9333	145.0667	450
	McIlwraith Ra.	CYP	-13.44	143.4333	700

[1] Central Queensland Coast, [2] Wet Tropics, [3] Cape York Peninsula.

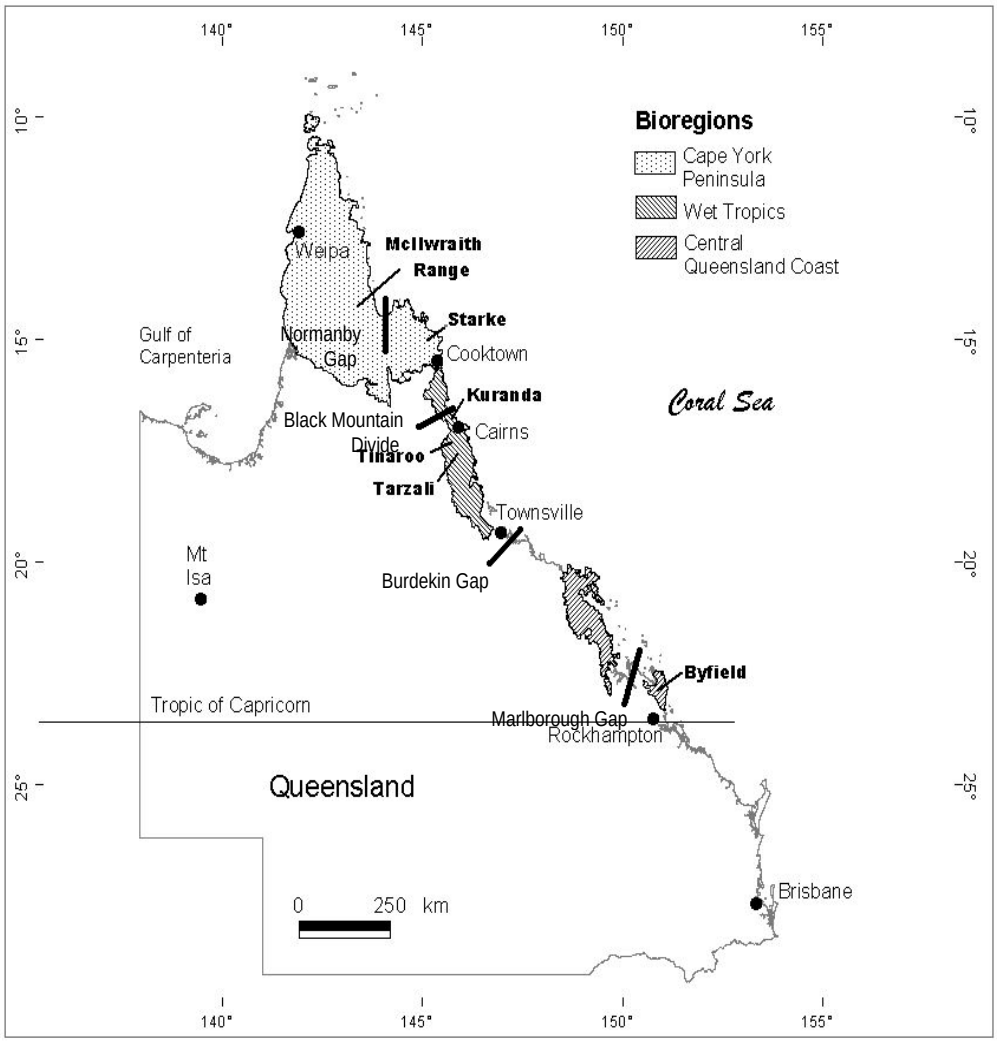


Figure 1.8 Map showing the location of the study populations of *Bowenia* and the intervening megatherm barriers (▬).

Due to the separation of the populations of *Bowenia* and difficulties in visiting them, the strategy employed was to intensively study *B. serrulata* and then use the information gained as a basis of the study of the northern taxa. Laboratory studies were conducted once a good understanding of the biology and ecology of *Bowenia* had been obtained. Voucher specimens of material examined are lodged in the Queensland Herbarium (BRI) and Tsukuba Botanic Garden Herbarium (TBG), Japan, and details of the collection data are presented in Table 1.3.

Table 1.3 Collection and acquisition numbers of taxa analysed in this study.

taxon	**Collection number/s**	**BRI** #	**TBG** #
Bowenia serrulata	GWW 69	Q668391	122889
B. spectabilis Tarzali	GWW 135	n/a	122893
B. spectabilis Kuranda	GWW 133	n/a	122898
B. spectabilis Tinaroo	GWW 134	Q668390	122895
B. spectabilis Starke	BSW 304[1]	Q662338	-
B. spectabilis McIlwraith Ra.	n/a[2]	-	-

[1] the material sampled was grown by seed collected by B.S. Wannan.
[2] only pinnules collected and a specimen not submitted to BRI - sample retained in JCU Collection.

1.6.1 Site climate data

The climate at a site dictates the type of vegetation present, some of its parameters, e.g. mean leaf size and proportion of leaves with entire margins, and in this study is pertinent to the discussion of pinnule morphology. The data presented in Table 1.4 is a compilation of Bureau of Meteorology records and data calculated by D. Gillieson of JCU using BIOCLIM (Busby 1991), as specific data for the Starke and McIlwraith Range sites was not available.

Table 1.4 Climate data for the *Bowenia* study population sites.

taxon	**mean annual rainfall** (mm)	**mean (%) rainfall May - Oct** (mm)	**mean annual temperature (MAT)** (ºC)	**min temp in coldest period** (ºC)	**annual temp range** (ºC)
Bowenia serrulata	1745	417 (23.9)	22.3	12.2	17.6
B. spectabilis Tarzali	3988	688 (17.3)	24.1	14.0	18.0
Tinaroo	1749	249 (15.2)	24.3	14.5	17.6
Kuranda	2088	352 (16.8)	24.5	16.3	15.1
Starke	>2000[1]	n/a[2]	25.2	17.9	14.1
McIlwraith	>2000[1]	n/a[2]	25.7	17.1	16.7

[1]these sites lie within the 2000 mm isohyet, mapped by BOM,[2] no specific data available.

The Central Queensland Coast and Wet Tropics sites all have a warm 'wet season', when ≥75% of rain falls, from November through April, and a 'dry season' in May through October. The Tarzali site on the eastern margin of the Atherton Tableland has higher rainfall than the others due to orographic effects on moisture-laden air sweeping up from the coastal lowlands. The Starke and McIlwraith Range sites are likely to have significantly higher rainfalls from those indicated in Table 1.4 as both rise above the surrounding landscape and support the only complex mesophyll rainforest, which requires >2500 mm of rainfall per annum (Tracey 1982), on Cape York Peninsula (Neldner 1998).

1.6.2 Study population vegetation community types

In this study the Specht (1970) classification of vegetation structure, based on life form, height, and projective foliage cover (*pfc*) of the tallest stratum, and Tracey (1982) classification of rainforest associations were used. Specht recognised four types of wooded vegetation based on decreasing *pfc* and these are described in Table 1.5.

Table 1.5 Types of wooded vegetation based on projected foliage cover (*pfc*) recognised by Specht (1982).

70-100%	30-70%	10-30%	<10%
closed-forest	open-forest	woodland	open-woodland

The Tracey (1982) classification of rainforest is based on rainfall and temperatures regimes, altitude, soil parent material, mean leaf length and surface-area of the dominant canopy species. Tracey used the mean size of the leaves (proscribed by Raunkiaer 1934) of the dominant strata to classify Australian tropical rainforests (Table 1.6) - megaphyll forests do not occur although individual plants with megaphyll leaves do.

Table 1.6 The size classes of leaves recognised by Tracey (1982) as occurring in Australian tropical rainforests.

leaf size category	length of lanceolate leaf (mm)	leaf area (mm^2)
mesophyll	125-250	4500-18225
notophyll	75-125	2025-4500
microphyll	25-75	225-2025
nanophyll	<25	25-225

Tracey divided rainforest types based on these leaf size classes into subtypes according to their vegetative complexity and location, i.e. lowland or upland, and mean rainfall and temperature and assigned them a numerical code from 1-13; e.g. 1a = lowland complex mesophyll vine forest, 1b = upland complex mesophyll vine forest, through 13 = vine forests with sclerophyll emergents and codominants. The value of these and the site climate data presented above are the climate and forest type and presence and/or form of *Bowenia* can be compared for the study population sites, and the likelihood of the presence of *Bowenia* in a particular vegetation type can be predicted as a result.

1.7 Other character classes considered in studies of the systematics of the cycads and potentially available for this study.

Recent advances in our knowledge of cycads and of analysis techniques suggest that use a more extensive suite of character classes may resolve outstanding phylogenetic and taxonomic problems relating to *Bowenia*. Such an approach may resolve the difficulties previously encountered using only morphological data as a multiple analysis approach is more powerful than that using a single character set (Hillis and Moritz, 1990).

The character classes available include chemical, plant-insect associations, karyology of the various populations, and the information contained in selected sequences of the plant genome. A brief review of the use of these character classes in studies of the systematics of cycads in general and *Bowenia* in particular is presented below. In addition, comment is made about the applicability and use of them in this study.

1.7.1 Chemical profiles of *Bowenia*

The use of chemical profiles in the determination of phylogenetic relationships of plants is widely used (Harborne 1988) and some higher-level classifications are characterised by a particular constituent, e.g. the presence of MAM glycosides in the Cycadales. A suite of chemical characteristics is available for consideration or has been used in analysis in the cycads and they include

- presence and concentration of phenolic compounds,
- concentrations of the MAM glycosides *cycasin* and *macrozamin*,
- concentration of β-methylamino-L-alanine (BMAA), and
- the type and concentration of allozymes, seed-coat carotenoids, mucilages, and cuticular waxes.

Flavonoids

Flavonoids are phenolic compounds derived through cycalisation of an intermediate from a cinnamic acid derivative and three malonyl CoA molecules. They function as a defense against herbivores, are found in all plants and are widely used in plant systematics as they are easily extracted and identified (Judd et al. 1999). Flavonoids are primarily used in assessing relationships among closely related species or in studies of infraspecific variation.

Wallace (1972) conducted a survey of benzoic and cinnamic acids in 22 species in the 10 then described genera of cycads and confirmed their presence in them but indicated that the results did not assist in resolving the taxonomy of the Order. Dossaji et al. (1975) reviewed the presence of 14 biflavonoids in 85 species across the same genera and distinguished the three families using them. Their work indicated *Cycas* was quite distinct, eight genera, including *Bowenia,* were closely related in Zamiaceae, and Stangeriaceae contained only *Stangeria* and was characterised by the absence of biflavonoids. Subsequently, Meurer-Grimes and Stevenson (1994) analysed 24 species in all 11 genera. A chemotaxonomic analysis of the Cycadales using the data supported the widely accepted result, based on morphological characteristics, of a previous cladistic analysis by Stevenson (1992).

Gadek (1982) reported on the presence of biflavonoids in the testa of eight species in five genera in Cycadaceae and Zamiaceae but did not sample *Bowenia* or *Stangeria*. Gadek's results are useful in considerations of the phylogenetics of the cycads as he found that the pattern of biflavonoids are uniform and specific in genera but also that there was little similarity in extracts from different portions of plants. Despite the studies detailed above and the apparent applicability of the biflavonoids for the task, there are no reports in the literature indicating the use of them in phylogenetic analyses of *Bowenia*.

Methylazoxymethanol (MAM) glycosides

The glycosides *cycasin*, *macrozamin*, and related forms, e.g. *neocycasin* in *Cycas*, differ in their structure in the sugar attached to a nitrogenous moiety (Osborne 1988a) and are ubiquitous in the Order. They are not toxic in this form to animals, but on digestion and hydrolisation, MAM, the toxic principal, is activated.

Studies by Moretti et al. (1983) showed that differences in total and relative amounts of macrozamin, but not cycasin, are sufficient to distinguish between genera, with *Cycas* quite distinct and *Bowenia* closer to *Stangeria* than indicated by analyses utilising biflavones. Concentrations of MAM glycosides in cycads vary widely and overlap in species and genera (Moretti et al. 1981), but are highest in *Bowenia.* The percentage fresh weight of macrozamin in ripe seeds of *B. serrulata* is 4.33 and 5.04 in *B. spectabilis,* and the species are clearly distinguished. There are no other data in the literature on any variation in the concentration of MAM glycosides in *Bowenia* or any other cycad species, nor is there is any record of analyses of them at an infraspecific level such as would be required in this analysis of *Bowenia*.

β-methylamino-L-alanine (BMAA)

BMAA is a neurotoxin found in varying amounts in all cycads (Norstog and Nicholls 1998). Analyses by Charlton et al. (1992) of levels of BMAA in 35 species in seven genera indicated that levels of BMAA varied widely across and within genera but with species-specific concentrations. The data supported previously recognised subgroups of species of *Cycas* and suggest that *Zamia* and *Ceratozamia* are more closely related to each other than to *Dioon*, which is postulated to be an ancestral member of the Zamiaceae (Stevenson 1992; Norstog and Nicholls 1998). Further data on the taxonomic utility of BMAA is not available, and while it is not pursued in this study in respect to *Bowenia*, more extensive studies appear warranted.

Allozymes

Allozymes are enzymes that differ in their electrophoretic mobility because of allelic differences and allozymic variation in or between populations is an indication of genetic diversity (Hartl 1988). Allozyme data has been used to examine the status of populations and putative species of cycads, e.g. of *Macrozamia* section *Parazamia* by Sharma et al. (1998, 1999), but the validity of estimates of enzyme allele polymorphism using electrophoresis techniques is debatable and the use of this type of data in phylogenetic analyses is problematic (Futuyma 1986; Hillis and Moritz 1990). The principal problem in the methodology is whether to transform the data to genetic similarities or differences or code it as presence-absence rather than frequency data.

Its use is particularly dubious in analyses that reveal low levels of allozyme heterozygosity as is typical in recently evolved or inbred populations (Schaal et al. 1991) or due to selection within a narrow range of environmental parameters (Jones and Forster 1994). Indeed, Sharma et al. (1998) observe that their results do not effectively reflect the phylogenetic relationships of species in *Parazamia* and that other molecular sequence, e.g., random amplified polymorphic DNA (RAPDs), may provide more variable markers. In addition, the applicability of allozyme data in taxonomic studies is limited, as speciation without divergence at enzyme loci and the converse has been shown to occur (Crawford 1989).

Seed-coat carotenoids

Carotenoids are isoprenoids that serve as light-harvesting molecules in photosynthesis, play a role in protecting prokaryotes from deleterious effects of light and act as yellow-red pigment in plants (Stryer 1988). There is a single reference in the literature to studies of seed-coat carotenoids of cycads; Bauman and Yokoyama (1976) considered the phylogenetic significance of seed-coat carotenoids in four genera in Zamiaceae and one species of *Cycas*, but not in *Bowenia* or *Stangeria*. They found some differences in the carotenoids in *Zamia* compared with other genera but concluded that the pigment conformed to a widespread 'magnolian' pattern that evolved >200 my ago and had remained constant through subsequent speciation in the cycads.

A comparison of the pigments in the species of *Bowenia* and *Stangeria* would be useful as the seed coats of species in Cycadaceae and Zamiaceae are yellow-orange in colour when mature while those of *Bowenia* are purple-lilac (Jones 2000). However, there are no references in Bauman and Yokoyama (1976) or elsewhere in the literature to infraspecific level analyses of carotenoids in cycad seed coats, and given the above data, the topic is not considered further in this study.

Mucilages

Cycads produce carbohydrate-rich mucilage that are particularly obvious when a plant is damaged and they deter feeding by many insects (Farrell et al. 1991). Stevenson and Siniscalco Gigliano (1989) examined the monosaccharide profile of 44 species in five Neotropical genera and found that they, but not species, could be distinguished.

There are no data in the literature on mucilages in *Bowenia* or any other cycad species and as there is no indication that they are useful in analyses at an infrageneric level, they are not considered in this study.

Waxes on cuticles of pinnules

The only published data on the use of the type and quantity of cuticular waxes in analyses of the systematics of cycads is by Osborne (1988) and Osborne et al. (1989). These authors examined species of *Encephalartos* and found they could be recognised by their cuticular wax profile, although, in common with biflavonoids, it differed in juvenile and adult plants. In addition, they found the profile of known hybrids to be intermediate to that of the parents. There are no other data in the literature in respect to this topic in cycads despite the fact the topic appears to have potential for use in studies of them.

1.7.2 Insect Associations of *Bowenia*

Futuyma (2000) observed that 'many traditional statements about the host associations of major taxa of insects are valid descriptions of [plant] evolutionary lineages' and 'some of the associations of insects lineages with plant lineages are astonishingly old'; this observation appears to describe the relationship of some cycads and insects particularly well. As cycads contain a suite of toxins including MAM glycosides (Moretti et al. 1983), β-methylamino-L-alanine [BMAA] (Vega and Bell 1967; Charlton 1992), biflavonoids (Dossaji et al. 1975; Meurer-Grimes and Stevenson 1994) and phytoecdysones (Harborne 1988) that discourage vertebrate, invertebrate, fungal and bacterial predators and pathogens, it is likely that associations will be of a specialised and long term nature (Mitter et al. 1991) and may be of use in delineating taxa.

Because of the above and precursory observations by the author, *Bowenia*-pollination-vector and *Bowenia*-insect relationships were examined closely in this study to see if any of them distinguished between species/populations of the genus. The questions considered in this section of the study were

- do some insect species distinguish between species of *Bowenia*? and
- are common insect associations across disjunct populations of *Bowenia* indicative of a close phylogenetic relationship of those populations?

1.7.3 Karyology of *Bowenia*

Another additional set of information available for consideration is contained in the chromosomes of the plants. Chromosomes contain the genome of an organism and their number, size, structure and banding patterns and are systematically informative (Futuyma 2000).

Sax and Beal (1934), Marchant (1968), Khoshoo (1961), Moretti (1990), Moretti et al. (1993) and Kokubugata et al. (2000, 2001) have previously reported on the karyology of cycads but only the latter dealt with infrageneric data. Sax and Beal (1934), Marchant (1968), Moretti (1990) and Moretti et al. (1993), found that all genera, except *Zamia,* have a constant chromosome number, and that while it varies in *Zamia,* polyploidy does not occur. Moretti et al. (1993) made the interesting observation that *Zamia* species morphologically adapted to more xeric conditions had higher chromosome numbers but that this situation did not apply across all Neotropical genera.

Kokubugata et al. (2000) provided an initial insight to the infrageneric status of species and populations of *Bowenia* and confirmed that *B. serrulata* and *B. spectabilis* had a chromosome number of 2n = 18, but could readily be separated on the basis of chromosome morphology. They also found that the karyology of Tinaroo plants with pinnules with serrate margins was not distinct from that of *B. spectabilis.* In further studies, Kokubugata et al. (2001) found that the karyotype of plants at Kuranda, also with pinnules with serrate margins, was similar to that of *B. spectabilis*.

In this study the karyology of *B. serrulata*, nominate *B. spectabilis* from Tarzali and putative *B. spectabilis* from Kuranda and Tinaroo, representing the full range of morphological variation expressed in *Bowenia,* were examined. The questions addressed in this section of the study were

- do all populations of *Bowenia* have the same chromosome number?
- is the karyology of nominate and putative *B*. *spectabilis* and *B. serrulata* the same or similar? and
- does the karyology of *Bowenia* suggest one, two or more species?

1.7.4 Molecular sequence data

DNA sequence data also has the potential to assist in the resolution of the systematic problems associated with Bowenia. Plants have three genomes (Table 1.7) and data from all three have been used in systematic and phylogenetic analyses of them (Judd et al. 1999).

Table 1.7 Details of the three genomes in a plant (from Judd et al. 1999).

genome	genome size (kbp[1])	mode of inheritance
chloroplast (cpDNA)	135-160	uniparental
mitochondrion (mDNA)	200-2500	uniparental
nuclear ribosomal (rDNA)	1.1×10^6 to 110×10^9	biparental

Data from the chloroplast genome, which is inherited in its entirety, is conservative and is not subject to recombination (Soltis and Soltis 1998), are useful for analyses of higher-level relationships. For example, Goremykin et al. (1996) used cpITS sequences in examining the relationship of Gnetales and angiosperms, and Chase et al. (1993) employed them, including data for *B. spectabilis*, in their analysis of the phylogenetics of the seed plants. However, in analyses at lower ranks, the use of more rapidly evolving regions is required and recent analyses have utilised information from the larger and more complex nuclear genome. The data in Figure 1.9 indicate the applicability of frequently used DNA sequences in phylogenetic reconstructions.

Figure 1.9 The taxonomic level of utility of selected genomic regions in phylogenetic reconstructions (modified from Avise 2000).

Two molecular data sets specifically relating to *Bowenia* are available. The first relates to the work by De Luca et al. (1995) who used chloroplast DNA (cpDNA) sequences in a broad analysis of the systematics of the cycads and a more detailed analysis of the systematics of *Dioon (*Zamiaceae*)*. Based on a sequence set from *B. spectabilis* they located the genus in a basal clade with *Stangeria* but they did not provide the sequence data and have not lodged it in the web-based *Genbank* database; as a result this data is not available for use or comparison in this study of the systematics of *Bowenia*.

The second set of sequence data for *Bowenia* are from analyses by Chaw et al. (2002) of the ITS region of *B. serrulata* and *B. spectabilis* and is of more interest in the context of this study. Chaw et al. (2002) lodged ITS sequence data for *B. serrulata;* nominate *B. spectabilis* and *Stangeria eriopus* with *Genbank*. An analysis of their results for *Bowenia* shows the sequences of the *Bowenia* species are 94.6% coincident, a level typical of sibling species, but the data do not address the status of the northern populations and, apart from confirming the existence of two species, do not further contribute to this study.

Data from the nuclear ribosome DNA cistron (rDNA) is particularly useful in phylogenetic analyses as it is derived from both parents and because the order of the genes is thought to be stable (Judd et al. 1999). The Internal Transcribed Spacer (ITS) region of the transcription units of the rDNA cistron do not code for protein synthesis, can accumulate numerous mutations and still function (Liston et al. 1996), evolve rapidly, and undergo concerted evolution which reduces sequence heterogeneity within genetically discrete populations (Dover 1982). As a result, they are ideal for use in phylogenetic analyses at genus, species and population levels (Baldwin et al. 1995; Liston et al. 1996).

1.8 Character classes used in this study of the phylogeny of *Bowenia*

After a review of the literature in respect to cycads and as a result of previous studies by the author and the off-campus and part-time mode of this study, the decision was made to examine morphology, insect associations, and karyology character classes in this consideration of the phylogenetics and infrageneric systematics of *Bowenia*.

The character classes used were chosen to

- re-examine morphological data and see if they define the currently recognised or more species of *Bowenia*, and
- if the morphological data support the hypothesis that pinnule size and shape reflects phenotypic variation in response to environmental parameters rather than discrete genotypes, and
- review results of studies of the biology of the genus and their possible use in phylogenetic analyses, and
- use characters from a range of classes, particularly those of a macro rather than micro nature, and discernable by the general observer.

1.9 Biogeographical analysis of *Bowenia*

Last, but by no means least, the results of the analysis based on the chosen data sets will be intersected with the distribution of extant *Bowenia* to see if they concur with what is 'on the ground', e.g. all members of one taxa on the same side of a geographical and/or climatic barrier. In addition, using the NLR hypothesis, the results obtained for the extant species can be applied to the extinct species and thus allow a closer consideration of the evolution of the members of the genus. For example, if the data for the extant species indicate that they are habitat and biophysical parameter-specific then support is given to the hypothesis that the taxa in the fossil record went extinct as the climate changed in the mid Miocene and conditions became untenable for them.

1.10 Summary

In this chapter, I have provided an introduction the cycads in general and *Bowenia* in particular and the status of knowledge of the biology and systematics of this genus. Details are presented of the difficulties experienced by systematists and management authorities in determining the number of extant species and the status of disjunct and morphologically atypical populations of plants in north Queensland. I then present a summary of character classes considered in this study.

1.11 Format of this thesis

The format of this thesis is,

- Chapter 1 provides an introduction to the study, describes the populations of *Bowenia* examined in this study and the biophysical parameters of the site where they occur and provides a brief introduction to the methods used in the study,

- Chapter 2 presents the results of the study of the morphology and morphological variation in *Bowenia*,

- Chapter 3 describes the reproductive biology of *Bowenia* and presents a detailed discussion of insect pollination in the genus,

- Chapter 4 presents details of a plant-insect association that provides evidence on the taxonomic status of the populations of *Bowenia,*

- Chapter 5 describes the chromosomes and karyology of *Bowenia,*
- Chapter 6 describes the infrageneric phylogeny, systematics and biogeography of *Bowenia*, and

- Chapter 7 presents a synthesis of the results of the study, recommendations for the management of *Bowenia*, and suggestions for further research on the genus.

- The Bibliography contain a list of the literature cited in the thesis, and
- The Appendices include copies of data sets analysed in the study and copies of papers resulting from it.

Chapter 2 Morphological variation in *Bowenia*

The use of morphological characteristics as a basis for the description of species and in phylogenetic analyses of plant taxa is commonplace. In 1912, Chamberlain used differences in pinnule and caudex morphology of *Bowenia* in central and north Queensland as the basis of the description of a second species, *B*. *serrulata*, for the southern population. He described *B. serrulata* as having pinnules with serrate margins and a many-branched caudex while *B. spectabilis* had pinnules with entire margins and a sparsely branched caudex. Chamberlain (1912) observed that 'the differences between the two are so pronounced that they should be recognised as distinct species'. However, realisation of the variability in morphological characteristics and of the value of comparative data sets in accurate circumscriptions of taxonomic groups (Hillis and Moritz 1990) has led to the inclusion of data from two or more character classes in more recent analyses.

The description by Chamberlain (1912) of *B*. *serrulata* solely on morphological characteristics needs to be reconsidered as *Bowenia* with pinnules with serrate margins and many branched caudices are also known from north Queensland (Jones 1993). This fact raises the questions of the taxonomic status of these plants and the systematics of the genus. A population of *Bowenia* at Tinaroo on the Atherton Tableland has been suggested (K.D. Hill, cited in Jones 1993) as a new species but was not described as such by him (Hill 1998) in a treatment of the Australian cycads in Volume 48 of the *Flora of Australia.* This population and another at nearby Kuranda are known amongst cycad fanciers as *B. Tinaroo* and *B. Kuranda* respectively (Gummow, pers. comm. 1998).

Specimens in the Queensland Herbarium from these populations and nearby Mt Haig were initially labelled as *B. serrulata* or *B. spectabilis* var. *serrata,* the latter the same as used by Bailey in 1883 for material from central Queensland. Some authors, e.g. Keto and Scott (1986), have referred plants from these populations to *B. serrulata.* In 1991 the HISPID name *Bowenia* sp. (Mt Haig L.W. Jessup 910) was applied to them, but in July 2000, on the basis of karyological work by Kokubugata et al. (2000), they were reassigned to *B*. *spectabilis* (Forster, cited in Holland et al. 2001).

The photocopy in Figure 2.1 of a Queensland Herbarium sheet of *Bowenia* collected at Downfall Creek, Tinaroo shows the sequence of nomenclatural determinations applied to the specimen.

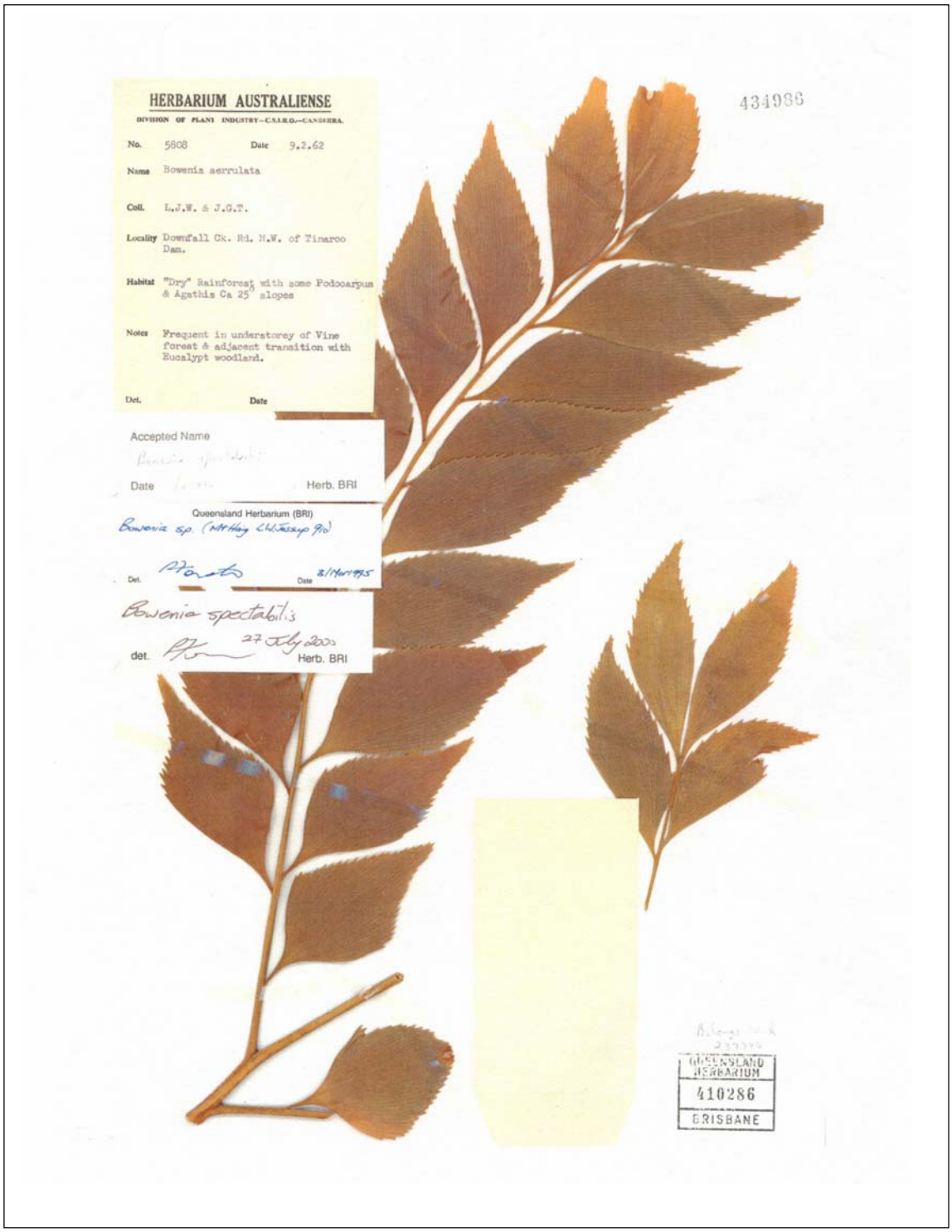


Figure 2.1 A photocopy of a herbarium sheet of *Bowenia* collected at Downfall Creek, Tinaroo, north Queensland showing (a) pinnules with serrate margins and (b) the sequence of nomenclatural determinations applied to the specimen.

The above data suggest the following questions,

- is variation in *Bowenia* pinnule morphology primarily due to phenotypic plasticity or genotypic variation? and
- are the northern populations with pinnules with serrate margins ecotypes within a species rather than genetically discrete varieties or subspecies?

These questions are important because there is a body of evidence, e.g. in Poaceae (Al-hiyaly et al. 1988), Caryophyllaceae (Schat et al. 1996) and Plumbaginaceae (Vekemans and Lefebvre 1997), that ecotypes are not morphologically well defined and may evolve at several locations. If this holds true for the gymnosperms, it is a possible explanation for the presence in north and central Queensland of apparently identical populations of *Bowenia*. If they are ecotypes of different species rather than genetically different varieties or subspecies, the use of pinnule morphology in the definition of *Bowenia* species is inappropriate and other characteristics must be sought for this purpose.

Two pieces of evidence indicate that *Bowenia* is capable of intraspecific morphological variation. The first is that leaves and pinnules on *B. serrulata* growing in typical shaded conditions are replaced with smaller '*sun'* leaves and pinnules after removal of the canopy cover (Wilson 1996). This example provides evidence of intra-generational phenotypic response related to a rapid environmental change. The second piece of evidence is that some *B. serrulata* have both multipinnate and bipinnate leaves (Wilson 1996).

Bowenia spp. with serrate-margin pinnules also occur in Eocene and Miocene deposits in southern Australia with an estimated mean annual temperature (MAT) of 17-20°C (Greenwood and Wing 1995) and an annual rainfall 500-1560 mm (Frakes 1998). These values are less than those where *B. serrulata* and putative *B. spectabilis* currently occur. However, Burnham et al. (2001) found temperatures estimated from fossil deposits of angiosperms consistently underestimate MAT in closed forest habitat by 2.5-5ºC, and thus the values for these *Bowenia* taxa may have been similar to those currently prevailing. This suggestion is supported by analyses by Christophel and Greenwood (1988), whose comparisons of leaf physiognomy of fossils and Nearest Living Relatives, including *Bowenia*, suggest that southern Australian forests in the Eocene were similar to those now extant in the Wet Tropics of Queensland.

This study of variation in pinnules can also contribute to our use of gymnosperm leaf physiognomy in the reconstruction of palaeoclimates and -ecologies, something to which they have not previously much contributed (Jordan 1997a). Indeed, Jordan warns of inaccuracies in such reconstructions and discusses contradictory data from Tasmanian Miocene deposits that include *Bowenia*. In contrast, studies by Hill and Carpenter (1991) and Hill (1994), of Tasmanian *Acmopyle* and *Dacrycarpus* (Podocarpaceae) from the Palaeogene, show that their leaf size and stomatal density were related to prevailing climatic conditions. If it can be shown that *Bowenia* responds with morphological variation to changes in environment, it can be postulated that extinct taxa did the same and that their morphology is indicative of conditions prevailing at that time.

The determination of the reason for the variation in pinnule morphology in north Queensland populations also provides an opportunity to consider the ontogeny of *B. serrulata.* If the variation is primarily due to intraspecific phenotypic plasticity then the following questions can be asked,

- did *B. serrulata* arise from a marginal population with serrate pinnule margins of an ancestor with entire pinnule margins?
- did this character state become genetically 'fixed' during the subsequent period of isolation to give rise to a second species? and
- did the ancestor of *B. serrulata* go extinct as changing climatic parameters during the mid-Miocene exceed its ecological tolerances?

Consideration of these questions will address Hill's (1998) assertion that the serrate-margined forms in the fossil record are derived from an entire-margined ancestor that may have persisted in mesic refugia during periods of adverse climate and habitat contraction. These data also question the use by Hill (1998) of pinnule margin morphology and then number of pinnae on leaves to distinguish two species of *Bowenia*.

The second morphological characteristic used in the discrimination of species of *Bowenia* was the form of the subterranean stem. Hooker (1863) described *B. spectabilis* as having a small and sparsely branched caudex, while Chamberlain (1912) described that of *B. serrulata* as being larger and many-branched.

However, botanists Sankowsky and Radke advise, (pers comm. 2000), that mature nominate *B. spectabilis* have large and many-branched caudices. If this is true, caudex morphology is not a good characteristic to use in the recognition and circumscription of species of *Bowenia*.

The use of pinnule and caudex morphology characteristics in the circumscription of species of *Bowenia* needs to be clarified before any re-analysis of the systematics of the genus. This situation is not unique in studies of the systematics of the cycads and some insights may be gained from parallels in *Zamia* in the Zamiaceae, a family to which *Bowenia* was previously assigned (Hooker 1863).

The systematics of this *Zamia* was initially based on pinnule morphology, despite variation in and among populations being well documented (Eckenwalder 1980; Newell 1983, 1985). Newell (1989) showed five morphologically distinct populations in Florida constituted a single species, three populations in Puerto Rico represented another two species, and that pinnule morphology is not a reliable analysis characteristic. Newell (1989) noted that 'further clarification of the taxonomy of the Caribbean *Zamia* will require additional characteristics' - this is the approach being taken to in this study.

2.1 Methods and materials

2.1.1 Leaf and pinnae morphometrics

As Hill (1998) used the number of pinnae on leaves to differentiate the species of *Bowenia*, this characteristic was re-examined to see if it does so reliably. Surveys of the number of pinnae on the largest leaves of 100 plants of nominate *B. spectabilis* at Tarzali and putative *B. spectabilis* at Tinaroo and 65 of *B. serrulata* was conducted. The mean number of pinnae on leaves in each sample was compared using a One-way Analysis of Variance (ANOVA) Test and a Tukey Multiple Comparison Test was used to show if any groupings of means occurred.

2.1.2 Pinnule morphology

Investigations of variation in pinnule morphology were conducted on nominate *B. spectabilis* from Tarzali, putative *B. spectabilis* from Kuranda and Tinaroo, and *B. serrulata.* Data were collected from both 'sun' and 'shade' leaves of *B. serrulata,* and from plants of nominate *B. spectabilis* with variable foliage, to determine the range of phenotypic variation in these species.

'Sun' leaves are adapted to high-light environments and are generally smaller, have thicker blades and cuticle, more heavily lignified epidermal cells, better developed palisade mesophyll and less intercellular space, they more efficient energy dissipators and lose less water per unit area at a given temperature than larger leaves. Shade leaves are adapted to low-light environments, they tend to be larger and thinner and less dense mesophyll cells. Shade leaves require less light to reach their CO_2 compensation point and they saturate at lower levels than do sun leaves. It has been demonstrated, e.g. by Smith and Noble (1977) in the desert shrub *Encelia farinose* Asteraceae, that sun and shade leaves grow in different light regimes on plants with the same genotype. In this experiment with *Bowenia,* the sample size of leaves from the McIlwraith Range and Starke populations was too small for analysis, but the full range of morphological variation known in the genus was included. The variation in size of pinnules of 'sun' and 'shade' leaves of *B. serrulata* and in different leaves on the same *B. spectabilis* plant were also compared and these data are included in the analyses and considered in the Discussion.

To determine if pinnule size and/or margin morphology is distinctive in populations of *Bowenia,* morphometric data was collected from four opposite ad- and abaxial pinnules from the second set of pinnae of leaves from 10 plants; i.e. 160 pinnules from each population. Pinnule length and width were measured to 0.5 mm, and the ratio of them calculated and compared. To ascertain if the surface area of pinnules is related to environmental parameters the mean value of it in each population was calculated using the formula $SA = \sqrt{LW}$ (Surface Area = square root of length multiplied by width), and examined using a multiple regression test for any correlation with mean annual rainfall, mean annual temperature and mean minimum temperature.

The number of margin serrations, or vascular trace apices that corresponds to serration apices in nominate *B. spectabilis* of both edges of pinnules were also counted and the ratio of them calculated and compared across populations to see if they can be distinguished by these metrics.

The mean dimensions of pinnules and their ratios, and number and ratio of adaxial (towards the leaf axis, forward) and abaxial (away from the leaf axis, trailing) serrations, and vascular traces in nominate *B. spectabilis,* were compared using One-way Analysis of Variance (ANOVA). The comparison was made to ascertain if there was a difference between them and a Tukey Multiple Comparison Test was used to show any groupings. Count data was checked for normality using Wilk-Shapiro Rankit Tests prior to analysis. The first sets of ANOVAs were conducted to see if the dimensions of their pinnules could differentiate the study populations. The second sets of ANOVAs were conducted to see if the number of ad- or abaxial serrations or ratio of them, on the margins of their pinnules, could differentiate these taxa.

2.1.3 Pinnule type distribution

To determine if pinnule morphology is primarily a result of phenotypic rather than genotypic variation and if populations represent ecotypes rather than distinct genotypes, three lines of inquiry were pursued,

- populations of *Bowenia* at Tarzali, Kuranda, Tinaroo and Byfield were surveyed for variations in pinnule morphology and size,
- pinnule morphology and habitat type of collections of *Bowenia* in the Queensland Herbarium HERBRECS database were examined, and
- data were collected from botanic gardens and private collectors to see if pinnule morphology varied in hybrids and plants grown in different environmental conditions.

2.1.4 Caudex morphology

To ascertain if caudex morphology in *Bowenia* conforms to the descriptions in the literature, and to check the claims made by Sankowsky and Radke, two caudices each of *B. serrulata* at Byfield and nominate *B. spectabilis* at Tarzali were excavated, photographed, and compared.

2.2 Results

2.2.1 Leaf and pinnae morphometrics

The mean (±SE) and range in number of pinnae on largest leaves from the sample populations is presented in Table 2.1. A one-way ANOVA showed the mean number of pinnae on the largest leaf was significantly different in the three populations (F = 62.871, DF = 2, P = <0.001). The Tukey Test showed the mean number was significantly different in three groups, i.e. nominate *B. spectabilis*, putative *B. spectabilis* and *B. serrulata*.

Table 2.1 Mean (± SE) and range in number of pinnae on the largest leaf of 100 plants of *B. serrulata*, nominate *B. spectabilis* and putative *B. spectabilis*.

population	**mean (±SE) number**
B. serrulata	11.25(0.26)
nominate *B. spectabilis*	8.24(0.13)
putative *B. spectabilis*	9.04(0.21)

2.2.2 Pinnule morphometrics

A summary of the morphometric data for the four populations of *Bowenia*, with two samples of *B. serrulata* to include 'sun' and 'shade' leaves, is presented in Table 2.2. Data for the Starke and McIlwraith Range populations are not included as sufficiently large sample sizes for analysis were not available.

Table 2.2 Mean (±SE) length, width, and ratio, and surface area of pinnules of three populations of *Bowenia spectabilis* and two of *B. serrulata*.

sample taxon	**mean** (±SE) **length** (mm)	**mean** (±SE) **width** (mm)	**l:w ratio**	**surface area** $\sqrt{LW}$ (mm^2)	**no of plants & pinnules sampled**
B. spectabilis					
Tarzali	107.77 (1.67)	23.78 (0.27)	4.59:1	246.9	10x16
Kuranda	105.58 (1.12)	30.63 (0.41)	3.5:1	314.7	10x16
Tinaroo	115.35 (1.40)	27.35 (0.39)	4.31:1	293.74	10x16
B. serrulata					
'shade' } Byfield	99.48 (1.41)	24.23 (0.46)	4.22:1	241.7	10x16
'sun'	86.1 (1.23)	25.5 (0.58)	3.46:1	236.6	5x10

Pinnule length

A one-way ANOVA of mean length of pinnules in the four populations showed significant differences among them (F = 21.502, DF = 3, P = <0.001) and the Tukey Test showed mean pinnule length was significantly different in three groups in increasing size of Byfield, Kuranda and Tarzali, and Tinaroo.

Pinnule width

A one-way ANOVA of the mean width of pinnules in the four populations showed significant differences among them (F = 66.22, DF = 3, P = <0.001) and the Tukey Test showed pinnule width was significantly different in three groups in increasing size of Tarzali and Byfield, Tinaroo, and Kuranda.

Length to width ratio of pinnules

A one-way ANOVA of length/width ratio of pinnules in the four populations showed significant differences in them (F = 57.919, DF = 3, P = <0.001) and the Tukey Test showed the ratio was significantly different in three groups in increasing number of Kuranda, Byfield and Tinaroo, and Tarzali.

Surface area of pinnules

The result of the multiple regressions show that 99.6% of the variation in surface area of pinnules was attributable to mean annual rainfall, annual temperature and minimum temperature (DF=3, F=861.41, R^2=0.996, P <0.001). The individual values of the three factors were

	Standardized Coefficient	*t*	P
Mean annual rainfall	-0.312	-14.194	<0.001
Mean annual temperature	0.395	5.902	<0.001
Mean minimum temperature	0.672	10.392	<0.001

Morphology of pinnule margins

The counts and ratios of the number of serrations on the adaxial and abaxial margins in three populations, and of vascular bundles in nominate *B. spectabilis,* are presented in Table 2.3.

Table 2.3 Number (±SE) of serrations or vascular bundle apices, and their ratio, in pinnules of four populations (but five samples) of *Bowenia.*

Sample population	mean (±SE) adaxial (#)	mean (±SE) abaxial (#)	ad:ab ratio	# pinnules sampled
Tarzali	14.36 (0.16)	9.0 (0.15)	1.61:1	10x16
Kuranda	16.89 (0.27)	13.61 (0.2)	1.24:1	10x16
Tinaroo	14.43 (0.18)	10.22 (0.13)	1.42:1	10x16
Byfield 'shade'	20.44 (0.33)	16.49 (0.29)	1.24:1	10X16
Byfield 'sun'	26.16 (0.32)	21.0 (0.34)	1.26:1	5X10

Adaxial serrations

A one-way ANOVA of the mean number of adaxial serrations on pinnules showed significant differences between populations (F = 138.163, DF = 3, P = <0.001). The Tukey Test showed the number of adaxial serrations was significantly different in three groups in increasing number of Tarzali and Tinaroo, Kuranda, and Byfield.

Abaxial serrations

A one-way ANOVA of the mean number of abaxial serrations on pinnules showed significant differences between populations (F = 286.674, DF = 3, P = <0.001). The Tukey Test showed the mean number of abaxial serrations was significantly different in all populations.

Adaxial to abaxial serration ratio

A one-way ANOVA of the ratio of ad- to abaxial serrations on pinnules showed significant differences between populations (F = 119.500, DF = 3, P = <0.001). The Tukey Test showed the ratio was significantly different in three groups in increasing size of Kuranda and Byfield, Tinaroo, and Tarzali.

Variation in pinnule size on plants

The values presented above indicate the range of pinnule variation found in the sample populations of *Bowenia* but do not show the full range of phenotypic variation that plants can express. This information is provided in the extreme values in data sets from plants with different size pinnules on different leaves on one plant, e.g. in *B. spectabilis*, and in plants from one population subjected to different light regimes, e.g. for *B. serrulata* ‘shade’ and ‘sun’ leaves.

Comparison of pinnule size on leaves on a plant of *B. spectabilis*.

The size of pinnules on different leaves of the same plant in *Bowenia* varies considerably. The morphometrics of pinnules on two leaves of a *B. spectabilis* growing in upland Complex Mesophyll Vine Forest (CMVF) at Tarzali are presented in Table 2.4 and a photograph of them in Figure 2.2.

Table 2.4 Mean length and width of pinnules on two leaves of a plant of nominate *B. spectabilis* at Tarzali.

leaf	# pinnules sampled	mean length (mm) (±SE)	mean width (mm) (±SE)
1	16	141.62 (1.69)	22.75 (0.52)
2	16	92.75 (2.74)	24.31 (0.69)

Pinnules were significantly longer (t = 15.80, DF = 30, P = <0.001) on one leaf than the other. There was no significant difference (t = -1.81, DF = 27.9, P = 0.0807) in the width of pinnules on the two leaves.

Figure 2.2 Photograph of a plant of *Bowenia spectabilis* with leaves with significantly different length pinnules growing in CMVF at Tarzali, Atherton Tableland. (Scale bar = 100 mm).

Comparison of pinnules on 'shade' and 'sun' leaves of *B. serrulata*

Pinnules were significantly longer (F = 26.210, DF = 1, P = <0.001) but not significant wider (F = 2.052, DF = 1, P = 0.154) or different in the ratio of length to width (F = 42.221, DF = 1, P = <0.001) on 'shade' leaves than on 'sun' leaves. There was a significant difference in the number of adaxial (F= 85.297, DF = 1, P = <0.001) and abaxial (F = 67.663, DF = 1, P = <0.001) serrations but no significant difference (F = 0.018, DF = 1, P = 0.893) in their ratio on pinnules of 'shade' and 'sun' leaves. Pinnules on shade leaves were longer but not proportionately wider or different in vasculature than those on sun leaves.

2.2.3 Pinnule type and distribution

Surveys of the six populations of *Bowenia* revealed that plants with pinnules with entire margins occur in areas with mesophyll and complex notophyll vine forest types. Plants with pinnules with serrate margins occur in simple notophyll, semi-deciduous mesophyll/notophyll vine forest and Vine forest with sclerophyllous emergents and their ecotones (Table 2.5).

Table 2.5 Site vegetation type and pinnule physiognomy in *Bowenia* in six populations.

sample population	complex meso/notophyll vine forest	simple notophyll vine forest	ecotone	vine forest with sclerophyllous emergents	open
McIlwraith Range	entire				
Starke	entire				
Tarzali	entire				
Kuranda	-	serrate	serrate		
Tinaroo	-	serrate	serrate	serrate	
Byfield	-	serrate	serrate	serrate	serrate

Data from collectors (Wannan, pers. comm. 2000; Forster, pers. comm. 2000; McDonald and Freeman, pers. comm. 2000) and from the intersect of collection locations with vegetation mapping (Anon, 1988; Neldner, in prep.) show that *Bowenia* at McIlwraith Range and Starke occurs in mesophyll forest types. All the collections have pinnules with entire margins and no plants with pinnules with serrate margins have been seen at these locations (Forster, pers. comm. 1999, Wannan, pers. comm., 1999).

The Tarzali site is in CMVF and only plants with pinnules with entire margins occur while Tinaroo has SNVF with *Araucaria* emergents and Vine forest with sclerophyllous emergents, and only plants with pinnules with serrate margins occur. The Kuranda site vegetation is CNVF, SNVF and Vine forest with sclerophyllous emergents and only plants with pinnules with serrate margins occur. At Byfield, the habitat is SDM/NVF and vine forest with sclerophyllous emergents and plants with pinnules with serrate margins occur in both.

A review of the Queensland Herbarium HERBRECS database reveals records of 19 collections of *B. serrulata* and 51 of specimens labelled *B. spectabilis*. Of the former, only two have details of the vegetation community at the collection site, both were from SNVF. Fourteen of the *B. spectabilis* collections have details of the vegetation community at the collection site; eight of 10 collections with pinnules with entire margins were from mesophyll and complex notophyll vegetation types, one was from SNVF and another from SNVF with *Araucaria* emergents. Three of four collections of *B. spectabilis* with pinnules with serrate margins were listed as from SNVF ± *Araucaria* emergents and one from Vine forest with sclerophyllous emergents (Table 2.6).

Table 2.6 Vegetation type and pinnule physiognomy of collections of *Bowenia* listed in the Queensland Herbarium HERBRECS database.

	total number of collections and (number) of records with habitat data	complex meso/notophyll vine forest	simple notophyll vine forest ± *Araucaria* emergents	vine forest with sclerophyllous emergents	open
B. serrulata	19 (2)	-	2	-	-
B. spectabilis	51				
entire margins	(10)	8	2	-	-
serrate margins	(4)	-	3	1	-

Data from botanic gardens and private collectors

These data are anecdotal but provide further insights into pinnule morphology variation in *Bowenia*. Lou Randal (pers. comm. 2000) of *Cycad Connections* in southeast Queensland advises that F1 hybrids of *B. serrulata* x *B. spectabilis* have leaves with pinnules with serrate margins (Figure 2.3a,b). Unfortunately, details of which species contributed the pollen and which contributed the ovule have been lost, and the order of the species given above is purely arbitrary.

In trials conducted in the 1990's at the Flecker Botanic Gardens, Cairns, 70% (*N* = 10) of F1 plants resulting from the pollination of ovules of nominate *B. spectabilis* with pollen from putative *B. spectabilis* at Tinaroo produced leaves with pinnules with serrate margins (Edwards, pers. comm. 2000).

Figure 2.3a,b Pinnules of an F1 hybrid of *B. serrulata* and *B. spectabilis* grown at *Cycad Connections* at Burpengary, southeast Queensland.

Photographs: L. Randall

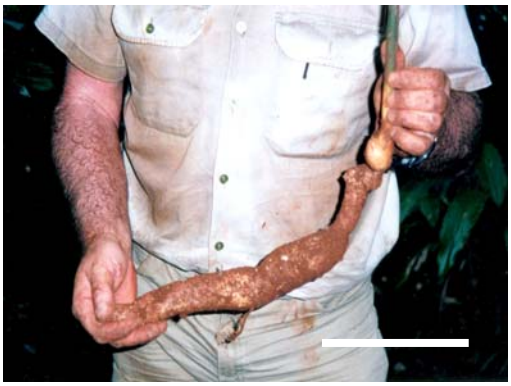
2.2.4 Caudex morphology

The caudices of multi-leaved *B*. *serrulata* excavated at Byfield were large and many branched, but those of nominate *B*. *spectabilis* included both large and branched and small and unbranched types (Table 2.7 and Figure 2.4a-c).

Table 2.7 Caudex and pinnule form and substrate type in populations of *Bowenia serrulata* and nominate *B. spectabilis*.

Sample population	caudex form	pinnule form	substrate type
Byfield	large and branched	serrate margin	sand, sandy loam
Tarzali	large and branched and small & sparsely branched	entire margin entire margin	fine red basalt soil

a.



b.

c.

Figure 2.4a,b,c Photos of caudices of (a) *Bowenia serrulata* at Byfield, and (b) and (c) of nominate *B. spectabilis* at Tarzali in north Queensland (scale bars = 300 mm).

2.3 Discussion

The data presented here indicate that the species of *Bowenia* cannot easily be distinguished using pinnule morphometric and morphological characteristics Variations in these characteristics are sufficient to distinguish the northern populations of putative *Bowenia spectabilis* with pinnules with serrate margins, but not without accurate measurements of repeated samples. The results indicate that pinnule morphology should not be used as a defining characteristic when issuing permits to harvest leaves of *B. serrulata*, as it is difficult to distinguish between them and those of some putative *B. spectabilis*, e.g. from the Tinaroo population, using the above criteria. The results also indicate that species of *Bowenia* cannot reliably be distinguished on the basis of size and morphology of the caudex of the plant with mature specimens of both having identical subterranean stems.

Leaf and pinnae morphometrics

The results indicate leaves of *Bowenia serrulata* and putative *B. spectabilis* cannot be distinguished from each other by the number of pinnae on the leaf unless the provenance of the collection is known. This result renders this metric useless for identification purposes in law enforcement situations and reduces the value of the data in taxonomic studies descriptions. The point of interest in the results is the increasing complexity of leaves in respect to number of pinnae; from nominate *B. spectabilis* with the lowest number and *B. serrulata* the highest, with putative *B. spectabilis* having an intermediate value. This trend is emphasised when the data in Wilson (1996) is included where a significant percentage of leaves of *B. serrulata* have 2nd order pinnules, i.e. polypinnate growth. It is inappropriate to suggest that these trends parallel those observed in angiosperms in response to increasingly arid environmental conditions but it does suggest a topic for later inquiry.

Pinnule morphology

The data indicate that variation in pinnule size in *Bowenia* is primarily phenotypic in nature; this is demonstrated particularly well by the variation found in leaves on the same plant of *B. spectabilis* and the rapid transition from ‘shade’ to significantly smaller ‘sun’ pinnules in *B. serrulata*.

The F1 hybrids of *B. serrulata* x *B. spectabilis,* and the presence of northern taxa with pinnules with entire and with serrate margins, suggest some genetic component in pinnule morphology. However, as Futuyma (1986) observes, leaf morphology has a polygenic inheritance with each locus contributing some slight amount to the total variation and that each genotype may be phenotypically variable as well. It would be difficult to perform genetic crosses that might determine the genotype of an individual, let alone the characteristics of a population or ecotype, and the change in form may be a switch to an alternative phenotype as a response to the change in the plant's environment.

Also of interest in this data is that while pinnule length in *Bowenia*, in all populations and individuals sampled, varies considerably, the proportionate width does not; this is demonstrated well in the data from 'shade' and 'sun' pinnules from *B. serrulata*. The results also indicate that pinnule morphometrics can be used to separate the study populations of putative *B. spectabilis* on the basis of all the metrics examined. Similarly, these populations can be separated from the initially similar *B. serrulata* using all of them except length to width and ad-to abaxial serrations ratios. However, although significant, the differences in these metrics are small and the populations can only be separated using accurate measurements of large samples – useful in phylogenetic and systematics studies but impractical for species management purposes.

Surface area of pinnules

The inquiry into any correlation between mean sizes of pinnule and climatic parameters shows that mean minimum temperature in the coolest period is the strongest determinant of surface area. This result conforms to those cited by Leigh (1999) in respect to leaf size in angiosperms in closed forest ecosystems.

The results assist in explaining why putative *B. spectabilis* at Kuranda, with high MAT and coolest period values, has the largest pinnules; nominate *B. spectabilis* at Tarzali and putative *B. spectabilis* at Tinaroo, with similar but lower MAT and coolest period temperatures, have similar sized pinnules, and the pinnules of *B. serrulata* are smallest in the populations sampled. Further work is required to see if the Starke and McIlwraith Range populations exhibit the same trends.

Caudex morphology

The data support the observations by Sankowsky and Radke (pers comm. 2000) that *B. spectabilis* exhibits the full range of caudex morphology reported for *Bowenia*. The reason for the predominance of reports of small and sparsely branched caudices in *B. spectabilis* may be that only a few mature plants have been examined fully. The results also concur with observations that the same situation occurs in *Stangeria eriopus* (Vorster and Vorster 1986; Jones 2002); Plants growing in grasslands have large branched stems while those growing in forested habitats often, but not always, have a simple and unbranched stem.

Distribution of and vegetation associated with *Bowenia*

The fact that most collections of nominate *B. spectabilis* are from lowland complex mesophyll vine forest supports the suggestion that this species requires constant warm and moist conditions to survive. These areas include those identified (Webb and Tracey 1981; Nix 1991b) as 'refugia' for ancient plant taxa - as such, they have had constant environmental conditions since the Eocene and *Bowenia* growing in them is likely to have direct links with the ancestral forms. The MAT and mean minimum temperature at Byfield are both lower than those in the other areas where *Bowenia* lives and this supports the hypothesis that *B. serrulata* is restricted to a small area of marginal habitat.

Summary

On the basis of the data presented in this chapter the questions posed in the text can be responded to as follows,

- pinnule size and morphology in *Bowenia* is phenotypically plastic and highly variable within plants and populations,
- the presence of serrations on pinnule margins is related to the prevailing, and possibly, previous, climatic parameters,
- populations of putative *B. spectabilis* are likely to be ecotypes rather than varieties or subspecies of nominate *B. spectabilis,* but at this time
- the status of northern populations of *Bowenia* with serrate margins remains unclear,
- it is difficult to differentiate plants from these populations from *B. serrulata* on the grounds of pinnule morphology, and
- caudex morphology is not distinctive at a species level.

The next step in this study is to examine other character classes that may assist in resolving the systematics of *Bowenia* and the status of northern populations with pinnules with serrate margins. The results of the first of these inquiries are presented in Chapter 3: Reproductive Biology.

Chapter 3 Reproductive biology

Cycads are dioecious gymnosperms whose structure, particularly in the Cycadaceae, indicates that they evolved as wind-pollinated taxa. Their age and the presence of a suite of toxins that are potent feeding deterrents to animals support the concept of anemophily in cycads. However, the production of sugar-rich pollination drops by ovules and thermogenic cones by male and female plants indicate an adaptation, at least in some species, to pollination by insects. This aspect of cycad biology is examined in this study because Zwölfer (1973) and Futuyma (2000) suggest that some insects closely associated with plant taxa reflect the diversity and phylogeny of their hosts.

Pollination in cycads has long been considered (Lawson 1926; Chamberlain 1935; Sporne 1971; Giddy 1984), to be anemophilic, despite indications to the contrary (Pearson 1906; Rattray 1913; Marloth 1914; Baird 1939). Recent studies (Norstog et al. 1986; Tang 1987b; Donaldson et al. 1995; Donaldson 1995; Terry 2001) suggest this hypothesis should be reconsidered, and Stevenson (1993), Norstog et al. (1986), Norstog and Nicholls (1998), and Hill (1998) indicate that all extant cycads may be insect pollinated. However, all taxa in which entomophilic pollination has been confirmed are in Zamiaceae and suggestions (Vorster 1995) of entomophily in Stangeriaceae and Cycadaceae, are unsubstantiated. The literature on the reproduction biology of *Bowenia* is restricted to a single paper by Lawson (1926). Lawson (1926) used laboratory investigations in Sydney, of cones posted from Rockhampton, to examine reproduction in *B. serrulata* and found no evidence of entomophily.

The suggestion that pollination in cycads is anemophilic is counter-intuitive in respect to *Bowenia* and other rainforest cycads. Studies (Bawa 1980; Bawa et al. 1985a,b) indicate that 97.5% of dioecious tropical plants are animal pollinated and that it is entomophilic in 90.2% of them. The complexity of rainforest ecosystems and environmental parameters of them, particularly rates of air movement, are not conducive to anemophily in cycads living beneath the canopy. Evidence for entomophily in rainforest cycads comes from Clark and Clark (1987) who found that pollination in the Neotropical *Zamia skinneri* Warscz. (Zamiaceae) is entomophilic.

Tang (1987a, 1993) reported thermogenesis and emission of aromatic volatiles in mature male and female cones of *B. spectabilis* and other cycads at the Fairchild Tropical Gardens, Florida, and hypothesised that it attracted insect pollination vectors to them. This hypothesis is supported by observations of angiosperms; particularly Araceae and Nymphaceae, where thermogenesis attracts the beetle and carrion fly pollination vectors (Proctor et al. 1996). Pellmyr and Thien (1986) suggested that olfactory pollinator attractants evolved from compounds that were initially herbivore deterrents. In 1991, Pellmyr et al. discovered that insect-pollinated archaic-angiosperms, e.g. *Zygogynum* in the Winteraceae (Thien et al. 1985) and gymnosperms shared the same classes of volatile compounds – this suggests the derivation of these classes from gymnospermous precursors. The putatively insect-pollinated taxa in Zamiaceae emit monoterpenes and short chain esters whilst the putatively wind-pollinated and widespread *Cycas rumphii* (Cycadaceae) emits alcohols, esters and ketones. Regrettably, Pellmyr et al. (1991) did not consider *Bowenia* or *Stangeria,* in the Stangeriaceae, in their analyses.

The aims of this portion of the study were to,

- describe the reproduction biology of *Bowenia*
- ascertain if pollination in *Bowenia* is entomophilic, and if so
- determine if the plant – pollination vector association is species - specific
- use this information to clarify the status of the northern populations of *Bowenia,* and
- provide data for use in the review of the systematics of the genus.

3.1 Methods and materials

This aspect of the study was approached at three levels due to the distance between the species and the difficulties in accessing far northern populations. Intensive studies of *B. serrulata* were instigated in 1991; a less intensive study of *B. spectabilis* commenced in 1994 and intensified in 1998, and data from Cape York Peninsula populations was obtained from other workers. Data obtained in the study of *B. serrulata* was used as an aide in planning the selective study of *B. spectabilis* populations.

3.1.1 Cone production and phenology

***Bowenia serrulata*:** forty plants at Byfield in central Queensland were identified using numbered aluminium tags tied with copper wire to a frond of the plant and later, to a steel post adjacent the plant. The plants were inspected once a month in the period 1991 through 1994, bimonthly in 1995 and 1996 and in November and March of 1997 and 1998. Details of the cone production and development, the timing of pollen dehiscence, the presence and activities of insects, and the production and dispersal of seed were recorded at each visit. Five hundred (500) plants were surveyed in the October or November of each year to ascertain the level of production and ratio of male and female cones.

***Bowenia spectabilis*:** Strategically timed trips were made to populations at Kuranda and Tinaroo in north Queensland in September and November of 1994 and 1995. Additional trips to the Tinaroo population were made in October 1998 and 1999. At these times details of cone production and development, the timing of pollen dehiscence, and the presence and activities of insects were recorded. Intensive studies were commenced at Tarzali in September 1998. Five hundred (500) plants were surveyed at Tinaroo in September and November of 1994, 98 and 99 and at Tarzali in 1998 and 1999 to ascertain the level of production and ratio of male and female cones.

P. Forster and K. McDonald and A. Freeman provided details of the pollination biology of the McIlwraith Range population in May 1992 and September 1999 respectively. Bruce Wannan of Kuranda supplied details of cone production in the Starke population of *Bowenia* in June 1996.

3.1.2 Cone Thermogenics and Emission of Volatiles

Cone thermogenesis was examined during the November 1998 reproduction cycle of *B. spectabilis* at Tinaroo. Probes attached to Stow Away XT1 data loggers (Hastings Data Loggers, Onset Computer Corporation) recorded the temperature at seven (7) minute intervals in dehiscing male and receptive female cones. The ambient air temperature was recorded by a probe and data logger located adjacent to the male cone. The data were downloaded to a Macintosh computer using the program *LogBook 2.01* (Onset Computer Corp. 1995) and graphed in *Excel 5* (Microsoft Corp. 1993).

The emission of volatiles by mature male cones of *B. serrulata* was determined using the technique of Kearns and Inoyue (1993) of placing them in clean, screw-top plastic tubes and sampling by nose at intervals of a few minutes for the presence of any obvious bouquet. Female cones were not sampled due to their low numbers and to reduce disturbance of local population dynamics. No further chemical analyses of the volatiles were undertaken.

3.1.3 Insect Associations and Entomophily

Early in the study, weevils (Coleoptera: Curculionoidea) were found breeding in the tissue of mature male cones and visiting receptive female cones of *B. serrulata* and nominate and putative *B. spectabilis*. An experiment was conducted to test if the weevils were pollination vectors of *Bowenia*. Insect exclusion devices (Figure 3.1) consisting of clear-plastic 3L bottles with the bottoms cut off and access ways cut into the lower edge were positioned over seven male cones of *B. serrulata* at Byfield and eight female cones of nominate *B. spectabilis* at Tarzali.

The apparatus prevented access to the cones by wind- and/or water-borne pollen but not of that carried by insects, and substantially reduced the likelihood of amphiphily, where pollen blown or washed to the exterior of the female cone is then carried inside by an insect agent. The exclusion devices were positioned over non-receptive females cones located by searching through the leaf litter at least one month before any pollen dehiscence by male cones in the study populations.

A steel mesh cage was placed over each apparatus and a similar number of untreated female cones of each species to prevent their disruption by animals and permit the collection of them six month later to compare fertilisation rates of ovules in treated and untreated cones. Insects collected from the interior of female cones were examined for the presence of pollen. Staff of the Australian National Insect Collection (ANIC) in Canberra identified the insects collected from the cones. Seed from the cones collected for analysis was returned to the study sites so as not to disturb local population dynamics. Comparisons of fertilisation rates of ovules in treated and untreated cones were performed on arcsine-transformed data using a Student's *t*-test.

Figure 3.1 Photograph of the exclusion apparatus and protective cage positioned over female cones of *Bowenia serrulata* and nominate *B. spectabilis.*

3.1.4 Seed Production, Viability and Dispersal

Details of fertilisation rates of ovules and seed production were recorded in female cones collected during the study. Seed germination trials were conducted on *B. serrulata* in Rockhampton in 1991, 92, 93 and 94 and on *B. spectabilis* in Atherton in 1998.

The sarcotesta was removed from all seeds except a control of 10 seeds of *B. serrulata* in 1992. The seeds were planted in coarse sand, the same substrate as at the point of collection, in trays in a controlled-environment greenhouse at the Central Queensland University, Rockhampton campus. The seeds were maintained in the same light, temperature and precipitation regime as experienced at the collection site at Byfield.

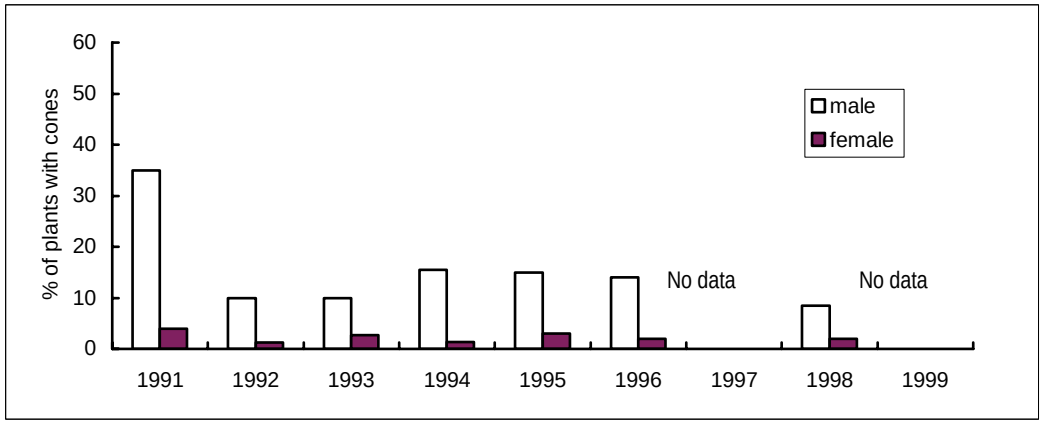
3.2 Results

3.2.1 Cone production and phenology

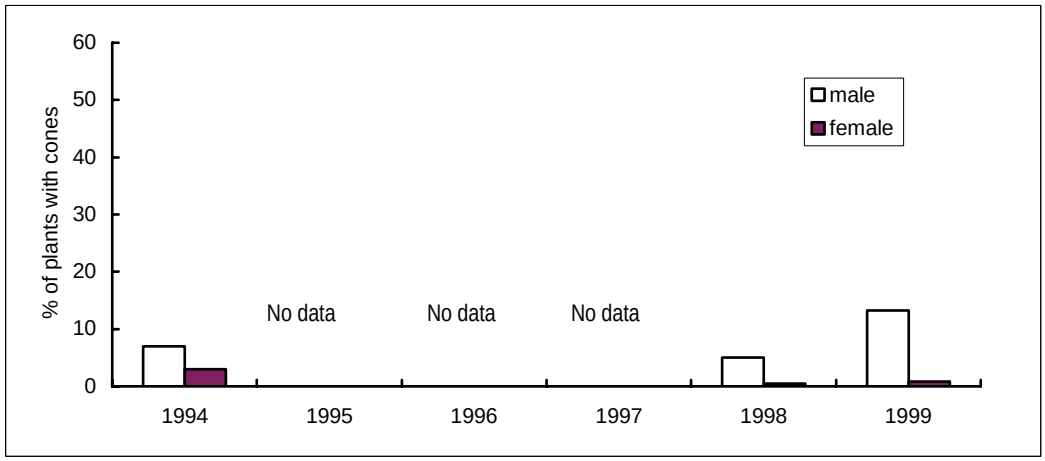
Cone growth commenced in the Dry Season, with male cones first obvious in August or September and about one month before female cones. Cones were produced earlier, and males dehisced pollen earlier, in populations in wetter locations, e.g. at Tarzali (3988 mm p.a.), than those in drier locations, e.g. at Tinaroo (1749 mm p.a.) and *B. serrulata* at Byfield (1745 mm p.a.). The coning phenology of *B. spectabilis* at Tinaroo and *B. serrulata* was similar. Male cones were produced more frequently on plants and in populations than female cones

Cone production in each population monitored varied widely, particularly in the number of male cones (*B. serrulata*, range 8.5-35%, putative *B. spectabilis*, range 5-13.2% and nominate *B. spectabilis*, range 1.8-50%). The production of female cones varied less (*B. serrulata*, range 1.25-4%, putative *B. spectabilis*, range 0.5-3% and nominate *B. spectabilis* range 0.18-5%) but no more than 5% of the plants in any population produced a cone in any year (Figure 3.2).

The number of male and female cones produced was correspondingly high in *B. serrulata* in one year (1991) but not thereafter, and was not paralleled in the other populations. The data do not indicate any masting events but the monitoring period is too short except in *B. serrulata* for such an event to be likely and the lack of data for 1997 and 1999 mean that it may have occurred but not been recorded.



a.



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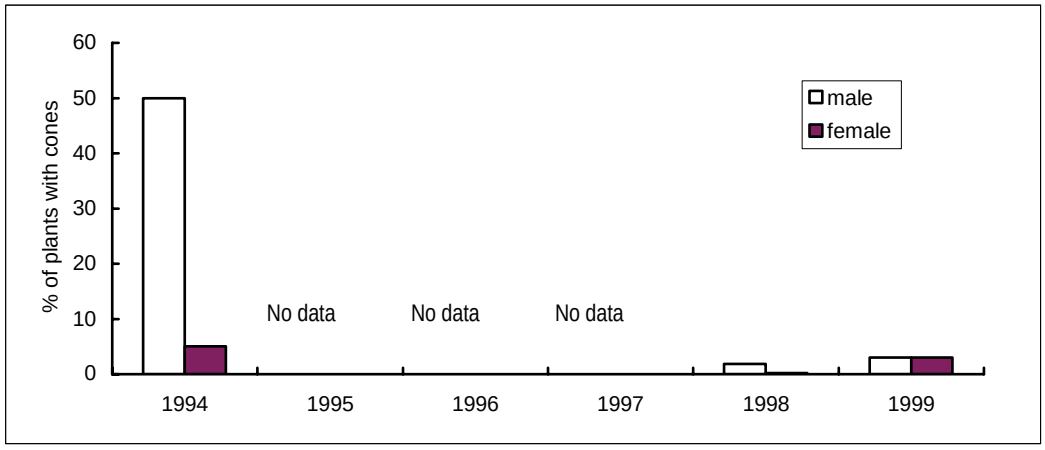


c.

Figure 3.2 Production of cones by (a) *Bowenia serrulata* in SEVT at Byfield, Central Queensland (7 years), (b) putative *B. spectabilis* in SNVF at Tinaroo, North Queensland (3 years) and (c) nominate *B. spectabilis* in CMVF at Tarzali, North Queensland (3 years) - (data are not available for all years).

Male Cones.

Male cones had a mean height of 100 (±2.74) mm (n = 5) at maturity (Figure 3.3). The microsporophylls changed from pale green to light yellow in colour and relaxed as the cones matured. The pollen in the sori on the abaxial-surface of the microsporophylls was released over a period of four or five days in the month prior to the onset of the Wet Season; this was mid-November through mid-December in *B. serrulata* in central Queensland, but at earlier dates in the northern populations. Torrential rain had an immediate effect on male cone phenology; it caused all mature cones in a population to immediately dehisce and to quickly disintegrate and decompose. The decomposition of the male cones was accelerated by the presence of *Miltotranes* weevils that burrow in and eat the tissue of the microsporophylls. Such an event occurred at Byfield in 1993 and in at Tarzali in 1998. Male cones commenced dehiscing pollen prior to the first female cone being receptive and continued until after the last female cone was receptive. In all years, substantial amounts of pollen fell to the leaf litter under or near cones (Figure 3.4).

Female cones.

Female cones were an average of 79 (±5.79) mm (n = 6) high when male cones dehisced their pollen (Figure 3.3). In receptive cones, the lower megasporophylls relaxed several millimetres for a period of 24-36 hours to allow the entry of pollen and/or pollination vectors. Female cones were often partially submerged in the soil or leaf litter at the time of pollination. A cone of putative *B. spectabilis* at Tinaroo was almost buried in the coarse granitic soil but a later analysis revealed 18 of 23 (78%) ovules were fertilised. The cones remained intact until the following mid-year, approximately 6-7 months after receptivity and in the middle of the Dry Season, and increased in height to 250 mm and weight to 1000 g during that period. The fertilised ovules (seeds) also increased in size and became visible between the megasporophylls and the sarcotesta of seeds changed in colour to pale lilac/blue as they matured. Few intact mature cones were found during the study, as they were broken apart by foraging animals once the seeds matured.

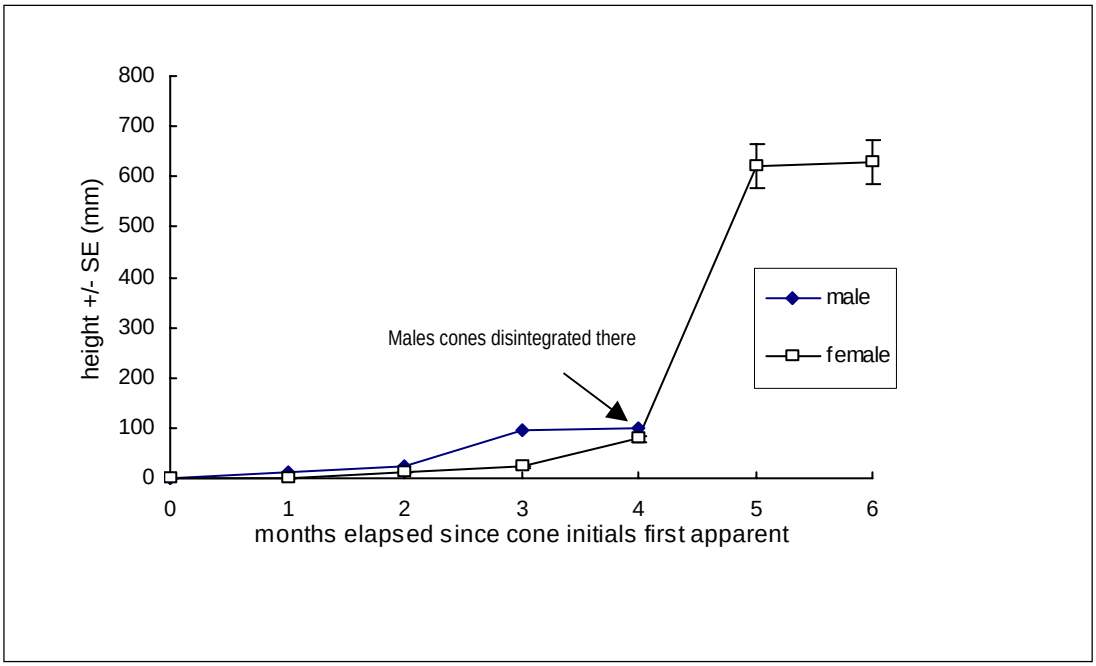


Figure 3.3 Height with age of male (n = 5) and female (n = 6) cones of *Bowenia serrulata* and mean height (mm) at pollen dehiscence and pollination.

Figure 3.4 *Bowenia serrulata* male cones dehiscing pollen – note the pollen on the leaf litter below the cones (scale bar = 40 mm).

Figure 3.5 Female cones of *Bowenia serrulata* (left to right) from receptive through recently pollinated to near mature (scale bar = 40 mm).

The best data set on cone production and phenology comes from the 40 plants of *B. serrulata* monitored at Byfield in central Queensland. Male plants frequently produced single male cones in successive years, some produced multiple (2 or 3) cones in one year but only one the next and a few plants produced multiple cones in successive but not subsequent years. Female cones were produced less frequently and in smaller numbers. Of ten plants that produced female cones in 1991-2, two produced cones in the second year, with one producing a single cone, and the other, two cones, in each season; both plants were located in high light regimes on the edge of a service road.

The data are insufficient to permit the determination of the sex ratio of plants in any population of either species but during eight years of observation of the 40 *B. serrulata* plants 18 (45%) produced cones. These results show that 14 (35%) of the plants are males and 4 (10%) are females, a 3.5:1 ratio.

3.2.2 Cone thermogenesis and Emission of volatiles

Cone thermogenesis

Mature male and female cones of putative *B. spectabilis* at Tinaroo were thermogenic and raised their temperatures 4-6°C above ambient between 1200 and 1400 hours (Figures 3.6, 3.7). The data for the female cone is incomplete as an unidentified rodent severed the probe lead on the first evening of recording; however, it is sufficient to demonstrate thermogenesis in the cone.

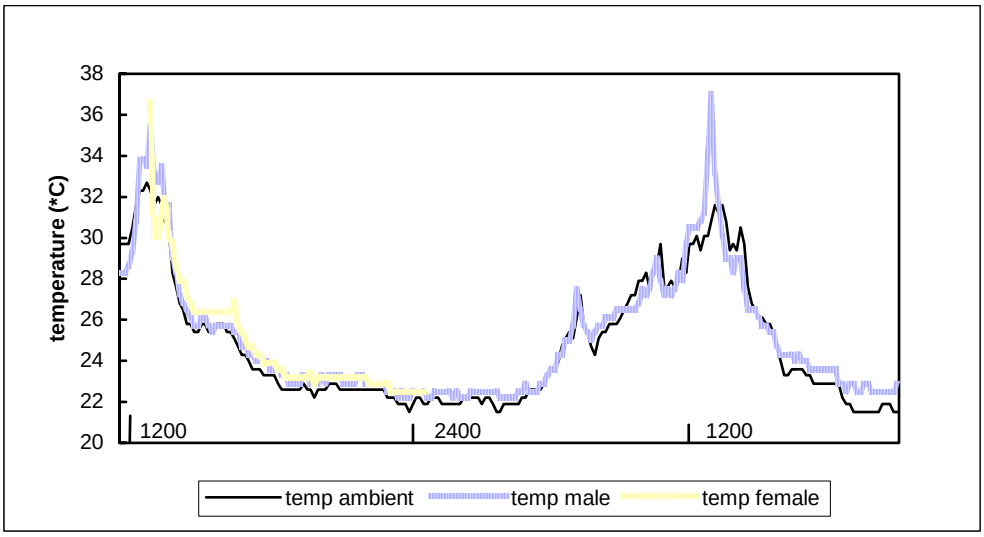


Figure 3.6 The ambient temperature and internal temperatures (°C) in a dehiscing male and a receptive female cone of putative *Bowenia spectabilis* at Tinaroo for 36 and 12 hours respectively.

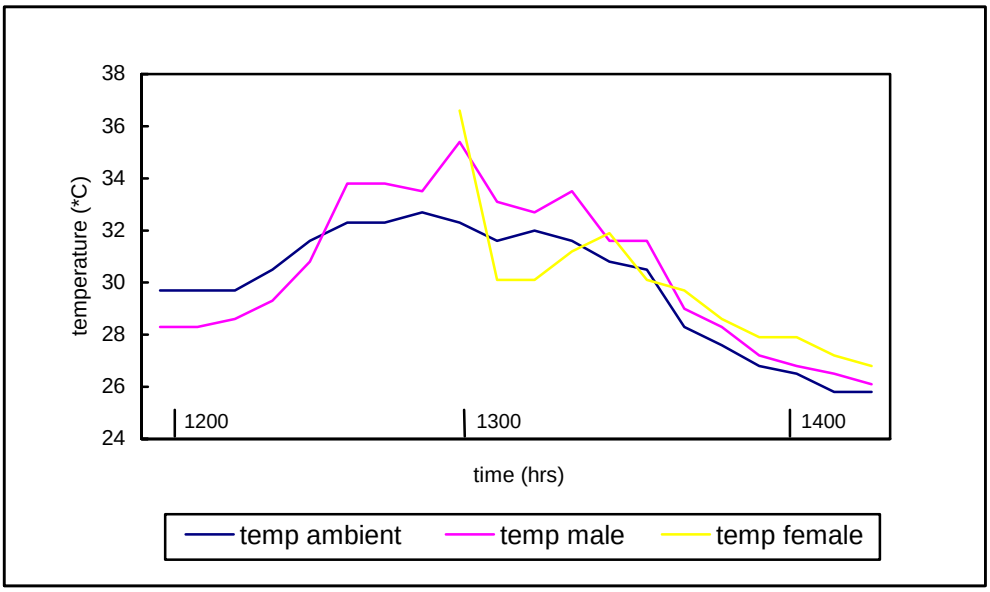


Figure 3.7 The ambient temperature and internal temperatures (ºC) in a dehiscing male and a receptive female cone of putative *B. spectabilis* at Tinaroo over a two-hour period.

Emission of Volatiles

The male cones of *B. serrulata* emitted a minty odour whilst dehiscing pollen but not at other times. Due to the method of sampling it was not possible to ascertain the chemical composition of the volatiles, if the emissions occurred on a cyclical basis, and if so if it corresponded with the period of elevated temperatures in the cones.

3.2.3 Insect associations

Weevils were found in 95% (n = 100) of dehiscing male cones of *B. serrulata* and in 98% (n = 50) of those of *B. spectabilis* (Figure 3.8). The weevils in *B. serrulata* were identified by Dr E C Zimmerman of the ANIC (pers. comm. 1994) as *Miltotranes subopacus* (Lea) and those in *B. spectabilis* as *M. prosternalis* (Lea) (Curculionidae: Curculioninae: Molytini).

Figure 3.8 *Miltotranes prosternalis* weevils on a male cone of putative *Bowenia spectabilis* at Tinaroo.

No other insect species were collected from the treated female cones of either *B*. *serrulata* or *B. spectabilis*. *Miltotranes prosternalis* weevils were also collected from the foliage of *B. spectabilis* at McIlwraith Range by Forster in 1992 and Freeman in September 1999. *M. prosternalis* was collected by the author from foliage and dehiscing male cones of putative *B. spectabilis* at Kuranda and Tinaroo in November 1994,95,98 and 99. In both *B. serrulata* and *B. spectabilis* the weevils were observed to copulate on male cones, oviposit into, and eat and pupate in the tissue of microsporophylls and cones axes of them (Figures 3.9a,b).

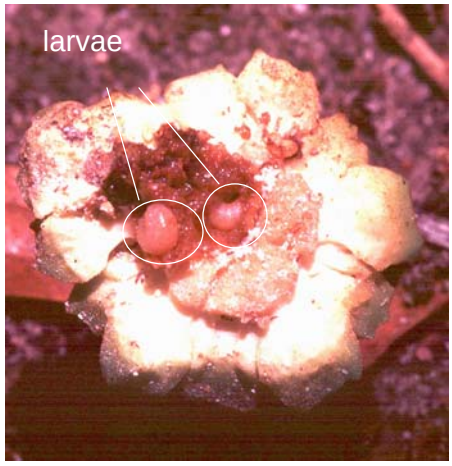
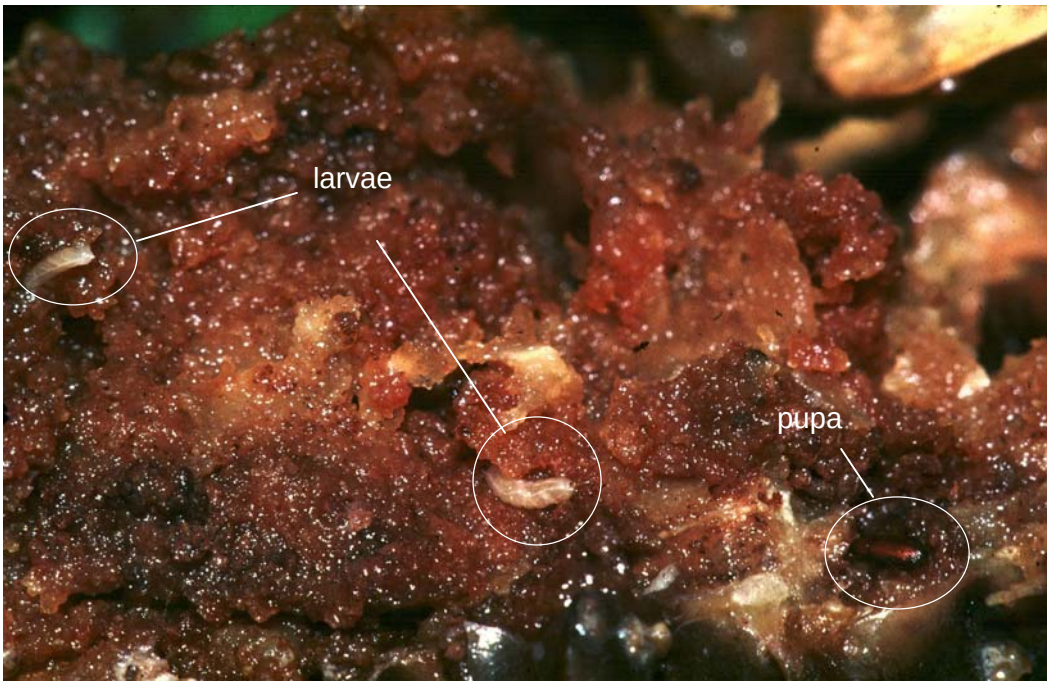


Figure 3.9a,b *Miltotranes subopacus* (Lea) weevil larvae and pupae in the tissue of a male cone of *Bowenia serrulata*.



Male cones of *B. serrulata* and both putative and nominate *B*. *spectabilis* were sectioned to reveal that the weevil larvae pupated in the distal portions of the microsporophylls and the cone axis, with one individual at each location. Adult weevils emerging from pupation exited the cone via the adaxial faces of the microsporophyll. The pollen sori on the abaxial face of microsporophylls were not damaged by the activities of the weevils.

The weevil was not observed eating or breeding in the tissue of female cones and only adult weevils were collected from them; scars on the axes and megasporophyll bases of female cones indicate that the weevils or another insect attempt to do so but without success. Weevils collected from the interior of female cones of both *Bowenia* species were found to be carrying pollen from male cones (Figure 3.10).

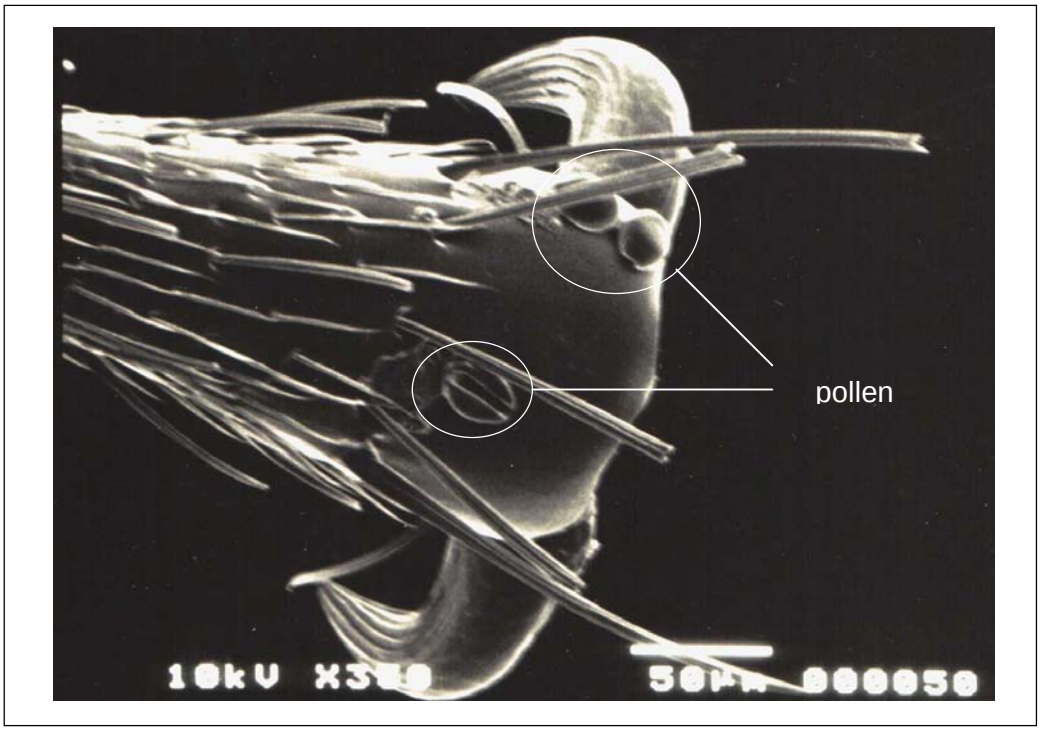


Figure 3.10 SEM micrograph showing *Bowenia serrulata* pollen on the tarsis of a *Miltotranes subopacus* weevil collected from the interior of a female cone.

Miltotranes weevils were not obvious at any site except when mature male cones were present, but searches of the topsoil about plants in the period between reproductive events revealed the presence of the weevils in pupal diapause (Figure 3.11).

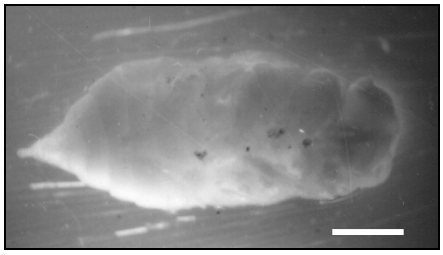


Figure 3.11 *Miltotranes subopacus* (Lea) weevil final instar pupa in diapause collected from the soil at the base of a *Bowenia serrulata* plant. Scale bar = 5 mm.

Miltotranes subopacus larvae collected with male cones of *B. serrulata* and *M. prosternalis* larvae collected with male cones of *B. spectabilis* and cultured at 22°C in the laboratory, pupated and emerged as adults after 7–10 days. The larvae were cannibalistic and the number of them in the culture decreased over time. Larvae were also cultured from eggs in mature male cones collected immediately prior to pollen dehiscence. Adult weevils provided with additional substrate of mature male cones produced a second generation that also successfully pupated.

3.2.4 Seed Production, Viability and Dispersal.

Seed production

Statistically significant data on the production of ovules and seed in both species was obtained from the cones in the experimental procedure to examine the likelihood of insect pollination where exclosures prevented the dispersal of seed. There was no significant difference in seed set in treated and untreated cones of *B*. *serrulata* (t = -0.32; D.F. = 21; P = 0.05) or *B. spectabilis* (t = -0.40; D.F. = 13; P = 0.05).

Seed viability

The greenhouse trials in 1991, 92, 93 and 94 of seed viability of *B. serrulata* indicated that 80% (n = 10), 90% (n = 20), 95% (n = 40) and 70% (n = 40) of seed was viable. Seed viability in *B. spectabilis* in 1998 was 80% (n = 20). Germination of seed in the nursery was synchronous with that of seed in the field. Seeds of both species had an infraseminal ('post-ripening') period of 3–7 months and a dissection series (Figure 3.12) of *B. serrulata* seeds during that period illustrates that embryo growth continued during this time.

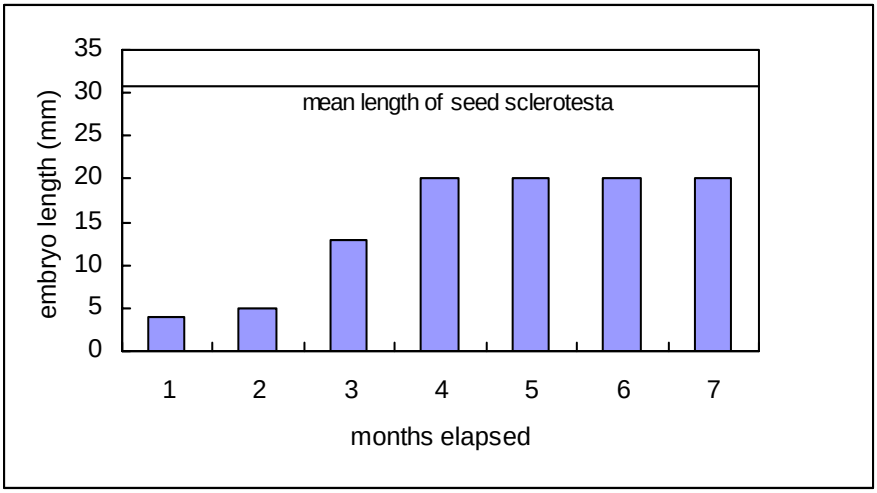


Figure 3.12 Length of *Bowenia serrulata* embryos at one-month intervals during the infraseminal (post-ripening) period prior to seed germination.

In those seeds that germinated, the sclerotesta decayed with time and immediately before germination was a thin and easily ruptured integument; this was more pronounced in *B. spectabilis* than *B. serrulata*. Germination of seed occurred in the period November through March (Figure 3.13) and coincident with the Wet Season when ambient temperatures, humidity, and soil moisture content, were all high. In the trial where the sarcotesta was left on seeds of *B. serrulata* (n = 10), none germinated in either the first or a second year and all rotted in the third.

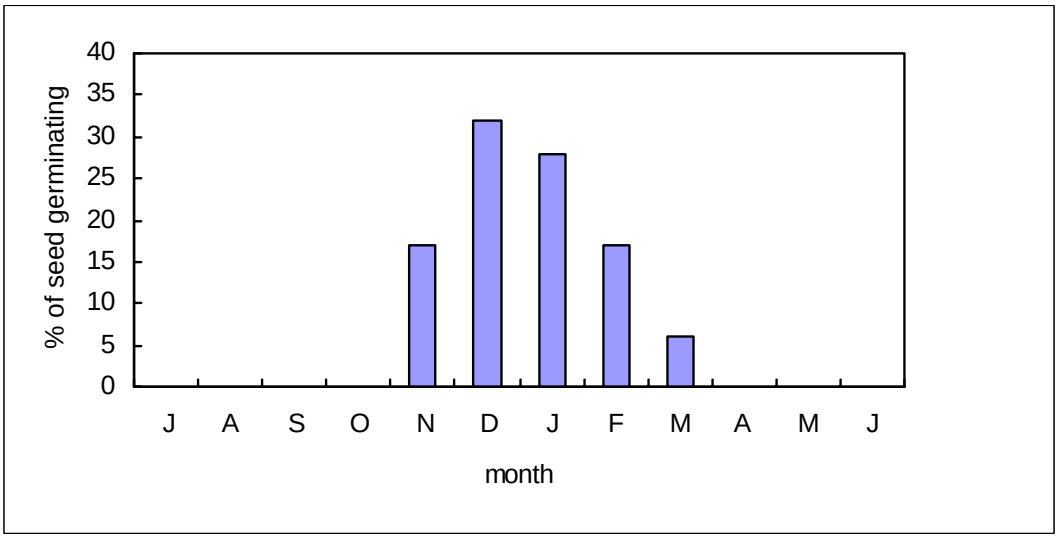


Figure 3.13 Month of germination in four years of seeds of *Bowenia serrulata* (n = 93) in the CQU Nursery.

Seed dispersal

The discovery of seed of *B. serrulata* and *B. spectabilis* in hollow logs and plants growing on tree stumps indicates dispersal by animals. Most seeds found had the fleshy sarcotesta removed and teeth marks on the sclerotesta indicated that an animal had removed it. Few seeds were found where the sclerotesta had been broached; one *B. serrulata* seed, from a cone covered by an exclusion device, had 50% of the endosperm eaten, with the teeth marks indicating a small animal had been responsible.

3.3 Discussion

3.3.1 Cone Production and Phenology

The data indicate that the reproduction cycle in *Bowenia* is similar across species and populations and is primarily related to seasonal factors. Cone growth occurs in the Dry Season, pollen dehiscence occurs in the low humidity and windy conditions prior to the onset of the Wet Season and seed germination in the Wet Season when conditions are optimal for seedling establishment. The regularly timed and synchronous production of cones maximises the likelihood of successful pollen transfer irrespective of the mechanism involved, but in the 'brood site pollination syndrome' described here it is fundamental to the success of the association with the specialist pollination vector. However, the synchronous production of cones and seeds risks the success of reproduction due to the possibility of heavy seed predation; and this matter is considered in 3.3.5 Seed Production, Viability and Dispersal.

There is a consistent bias in the production of male cones in *Bowenia,* as in other cycad species, e.g. *Zamia pumilla* (Tang 1987b) and *Z. skinneri* (Clark and Clark 1987), which grow in habitats similar to that of *B. serrulata* and *B. spectabilis,* respectively. This bias increases the chances, due to the quantity of pollen produced, of successful pollination of the ovules in the relatively scarce female cones, but both the results of Tang (1987b) and those in this study indicate that an insect vector is required for this to occur.

The 3.5:1 ratio of male to female cones recorded for *B. serrulata* in this study is the same as that found in *Z. pumilla* by Tang (1987b). It is much lower than that in *Z. skinneri,* where Clark and Clark (1987) reported that female plants produce a cone on average only every 16-18 years.

Clark and Clark (1987) noted that the energy cost of the production of a female cone is much greater than that of a male cone and that light is a limiting resource for ground-dwelling species in the complex rain forest in which *Z. skinneri* grows. The latter concurs with my observations that female cones occur more frequently on plants in better lit locations but further quantitative observations of cone production in different light regimes are required to determine the specific effects of light on cone production.

3.3.2 Cone Thermogenics and Emission of Volatiles

Cone thermogenics

The results obtained in the field study correspond with those obtained by Tang (1993) from plants at Fairchild Botanic Gardens. Whilst it would be desirable to extend the sample size and number, the evidence suggests that all dehiscing male or receptive female *Bowenia* cones are thermogenic. The question arises, why are cycad cones thermogenic? Thermogenicity in *Bowenia* raises the temperature of the cone above ambient and contrasts it to the surrounding vegetation and probably also assists in the emission of volatiles. Thermogenicity in the reproductive parts of other plant groups, e. g. the aroids (Araceae), assists in the emission of aromatic volatiles and the attraction of insect pollination vectors (see Raven et al. 1995) and the evidence from this study indicates that the same occurs in *Bowenia*.

A second question that arises is 'why are female *Bowenia* cones thermogenic?' as the reason for it is not immediately apparent. Male cones offer food and a brood site reward to visiting weevils but the female cone does not offer the first and only a little of the latter in the form of a pollination drop at each micropyle. It is likely that thermogenesis in the female cone also facilitates the release of volatiles to attract weevils to it - the ruse is that it is not apparent to the insect what sex the cone is until it visits it. It can be hypothesised that once the weevil is inside the female cone the sugars in the pollination drop are sufficient to attract it to the micropyle and thus to effect pollination.

Emission of volatiles

The detection of the emission of volatiles by male *Bowenia* cones supplements the data compiled by Pellmyr et al. (1991) and Tang et al. (1987), but further analysis, particularly of the constituents of the volatiles of *Bowenia*, is required.

Thermogenesis in cones of *Bowenia,* which facilitates the emission of volatiles, together with the discovery of an insect pollination vector using the male cone as a brood site suggest that volatiles play a role in pollinator attraction. In *Bowenia,* it is likely that the toxic nature of the plant tissue has allowed only a very specialised insect pollination association to evolve.

3.3.3 Insect Associations and Entomophily

The results in this study demonstrate that pollination in *Bowenia* is entomophilous, species-specific and possibly coevolutionary. It is the first record of entomophily in the Stangeriaceae and extends records of it to the second of the three families of cycads. The results, when added to the other less rigorous but indicative data in the literature, support the hypothesis that pollination in all extant taxa of cycads is entomophilous. In light of the previously widely held view that pollination in cycads is anemophilous, the question arises as to why *Bowenia* has an entomophilous pollination strategy.

Four factors dictate the likely success or otherwise of anemophily in *Bowenia*;

- the density, distribution and productivity of male cones,
- their spatial relationship to female cones,
- the rate of fall of pollen in calm air, and
- the distance pollen will travel in moving air from a point source.

The first and second factors are outside the ambit of this study but should be pursued in an ecologically orientated study. However, consideration of factors three and four is pertinent to this study.

Proctor et al. (1996) indicate that for pollen to reach a stigma of area $1mm^2$, approximately that of the micropyle of a *Bowenia* ovule, every square metre of habitat must receive $\geq 1 \times 10^6$ units of pollen. The amount of pollen produced must be high and the receptive stigma optimally positioned for this to occur.

The former is true in *Bowenia* but the latter is not, as the micropyle of the ovule is contained in a cone that is frequently wholly or partially submerged in the leaf litter or soil at the time of pollination. For wind pollination to be successful air movement must be sufficient to transport the pollen to the receptive area.

Data in the literature (see Proctor et al. 1996) indicate that with an air speed of 4 m sec^{-1}, pollen travels a distance approximately the square of the source height in trees species but less in herbaceous species (Wright 1953, *cited in* Proctor et al. 1996). For an 80 -100 mm high *Bowenia* cone, this distance is at most 6.4-10 metres, and likely to be much less. Other data (p270 Proctor et al. 1996) indicate that in calm air, pollen falls between 20 and 400mm sec^{-1}, depending on its size, with larger grains falling faster. As cones of *Bowenia* are found in an environment where air movement rarely exceeds 0.05 m sec^{-1} (Leigh 1999), this suggests another pollination strategy is employed and the results presented in this chapter indicate that it is entomophily.

Prior to this study, *M. subopacus* had been known only from a female collected at Byfield in 1926. Zimmerman (1994) erected *Miltotranes* for species previously assigned to *Tranes* that are associated with *Bowenia*. The fact that *Miltotranes* breed in the tissue of the male cone, aestivate in the soil between reproductive cycles of the host species, and have only been collected in association with *Bowenia* species, suggests a species-specific and possibly a coevolutionary association between them. The plants provide food, a brood site and shelter to the weevils, and in return they provide a pollination service to the plants. This conforms to the 'specialised brood substrate' pollination strategy (Proctor et al. 1996) and as such, joins the better-known examples in *Ficus* (Moraceae) (Wiebs 1963; Herre et al. 1996; Dixon et al. 2001) and *Yucca* (Agavaceae) (Tyre and Addicott 1993) and in Zamiaceae (Norstog et al. 1986, 1989).

Oberprieler (1995a,b) suggested that the degree of intimacy in cycad-weevil associations is indicative of its evolutionary status. The weevil tribe Molytini that includes *Tranes* and *Miltotranes* is regarded as primitive and unspecialised (Oberprieler 1995a), and Forster et al. (1994) note that strict host specialisation has not occurred in *Tranes* with ‘two species present on a wide range of *Macrozamia* and *Lepidozamia* species’. Oberprieler (1995b) further suggested that the association between *Bowenia* and *Miltotranes* is comparatively recent; the data presented here indicate that the association is of greater antiquity and more specialised than Oberprieler suggests. The presence of *M. prosternalis* in all northern populations of *Bowenia* except for Starke, for which no data is available, supports the hypothesis that all plants in northern Queensland are *B. spectabilis*.

Studies by Kokubugata et al. (2000, 2001) indicate that little genetic differentiation has occurred in several northern populations of *Bowenia*, however Oberpreiler (pers comm. 2000) has advised me that the *Miltotranes* weevils collected from *B. spectabilis* in the McIlwraith Range are slightly different in respect to colour and markings to those collected elsewhere in the Wet Tropics but that he has not had the time to determine if they warrant description as a new species. These data suggest that some differentiation between the populations of weevils has occurred and it will be interesting to see if it is paralleled in the *Bowenia* populations and a coevolutionary association can be demonstrated.

The species-specific and obligate entomophilous nature of pollination in *Bowenia* also explains why fertilisation rates in cultivated plants, even those in close proximity and particularly those that have been subject to quarantine inspection, is extremely low (Randall, pers. comm. 1999). The majority of the plants are grown from seed and the specialised pollination vector, which generally resides in the soil around the plants, is not available to them.

The plant-weevil association described here is very similar to that described by Norstog et al. (1992) for the Neotropical *Zamia furfuracea* (Zamiaceae) and the snout weevil *Rhopalotria mollis* (Sharp) (Belidae: Oxycoryninae: Allocorynini). Crowson (1991) notes that this weevil's 'lineage and association with cycads is generally assumed to be quite old' and this association conforms to Oberprieler's (1995b) definition four (4) of one where 'the inner and more protected structures of the cone are utilised for larval development'. The results of this study demonstrate that the same associations occur in *Bowenia.*

A phylogenetic analysis of the cycads by Stevenson (1990) indicates that the Stangeriaceae diverged from a common ancestor prior to the Zamiaceae, this means that *Miltotranes* has had an equal or greater length of time in which to adapt to *Bowenia*. This suggests parallel evolution in *Rhopalotria mollis* and *Miltotranes subopacus* and *M. prosternalis*, as they sought to accommodate similar problems in a similar manner; further studies are required to ascertain if this is so.

3.3.4 Seed Production, Viability and Dispersal

Seed Production

Terborgh (1986) observed that the reproductive cycle of many tropical species in environs with minimal climatic variation is highly regular and not apparently related to season and thus must be related some other factors. This is the case in other rainforest cycad species, e.g. *Zamia skinneri* (Clark and Clark 1987), and it is appropriate to consider if the same applies in *Bowenia* and if there are any 'costs' to it of a very regular reproductive cycle.

The negative effect of having all seed mature at the same time is that seed predators have the opportunity to remove or damage the entire crop. A response to this problem that is widely used by plants is to produce more seed than the predators can take and thus ensure that some will survive to germinate – this is 'the predator satiation' model' proposed by Janzen (1981). Regularly reproducing plants use a 'mast seeding' strategy where low reproduction effort events are irregularly interspersed with high productivity events. This strategy ensures that seed predators do not acclimate to reproductive events and that more seed is produced than they can predate when reproduction does occur. The data presented here do not give evidence of mast seeding in *Bowenia* but due to the incomplete nature of the data sets, the possibility of it cannot be excluded. An additional factor that must be considered in respect to *Bowenia* is that the seeds are very toxic and seed predation is lower (Harrington et al. 1997; this study) than many other rainforest species. Mast seeding has been demonstrated in *Macrozamia* (Ballardie & Whelan 1986) and *Encephalartos* in the Zamiaceae (Donaldson 1993), but both these taxa produce seeds that are much less toxic than those of *Bowenia.*

Seed viability

The high levels of seed viability in *B. serrulata* and *B. spectabilis* reported in this chapter indicate that the insect pollination strategy being employed by the plants is successful and that seed production is not pollen limited. The failure in the trials of *B. serrulata* seeds with intact sarcotesta to germinate demonstrates that environmental conditions are insufficient to effectively remove the germination-inhibiting mucilage-rich sarcotesta, and the importance in seed germination of animals in removing the sarcotesta.

The very low level of seed predation due to their toxicity allows the successful infraseminal (after-ripening) growth of the embryo. Eames (1961) notes that infraseminal growth is most common in more 'primitive' angiosperm taxa, e.g. some Araceae, and herbaceous geophilous genera, e.g. *Clematis*, *Crocus* and *Trillium,* but that the reasons for it are not well understood; there is no discussion of it in the gymnosperms in the literature

Three questions present themselves in respect to infraseminal growth in *Bowenia*,

- is the habit a relic of a previous reproductive practice?
- is the energy cost of maintaining a large female cone too great to be sustained?, and
- does seed abscission at this time allow the plant to commence another reproduction event?

None of these has an immediately obvious answer and the opportunity exists for further inquiry on this subject.

Seed dispersal

This study did not reveal which animal species eat the sarcotesta or disperse the seeds of *B. serrulata* but anecdotal data (J. Winter pers. comm. 1998) and data in Dennis (2002) indicate that macropods, particularly the Musky Rat-kangaroo *Hypsipyrmnodon moschatus,* disperse the seeds of *B. spectabilis* but do not eat them.

3.4 Systematics Analysis

The data reported in this chapter provide information that can be utilised in the consideration of the status of the disjunct northern populations of *Bowenia* and of the systematics of the genus. The pollination biology data support the hypothesis that there are two extant species of *Bowenia*, i.e. *B. spectabilis* and *B. serrulata*, with *M. prosternalis* being the obligate pollination vector of the former and *M. subopacus* the obligate pollination vector of the latter. The ubiquity of the presence of *M. prosternalis* in the northern populations supports the hypothesis that they are disjunct occurrences of *B. spectabilis.*

Chapter 4 Other Insect Associations

In Chapter 3, the weevils *Miltotranes subopacus* (Lea) and *M. prosternalis* (Lea), were demonstrated to be the obligate pollination vectors of *Bowenia serrulata* and the northern populations of *Bowenia* currently referred to *B. spectabilis.* These data support the hypothesis that there are only two extant species of *Bowenia*. In this chapter, a second insect association that appears to distinguish between the species and populations of *Bowenia* is described.

Once again, the reason for this part of the study is because of the suggestion by Zwölfer (1973) and Futuyma (2000) that some insects closely associated with plant taxa reflect the diversity and phylogeny of their hosts. A recent demonstration of the use of this concept to distinguish between species and hybrids is the study by Ishida et al. (2003) of *Quercus crispula* Blume and *Q. dentata* Thunberg (FAGACEAE). These species hybridise in the wild and exhibit continuous variation in morphological characteristics between the parent taxa. Ishida et al. (2003) used multivariate analyses of morphological and molecular (amplified fragment length polymorphism [ALFP]) data, and the presence or otherwise of leafminer (*Phyllonorycter*; Gracillariidae; Lepidoptera) known to be specific to the parent taxa in an attempt to differentiate between the parent taxa, hybrids and backcrosses. They found the morphological and insect-association data to be more reliable in this task than the molecular data.

Toxins in plants are typically low-cost molecules that are by-products of metabolic activities and are often stored in maturing cells (Harborne 1988). Cycads contain a suite of toxins including flavonoids (Dossaji et al. 1975; Meurer-Grimes and Stevenson 1994), non-protein amino acids (β-methylamino-L-alanine (BMAA)) (Charlton et al. 1992) and methylazoxymethanol (MAM) glycosides (Moretti et al. 1983) that discourage vertebrate, invertebrate, and fungal and bacterial predators and pathogens (Siniscalco Gigliano 1990). The presence of mercury-containing glycosides is restricted to the cycads in the 'higher' plants and *Bowenia* contains the highest levels of them (Moretti et al. 1983). In addition, some cycads contain phytoecdysones, e.g. *cycasterone* in *Cycas* (Harborne 1988), which disrupt the development of insects that feed on them. The metabolic pathways for the major groups of secondary plant compounds are shown in Figure 4.1.

Figure 4.1 Metabolic pathways for major groups of secondary compounds with principal building blocks from primary pathways shown in boxes (adapted from Lindroth 1989).

The small number of animals that eat cycads and the often-specialised association of them demonstrate the effectiveness of these deterrents to feeding. Well-documented associations include the Leopard Magpie Moth *Zereopsis leopardina* with *Encephalartos* sp. and *Stangeria* eriopus (Donaldson 1995) in Africa and the Hairstreak Blue Butterfly *Eumaeus atala florida* and Tiger Moth *Seirarctia echo* with *Zamia pumilla* (Rawson 1961; Teas 1967, Teas et al.1966; Rothschild 1973) and *Dioon edule* (Vovides 1990) in the Americas. The larvae of these species metabolise the MAM glycosides in the cycads and use an aglycone, *cycasin,* as a defence against predators (Nash et al. 1992).

Other insects that accommodate and/or avoid the effects of toxins in cycads include the beetles that pollinate some taxa (Norstog et al. 1986; Norstog and Fawcett 1989; Wilson 1993a, 2001, 2002a,b). For example, the weevil *Rhopalotria mollis* that pollinates *Zamia furfuracea* avoids the toxins in male cones by ingesting and excreting intact, the idioblastic cells that contain them (Norstog and Fawcett 1986; Norstog et al. 1995).

Chrysomelid beetles that feed on cycads also assimilate toxins (Pasteels et al. 1982, 1984, 1986) and use them, in combination with aposematic colouration, for defense purposes. The chrysomelids date from the Jurassic (Crowson 1981) and Farrell (1998) suggests they were associated with conifers and cycads prior to the evolution of the angiosperms. Crowson (1981) suggests that the separation of the Chrysomelidae and the Cerambycidae within the Chrysomeloidea dates from the late Jurassic and may be linked to an adaptation to cycads or conifers as food plants. Contemporary fossil records of cycads and chrysomelids from Mesozoic lake deposits in Central Asia (Medvedev 1968) and the current number of host-specific interactions suggest their association is of considerable antiquity (Windsor et al. 1999).

The Criocerinae is a basal pan-tropical subfamily of the Chrysomelidae (Schmitt 1998) and species of *Lilioceris* in the subfamily are recorded as feeding on taxa in all three families of cycads (Schmitt 1988; Wilson 1993b; Forster and Machin 1994; Jolivet and Hawkeswood 1995) and Hawkeswood (1990) and Monteith (1991) document *L. nigripes* (Fabricius) feeding on *B. spectabilis.*

Early in this study, it was observed that *L. nigripes* larvae did not eat the foliage of *B. serrulata,* although they ate the foliage of *Cycas ophiolitica* growing nearby. As the distribution of *L*. *nigripes* includes that of *Bowenia* (Oberpreiler pers. comm. 2000) and bearing in mind the comment by Futuyma (2000) that 'many traditional statements about the host associations of major taxa of insects are valid descriptions of [plant] evolutionary lineages' the observations were continued to see if they provide an insight into the systematics of *Bowenia*. The rationale was that other studies of plant-insect associations, (see Kircher and Heed 1970; Wood 1982; Herre et al. 1996; Dixon 2001; Dixon et al. 2001), have assisted in delineating existing and flagging previously unknown plant species.

This aspect of the study sought to clarify the previous incidental observations of *L. nigripes* feeding on *Bowenia* and see if the beetle really did avoid *B. serrulata* while feeding on *B. spectabilis*. Two procedures were designed to answer these questions: field observations to ascertain the association of *L. nigripes* and *Bowenia*, and an experiment to ascertain if *L. nigripes* distinguishes between *B. spectabilis* and *B. serrulata* or nominate and putative *B. spectabilis*.

4.1 Methods and materials

4.1.1 Field studies

Plants of *B. serrulata* at Byfield were surveyed for the presence of phytophagous insects in each month in the years 1992, 1993 and 1994 and immediately pre- and post wet season, in 1995 and 1996. Plants of nominate *B. spectabilis* at Tarzali and putative *B. spectabilis* at Tinaroo and Kuranda were surveyed immediately pre- and post wet season in 1994, 1998, 1999 and 2000. In addition, other field workers familiar with *L. nigripes* and *Bowenia* were questioned on any observations they had of interactions of the two species, and opportunistic records of *L. nigripes* on other cycad species were collected.

4.1.2 Laboratory studies

The experiment was designed in two parts, the first to see if *L. nigripes* beetles previously feeding on *B. spectabilis* would feed on the foliage of *B. serrulata* or *Cycas ophiolitica* in the absence of the preferred substrate. In addition, a test was conducted to see if beetles feeding on *Cycas ophiolitica* which grows near *B. serrulata,* would also feed on that species if the need arose. The second part of the experiment was designed to determine if *L. nigripes* beetles previously feeding on *B. spectabilis* would feed on the foliage of *Cycas* species growing near it, and if beetles feeding on them could or would feed on *B. spectabilis.* Acceptance of the alternate food would indicate that no physiological feeding barrier existed and that the weevil could utilise either substrate. In each case, glabrous juvenile foliage was provided to the beetles as previous observations (Wilson, unpubl. data) indicated that this was most palatable to the species.

Part 1 Ten adult *Lilioceris nigripes* beetles were collected from the foliage of nominate *B. spectabilis* at Tarzali and five of them placed in each of two 1000 mL glass beakers with a fine gauze cover fastened with an elastic band. A 50 mL container of water was placed in each beaker during the experiment to maintain humidity levels for both the insects and the plants. Three pinnules of *B. serrulata* were introduced into one jar and three of *C. ophiolitica* into the other, on five days; the material from the previous day was removed at this time and the amount eaten recorded. In addition, ten *L. nigripes* collected from *C. ophiolitica* and managed in the same way were offered foliage of *B. serrulata*.

Part 2 Five adult *L. nigripes* leaf beetles were collected from putative *B. spectabilis* and *Cycas m. banksii* and placed in 1000 mL jars containing *C. m. banksii* and putative *B. spectabilis* from Starke respectively. These insects were offered three fresh pinnules of these species on five days, the material from the previous day was removed at this time and the amount eaten recorded.

In all cases the amount of foliage of the cycad species eaten by the beetles in the previous 24 hours was scored on a scale of 0 – 3, where

0 = none eaten	2 = 5-50% eaten
1 = sample bites only	3 = >50% eaten

4.2 Results

4.2.1 Field studies

Bowenia serrulata No *Lilioceris nigripes* were found on *B. serrulata* during forty surveys. The small amount of damage to the foliage of *B. serrulata* during the survey period was caused by the longicorn beetle *Sybra centurio* Pascoe, which ate only dry pinnules of senescing leaves; these activities will be described elsewhere (Wilson, in prep.). *L. nigripes* was found feeding on *Cycas ophiolitica* (Cycadaceae) growing one kilometre from the study site but not on *Macrozamia miquellii* (Zamiaceae) growing in a forest ecotone immediately adjacent.

Bowenia spectabilis *Lilioceris nigripes* was found eating pinnules of juvenile leaves of nominate *B. spectabilis* at Tarzali and putative *B. spectabilis* at Tinaroo and Kuranda in September and October prior to the onset of the wet season. *L. nigripes* was one of two insect species found feeding on *B. spectabilis*; the other was a longicorn beetle, *Dihamus* sp., seen twice at night feeding on nominate *B. spectabilis* at Tarzali. Adult *L. nigripes* were observed to copulate and oviposit on the foliage of *B. spectabilis*. Activity by early instar larvae was restricted to apical and mid sections of glabrous juvenile pinnules and they were most often found on the underside of pinnules. Late instar and adult beetles browsed both surfaces of glabrous juvenile and mature pinnules (Figures 4.2, 4.3) and usually removed all but the lower third of the pinnule. When disturbed, adult beetles either dropped to the ground and feigned death or flew away. No predation of larvae or adult beetles by birds or other insect species was seen during the study.

Figure 4.2 Late instar larvae of *Lilioceris nigripes* feeding on mature pinnules of nominate *Bowenia spectabilis* at Tarzali, northeast Queensland.

Figure 4.3 Adult *Lilioceris nigripes* feeding on tomentose juvenile pinnules of nominate *Bowenia spectabilis* at Tarzali in northeast Queensland.

The inquiries of other field workers revealed Paul Forster of the Queensland Herbarium and Alistair Freeman of the Queensland Parks and Wildlife Service had both seen adult *L. nigripes* feeding on young foliage of *B. spectabilis* at McIlwraith Range. The results of the field surveys and these inquires are presented in Table 4.1.

Table 4.1 Summary of the presence of *Lilioceris nigripes* on the foliage of *Bowenia serrulata* and *B. spectabilis* growing in their natural habitat.

	Bowenia* foliage type and presence of *Lilioceris nigripes					
	emerging and tomentose		juvenile but glabrous		mature	
	larvae	adult	larvae	adult	larvae	adult
B. serrulata	–	–	–	–	–	–
B. spectabilis	–	–	Y	Y	Y (final instar)	Y

Lilioceris nigripes was also found feeding on juvenile foliage of *Cycas m. banksii* growing in a forest ecotone immediately adjacent the Kuranda and Tinaroo sites and on *Cycas platyphylla* and *C. cairnsiana* that grow contiguously to the west of *B. spectabilis* and *Cycas media banksii*.

An observation of interest made during the fieldwork is the disjunct effects of the beetle on plants of both *B. spectabilis* and *Cycas* species. In one plant of a *Cycas* a whole cohort of leaves or all the pinnules on a leaf of *B. spectabilis* will be substantially damaged but slightly younger or older leaves on adjacent plants will be untouched.

4.2.2 Laboratory Studies

Part 1 *Lilioceris nigripes* beetles collected from *B. spectabilis* did not eat the glabrous juvenile foliage of *B. serrulata* but did eat that of *C. ophiolitica* (Table 4.2a). *L. nigripes* collected from *C. ophiolitica* did not eat the glabrous juvenile foliage of *B. serrulata* (Table 4.2b).

Table 4.2a Feeding over five days by adult *Lilioceris nigripes* beetles collected on nominate *Bowenia spectabilis* on glabrous juvenile foliage of *B*. *serrulata* and *Cycas ophiolitica.*

Day of trial	*B. serrulata*	*C. ophiolitica*
1	1	2
2	0	3
3	0	3
4	0	3
5	0	3

0 = none eaten, 1 = sample bites only, 2 = <50% eaten, 3 = >50 % eaten.

Table 4.2b Feeding over five days by adult *Lilioceris nigripes* beetles collected from *Cycas ophiolitica* on glabrous juvenile foliage of *Bowenia serrulata.*

Day of trial	*B. serrulata*
1	1
2	1
3	1
4	1
5	1

0 = none eaten, 1 = sample bites only, 2 = <50% eaten, 3 = >50 % eaten.

Part 2 *Lilioceris nigripes* collected from nominate *B. spectabilis* readily ate the foliage of *Cycas m. banksii,* but beetles collected from this species only sparingly ate the foliage of putative *B. spectabilis* from Starke (Table 4.3) and only on the last day of the trial when hunger may have over ruled a normal inhibitions to feeding on the species.

Table 4.3 Feeding over five days by adult *Lilioceris nigripes* collected from nominate *Bowenia spectabilis* on glabrous juvenile foliage of *Cycas m. banksii* and from *Cycas m. banksii* on the foliage of putative *B. spectabilis* from Starke.

Day of trial	(a) *C. m. banksii*	(b) putative *B. spectabilis*
1	2	1
2	3	0
3	3	0
4	3	1
5	3	2

0 = none eaten, 1 = sample bites only, 2 = <50% eaten, 3 = >50 % eaten.

4.3 Discussion

The results indicate that *L. nigripes* distinguishes two chemotypes of *Bowenia* that conform to the recognised species but does not distinguish nominate and putative *B. spectabilis.* The latter adds weight to the hypothesis that all the plants in North Queensland are one, morphologically variable, species.

The results of the laboratory experiments confirm the field observations in respect to *Bowenia* and suggest that some deterrent to feeding is present in *B. serrulata* but absent or present at lower levels in *B. spectabilis*. That *L. nigripes* is readily able to move from *B. spectabilis* to *Cycas* species as a food source, and able, but less readily, to move from *Cycas* to *B. spectabilis* provides some indication of the way that this insect species adapted to feeding on *Bowenia*.

Jolivet and Hawkeswood (1995) indicated that allotrophy is unusual in chrysomelids, and as *Cycas* is c.90 my older than *Bowenia* (Jones 1993) and as chrysomelids date from the Jurassic (Farrell 1998), it is likely that *L. nigripes,* or a precursor, adapted to feeding on *Cycas* or another cycad extant at the time and later moved to *Bowenia.* There is some support for this in the fact that *L. nigripes* collected from *C. m. banksii* only fed on the foliage of *B. spectabilis* after three days. This behaviour suggests they were driven to do so by hunger but also indicates that they were able to tolerate the differences in the composition and/or concentrations of toxins in *Bowenia* if they have to – this is in contrast to the situation with beetles from *C. ophiolitica* offered *B. serrulata*. The results of this study do not explain why *L. nigripes* doesn't feed *on B. serrulata*, but a chemical agent is likely to be involved. This subject warrants further inquiry, as leaf beetles are important as pests of food crops, and such an effective deterrent may have commercial applications.

An interesting aspect of the results is the short window of opportunity *L. nigripes* has for browsing on the foliage of *Bowenia.* The early instars of *L. nigripes* only feed on glabrous foliage of *B. spectabilis* and as this species produces single, fast-growing, leaves on an irregular basis, the beetle often does not find them in time to oviposit and feed on them. This explains why some leaves in a population are severely damaged while others are intact. However, the insect is able to maintain its metapopulation by using the foliage of other cycad taxa, e.g. *Cycas,* as an alternate food supply. These species flush regularly before the onset of the wet season and aseasonally, after fires, and young foliage is usually available to the beetles which are powerful fliers and highly mobile.

Nymphosis in *Lilioceris* beetles occurs in cocoons in the ground and they emerge from it after 6 or 12 months (Schmitt 1998). Whilst further research is required to ascertain the full details of the life cycle of *L. nigripes*, these periods are sufficient to bridge between foliage production events in their preferred food species and it seems likely that they survive periods when food is not available in this way.

The results presented here bear striking parallels with those of studies of cycads in South Africa and Central America by Windsor et al. (1999), which gives further credence to the suggestions made in the text above.

The first parallel is the distinguishing of the grassland and forest 'forms' of *Stangeria eriopu*s by the Geometrid Moth *Callioratis millari* (Staude, cited in Crouch et al. 2000) – this species of cycad is morphologically variable and there have been suggestions, albeit not accepted by taxonomists (Dyer 1966; Vorster and Vorster 1986) that it comprises two closely allied species. The results of studies currently in train in South Africa of *Stangeria* will be most interesting if they determine that two species do indeed exist.

The second parallel is in Central America, where the feeding behaviour of species of the subfamily Aulascoscelinae, which is of similar antiquity and phylogenetically close to the Criocerinae (Jolivet 1988), on species of *Zamia* and introduced *Cycas rumphii* (Windsor 1999) is remarkably similar to that of *Lilioceris* on *Bowenia* and *Cycas.* The beetles move *en masse* and defoliate young leaves of plants of several species and then move on, and distinguish between two species, *Z. skinneri* and *Z. neurophyllidia*, that were initially considered to be conspecific.

4.4 Summary

The value of evidence from the studies described in Chapter 3 and this chapter in resolving the systematics of *Bowenia* is summarised in Table 4.4.

Table 4.4 Summary of the value of evidence from insect association character classes on the resolution of the systematics of *Bowenia*.

character class	character	*B. serrulata*	putative *B. spectabilis*	nominate *B. spectabilis*
Insect association	pollination vector	informative	informative	informative
	phytophagous insects	informative	informative	informative

These data will considered, in concert with genetic data reported in Chapter 5, in Chapter 6: Phylogenetic analysis, biogeography and systematics. The key term here is 'in concert'; I am not suggesting that either association, but particularly the latter, is sufficient in itself to reliably distinguish species of *Bowenia*. I recognise that there is the potential for much more work on the *Bowenia-Lilioceris* association, particularly the chemistry involved, and that more extensive sampling and larger sample sizes would assist in definitively describing the relationship.

Chapter 5 Chromosomes and Karyology

The number, size and structure (collectively named the karyotype) and the banding patterns of chromosomes are systematically informative (Futuyma 2000). The chromosome number and morphology is usually distinct and constant at a species level, and, although exceptions are quite common (Futuyma 2000), can often be used to distinguish between taxa when other characters, e.g. morphology, may not be definitive. In this chapter the karyology of *Bowenia serrulata* and nominate and putative *B. spectabilis* are reported and then examined to see what relationships between *B. serrulata* and *B. spectabilis*, and nominate and putative *B. spectabilis,* are indicated and if they provide some insight to evolution and speciation in *Bowenia*.

A frequently cited example of the use of chromosome data to resolve a taxonomic problem is in the monotypic *Tolmiea* (Saxifragaceae). Soltis and Rieseberg (1988) found plants with identical morphology and similar allozyme frequencies, floral anthocyanins, foliar flavonoids, karyotypes and chromosome banding patterns (Soltis and Soltis 1989), and 5S and 18-25S ribosomal RNA genes (Soltis and Doyle 1987) growing in different areas of western America were autopolyploids of 2*n* = 14 and 28 respectively. However the 2*n* = 28 tetraploids are more heterozygous than the 2n = 14 diploids (0.23 vs. 0.07) and have alleles at some loci not present in the diploid plants. Considering their ecological disparity, partial post-pollination isolation and differences in chromosome number, in the context of the Ecogenetic Species Concept (Levin 1999) that I have adopted for this study, the two are recently diverged species.

Studies of chromosomes can assist in delineating taxa; an example is in *Lantana* (Verbenaceae), tropical shrubs that are taxonomically confusing due to their tendency to hybridise, frequent polyploidy, and poorly resolved generic limits (*x* = 11 in section *camara* and *x* = 12 in section *calliorea*) (p148 Judd et al. 1999). In Florida, where *Lantana* is a woody weed, morphological studies indicated the presence of the native *L. depressa* and an introduced tetraploid, *L. camara*. However, karyological studies by Sanders (1987) revealed that *L. depressa* consisted of three diploid varieties, each of which hybridise with *L. camara*. Only after these facts became apparent could the variability observed in the plants be explained and plans to control some populations implemented.

Variation in Chromosomes

Variation in plant chromosomes is caused by mutation or during genetic recombination. The former ranges from point mutations in the DNA sequence, through insertions, duplications or deletions, to inversions of parts or whole chromosomes (Sessions 1990) depicted in Figure 5.1.

Figure 5.1 Diagram showing the sources of alterations of chromosome structure (Arrows indicate where the chromosomes break and the shaded regions symbolise the genes affected by the chromosomal rearrangement): (a) deletion removes a chromosomal segment, (b) duplication repeats a segment, (c) inversion reverses a segment and (d) a translocation moves a segment from one chromosome to another, nonhomologous one (adapted from Campbell, 1993, p292).

Genetic recombination most frequently occurs during meiosis when haploid reproductive cells are formed and chromosome segments are exchanged. The source of recombination material may be via

1. sexual reproduction, or
2. the formation of genetically different gametes either via
    a. independent segregation of nonhomologous chromosomes, or
    b. crossing over between homologous chromosomes.

The addition or loss of chromosomes is termed aneuploidy and results in an abnormal chromosome number, e.g. *x* = 11 in section *camara* detailed above. The presence of three or more sets of chromosomes is termed polyploidy and is particular common in the grass family, e.g. in domestic wheat *Triticum aestivum* where 2*n* = 6x = 42. Differences in chromosome number between species resulting from mutation or genetic recombination often leads to reduced fertility of hybrids and the creation of a species boundary.

Previous studies of the karyology of cycads

The diploid number of chromosomes in plants ranges from 4 to c. 250 in angiosperms and extends to 1200 in ferns, but in cycads is limited from 16 to 28. In addition, cycad chromosomes are larger than those in angiosperms, ferns and most other gymnosperms, and this, coupled with the low numbers, makes karyology a useful tool in the study of cycad systematics. Sax and Beal (1934), Marchant (1968), Khoshoo (1961), Moretti (1990) and Kokubugata et al. (2000, 2001) have previously reported on the karyology of cycads and the results of their work are presented in Table 5.1.

Table 5.1 The diploid chromosome numbers of the 11 extant genera of cycads.

Diploid number	genera
16	*Ceratozamia, Stangeria, Zamia* (part)
18	*Bowenia, Chigua, Dioon, Encephalartos, Lepidozamia, Macrozamia, Zamia* (part).
22	*Cycas*
26	*Microcycas*
22, 23, 24, 25, 26, 27, 28	various *Zamia*

Sax and Beal (1934), Marchant (1968), Moretti (1990) and Moretti et al. (1993), found that all genera, except *Zamia,* have a constant chromosome number, and that while it varies in *Zamia,* polyploidy does not occur. Moretti et al. (1993) made the interesting observation that *Zamia* species morphologically adapted to more xeric conditions had higher chromosome numbers but that this situation did not apply across all Neotropical genera. Norstog and Nicholls (1999) suggested the base chromosome number of cycads is *x* = 9.

Moretti (1990) proposed, on grounds of chromosome number and structure, that *Cycas*, *Bowenia, Dioon* and *Stangeria* were the basal extant cycads. This proposal supports the widely accepted phylogenetic analyses by Johnson (1959) and Stevenson (1992) based on morphological character sets. The fact that 2n in *Bowenia* and *Dioon* is 18, but 16 in *Stangeria* and 22 in *Cycas*, suggests that the former taxa may be more closely related than the latter taxa and based on an $x = 9$ with a deletion in *Stangeria*, but that *Cycas* is more distantly related. This is consistent with analyses that consistently show *Cycas* constitutes a monotypic family of great antiquity and may not be closely related to other extant cycads.

The data in Figure 5.1 show that *Zamia*, the most specious and morphologically variable genus, and the likely recent progenitor of *Chigua,* also has the most variable diploid chromosome number. This conforms to the hypothesis that actively speciating genera will have the greatest chromosome variation.

These data indicate that an examination of the various taxa of *Bowenia* might usefully be undertaken as an aide in resolving the systematics of the genus. The questions considered in this aspect of the study were

- do all populations of *Bowenia* have the same chromosome number?
- is the karyology of nominate and putative *B*. *spectabilis* and *B. serrulata* similar? and
- the karyology of *Bowenia* suggest one, two or more species?

If the data indicate no difference in karyology between what is currently considered *B. serrulata* and *B. spectabilis* then, in concert with the previously described morphological vagility, the two might be considered ecotypes of one species. Similarly, if the karyology of nominate and putative *B. spectabilis* is similar, support is gained for the hypothesis that the two are morphological variations in the one species.

5.1 Methods and materials

The chromosomes of specimens of *B. serrulata*, nominate *B. spectabilis,* and putative *B. spectabilis* from Kuranda and Tinaroo were examined using the orcein stain and squash technique (Sessions 1982). This method of examination is particularly applicable to chromosomes of cycads as they are large in comparison and fewer in number than those in many other plant taxa. The collection sites and accession numbers of these specimens are presented in Table 5.2.

Table 5.2 Source and accession number of *Bowenia* material used in the analysis of chromosome number and karyotype.

taxon	source	accession number
Bowenia serrulata	Byfield, central Queensland	TBG122889
B. spectabilis	Mount Bellenden Kerr, North Queensland	TBG122893
B. spectabilis 'Kuranda'	Saddle Mountain, Kuranda, North Queensland	TBG122898
B. spectabilis 'Tinaroo'	Black Mountain, Tinaroo, North Queensland	TBG122895

TBG: Tsukuba Botanic Gardens.

To obtain chromosomes suitable for examination young leaflets were harvested and pre-treated in 4nM 8-hydroxyquinoline at 4º C for 8 hours, fixed in acetic acid (1:3) for 24 hours, and then stored in 70% ethanol at -20ºC. The leaflets were macerated in 45% acetic acid at 60ºC for 3 minutes and then stained in 2% acetic-orcein at room temperature for 4 hours. The stained sample was squashed in 45% acetic acid between a cover slip and glass slide, and examined and photographed at 1000X magnifications using light microscopy.

Three mitotic metaphase plates per taxon were examined and the chromosome number and mean length and arm ratio was calculated for each. The chromosomes were then aligned from longest to shortest and ideograms for each taxon were constructed. Chromosomes were classified using the protocol of Levan et al. (1964), where the position of the centromere is defined by the ratio of the arm lengths (Table 5.3) and described using the terminology in Figure 5.2.

Table 5.3 Terminology for chromosome morphology based on the position of the centromere.

Centromere index	terminology
0.00-0.12	telocentric = t
0.13-0.25	subtelocentric = st
0.27-0.38	submetacentric = sm
0.39-0.50	metacentric = m

Figure 5.2 Diagram showing the commonly used terminology for chromosome morphology and position of bands. M, metacentric; SM, submetracentric; ST, sublelocentric; T, telocentric; c, centrometric; pc, pericentric; i, interstitial; sc, secondary constriction; t, telomeric. (adapted from Hills and Moritz 1990, p160).

5.2 Results

All of the four *Bowenia* taxa examined had a chromosome number of 2n = 18 with chromosomes 11 and 12 in each sample characterised by the presence of microsatellite structures (Figure 5.3 and 5.4).

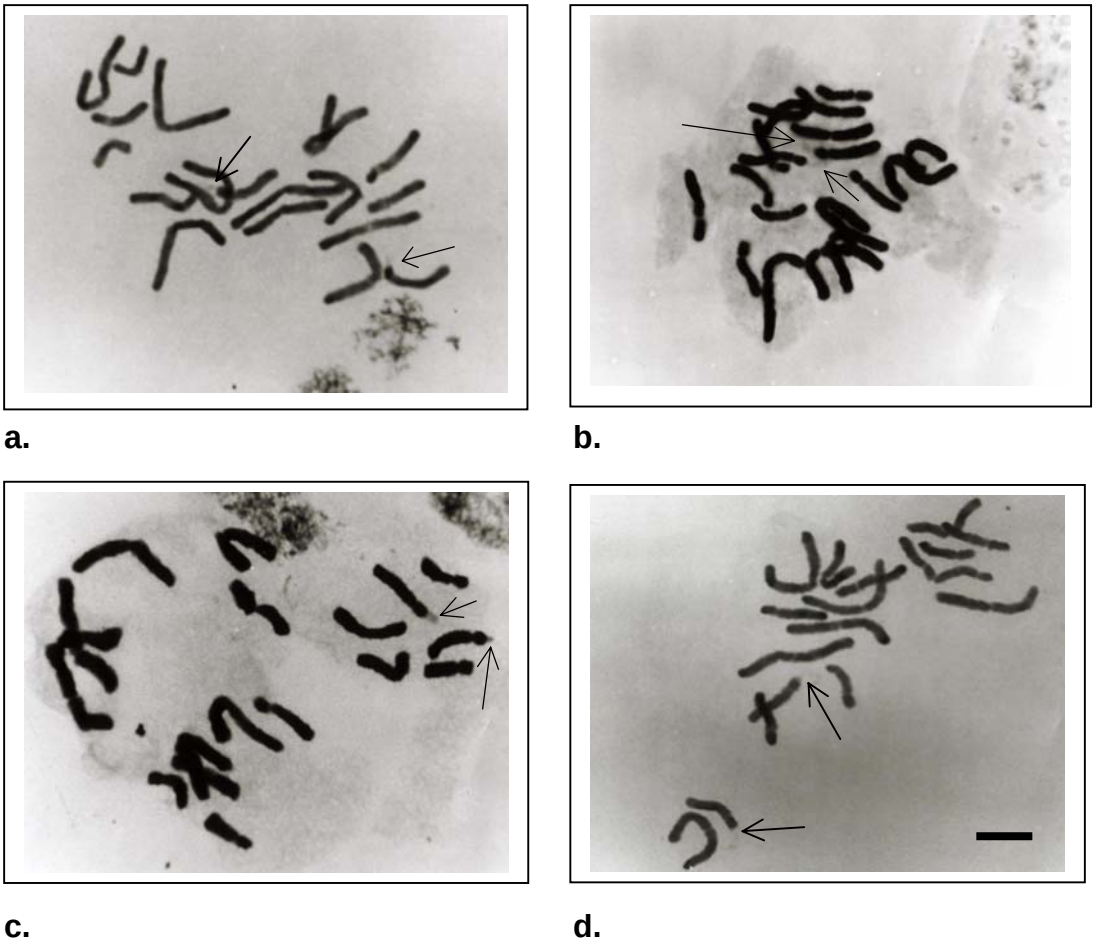


Figure 5.3 Photomicrographs of chromosomes at mitotic metaphase in somatic cells from plants of (a) *B. serrulata* from Byfield and (b) nominate *B. spectabilis* from Mount Bellenden Kerr and (c and d) putative *B. spectabilis* from Kuranda and Tinaroo, respectively. The arrows indicate microsatellite structures. Scale bar = 10µm.

The karyotype data in Table 5.4 shows two discrete karyological profiles for *Bowenia* that on basis of chromosome structure alone distinguishes central and north Queensland taxa. *Bowenia serrulata* has a 10m+3sm+5st chromosome structure, and the northern populations, i.e. nominate *B. spectabilis* and putative *B. spectabilis* from Kuranda and Tinaroo all have an 8m+6sm+4st structure.

The greatest difference between the two is on chromosomes number 9 and 10, which in *B. serrulata* have a metacentric structure but in the northern populations have a submetracentric structure.

Table 5.4 Mean lengths and arm ratios of chromosomes of *Bowenia serrulata* and nominate and putative *B. spectabilis*.

Chromosome no.	*B. serrulata*		nominate *B. spectabilis*		*B. spectabilis* 'Kuranda'		*B. spectabilis* 'Tinaroo'	
	L	R (F)	L	R (F)	L	R (F)	L	R (F)
1	26.6	1.2 (m)	23.7	1.2 (m)	27.3	1.3 (m)	29.3	1.0 (m)
2	25.8	1.1 (m)	23.6	1.1 (m)	25.6	1.1 (m)	29.1	1.1 (m)
3	25.2	1.2 (m)	22.4	1.3 (m)	25.5	1.2 (m)	27.7	1.1 (m)
4	25.0	1.2 (m)	22.2	1.1 (m)	24.5	1.0 (m)	27.3	1.0 (m)
5	24.6	1.1 (m)	20.9	1.2 (m)	24.5	1.3 (m)	27.2	1.3 (m)
6	24.3	1.3 (m)	20.8	1.1 (m)	24.1	1.1 (m)	25.8	1.1 (m)
7	24.2	1.0 (m)	20.3	1.0 (m)	23.2	1.2 (m)	25.2	1.1 (m)
8	23.2	1.1 (m)	18.2	1.1 (m)	21.6	1.1 (m)	23.2	1.3 (m)
9	22.4	1.1 (m)	13.0	2.1 (sm)	16.7	3.4 (st)	17.2	3.5 (st)
10	18.5	1.0 (m)	12.9	4.0 (st)	16.1	2.4 (sm)	16.2	4.2 (st)
11	14.4	4.0 (st)	12.6	2.2 (sm)	15.9	3.1 (st)	15.5	2.4 (st)
12	14.3	3.8 (st)	12.5	2.6 (sm)	15.2	2.9 (sm)	14.9	3.4 (st)
13	13.9	4.6 (st)	12.5	4.4 (st)	14.1	3.1 (st)	14.6	2.2 (sm)
14	12.4	2.8 (sm)	12.2	2.8 (sm)	14.0	2.4 (sm)	14.4	2.6 (sm)
15	11.8	2.7 (sm)	12.2	3.7 (st)	13.8	2.8 (sm)	14.3	2.5 (sm)
16	11.7	3.2 (st)	12.1	2.9 (sm)	12.5	3.2 (st)	13.8	3.3 (sm)
17	11.5	3.1 (st)	11.6	2.9 (sm)	12.5	2.6 (sm)	13.8	2.4 (sm)
18	11.3	2.4 (sm)	11.2	3.3 (st)	12.3	2.4 (sm)	12.6	2.8 (sm)

L = chromosome length (µm), R = arm ratio, F = chromosome form, m = median centromere, sm = submedian centromere and st = subterminal centromere.

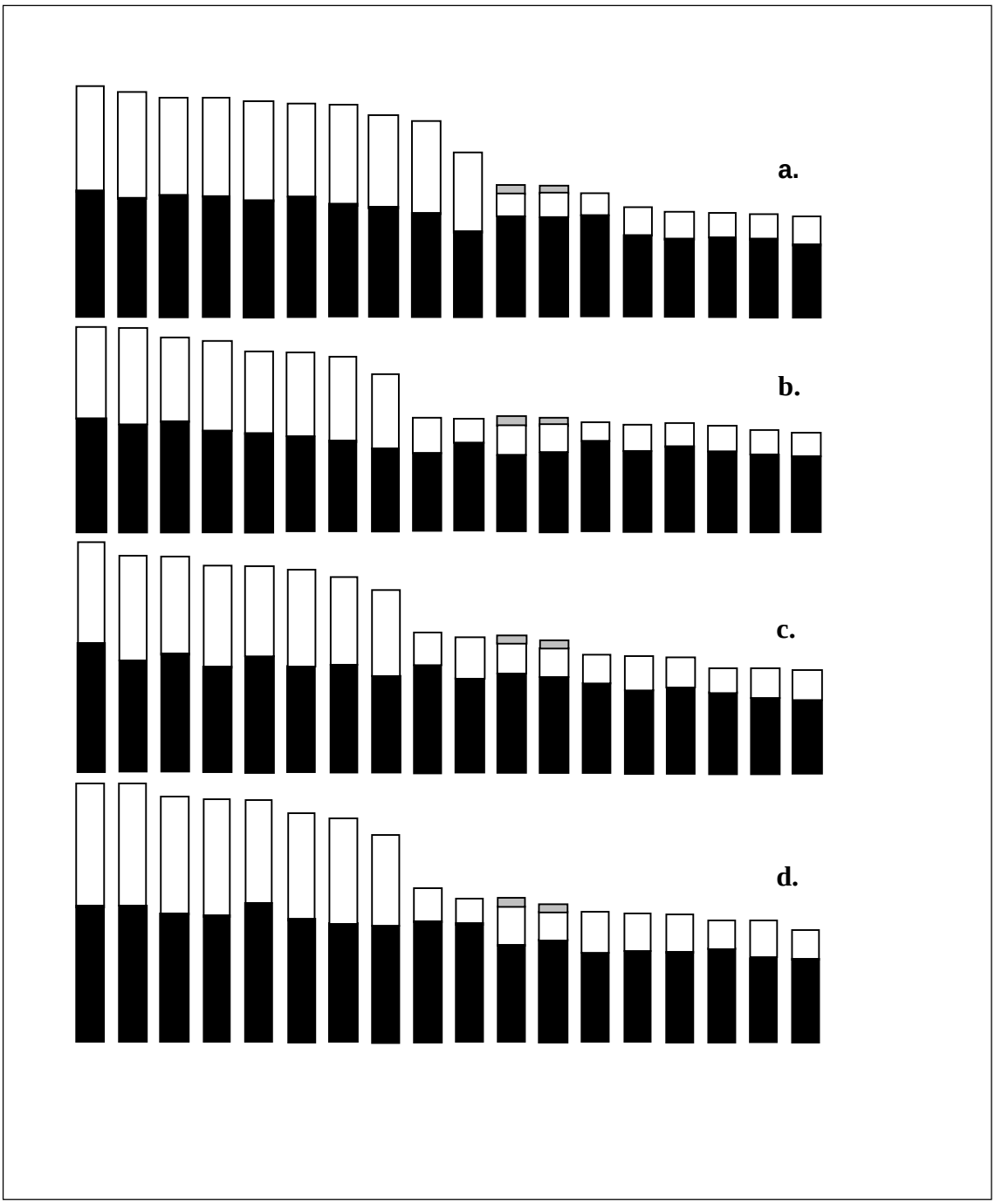


Figure 5.4 Ideograms of the chromosomes of somatic cells of (a) *B. serrulata* from Byfield; (b) nominate *B. spectabilis* from Mount Bellenden Kerr and (c and d) putative *B. spectabilis* from Kuranda and Tinaroo respectively.

5.3 Discussion

The results presented above are subject to some limitations and it is appropriate that these are considered before proceeding with a discussion of them. The first limitation is the extent of sampling; only four of the six populations were sampled, as suitable material from the Starke and McIlwraith Range populations was not available during the study period. As the specimens analysed represented both species and nominate and putative *B. spectabilis,* the assumption has been made that the range of genetic variation related to differences in morphology has been included. The second limitation is the small number of samples analysed; for best and most representative results, multiple collections should have been made. Each of these points needs to be addressed in any further study of the karyology of the genus.

5.3.1 Chromosome number

The 2n = 18 chromosome number of *B. serrulata* and nominate *B. spectabilis* reported here for corresponds with that previously reported by Sax and Beal (1934), Khoshoo (1961), Marchant (1968) and Moretti (1990). The value of the reconfirmation of the chromosome number of the three representatives of *Bowenia* is that it dispenses with any suggestion of a variable number in different taxa (species) as has been reported in *Zamia*.

5.3.2 Morphology

The similarity of the karyotype data of the Tarzali, Tinaroo and Kuranda populations supports the concept of taxonomic synonymy of them and therefore of a morphologically variable *B. spectabilis*. The most likely reason for this karyotypic homogeneity is that within the life span of extant plants these populations would have been contiguous or nearly so, and their pollination vector, *Miltotranes prosternalis,* which is able to fly extended distances, could maintain gene flow between these now disjunct populations. This situation contrasts sharply with that between *B. spectabilis* and *B. serrulata*, where their large physical separation and the obligate and species-specific nature of their pollination, suggest that any genetic differentiation between them is due to mutation and natural selection.

The data on the rate of genetic evolution in cycads is highly variable; Moretti (1990) reports it appears particularly low in *Cycas*, but variable in others, e.g. *Zamia.* The former genus is particularly long-lived and Pangaean in origin but now spread across both hemispheres and several botanical regions whereas *Zamia* is Gondwanan and is restricted to the Americas (Jones 1993). The prevailing wisdom has been that variation is high in *Zamia* because it is actively speciating as it adapts to the numerous niches available in its area of distribution and because that area is geologically active and new niches have/are evolving at a rapid rate. Conversely, *Cycas* is thought to have a more restricted potential for morphological variation and stabilised genetically in long existing habitats.

I suggest the latter in particular is erroneous in so far as the genus occupies a range of habitat types, from complex rainforest to arid lands, and exhibits substantial morphological variation, e.g. subterranean caudices and divided leaves in *C. micholtzii* versus erect stems and pinnate leaves in most species. Furthermore, there is evidence (Jones 2002), that *Cycas* in Queensland is actively speciating – certainly the distinction between taxa in the *C. media* complex is indistinct (Forster, pers. comm. 2002). I believe it is likely that views on the rate of genetic evolution in *Cycas* will have to be reviewed after closer examination of the Australian taxa.

In respect to *Bowenia* the palaeoclimatological evidence indicates the period of separation of *B. serrulata* and *B. spectabilis* to be 12-15 million years (Adams 1992; Truswell 1993). These data provide an opportunity to examine the change in their karyotypes over that time; any differences will be due to mutation and selection due to stochastic effects. The results reported here for *Bowenia* indicate very little difference in the karyology of the two long-separated taxa. This may best be explained by the fact that the distribution and habitat type of the living and fossil taxa suggests they occur or occurred in refugia with similar climatic parameters that is likely to select for homogeneity. The small degree of difference in the karyology of *B. serrulata* and *B. spectabilis* indicates that the rate of genetic change in this genus is extremely low; this observation concurs with those of Moretti et al. (1993) in meso-American Zamiaceae, Schama et al. (1999) in the *Macrozamia heteromera* clade in Zamiaceae, and Gonzales et al. (2002) in *Ceratozamia* (Zamiaceae).

The presence of microsatellites on chromosomes 11 and 12 in the sampled populations adds further weight to the suggestion that some or all cycads may have ‘sex’ chromosomes. The possibility of sex chromosomes in *Cycas* has previously been suggested (Jones 2002) and further work in needed to clarify the structure and function of the features in *Bowenia.* Regrettably the gender of the plants sampled is not known so no insight can be gained from that source.

The karyotypes described here for *B. serrulata* and *B. spectabilis* differ from those described by Sax and Beal (1934) who reported a 12m+6st morphology of the chromosomes of the former, and Moretti (1990) who reported the same structure for the latter but did not report on *B. serrulata*. These data suggest that Moretti may have confused the two species or inadvertently reported for the wrong species; as Moretti (1990) reported his accession numbers it should be possible to confirm, by reanalysis, if this possible error actually occurred.

The results presented in this chapter serve three functions. The first is to provide direct evidence to this study of the systematics, the second is to provide an indication of the rate of genetic change in the genus, and the third is to provide two character classes, i.e. chromosome number and chromosome morphology, for use in later phylogenetic analyses.

Chapter 6 Phylogenetic analysis, biogeography and systematics

In this chapter I describe the phylogeny, biogeography and systematics of *Bowenia* in an attempt to determine the number of species and the taxonomic status of disjunct populations in the genus and summarise the biology, ecology and relationships of them. The phylogenetic analysis is based in part on the character data described in the previous chapters of this thesis and in part on data recorded in the published literature on cycads. The biogeography of the species of *Bowenia* recognised in the phylogenetic analysis is explored in light of geological, climatological and ecological changes that have occurred during the life of the genus, to explain the processes that have given rise to the extant species and to explain the distribution of them. The systematics presented here of the genus is a synthesis of the results of the phylogenetic and biogeographical analyses and data described in this document and in the literature and provides an overview of the biology, diversity, relationship (classification) and taxonomy of the species recognised.

6.1 Phylogenetic analysis

A phylogenetic approach was adopted in this study as it describes the evolutionary chronicle of *Bowenia* from the fossil evidence to that contained in the chromosomes in populations and the environmental and ecological changes and parameters that have acted on individuals, demes and species over time. It is important to note that in this study the species resulting from the analysis are an ontological issue recognised using the criteria of the Ecogenetic Species Concept (ESC) (Levin (2000) while the analysis process is epistemological - the analysis technique should not be confused with the Phylogenetic Species Concept (PSC) elucidated by Nixon and Wheeler (1990).

Phylogenetic analyses are attempts to arrange organisms into groups on the basis of their evolutionary relationships (Judd et al. 1999) and build on the concepts proposed by Darwin (1859, 1871) and subsequently elaborated by generations of biologists but most notably Hennig (1913-1976), Zimmerman (1892-1980), and Wagner (b. 1920). Phylogenetic systematists echo Darwin in that they focus on the processes of descent with modification and separation of lineages (de Queiroz and Gauthier 1992) and document the evolutionary processes that result in the present diversity, e.g. Lack (1947) and Grant (1986, 1999) in respect to Darwin's finches on the Galapagos Islands.

The underlying principals observed in phylogenetic analyses are,

1) the taxa described are monophyletic; that is, have a common ancestor, and form discrete clades recognised by a shared derived character state (synapomorphy) in all their members, and
2) that evolution has and is occurring.

In addition, the analyses observe Ockam's razor; i.e. 'the simplest explanation of the data is most likely to be correct'; this principal of simplicity is referred to as parsimony. The most parsimonious tree resulting from a phylogenetic analysis is the one involving the least number of evolutionary changes.

The adoption of a phylogenetic approach contrasts with the possible choice of phenetic or phyletic approaches. Phenetic analyses are based on the overall similarities of species, which are separated from others by a gap in variation, without consideration of evolutionary processes and phylogeny in the analysis; for example, apparently similar taxa, e.g. cacti and morphologically and ecologically similar euphorbs, can be assigned to the same but a polyphyletic group not related by way of a common ancestor taxa, using this methodology. An example of the use of a phenetic approach to the classification of cycads is in the use of allozyme data, for example by Sharma et al. (1998, 1999) in their studies of species in *Macrozamia* section *Parazamia*; their results distinguished 'genetic' species and groups within these species but did not explain the relationships (phylogeny) of them. On the other hand, phyletic analyses assume transformation of genetic and phenotypic properties of organisms within a lineage without any splitting of the lineage (Eldridge 1989). They are rejected in this study as inappropriate to explain the long physical separation of a previously more wide distributed lineage in *Bowenia* and not sufficiently considerate of the temporal aspects in the ontogeny of the genus.

The analysis described here was conducted using data from populations of *B. serrulata* and nominate and putative *B. spectabilis* and *Stangeria eriopus* (Kunze) Baillon as a comparative outgroup. Outgroups are included in phylogenetic analyses to allow the production of a 'rooted tree' and thus allow the inference of evolutionary processes in the taxa being considered and are chosen on the basis of being the closest unequivocally distinct relative.

Stangeria eriopus was chosen as the 'outgroup' in this analysis as it is considered (Stevenson 1992) most closely related, i.e. a sister group', to *Bowenia* (Figure 6.1), being included in Stangeriaceae in the subfamily Stangerioideae (Johnson 1959, Stevenson 1992).

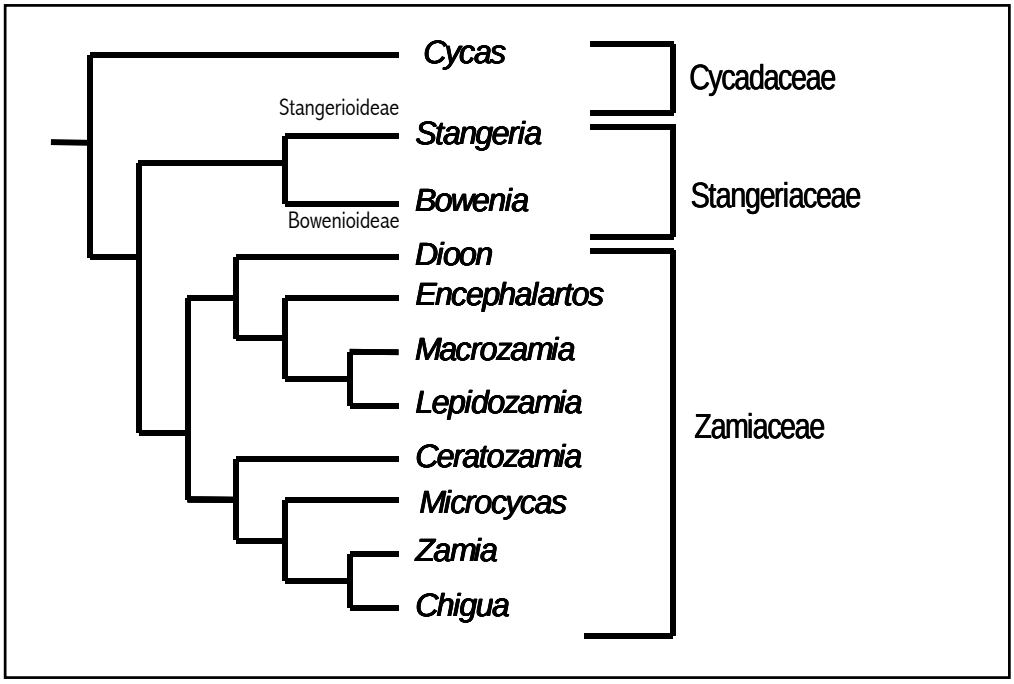


Figure 6.1 Phylogeny of the genera and families of extant cycads showing the relationship and subfamilies of *Bowenia* and *Stangeria* (from Stevenson 1992).

As this study is concerned with the infrageneric systematics of *Bowenia* including the status of the nominate *B. spectabilis,* it is initially necessarily to review how the currently recognised species are described in the literature. The description of *Bowenia* by Hill (1998) in Volume 48 of the *Flora of Australia* and of *Stangeria eriopus* in Vorster and Vorster 1986, Jones (1993) and Crouch et al. (2000) is used for this purpose. The morphological characteristics of the species have been extracted from the descriptions in the literature and are listed in Table 6.1 to allow their comparison. Of the 24 characters listed, only four record any variation between the putative species of *Bowenia* and the potential for distinguishing between them. With the exception of the data for pinnules, which occur in the bipinnate *Bowenia* but not in the pinnate *Stangeria* and thus cannot be compared, the morphological characteristics of the two genera are similar. These data indicate why, on bases of morphological characteristics, the two genera are often both assigned to Stangeriaceae.

Table 6.1 Morphological characteristics of *B. spectabilis* and *B. serrulata* (from Hill 1998) and *Stangeria eriopus* (from Vorster & Vorster 1986; Jones 1993 and Crouch et al. 2000) and their potential for differentiating the species of *Bowenia.*

character	**species**			
	B. spectabilis	***B. serrulata***	***S. eriopus***	**potential for differentiation of the species of *Bowenia* (Y/N)**
Stem	≤10 cm diam.	≤25 cm diam.	≤20 diam.	Y
# of leaf- and cone-bearing branches	1-5	5-20	5-20	Y
Leaves in crown	1-7	6-16	6-20	Y
Pinnae	4-10	6-16	10-40	Y
Petiole length	≥100 cm	≥100 cm	10-80 cm	N
Pinnae length	100-200 cm	100-200 cm	n/a	N
Pinnae width	≤100 cm	≤100 cm	n/a	N
Pinnule shape	obliquely lanceolate, decurrent at base	obliquely lanceolate, decurrent at base	n/a	N
Pinnule number	7-30 per pinnae	7-30 per pinnae	n/a	N
Pinnule length	7-15 cm	7-15 cm	n/a	N
Pinnule width	1.5-4 cm	1.5-4 cm	n/a	N
Pollen cone	stalked	stalked	stalked	N
Pollen cone shape	ovoid	ovoid	ovoid	N
Pollen cone size	5 x 25 cm	5 x 25 cm	4 x 25 cm	N
Sporophyll	broadly cuneate, distally dilated and truncate	broadly cuneate, distally dilated and truncate	triangular to rhomboid; distally dilated and truncate	N
Female cone	sessile	sessile	stalked	N
Cone shape	ovoid to globose	ovoid to globose	ovoid to ellipsoid	N
Cone size	10 x 10 cm	10 x 10 cm	8.3 x 6.1 cm	N
number of sporophyll	c. 8 ranks	c. 8 ranks	c. 8 ranks	N
shape of sporophyll	expanded ends, ± hexagonal	expanded ends, ± hexagonal	expanded ends, ± hexagonal	N
size of sporophyll	30-55 mm wide	30–55 mm wide	35-60 mm wide	N
size of sporophyll	c. 15 mm tall	c.15 mm tall	c. 15 mm tall	N
seeds	radiospermic, ≤ 32 x 18 mm	radiospermic ≤ 32 x 18 mm	radiospermic 33.5 x 22.5	N

In addition, Hill (1998) notes that plants with pinnules entire or with a few irregularly lacerate are *B. spectabilis* and notes 'individuals with serrate pinnae (sic) occur occasionally in most populations throughout the range of this species'. This do not recognise the status of extensive populations of plants with all pinnules serrate that exist in north Queensland.

6.1.1 Methods and materials

A phylogenetic analysis using the twelve characters in the four character classes shown in Table 6.2 was conducted of six populations of *Bowenia*, i.e. *B. serrulata*, nominate *B. spectabilis* from Tarzali and putative *B. spectabilis* from Tinaroo and Kuranda on the Atherton Tableland and Starke and McIlwraith Range on Cape York Peninsula and a *Stangeria eriopus* outgroup. The populations of *Bowenia* included in the analysis represented the range in morphological variation and distribution of the genus. The character classes were selected after consideration of the data and results presented in this thesis and a review of data in the literature. Not all characters were analysed for all taxa, as information for some characters and character states was not available; e.g. for the Starke and McIlwraith Range populations, which are poorly collected and were not visited during the study. In addition, due to the fact that only *Bowenia* in the cycads has pinnules (2nd order leaflets) data relating to them could not be compared with equivalent data for the outgroup.

Table 6.2 A summary of the character classes and characters used in this phylogenetic analysis of *Bowenia* and a *Stangeria eriopus* outgroup.

Class	character	
Morphology	1	Leaf form: pinnate or polypinnate
	2	Leaf form: number of pinnae on leaf
	3	Pinnule form: entire or serrate margins
	4	Pinnule morphometrics: length
	5	" " : width
	6	" " : length to width ratio
	7	" " : number of adaxial serrations
	8	" " : number of abaxial serrations
	9	" " : ratio of number of ad- to abaxial serrations
Pollination	10	Pollination mechanism : anemophilic or entomophilic and identity of insect vector
Insect Herbivory	11	Taxa browsed or not by *Lilioceris* sp. leaf beetles.
Karyology	12	Chromosome number and morphology

6.1.2 Characters used in the phylogenetic analysis

Character 1 – Leaf form: pinnate or polypinnate

Bowenia is unique in the cycads in that all species have polypinnate foliage; *Stangeria eriopus* and all other extant taxa, except *Cycas debaoensis* and *C. multipinnata* in the Cycadaceae, which are bipinnate, have pinnate foliage (Jones 2002).

It is difficult to determine which state in the characteristic in the cycads is ancestral (plesiomorphic) and which is derived (synapomorphic). The reason for this lies in the likely origin and the great age of the cycads. For example, the progymnosperms, from which the cycads are thought to have evolved (Stewart 1983; Crane 1985; Doyle and Donoghue 1986; Beck and Wright 1988), included taxa with pinnate and bipinnate foliage, e.g. *Archaeopteris* (Beck 1962a), and either might be the ancestral condition. In addition, the fossil record of cycads and distinct identities of the genera suggests that some are not closely related and that intermediate forms that may assist in the resolution of the phylogenetics of the order are long extinct and not yet found. The answer to this conundrum on the plesiomorphic condition in foliage morphology in cycads remains elusive and further consideration of it is outside the parameters of this study; accordingly the states for this character are assigned a presence/absence status and not polarity inferring ancestral or derived status.

Data presented in Chapters 1 and 2 and in Wilson (1996) indicate that leaves in *B. serrulata* have bi- and/or tripinnate foliage but that the latter does not occur in either nominate or putative *B. spectabilis* including the Starke and McIlwraith Range populations, which have only bipinnate foliage. Further, the data (Wilson 1996) indicate that bi- and tripinnate leave occur on the same plant and thus the latter are an indication of variation within the species. As a result, this difference in foliage in the central and north Queensland taxa may be phylogenetically informative. In this analysis the states for Character 1 are,

0. leaves pinnate
1. leaves bipinnate.
2. bipinnate and/or tripinnate.

Character 2 – number of pinnae on a leaf

Hill (1998) used the mean number of pinnae on leaves and the physiognomy of margins of pinnules in a binary key to differentiate nominate and putative *B. spectabilis* and *B. serrulata.* Hill indicated that *B. serrulata* had significantly more pinnae on a leaf than both nominate and putative *B. spectabilis* and as a result the latter could be distinguished from *B. serrulata* using this metric. The veracity of this method of distinguishing different taxa of *Bowenia* was examined and reported in Chapter 2.

Counts of pinnae found that comparison of the mean number of them is of use in separating *B. serrulata* and putative *B. spectabilis* when the provenance of the collection is unknown, as the range in number, 5-16 and 6-16 respectively, is nearly identical. However, the result of a one-way ANOVA shows a significant difference ($F = 62.871$, $DF = 2$, $P = <0.001$) in mean number of pinnae on leaves of *B. serrulata* and nominate and putative *B. spectabilis* when a sample size of leaves from 100 plants is considered. This result indicates the mean number of pinnae on a leaf may have some value, with other characters, in a phylogenetic analysis of *Bowenia* and it is included for that reason.

The foliage of *Stangeria eriopus* is pinnate and Vorster and Vorster (1985) and Jones (2002) indicate the number of pinnae (1st order leaflets) on a leaf ranges from 10-50 and as a result, a nominal mean value of 30 was assigned for this for this species. In this analysis the states for Character 2 were assigned rank values without inference of polarity and are,

0. mean number of pinnae = 8.24 (i.e. nominate *B. spectabilis*)
1. mean number of pinnae = 9.04 (i.e. putative *B. spectabilis*)
2. mean number of pinnae = 11.25 (i.e. *B. serrulata*).
3. mean number of pinnae = c.30 (i.e. *Stangeria eriopus*).

Character 3 - Pinnule form: entire or serrate margins

The margins of pinnules of nominate *B. spectabilis*, which occurs in mesophyll vegetation types, are entire (Jones 1993; this study). In contrast, those in populations of putative *B. spectabilis* and of *B. serrulata*, in notophyll and tall wet forest environs with a more variable and stressful climate, are serrate margin (this study).

The margins of pinnules of fossil *Bowenia,* which palaeoclimate and palaeoecological data indicate occurred in habitats and environmental conditions similar to those of *B. serrulata* (Hill 1978, 1999), are serrate (Hill 1978, 1998; Scriven 1993; Christophel, pers. comm. 1999). The foliage of *Stangeria eriopus* is pinnate and pinnules (2nd order leaflets) do not occur, and as the characteristic is not expressed, it is not available for comparison.

In this study, pinnules with entire margins, is considered the ancestral character state; this concurs with the view expressed by Hill (1998). The are two reasons for this decision; the first is that the core distribution of nominate *B. spectabilis* is in complex mesophyll vine forest in mesotherm refugia that have persisted since the Mesozoic and allowed some taxa, including *Bowenia*, to survive environmental changes over the intervening period (Webb and Tracey 1961; Nix 1991). Therefore, it is considered the form of nominate *B. spectabilis*, the taxon that currently exists in these refugia, will be most like the ancestral type. The second reason for the decision is the morphological data in Chapter 2, which demonstrates the phenotypic plasticity in *Bowenia*, the insect-association data in Chapters 3 and 4, which shows that they do not distinguish between nominate and putative *B. spectabilis*, and karyological data in Chapter 5 that indicates that nominate and putative *B. spectabilis* are not genetically different, that indicate serrate morphology of pinnules in putative *B. spectabilis* is primarily the result of intraspecific phenotypic variation, rather than genetic differentiation, in response to more stressful environmental conditions.

In contrast, in *B. serrulata,* pinnule with serrate margins is considered a derived and stable character state for the following reasons. (a) *B. serrulata* with pinnules with entire margins have not been found despite extensive searches (Chapter 2), (b) F1 hybrids of nominate *B. spectabilis* and *B. serrulata* also have pinnules with serrate margins (Chapter 2), and (c) studies show significant karyological differences between *B. serrulata* and *B. spectabilis* (Chapter 5).

In this analysis, and so as not to prejudge the phylogenetic status of putative *B. spectabilis*, the designated states for Character 3 are,

0. pinnule margins entire
1. pinnule margins serrate
5. characteristic not expressed as pinnules not present.

Characters 4 – 9: Pinnule morphometrics

The results of one-way Analysis of Variance (ANOVA) and Tukey Tests analyses (Table 6.3, 6.4) indicate that the four populations sampled, i.e. *B. serrulata*, nominate *B. spectabilis* from Tarzali and putative *B. spectabilis* from Kuranda and Tinaroo, can be differentiated by some morphometrics of the pinnules (see Appendix I for the raw data used in the analyses).

The metrics considered were length, width and length-width ratio of pinnules, and number of adaxial and abaxial serrations and ratio of them on pinnules with serrate margins and of vascular bundles apices on those, i.e. nominate *B. spectabilis*, that have entire margins. In respect of the latter, the assumption was made, after an inspection of 100 pinnules each of *B. serrulata* and putative *B. spectabilis,* was serrations on the margins of pinnules are directly related to the number of vascular bundle apices. Significant sample sizes were not available from the Starke and McIlwraith Range populations and as a result data for them are not included.

Table 6.3 Results of a one-way ANOVA of differences in pinnule morphometrics in four populations of *Bowenia*.

pinnule metric	analysis	F	DF	P
length	1 – way ANOVA	21.502	3	<0.001
width	1 – way ANOVA	66.22	3	<0.001
Length-width ratio	1 – way ANOVA	57.919	3	<0.001
Number of adaxial serrations	1 – way ANOVA	138.163	3	<0.001
Number of abaxial serrations	1 – way ANOVA	286.674	3	<0.001
Ratio of adaxial to abaxial serrations	1 – way ANOVA	119.500	3	<0.001

For the purpose of the analysis, the states for pinnule morphometric data were assigned rank values, with the measurements reported including plus and minus the standard error in each case and the ratios as raw scores. As pinnules are not present in *S. eriopus* the character state for these characters is again scored as 5 = characteristic not expressed.

Table 6.4 Rank grouping by Tukey Test analysis of differences in pinnule morphometrics in four populations of *Bowenia*.

	rank			
pinnule characteristic	0	1	2	3
4 length	Byfield >98.4-100.9<	Kuranda and Tarzali >104.46-109.44<	Tinaroo >113.95-116.75<	–
5 width	Tarzali and Byfield >23.51-24.69<	Tinaroo >24.96-27.74<	Kuranda >30.22-31.04	–
6 L to W ratio	Kuranda 3.5	Byfield and Tinaroo 4.2 and 4.4	Tarzali 4.6	–
7 adaxial serrations	Tarzali and Tinaroo >14.2-14.61<	Kuranda >16.62-17.13<	Byfield >20.11-20.77<	–
8 abaxial serrations	Tarzali >8.85-9.15<	Tinaroo >10.09-10.35<	Kuranda >13.41-13.81<	Byfield >16.20-16.78<
9 Ad- to abaxial ratio	Kuranda and Byfield 1.27 and 1.27	Tinaroo 1.42	Tarzali 1.63	–

Stem morphology

Chamberlain (1912) used the size and branching of the subterranean stem to distinguish *B. spectabilis* and *B. serrulata*; describing the stem of the former as small and sparsely branched and that of the later as large and many branched. In contrast, in observations of mature *B. spectabilis,* botanists Garry Sankowsky and Peter Radke, found these plants had subterranean stems identical in form to those of *B. serrulata*.

As a result of these observations, an investigation of this characteristic was undertaken in this study. The results (Table 6.5) confirm the observations of Sankowsky and Radke in respect to *Bowenia* and concur to those by Vorster and Vorster (1986) and Jones (2002) of a similar situation in *Stangeria eriopus* with plants in grasslands having large branched stems while those in forested habitats often, but not always, have a simple and unbranched stem.

Table 6.5 Caudex and pinnule form and substrate type in *Bowenia serrulata* and nominate *B. spectabilis*.

Sample population	caudex form	pinnule form	substrate type
Byfield	large and branched	serrate margin	sand, sandy loam
Tarzali	large and branched	entire margin	fine red basalt soil
	and		
	small and sparsely branched	entire margin	

The results indicate that stem morphology in *Bowenia* is variable within species and does not reliably distinguish *B. spectabilis* and *B. serrulata* as had previously suggested by Chamberlain (1912). It is apparent that further work is required on this subject and that many more mature plants, particularly of nominate and putative *B. spectabilis*, need to be excavated to more adequately determine the variation in stem morphology. In the current context, stem morphology cannot be used as a character as it is uninformative in that it does not contribute to our knowledge of infrageneric relationships in *Bowenia*; for this reason the character is not included in the phylogenetic analysis.

Character 10: Pollination

The studies described in Chapter 3 show that pollination in *Bowenia* is entomophilic. In contrast, comprehensive field studies have failed to find any sign of entomophily in *Stangeria eriopus* and pollination in this species is presumed (Vorster and Vorster 1986; Donaldson 2001; Crouch et al. 2000), to be anemophilic. However, Jones (2002) notes that plants removed from their natural habitat can produce fertile seed and that it has been suggested that a generalist rather than host-specific insect pollination association may exist. It may be the *ex situ* pollination recorded in this species is entirely opportunistic as both Crouch et al. (2000) and Jones (2002) indicate that neither male nor female cones are thermogenic or produce aromatic volatiles likely to attract an insect pollination vector. As a result of the above data, for the purposes of this analysis, pollination in *S. eriopus* is regarded as anemophilic.

Another character that work presented in this thesis indicates may have value in a phylogenetic analysis of *Bowenia* is the identity of the pollination vector. The results of work described in Chapter 3 show that the obligate pollination vector of *B. serrulata* is the weevil *Miltotranes subopacus* (Lea) and that of nominate and putative *B. spectabilis* is *M. prosternalis* (Lea), and that both species are involved in ‘brood site reward’ pollination syndrome with their hosts.

Zimmerman (1994) indicated that *Miltotranes* weevils only occur in species-specific associations association with *Bowenia,* and this fact and that they belong to a basal clade of weevils dating from the Jurassic (Crowson 1991), have adapted to the reproductive cycle and toxins contained in their hosts, all indicate that the association is of considerable antiquity.

As such, and coupled with other examples in the literature, e.g. Tang (1987a), Norstog et al. (1986), Norstog and Fawcett (1989) and Terry (2002), of species-specific entomophily in cycads, these conditions conform to those elucidated by Futuyma (2000) as being indicative of the evolutionary history of the host.

Insect pollination in cycads is not exclusive to *Bowenia*, already being well described for several taxa in the Zamiaceae (Norstog and Stevenson 1980; Tang 1987b; Norstog et al. 1986, 1992; Donaldson et al. 1995; Terry 2001). However, as this study has demonstrated insect-mediated pollination in *Bowenia* to be species-specific the different states of the characteristic may be phylogenetically informative and as a result are included in the analysis.

In this study the character state of anemophilic pollination is considered the plesiomorphic condition and entomophilic pollination is considered the derived condition. In this analysis the states for Character 10 are,

0. pollination anemophilic
1. pollination entomophilic and the vector is *Miltotranes subopacus* (Lea)
2. pollination entomophilic and the vector is *M. prosternalis* (Lea).

Character 11: Insect Herbivory

The results of an inquiry of the specificity of phytophagous insect-*Bowenia* associations reported in Chapter 4 show the leaf beetle *Lilioceris nigripes* (Fabricius) (Coleoptera: Chrysomelidae: Criocerinae) browses on the foliage of nominate and putative *B. spectabilis* but not *B. serrulata*. This selective feeding is despite the fact the range of *L. nigripes* overlaps that of *Bowenia* and that it browses on *Cycas ophiolitica* growing nearby. In addition, the results of the feeding trials conducted to determine the acceptability of foliage from northern and southern populations of *Bowenia* (this study) indicate that *L. nigripes* will not browse *B. serrulata* even in the absence of other foodstuff.

The above data indicate that *L. nigripes* distinguishes two chemotypes/species of *Bowenia* and does not differentiate nominate and putative *B. spectabilis*. Support for this hypothesis is provided by studies by Windsor et al. (1999) in Central America where a species of Aulascoscelinae leaf beetle, of similar antiquity and phylogenetically close to *Lilioceris* (Jolivet 1988), distinguishes between *Z. skinneri* and *Z. neurophyllidia,* species initially considered conspecific but more recently found to be discrete (Jones 2002).

The genus *Lilioceris* also occurs in South Africa (Schmitt 1988), and although *L. nigripes* does not, the opportunity exists for another species to adapt to feeding on *S. eriopus*. However, extended and careful *in situ* observations show that beetles of this genus do not feed on *S. eriopus* (Crouch et al. 2001*)*.

After consideration of the above data, in this analysis the states for Character 11 are,

0. not browsed by *Lilioceris*
1. browsed by *L. nigripes.*

Character 12 - Karyology

The chromosome number and morphology is usually distinct and constant at a species level, and, although exceptions are quite common (Futuyma 2000), can often be used to distinguish between taxa when other characters, e.g. morphology, may not be definitive. In addition, cycad chromosomes are larger than those in angiosperms, ferns and most other gymnosperms, and this, coupled with the low numbers (*2n* from 16 to 28), makes karyology a useful tool in the study of cycad systematics. Data presented in Chapter 5 show that the diploid chromosome number in *Bowenia serrulata* and nominate and putative *B. spectabilis* is 18. This concurs with the results previously published in Sax and Beal (1934), Khoshoo (1961), Marchant (1968), Moretti (1990) and Kokubugata et al. (2000, 2001). In contrast, other data in the same literature and in Kokubugata and Hill (2002) show that 2*n* = 16 in *Stangeria eriopus*

The 2*n* values of 18 and 16 for *Bowenia* and *Stangeria* are states in this character class but in this analysis have not been defined as ancestral or derived. This is because the basal chromosome number, *x*, for the cycads is debatable and which value one adopts affects the assigning of ancestral and derived appellations. The reason for this is as follows; Norstog and Nicholls (1997) suggested *x* = 9 in the cycads, this concurs with a base haploid number of 9 in *Bowenia* and in Zamiaceae, but not with assumed base haploid values of 8 in *Stangeria* (2*n* = 16) and 11 in *Cycas* (2*n* = 22) respectively. This presents a conundrum if, as widely accepted (Doyle and Donoghue 1986; Crane 1988; Stevenson 1992; Stewart and Rothwell, 1993), Cycadaceae is the most ancient of cycads and Cycadales is monophyletic, then *x* = 11 is the ancestral base number and all others, including *x* = 9, derived.

This is at odds with Marchant (1968) who suggests that higher chromosome numbers are derived from lower numbers, but in accordance with the observation by Moretti et al. (1988), that arid-adapted species in the most specious and morphologically variable genus, i.e. *Zamia*, have higher base numbers. An equally likely explanation, especially given the distinct nature of Cycadaceae but the more equivocal delineation of Stangeriaceae and Zamiaceae, is that they are not closely related and indicative precursory and/or intermediate species are long since extinct. This impasse, the resolution of which is outside the parameters of this study, is therefore, the reason for not inferring ancestral and derived states in this characteristic.

The chromosome number of 2*n* = 18 reported for *B. serrulata* and nominate and selected putative *B. spectabilis* discounts any possibility of different chromosome numbers for different populations of *Bowenia*. It was necessary to establish this fact as data in the literature show that the chromosome number for *Zamia* varies with species and there was the potential that the same may have occurred in *Bowenia*.

The data reported in Chapter 5 also show the karyotypes of nominate *B. spectabilis* and putative *B. spectabilis* from Kuranda and Tinaroo is 8m+6sm+4st while that of *B. serrulata* is 10m+3sm+5st. These data support the concept of just two species of *Bowenia* and that nominate and all the tested putative *B. spectabilis* are sympatric.

Karyological data are potentially phylogenetically informative and as a result those for *Bowenia* are included in the following analysis. Kokubugata et al. (2001) describe the karyotype of *Stangeria eriopus* but as *2n* in *Stangeria* and *Bowenia* is different (Sax and Beal 1934; Marchant 1968; Moretti 1990; Kokubugata et al. 2000, 2001, this study), the data are not comparable and are not included in this analysis.

In this analysis the states for Character 12 are,

0. diploid chromosome number = 16
1. diploid chromosome number = 18 and karyotype = 8m+6sm+4st
2. diploid chromosome number = 18 and karyotype = 10m+3sm+5st.

6.1.3 Phylogenetic analysis

The characters and character values described above and listed in Table 6.6 were included in the analysis of the infrageneric phylogenetics of *Bowenia.*

Table 6.6 Characters and character states for six populations of *Bowenia* and *Stangeria eriopus* in the analysis of the infrageneric phylogenetics of *Bowenia*.

	Character numbers and states											
taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Bowenia serrulata	2	2	1	0	0	1	2	3	0	1	0	2
B. spectabilis Tarzali	1	0	0	1	1	2	0	0	2	2	1	1
B. spectabilis Tinaroo	1	1	1	2	2	1	0	1	1	2	1	1
B. spectabilis Kuranda	1	1	1	1	1	0	1	2	0	2	1	1
B. spectabilis Starke	1	9	0	9	9	9	9	9	9	9	1	9
B. spectabilis McIlwraith	1	0	0	9	9	9	9	9	9	2	1	9
Stangeria eriopus	0	3	5	5	5	5	5	5	5	0	0	0

9 = missing data

The analysis was conducted using the program PAUP (Phylogenetic Analysis Using Parsimony) +Version 4 (Swofford 2000) on a Macintosh desktop computer. Due to the small size of the data set and the resulting lack of constraints on processing time an exhaustive search option with retention of the ten most parsimonious trees setting was used in the analysis. The options applied in the analysis were, branches collapsed if maximum branch length was zero, 'MULTREES' option allowing the generation of multiple trees in effect and topological constraints on them not enforced. No characters were 'weighted' in comparison with any other as they were all considered to be of equal status.

The multiple results of the initial analysis were subjected to a strict consensus analysis to obtain a final phylogenetic tree that contains only those monophyletic groups common to all trees. Finally, in order to determine support for the strict consensus tree, a 50% majority–rule bootstrap analysis, with a simple addition sequence, tree-bisection-reconnection (TBR) branch-swopping algorithm and 'MULTREES' options in effect, was conducted.

6.1.4 Results of the phylogenetic analysis

The exhaustive search of the unweighted character data in Table 6.6 yielded nine similarly parsimonious trees, each of 30 steps. The strict consensus analysis of the nine trees resulted in the tree of 30 steps presented in Figure 6.2 and the bootstrap analysis provided the support values indicated on the tree.

Three of the twelve characters were found to be parsimony informative in each analysis. They are Character 1: leaf form: pinnate or polypinnate, Character 10: pollination mechanism; anemophilic or entomophilic and identity of insect vector, and Character 12: chromosome number and morphology The descriptive data for these analyses are presented in Table 6.7 and a list of changes in character states is presented in Appendix 1.

The strict consensus analysis shows that *Bowenia* is monophyletic, i.e. constitutes a clade containing all descendents of a common ancestor. In addition, the genus contains two clades; the first containing what in this study has been identified as *B. serrulata,* and the second, all of what previously were referred to as nominate and putative *B. spectabilis.* These clades are distinguished by differences in Characters 11 – taxa browsed or not by *Lilioceris* sp. leaf beetles and Character 12 – chromosome number and morphology. The *B. spectabilis* clade includes two groups; nominate *B. spectabilis* from Tarzali, Starke and McIlwraith Range with pinnules with entire margins in one, and putative *B. spectabilis* from Kuranda and Tinaroo, with pinnules with serrate margin, in the other.

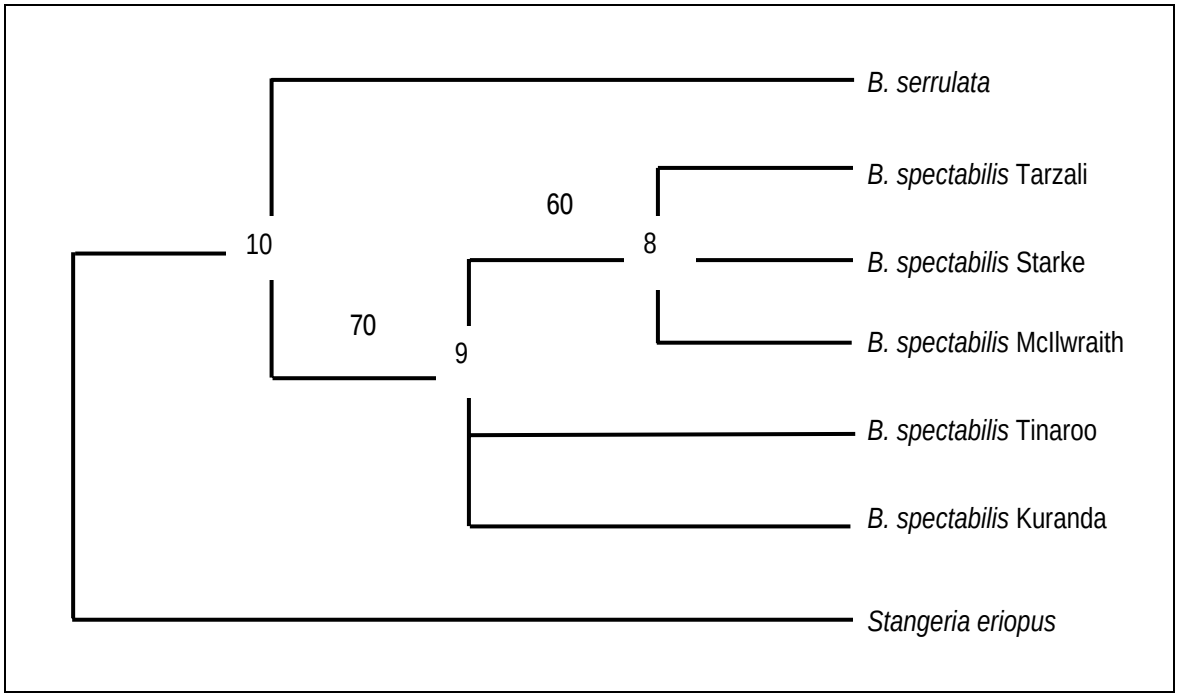


Figure 6.2 A strict consensus of nine trees obtained in an exhaustive analysis of twelve characters of six populations of *Bowenia* and a *Stangeria eriopus* outgroup. Tree length = 30, CI = 1.0000 excluding uninformative characters. Bootstrap values shown above the branches and node numbers on the lines.

Table 6.7 Descriptive data for exhaustive search and consensus trees of twelve characters in six populations of *Bowenia* and a *Stangeria eriopus* outgroup.

	descriptive data
Exhaustive search	Number of trees evaluated = 945 9 of 30, 94 of 31, 212 of 32, 326 of 33 and 304 of 304 steps Score of best tree found = 30 Score of worst tree found = 34 Number of trees retained = 9 Frequency distribution of tree scores: mean = 32.869841, SD = 1.006324, g1 = -0.547467, g2 = -0.547338
Consensus search	Tree length = 30 Consistency Index (CI) = 1.0000 Homoplasy index (HI) = 0.0000 CI excluding uninformative characters = 1.0000 HI excluding uninformative characters = 0.0000 Retention Index (RI) = 1.0000 Rescaled consistency index (RC) = 1.0000

6.1.5 Discussion of the phylogenetic analysis results

The phylogenetic analysis resolves the some aspects of the infrageneric relationships of *Bowenia*, e.g. it confirms the presence of two distinct clades, one conforming to the described species *B. serrulata* and another including nominate and putative *Bowenia* in north Queensland conforming to the concept of *B. spectabilis* as defined by Queensland Herbarium taxonomists. The bootstrap analysis values of 70 and 60% respectively provide moderate support for the results depicted in Figure 6.2. The analysis also shows that the two clades cannot reliably be distinguished on the basis of the morphological characteristics examined in this study and on which the two named species were initially differentiated. This fact compounds problems experienced by systematists and management authorities, as material from plants with pinnules with serrate margins of unknown provenance cannot reliably be assigned to a species using morphological metrics.

Of particular interest is the analysis result that separately groups those plants in north Queensland with pinnules with entire margins and those with serrate margins, and indicates the former are derived from the latter. This infers the common ancestor of this clade and the genus, had pinnules with entire margins. This inference is counter to that of Hill (1998) who considered the ancestral form had pinnules with entire margins, but is in accord with the evidence in the fossils, all of which have pinnules with serrate margins.

The inference is also counter to the hypothesis put in this study that ‘nominate’ *B. spectabilis* is likely to be most similar to the ancestral type as it occurs in mesophyll vine forests in areas of habitat that are recognised as refugia from climate and associated vegetation change that have occurred since the mid-late Miocene (Adam 1992; Truswell 1993). In addition, as the differences between ‘nominate’ and ‘putative’ *B. spectabilis* identified in the analysis, i.e. number of pinnae on leaves, length to width ratio and ratio of adaxial to abaxial serrations on pinnules, are phenotypically variable (this study), further investigations are required to determine the character state of the ancestral type.

Also of interest in this analysis is that the two clades of *Bowenia* are differentiated by phytophagy or otherwise by *Lilioceris nigripes*, pollination by different species of *Miltotranes* weevils, and karyology. Conversely, the similarity of these same characters results in ‘putative’ and ‘nominate’ *B. spectabilis* in north Queensland being nested together.

These differences both support the hypothesis that the *B. serrulata* and *B. spectabilis* clades have been separated for an extended period and suggest chemical differences in the clades. Further investigation of the latter is warranted as the results may both add to previous incomplete investigations where *Bowenia* had not been considered and provide data that can be included in future phylogenetic analyses.

The reasons for the failure to answer all of the phylogenetic questions posed in this study are manifold but several are immediately apparent and they are indicated by the small number of characters included in the phylogenetic analysis, the very small number, three only, of informative characters in the analysis, and the substantial lack of character data for the Starke and McIlwraith Range populations. In addition the number of character classes used in the analysis was less than desirable and should have contained, at minimum, molecular sequence data sets, which are likely to both have resolved the conundrum on the status of northern populations and also permitted a better estimation of the time of divergence and/or separation of taxa and populations of *Bowenia* and a reconsideration of the closeness the genus to *Stangeria*.

The lack of data for Starke and McIlwraith Range populations is both regrettable and costly for this analysis. While access to the former population is extremely difficult and only a little less so for the latter, karyological and molecular sequence data which can be obtained from small sample numbers may have allowed the questions of relationship and duration of isolation of the populations and to other north Queensland populations to be answered.

6.2 Biogeography of *Bowenia*

This aspect of the analysis was designed to ascertain if the results of the phylogenetic analysis depicted in the consensus tree concur with the geographic distribution of the taxa in the analysis. If the analysis presented above is correct and *Bowenia* and *Stangeria eriopus* constitute monotypic and monophyletic clades and central and northeast Queensland populations are phylogenetically distinct from each, and these groups can be mapped by region of occurrence on the cladogram, additional support is gained for the analysis.

6.2.1 Methods and materials

The results of the phylogenetic analysis and the distribution of the taxa in the analysis were compared using data on the distribution of the extant *Bowenia* and *Stangeria eriopus* extracted from Jones (2002), the Queensland Herbarium HERBRECS database, and from this study. The region of distribution of each taxon and location of intervening megatherm climatic barriers was drawn on the cladogram of the results of the phylogenetic analysis. The Black Mountain Divide is drawn as a dashed line as it more variable than the others and subject to making and breaking in short, (00's of years), periods of time

6.2.2 Results

The result of the comparison of the phylogenetic relationship and the distribution of taxa of *Bowenia* and *Stangeria eriopus* is shown in Figure 6.3. The regions of distribution match the two taxonomic groups of *Bowenia* and one of *S. eriopus* indicated in the phylogenetic analysis. The megatherm barrier of greatest age, the Burdekin Gap, separates *B. serrulata* from the remaining taxa of *Bowenia*; the next oldest barrier, the Normanby Gap, separates the McIlwraith Range population from all others in northeast Queensland, and the Black Mountain Divide separates the Starke population from all others in the Wet Tropics.

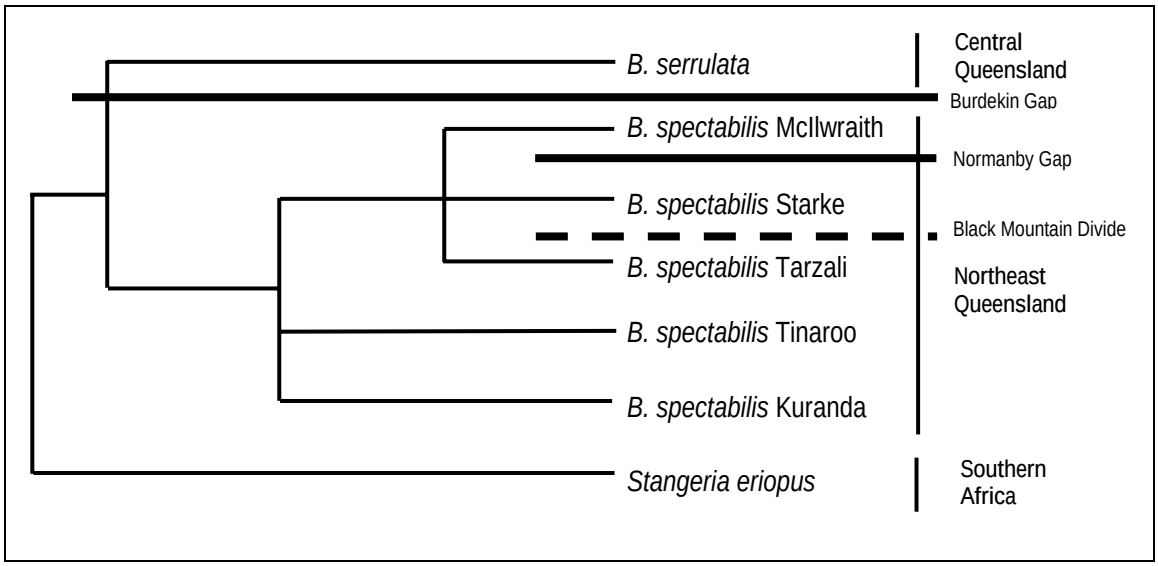


Figure 6.3 Comparison of the phylogenetic relationships and (a) distribution of *Bowenia* and *Stangeria eriopus* and (b) the location of megatherm barriers ▬.

6.2.3 Discussion of the biogeography of *Bowenia*

The comparison of the phylogenetic units defined in the analysis and the areas of distribution of *Bowenia* and *Stangeria eriopus* show the two correspond to the data in the literature and this study. In addition, evidence from the intervening megatherm barriers supports the separation of *B. serrulata,* or a precursor or a population of what was to evolve into *B. serrulata*, from taxa in the north since the onset of mid-late Miocene drying and the establishing of the Burdekin Gap. Further, the results in respect to *B. spectabilis* with pinnule with entire margins suggest this morphological form evolved prior to the establishing of the Normanby Gap in the late Miocene and has persisted in mesic refugia on either side of it. The results also support the inference in the fossil record; that is, as all material found at five sites across south eastern Australia has pinnules with serrate margins, the prevailing habitat was similar to that in locations where *B. serrulata* and putative *B. spectabilis* now occur, i.e. notophyll vine forest and tall wet forest and their ecotones.

6.3 Systematics of *Bowenia*

'Systematics is the science of organismal diversity…and entails the discovery, description, and interpretation of biological diversity as well as the synthesis of information on diversity in the form of predictive classification systems' (Judd et al. 1999). The results of the phylogenetic analysis of *Bowenia* summarised in Figures 6.2, the biogeographic data in Figure 6.5, and the data presented in this thesis, allow the systematics of the genus to now be reconsidered.

Applying the concepts of the Ecogenetic Species adopted in this study the data and results of analyses presented in this thesis support the recognition of two species of *Bowenia*. The phylogenetic analysis indicates two clades in *Bowenia* that correspond to the currently recognised *B. serrulata* (W. Bull) and *B. spectabilis* Hook. ex Hook. f.. The clades are phenotypically variable and cannot be distinguished solely on the basis of morphological differences but can be distinguished by differences in the identity of the insects that pollinate them, the insects that eat them, and in their karyology. *Bowenia* with entire and with serrate margins in northeast Queensland are phenotypic variations (ecotypes) within *B. spectabilis.*

While the results reported in this thesis reduce the value of pinnule morphology and negate the value of caudex size and structure as characters in the circumscription of the species of *Bowenia*, they provide evidence of genetic, chemical and ecological differences in them.

The phylogenetic analysis conducted in this study did not include genetic or molecular data for all populations that would have allowed a determination of the periods of separation of the species and populations and a comparison with dates given for the megatherm gaps that currently separate them. The small difference in the karyology of the two species separated for this period suggests the rate of change due to natural selection and mutation has been very low; this is indicative of slowly evolving species in a constant habitat and supports the suggestions of extended mean species duration times in cycads, i.e. 54 Ma, (Levin and Wilson 1976) and >5.5 Ma, (Niklas, Tiffney and Knoll 1983).

The biogeographic analysis provides supporting evidence for the above observations in respect to slow genetic change and the persistence of ecotypes in refugial areas of constant climatic parameters and habitat type. The fossil record provides additional support for the latter concept, where species previously extant in the same habitat became extinct as the climate changed.

6.4 Resolution of remaining phylogenetic and systematics problems

The studies described in this thesis did not definitively resolve the status of what were described in the study as putative *B. spectabilis*, and in particular, they did not adequately consider the status of the populations at Starke and McIlwraith Range.

The known period of isolation of the latter two populations and the suggestion by Ralph Oberpreiler of the Australian National Insect Collection (ANIC) that *Miltotranes prosternalis* (*sic*) weevils associate with the McIlwraith Range population appeared to be morphologically different from those in the Wet Tropics populations, raises interesting research opportunities.

The areas of endeavour that appear to have greatest potential in resolving the remaining phylogenetic and systematics questions are those of genetics and molecular sequencing. The activities that should be considered for attention are,

1. the sampling of all populations for material for the analysis of rDNA Internal Transcribed Spacer (ITS) sequence data,
2. the expansion of the analysis of the karyology of the populations to include representatives of the Starke and McIlwraith Range populations, and
3. The resampling of larger numbers of plants in populations whose to data set is small.

To undertake these tasks would require some good forward planning due to difficulties in accessing the Starke and McIlwraith Range populations but is necessary for the resolution of remaining phylogenetic and systematics problems.

Chapter 7 Summary and Management Recommendations

This is the final chapter of this thesis and the contents summarise what has been learned about *Bowenia* in this study and how that data can be used. I present a synthesis of the data and findings presented in the previous chapters and discuss the reasons and make recommendations for the management of *Bowenia*.

7.1 Summary

The data in this thesis show that *Bowenia* comprises two species, *B. serrulata* (W. Bull) Chamberlain and *B. spectabilis* Hook. ex J.D. Hook. The former is restricted in distribution to central Queensland and the latter to northeast Queensland and they are separated by the megathermal Burdekin Gap. Disjunct populations of plants in northeast Queensland are all *B. spectabilis* and populations with different pinnule morphology are ecotypes of that species.

The contents of Chapter 1 introduce the cycads in general and *Bowenia* in particular, and previous studies of them and the problems encountered by systematists in assigning genus to a family. The contents of Chapter 2 describe the strategies and general methods employed in this study and gives reasons for their use and the results of similar methods in studies of cycads.

The studies described in Chapter 2 show the species of *Bowenia* have phenotypically variable leaf, pinnule and stem morphology and cannot easily be separated using these characteristics. *Bowenia serrulata* has pinnules with serrate margins and bi- and/or polypinnate foliage, and *B. spectabilis* has plants with bipinnate foliage and pinnules with entire and serrate margins. They also show that *Bowenia spectabilis* with pinnules with entire margins occurs in mesophyll vine forest environs that have warm and constantly moist conditions and core areas that are coincident with refugia that extend back to the Mesozoic. *Bowenia serrulata* and *B. spectabilis* with pinnules with serrate margins occur in less complex forest types with less mean rainfall, a greater mean temperature range and lower mean minimum temperatures.

The data in Chapter 3 demonstrate that *Bowenia* is dioecious, with pollen dehiscence in male cones and receptivity in female cones occurring immediately prior to the wet season. Male cones are produced more frequently than female cones in both species.

The data also show that pollination is in *Bowenia* is obligately entomophilic; the vector in *B. serrulata* is *Miltotranes subopacus* (Lea) (Coleoptera: Curculionoidea: Molytini) and the vector in *B. spectabilis* is *M. prosternalis* (Lea). The plant–pollinator association is species-specific with the insect involved in a 'brood site reward' association with the plant.

The studies described in Chapter 4 show the two species of *Bowenia* are also distinguished by the fact that the leaf beetle *Lilioceris nigripes* (Coleoptera: Chrysomelidae), whose range overlaps that of the genus, feeds on the foliage of *B. spectabilis* but not on *B. serrulata*. The reasons for the differentiation of the two species by the beetles was not ascertained during the study but is presumed to be due to differences in chemistry in them.

The contents of Chapter 5 describe the chromosome number and karyology of representatives of *B. serrulata* and nominate and putative *B. spectabilis*. The two species have a diploid chromosome number of 2*n* = 18; *B. spectabilis* has a karyological morphology of 8m+6sm+4st, while *B. serrulata* has a morphology of 10m+3sm+5st. The data indicate that little variation has occurred in the genetics of the species despite the long period of separation of them. This observation conforms to suggestions by Levin and Wilson (1976) and Niklas et al. (1983), that speciation in cycads is a slow process and that species are long lived. In this thesis I suggest that another reason for the small amount of genetic differentiation is that the taxa are restricted to very stable environments that exert little selective pressure on them.

The phylogenetic analysis presented in Chapter 6 supports the existence of two extant species which conform to the currently describe *B. serrulata* and *B. spectabilis*. The consideration of the biogeography in this chapter shows that the distribution of the species concurs with the parameters of the accepted species. The contents of the systematics section of this chapter summarise the status of the two species of *Bowenia* but emphasis that the data set used in the phylogenetic analysis was severely limited and that a definitive description of the infrageneric phylogenetics and systematics of the genus cannot be presented without additional work.

7.2 Management Recommendations

7.2.1 Conservation biology

The species of *Bowenia* are listed in the Queensland Nature Conservation Act 1992 as COMMON and are included in national parks and reserves in that state (Forster 2002). The data collected during this study indicate that, despite being subject to both legal and illegal collecting, the species are adequately protected and that all populations are currently reproducing well.

The only perceivable threat to species of *Bowenia* is the effects of global climate change with widely- accepted projections (CSIRO 1996) inferring that the areas of distribution of the species are likely to become warmer and dryer in the next 75 years. While this change has been suggested as likely to cause retractions in the range or possible extinction of some high altitude species of fauna in the wet tropics of Australia, e.g. the Lemuroid Possum (Kanowski 2001), modelling by Hilbert et al. (2001) indicates that the area of complex mesophyll vine forest, the core vegetation type of *Bowenia*, will not be substantially reduced. However, the modelling by Hilbert et al. (2001) indicate that the area of notophyll vine forest will be reduced and as a result the two populations of *Bowenia*, the small and relictual one of *B. spectabilis* at Starke and the population of *B. serrulata* at Byfield, already occupying an area of marginal climatic parameters, are more likely to be affected by warming and drying associated with climate change in Queensland.

7.2.2 Commercial harvesting

The commercial harvesting under licence from the Queensland Environmental Protection Agency (EPA) is a vexatious topic. Cycads are totally protected under the Queensland Vegetation Management Act 1999 and there is no need for permits to be issued for the harvesting of leaves or of seed material, as technically, there should not be any harvest. Both species of *Bowenia* are currently listed under Queensland legislation as 'COMMON' and are included in Appendix II of CITES, a status that permits trade under licence; although there are plans (Forster, pers. comm. 2002) to raise the rating to Appendix I, which will preclude any trade in them.

However, the EPA realises that

- a ‘cottage’ industry in harvesting leaves of *Bowenia serrulata* from freehold, lease-hold and crown lands exists and a demand for the foliage continues in the domestic ‘cut flower’ industry, and
- Queensland Forestry has also harvested and sold seed of *B. serrulata* from its *Pinus* spp. plantations at Byfield in Central Queensland.

The EPA is responsive to these activities and the limited harvesting of cycad seed for nursery propagation purposes, and is attempting to balance these demands with the conservation of the taxa involved. The situation has become more complex in the last several years with expressions of interest from overseas, particularly Japan, in importing leaves of *Bowenia*, and applications to harvest larger quantities of leaves of *B. spectabilis* for export.

This raises the questions of (1) how such activities can adequately be policed by the Environmental Protection Agency considering their limited resources, and (2) if the wild grown stocks can sustainably support the intended harvest? This is particularly the case given that the leaves of *B. serrulata* cannot easily be distinguished from those of putative *B. spectabilis* and the possibility and/or temptation to illegally collect the latter and sell it as the former. As a result, and considering the contents of the Nature Conservation Act 1992, I now make the following recommendations for the management of *Bowenia* in Queensland.

1. The harvesting for commercial purposes of the whole or parts of any species of *Bowenia*, either currently described or described in future, be prohibited,
2. That permits to collect whole or parts of *Bowenia* for sale from lands of all tenure types not be issued after a set date and that existing permits not be renewed on expiry,
3. That sufficient notice be given of the intent in part 2, to allow parties currently working under permit to collect and to establish, should they so desire, accredited and licensed propagation facilities or contracts with licensed and accredited nurseries to permit them to continue their business from material grown at that facility, and
4. That collecting of parts of *Bowenia*, including leaves and seed, for scientific purposes continue to be allowed under permit issued by the Queensland Environmental Protection Agency or any succeeding government authority.

The strategy implied by the above suggestions is to allow an easily monitored and tightly controlled ‘cottage’ industries based on cultivated plants and rigorously apply the Act in all other circumstances.

Appendix I

Data from Chapter 2 Morphological variation in *Bowenia*

2.1 Morphometric data from pinnules from samples collected from each population of *Bowenia*.

Sites:	1 = Byfield	2 = Tarzali	3 = Tinaroo	4 = Kuranda	5 = Starke		6 = McIlwraith		
Site #	**Plant #**	**Pinnae #**	**Pinnule #**	**length (mm)**	**width (mm)**	**ratio**	**# adaxial serrations**	**# abaxial serrations**	**ratio**
1	1	1	1	110.5	24.5	4.51	22	17	1.29
Byfield			2	127.5	26	4.90	22	15	1.47
			3	123.5	26	4.75	20	16	1.25
			4	120	22.5	5.33	21	16	1.31
			5	110	27	4.07	21	17	1.24
			6	123	24	5.13	21	16	1.31
			7	134.5	26	5.17	22	18	1.22
			8	125.5	24.5	5.12	20	16	1.25
		2	1	109.5	26.5	4.13	20	15	1.33
			2	124	28.5	4.35	23	17	1.35
			3	96	19	5.05	20	18	1.11
			4	102.5	21	4.88	19	16	1.19
			5	111.5	28	3.98	18	18	1.00
			6	120.5	27	4.46	23	18	1.28
			7	90	18.5	4.86	19	17	1.12
			8	121	23.5	5.15	21	16	1.31
	2	1	1	97	19.5	4.97	20	15	1.33
			2	103.5	19.5	5.31	20	17	1.18
			3	105.5	20	5.28	21	17	1.24
			4	105.5	19.5	5.41	23	16	1.44
			5	99	21.5	4.60	21	21	1.00
			6	100.5	21	4.79	19	18	1.06
			7	100.5	20.5	4.90	21	16	1.31
			8	102	19	5.37	19	16	1.19
		2	1	92	16.5	5.58	18	15	1.20
			2	99	18.5	5.35	21	17	1.24
			3	97	18	5.39	21	17	1.24
			4	96	17	5.65	24	16	1.50
			5	95.5	19.5	4.90	18	17	1.06
			6	93	19	4.89	22	19	1.16
			7	92	15.5	5.94	19	15	1.27
			8	91.5	16	5.72	21	14	1.50
	3	1	1	80	25	3.20	21	14	1.50
			2	88	25	3.52	19	15	1.27
			3	91	24.5	3.71	20	19	1.05
			4	90	23.5	3.83	20	18	1.11
			5	83	26	3.19	20	19	1.05
			6	83.5	24.5	3.41	20	18	1.11
			7	86.5	24.5	3.53	20	19	1.05
			8	91	23	3.96	20	18	1.11
		2	1	84	26	3.23	24	17	1.41

			2	88	24.5	3.59	22	17	1.29
			3	92	24.5	3.76	22	20	1.10
			4	90.5	23.5	3.85	22	18	1.22
			5	80	27	2.96	20	18	1.11
			6	83	25	3.32	18	17	1.06
			7	86	22.5	3.82	22	17	1.29
			8	87	23	3.78	20	16	1.25
	4	1	1	93.5	21.5	4.35	16	10	1.60
			2	95.5	21	4.55	19	10	1.90
			3	97.5	20	4.88	16	9	1.78
			4	96	20	4.80	17	10	1.70
			5	91.5	18.5	4.95	15	10	1.50
			6	95	21	4.52	15	10	1.50
			7	102.5	21	4.88	16	10	1.60
			8	104.5	21	4.98	15	11	1.36
		2	1	101.5	19	5.34	18	11	1.64
			2	107	18	5.94	15	10	1.50
			3	109	17.5	6.23	15	10	1.50
			4	105.5	17	6.21	14	10	1.40
			5	93.5	17.5	5.34	15	12	1.25
			6	102	19.5	5.23	17	11	1.55
			7	105	17.5	6.00	16	11	1.45
			8	105.5	17.5	6.03	15	10	1.50
	5	1	1	125	32	3.91	25	21	1.19
			2	123.5	37	3.34	19	19	1.00
			3	123.5	32	3.86	24	19	1.26
			4	125	27.5	4.55	24	19	1.26
			5	114.5	28.5	4.02	19	18	1.06
			6	119.5	30	3.98	22	19	1.16
			7	112.5	26.5	4.25	19	17	1.12
			8	114	29	3.93	21	18	1.17
		2	1	99.5	28	3.55	21	17	1.24
			2	108.5	28.5	3.81	22	15	1.47
			3	112.5	24.5	4.59	21	17	1.24
			4	105.5	29	3.64	22	19	1.16
			5	117.5	31.5	3.73	23	20	1.15
			6	129.5	29.5	4.39	20	19	1.05
			7	128.5	31	4.15	21	20	1.05
			8	121.5	29.5	4.12	23	16	1.44
	6	1	1	120	32.5	3.69	23	22	1.05
			2	121	34	3.56	29	23	1.26
			3	126.5	33.5	3.78	28	24	1.17
			4	121.5	32	3.80	27	20	1.35
			5	122	33.5	3.64	26	22	1.18
			6	119	35	3.40	29	22	1.32
			7	125.5	33	3.80	26	22	1.18
			8	129	32	4.03	25	19	1.32
		2	1	101.5	28	3.63	21	18	1.17
			2	117	29.5	3.97	22	20	1.10

			3	111	31.5	3.52	26	22	1.18
			4	111.5	31	3.60	22	18	1.22
			5	104.5	31	3.37	23	21	1.10
			6	107.5	30	3.58	25	21	1.19
			7	116.5	30.5	3.82	24	22	1.09
			8	119	30.5	3.90	27	22	1.23
	7	1	1	79	21.5	3.67	19	16	1.19
			2	86	23.5	3.66	15	13	1.15
			3	81	21.5	3.77	19	15	1.27
			4	78	20.5	3.80	16	13	1.23
			5	63.5	26.5	2.40	18	11	1.64
			6	76	21.5	3.53	16	15	1.07
			7	83.5	21	3.98	17	11	1.55
			8	79.5	19	4.18	18	13	1.38
		2	1	77.5	23	3.37	19	13	1.46
			2	79	23	3.43	19	13	1.46
			3	79.5	21	3.79	20	12	1.67
			4	76.5	19.5	3.92	16	14	1.14
			5	70	24.5	2.86	17	15	1.13
			6	75.5	21.5	3.51	17	15	1.13
			7	81.5	22	3.70	19	14	1.36
			8	78	19	4.11	17	12	1.42
	8	1	1	91.5	24	3.81	16	15	1.07
			2	96	23.5	4.09	19	13	1.46
			3	98.5	22.5	4.38	20	16	1.25
			4	93.5	20.5	4.56	16	16	1.00
			5	89	23.5	3.79	18	15	1.20
			6	96.5	27	3.57	18	16	1.13
			7	91.5	20.5	4.46	16	15	1.07
			8	87.5	20.5	4.27	15	17	0.88
		2	1	91	22.5	4.04	18	18	1.00
			2	86	22.5	3.82	16	18	0.89
			3	86	21	4.10	18	14	1.29
			4	80.5	17	4.74	11	13	0.85
			5	83	22	3.77	18	16	1.13
			6	91	21.5	4.23	18	15	1.20
			7	91	20	4.55	16	15	1.07
			8	88.5	23	3.85	16	17	0.94
	9	1	1	117	35.5	3.30	30	20	1.50
			2	118	34	3.47	33	20	1.65
			3	116	37	3.14	30	24	1.25
			4	119.5	35	3.41	31	20	1.55
			5	115	33.5	3.43	25	22	1.14
			6	117.5	31.5	3.73	27	21	1.29
			7	117.5	31.5	3.73	25	20	1.25
			8	115.5	31	3.73	26	19	1.37
		2	1	121	36	3.36	21	23	0.91
			2	128	37	3.46	35	23	1.52
			3	129	34.5	3.74	30	22	1.36

			4	116	28.5	4.07	24	22	1.09
			5	112.5	33.5	3.36	25	21	1.19
			6	119	32.5	3.66	27	22	1.23
			7	124	34	3.65	28	25	1.12
			8	124	32	3.88	30	25	1.20
	10	1	1	75.5	18	4.19	18	14	1.29
			2	81.5	20.5	3.98	17	11	1.55
			3	81	21	3.86	21	15	1.40
			4	73	16	4.56	17	15	1.13
			5	65.5	17	3.85	17	14	1.21
			6	70.5	16	4.41	18	14	1.29
			7	72.5	16	4.53	17	14	1.21
			8	71	15	4.73	17	12	1.42
		2	1	75	17	4.41	17	13	1.31
			2	75.5	16.5	4.58	18	15	1.20
			3	72.5	20.5	3.54	17	14	1.21
			4	68.5	13	5.27	16	12	1.33
			5	68	16	4.25	18	16	1.13
			6	71	15	4.73	16	14	1.14
			7	72.5	14.5	5.00	18	13	1.38
			8	69	13	5.31	17	12	1.42
4	1	1	1	102	32	3.19	18	15	1.20
Kuranda			2	101	31	3.26	18	15	1.20
			3	102	29	3.52	17	14	1.21
			4	105	28	3.75	18	14	1.29
			5	105	32	3.28	17	16	1.06
			6	98	28	3.50	20	14	1.43
			7	99	31	3.19	18	17	1.06
			8	102	27	3.78	19	14	1.36
		2	1	101	26.5	3.81	20	12	1.67
			2	96.5	26	3.71	18	16	1.13
			3	98	25.5	3.84	15	17	0.88
			4	97	25	3.88	17	15	1.13
			5	101	29.5	3.42	17	15	1.13
			6	101.5	29	3.50	20	16	1.25
			7	95.5	27	3.54	19	15	1.27
			8	92	25	3.68	18	14	1.29
	2	1	1	95	24.5	3.88	12	11	1.09
			2	92	24	3.83	12	11	1.09
			3	94	22.5	4.18	12	9	1.33
			4	100	23.5	4.26	11	10	1.10
			5	98	25	3.92	15	11	1.36
			6	95	25	3.80	13	10	1.30
			7	95	24	3.96	12	10	1.20
			8	95	23	4.13	12	10	1.20
		2	1	96	24.5	3.92	12	10	1.20
			2	96	24.5	3.92	11	10	1.10
			3	102	24	4.25	12	11	1.09

			4	101	25	4.04	12	9	1.33
			5	100.5	25.5	3.94	12	12	1.00
			6	102	26.2	3.89	11	13	0.85
			7	99	24.5	4.04	14	9	1.56
			8	100	26	3.85	13	11	1.18
	3	1	1	90	24	3.75	11	11	1.00
			2	97	22.5	4.31	12	10	1.20
			3	94	22	4.27	11	11	1.00
			4	102	25.5	4.00	13	11	1.18
			5	99	24	4.13	10	10	1.00
			6	101	22.5	4.49	12	10	1.20
			7	99	22	4.50	13	12	1.08
			8	92	21	4.38	12	10	1.20
		2	1	91	24	3.79	14	10	1.40
			2	95	23	4.13	12	12	1.00
			3	109	21.5	5.07	12	10	1.20
			4	104	25	4.16	15	11	1.36
			5	92	24	3.83	11	12	0.92
			6	95	22	4.32	12	11	1.09
			7	93.5	22	4.25	13	10	1.30
			8	95	19	5.00	9	9	1.00
	4	1	1	96	32.5	2.95	20	15	1.33
	(7)		2	95	35	2.71	17	15	1.13
			3	105	35	3.00	20	13	1.54
			4	105	35	3.00	21	17	1.24
			5	93	35.5	2.62	20	15	1.33
			6	100	31.5	3.17	17	15	1.13
			7	104.5	33	3.17	19	12	1.58
			8	109	31.5	3.46	19	13	1.46
		2	1	95	33.5	2.84	22	17	1.29
			2	101	30	3.37	21	18	1.17
			3	106	31	3.42	21	16	1.31
			4	108	31.5	3.43	23	17	1.35
			5	100	31.5	3.17	22	18	1.22
			6	105	33	3.18	22	16	1.38
			7	100.5	30.5	3.30	17	16	1.06
			8	107	31.5	3.40	22	17	1.29
	5	1	1	95.5	34	2.81	18	17	1.06
	(9)		2	107	36	2.97	21	16	1.31
			3	118	37.5	3.15	20	17	1.18
			4	116	36	3.22	18	19	0.95
			5	103.5	33.5	3.09	19	16	1.19
			6	111	37	3.00	21	19	1.11
			7	122	38.5	3.17	21	19	1.11
			8	125	39	3.21	23	14	1.64
		2	1	102	38.5	2.65	21	16	1.31
			2	117	34.5	3.39	20	18	1.11
			3	114	39	2.92	25	21	1.19
			4	111.5	38	2.93	23	19	1.21

			5	98	32.5	3.02	18	16	1.13
			6	110	35.5	3.10	22	16	1.38
			7	114	35	3.26	22	16	1.38
			8	115	37.5	3.07	23	16	1.44
	6	1	1	117	28.5	4.11	14	11	1.27
	(9)		2	117	32	3.66	14	12	1.17
			3	119	30	3.97	14	11	1.27
			4	125	30	4.17	15	11	1.36
			5	117	33	3.55	16	11	1.45
			6	124	31	4.00	14	11	1.27
			7	116	31	3.74	15	11	1.36
			8	126	31	4.06	14	12	1.17
		2	1	117	32	3.66	14	12	1.17
			2	126	33	3.82	16	12	1.33
			3	126	32	3.94	15	11	1.36
			4	116	30.5	3.80	16	11	1.45
			5	116	30	3.87	13	10	1.30
			6	116	32	3.63	15	12	1.25
			7	131	31.5	4.16	14	13	1.08
			8	129	31.5	4.10	15	12	1.25
	7	1	1	117	31	3.77	19	16	1.19
	(10)		2	121	31.5	3.84	16	15	1.07
			3	126	32.5	3.88	18	14	1.29
			4	124	31.5	3.94	20	15	1.33
			5	103	29	3.55	18	13	1.38
			6	106	29	3.66	14	13	1.08
			7	115	30.5	3.77	19	11	1.73
			8	117	30	3.90	18	15	1.20
		2	1	101	29	3.48	17	14	1.21
			2	114	28.5	4.00	19	14	1.36
			3	114	29	3.93	20	13	1.54
			4	118	30	3.93	19	13	1.46
			5	86	26	3.31	14	14	1.00
			6	98	28	3.50	16	14	1.14
			7	115	30	3.83	18	13	1.38
			8	115	30	3.83	19	14	1.36
	8	1	1	128	45.5	2.81	19	17	1.12
	(9)		2	130	43.5	2.99	18	13	1.38
			3	119.5	40.5	2.95	16	14	1.14
			4	115	36.5	3.15	15	13	1.15
			5	117	41	2.85	19	15	1.27
			6	125	37	3.38	19	11	1.73
			7	132.5	48	2.76	15	13	1.15
			8	125	34.5	3.62	13	13	1.00
		2	1	115	36	3.19	17	12	1.42
			2	130	42	3.10	21	11	1.91
			3	138	42	3.29	19	12	1.58
			4	135.5	39.5	3.43	17	12	1.42
			5	110	37	2.97	15	15	1.00

			6	113	34.5	3.28	15	12	1.25
			7	125	36	3.47	14	13	1.08
			8	136	41	3.32	18	15	1.20
	9	1	1	116	33.5	3.46	20	16	1.25
	(7)		2	121	34.5	3.51	19	15	1.27
			3	120	33.5	3.58	21	16	1.31
			4	120	34	3.53	20	17	1.18
			5	116	32.5	3.57	18	15	1.20
			6	111.5	33.5	3.33	20	12	1.67
			7	109	30	3.63	20	15	1.33
			8	112	32.5	3.45	21	17	1.24
		2	1	104	32	3.25	20	15	1.33
			2	112	32.5	3.45	18	15	1.20
			3	111	34.5	3.22	20	17	1.18
			4	106	32.5	3.26	22	16	1.38
			5	99	33	3.00	19	15	1.27
			6	113.5	32	3.55	18	15	1.20
			7	111	33	3.36	20	17	1.18
			8	110	33	3.33	21	16	1.31
	10	1	1	72	27.5	2.62	15	14	1.07
	(12)		2	78	28	2.79	17	14	1.21
			3	78	30	2.60	20	14	1.43
			4	82	28	2.93	16	13	1.23
			5	79	28	2.82	17	14	1.21
			6	80	29	2.76	16	14	1.14
			7	83	30	2.77	18	14	1.29
			8	84	28	3.00	16	12	1.33
		2	1	78	31	2.52	17	14	1.21
			2	80	31	2.58	18	14	1.29
			3	73	30	2.43	18	10	1.80
			4	82	29	2.83	16	14	1.14
			5	81	27	3.00	14	13	1.08
			6	85	25	3.40	15	12	1.25
			7	77	27	2.85	13	14	0.93
			8	72	26.5	2.72	17	12	1.42
2	1	1	1	106	24	4.42	13	10	1.30
Tarzali	(9)		2	106	23	4.61	14	9	1.56
			3	100	21	4.76	14	10	1.40
			4	102	20	5.10	15	10	1.50
			5	100	24	4.17	15	9	1.67
			6	108	24	4.50	13	9	1.44
			7	114	23	4.96	14	9	1.56
			8	112	25	4.48	14	10	1.40
		2	1	95	21	4.52	13	10	1.30
			2	103	22	4.68	13	10	1.30
			3	109	24	4.54	13	10	1.30
			4	105	23	4.57	13	9	1.44
			5	105	24	4.38	12	11	1.09

			6	104	24	4.33	15	9	1.67
			7	110	24	4.58	15	9	1.67
			8	105	23	4.57	14	10	1.40
	2	1	1	104	23	4.52	14	10	1.40
	(9)		2	112	24	4.67	16	10	1.60
			3	115	24	4.79	17	9	1.89
			4	109	23	4.74	17	8	2.13
			5	107	23	4.65	14	8	1.75
			6	106	25	4.24	14	8	1.75
			7	118	24	4.92	14	9	1.56
			8	110	23	4.78	14	8	1.75
		2	1	112	22	5.09	11	7	1.57
			2	122	24	5.08	14	10	1.40
			3	132	24	5.50	15	9	1.67
			4	133	25	5.32	13	10	1.30
			5	125	25	5.00	16	9	1.78
			6	126	26	4.85	14	11	1.27
			7	131	26	5.04	13	9	1.44
			8	130	25	5.20	14	8	1.75
	3	1	1	92	26	3.54	18	10	1.80
	(9)		2	100	26	3.85	15	11	1.36
			3	108	25	4.32	15	13	1.15
			4	90	23	3.91	15	11	1.36
			5	93	23	4.04	19	9	2.11
			6	101	26	3.88	18	12	1.50
			7	96	21	4.57	18	8	2.25
			8	80	20	4.00	16	8	2.00
		2	1	69	26	2.65	16	10	1.60
			2	95	25	3.80	20	10	2.00
			3	95	26	3.65	17	10	1.70
			4	78	18	4.33	16	10	1.60
			5	82	24	3.42	18	15	1.20
			6	100	29	3.45	17	12	1.42
			7	95	25	3.80	17	15	1.13
			8	110	26	4.23	17	12	1.42
	4	1	1	93	31	3.00	16	9	1.78
	(7)		2	96	33	2.91	19	14	1.36
			3	102	33	3.09	19	13	1.46
			4	93	30	3.10	15	10	1.50
			5	94	28	3.36	16	10	1.60
			6	99	29	3.41	14	10	1.40
			7	102	30	3.40	14	8	1.75
			8	102	30	3.40	14	9	1.56
		2	1	97	28	3.46	16	9	1.78
			2	109	32	3.41	18	13	1.38
			3	107	33	3.24	17	12	1.42
			4	107	31	3.45	18	11	1.64
			5	96	35	2.74	16	13	1.23
			6	104	36	2.89	16	10	1.60

			7	107	33	3.24	17	12	1.42
			8	105	33	3.18	18	12	1.50
	5	1	1	145	24	6.04	15	9	1.67
	(7)		2	154	25	6.16	16	9	1.78
			3	147	22	6.68	15	7	2.14
			4	138	19	7.26	14	6	2.33
			5	137	24	5.71	14	10	1.40
			6	139	24	5.79	16	7	2.29
			7	140	24	5.83	15	7	2.14
			8	144	20	7.20	14	7	2.00
		2	1	143	25	5.72	15	9	1.67
			2	155	24	6.46	15	8	1.88
			3	138	23	6.00	16	8	2.00
			4	132	20	6.60	13	6	2.17
			5	145	24	6.04	13	6	2.17
			6	141	24	5.88	15	8	1.88
			7	138	23	6.00	17	7	2.43
			8	130	19	6.84	12	5	2.40
	6	1	1	76	21	3.62	13	8	1.63
	(10)		2	79	21	3.76	12	7	1.71
			3	81	21	3.86	15	7	2.14
			4	85	22	3.86	14	9	1.56
			5	73	20	3.65	15	7	2.14
			6	75	19	3.95	12	7	1.71
			7	78	21	3.71	14	8	1.75
			8	73	20	3.65	14	9	1.56
		2	1	76	21	3.62	12	9	1.33
			2	75	19	3.95	14	9	1.56
			3	77	19	4.05	13	9	1.44
			4	75	19	3.95	14	8	1.75
			5	73	21	3.48	12	7	1.71
			6	69	20	3.45	11	8	1.38
			7	67	19	3.53	11	8	1.38
			8	70	20	3.50	13	8	1.63
	7	1	1	108	25	4.32	15	9	1.67
	(8)		2	116	23	5.04	13	8	1.63
			3	116	24	4.83	13	7	1.86
			4	112	22	5.09	12	7	1.71
			5	107	26	4.12	15	8	1.88
			6	118	23	5.13	14	10	1.40
			7	102	25	4.08	14	9	1.56
			8	80	17	4.71	12	9	1.33
		2	1	112	24	4.67	16	9	1.78
			2	116	24	4.83	15	10	1.50
			3	115	23	5.00	14	8	1.75
			4	113	21	5.38	13	10	1.30
			5	112	23	4.87	15	11	1.36
			6	114	23	4.96	15	9	1.67
			7	100	19	5.26	12	7	1.71

			8	98	20	4.90	13	8	1.63
	8	1	1	122	24	5.08	15	10	1.50
	(8)		2	125	24	5.21	18	10	1.80
			3	117	23	5.09	17	10	1.70
			4	125	23	5.43	14	12	1.17
			5	119	25	4.76	16	11	1.45
			6	132	23	5.74	15	11	1.36
			7	126	22	5.73	14	10	1.40
			8	124	22	5.64	11	8	1.38
		2	1	119	24	4.96	15	9	1.67
			2	127	23	5.52	16	10	1.60
			3	131	24	5.46	14	10	1.40
			4	124	23	5.39	16	11	1.45
			5	114	23	4.96	13	10	1.30
			6	121	24	5.04	16	11	1.45
			7	130	23	5.65	15	9	1.67
			8	124	23	5.39	15	11	1.36
	9	1	1	107	23	4.65	11	7	1.57
	(9)		2	11	21	0.52	12	7	1.71
			3	104	20	5.20	10	5	2.00
			4	116	20	5.80	11	6	1.83
			5	106	21	5.05	10	5	2.00
			6	115	21	5.48	10	5	2.00
			7	111	20	5.55	8	6	1.33
			8	92	19	4.84	10	6	1.67
		2	1	135	26	5.19	10	8	1.25
			2	138	26	5.31	15	8	1.88
			3	138	26	5.31	14	9	1.56
			4	132	23	5.74	12	8	1.50
			5	130	26	5.00	14	8	1.75
			6	135	26	5.19	15	9	1.67
			7	130	25	5.20	14	10	1.40
			8	132	25	5.28	14	8	1.75
	10	1	1	86	25	3.44	13	10	1.30
	(10)		2	98	23	4.26	13	8	1.63
			3	98	21	4.67	17	7	2.43
			4	98	20	4.90	13	7	1.86
			5	92	21	4.38	13	8	1.63
			6	85	20	4.25	12	7	1.71
			7	92	20	4.60	12	8	1.50
			8	102	21	4.86	14	8	1.75
		2	1	85	24	3.54	13	8	1.63
			2	106	26	4.08	14	12	1.17
			3	102	21	4.86	12	8	1.50
			4	103	24	4.29	13	7	1.86
			5	99	26	3.81	13	8	1.63
			6	103	25	4.12	15	8	1.88
			7	107	25	4.28	15	6	2.50
			8	95	24	3.96	15	8	1.88

Byfield			2	75	30	2.50	22	22	1.00
'sun'			3	80	30	2.67	24	24	1.00
			4	75	25	3.00	26	20	1.30
			5	90	30	3.00	24	22	1.09
			6	100	35	2.86	29	25	1.16
			7	95	30	3.17	29	20	1.45
			8	95	30	3.17	23	23	1.00
			9	95	25	3.80	25	21	1.19
			10	85	25	3.40	23	19	1.21
	2	1	1	85	30	2.83	25	22	1.14
			2	85	30	2.83	27	25	1.08
			3	90	30	3.00	26	21	1.24
			4	90	25	3.60	20	17	1.18
			5	95	30	3.17	26	22	1.18
			6	95	30	3.17	29	21	1.38
			7	80	25	3.20	21	17	1.24
			8	70	30	2.33	22	24	0.92
			9	70	30	2.33	28	21	1.33
			10	75	25	3.00	23	23	1.00
	3	1	1	100	25	4.00	27	18	1.50
			2	95	25	3.80	28	20	1.40
			3	85	20	4.25	27	18	1.50
			4	100	25	4.00	28	20	1.40
			5	90	20	4.50	27	20	1.35
			6	90	20	4.50	24	17	1.41
			7	90	25	3.60	24	21	1.14
			8	80	20	4.00	26	20	1.30
			9	90	20	4.50	27	20	1.35
			10	85	25	3.40	25	19	1.32
	4	1	1	90	25	3.60	27	22	1.23
			2	70	25	2.80	26	23	1.13
			3	90	25	3.60	32	22	1.45
			4	80	25	3.20	29	23	1.26
			5	90	30	3.00	33	23	1.43
			6	80	25	3.20	24	18	1.33
			7	75	20	3.75	27	22	1.23
			8	80	25	3.20	29	22	1.32
			9	70	25	2.80	30	22	1.36
			10	75	25	3.00	30	23	1.30
	5	1	1	95	20	4.75	27	20	1.35
			2	85	25	3.40	27	20	1.35
			3	90	20	4.50	24	18	1.33
			4	95	25	3.80	26	21	1.24
			5	95	25	3.80	27	22	1.23
			6	90	20	4.50	22	20	1.10
			7	90	20	4.50	27	18	1.50
			8	90	20	4.50	28	18	1.56
			9	75	20	3.75	26	23	1.13

			10	75	25	3.00	25	19	1.32
3	1	1	1	120	36	3.33	17	13	1.31
Tinaroo	920mm		2	120	36	3.33	18	11	1.64
	8		3	120	35	3.43	16	12	1.33
			4	120	33	3.64	15	10	1.50
			5	110	36	3.06	15	11	1.36
			6	117	35	3.34	16	11	1.45
			7	114	35	3.26	15	10	1.50
			8	111	31	3.58	14	10	1.40
		2	1	125	36	3.47	19	11	1.73
			2	123	35	3.51	17	11	1.55
			3	118	34	3.47	16	12	1.33
			4	117	33	3.55	17	11	1.55
			5	109	35	3.11	17	12	1.42
			6	111	35	3.17	18	10	1.80
			7	115	30	3.83	15	11	1.36
			8	113	30	3.77	15	10	1.50
	2	1	1	109	26	4.19	18	12	1.50
	810mm		2	114	25	4.56	16	11	1.45
	12		3	111	25	4.44	15	12	1.25
			4	118	25	4.72	17	11	1.55
			5	113	24	4.71	18	12	1.50
			6	115	25	4.60	17	11	1.55
			7	114	27	4.22	17	11	1.55
			8	117	25	4.68	15	11	1.36
		2	1	112	24	4.67	17	12	1.42
			2	101	25	4.04	15	11	1.36
			3	105	23	4.57	17	12	1.42
			4	109	21	5.19	14	11	1.27
			5	109	25	4.36	17	13	1.31
			6	107	24	4.46	17	11	1.55
			7	105	24	4.38	16	11	1.45
			8	102	25	4.08	17	11	1.55
	3	1	1	123	29	4.24	15	12	1.25
	710mm		2	119	28	4.25	15	12	1.25
	10		3	119	28	4.25	18	11	1.64
			4	122	30	4.07	15	11	1.36
			5	110	28	3.93	15	10	1.50
			6	120	30	4.00	13	10	1.30
			7	131	32	4.09	15	11	1.36
			8	128	30	4.27	15	11	1.36
		2	1	101	24	4.21	13	9	1.44
			2	117	25	4.68	15	9	1.67
			3	115	27	4.26	15	10	1.50
			4	124	29	4.28	14	13	1.08
			5	92	26	3.54	13	11	1.18
			6	115	29	3.97	14	10	1.40
			7	119	30	3.97	15	11	1.36

			8	126	25	5.04	12	10	1.20
	4	1	1	125	35	3.57	16	12	1.33
	740mm		2	131	35	3.74	16	12	1.33
	8		3	142	35	4.06	17	10	1.70
			4	130	34	3.82	16	13	1.23
			5	135	31	4.35	15	11	1.36
			6	139	32	4.34	15	11	1.36
			7	133	32	4.16	15	12	1.25
			8	121	28	4.32	12	11	1.09
		2	1	137	35	3.91	17	11	1.55
			2	137	32	4.28	13	10	1.30
			3	137	28	4.89	12	12	1.00
			4	134	28	4.79	12	10	1.20
			5	126	32	3.94	16	11	1.45
			6	135	34	3.97	15	12	1.25
			7	125	27	4.63	14	9	1.56
			8	135	29	4.66	12	9	1.33
	5	1	1	93	31	3.00	16	11	1.45
	630mm		2	89	29	3.07	15	9	1.67
	7		3	88	27	3.26	13	10	1.30
			4	90	25	3.60	11	8	1.38
			5	85	27	3.15	13	10	1.30
			6	84	27	3.11	14	10	1.40
			7	85	27	3.15	12	9	1.33
			8	89	23	3.87	11	8	1.38
		2	1	93	30	3.10	14	14	1.00
			2	95	30	3.17	15	10	1.50
			3	86	27	3.19	14	10	1.40
			4	83	25	3.32	12	9	1.33
			5	85	27	3.15	15	9	1.67
			6	87	27	3.22	15	9	1.67
			7	90	28	3.21	13	10	1.30
			8	89	26	3.42	13	10	1.30
	6	1	1	123	31	3.97	12	9	1.33
	1140mm		2	125	31	4.03	13	8	1.63
	10		3	129	30	4.30	12	10	1.20
			4	127	28	4.54	12	9	1.33
			5	120	33	3.64	14	10	1.40
			6	123	32	3.84	13	10	1.30
			7	133	31	4.29	13	9	1.44
			8	121	30	4.03	13	10	1.30
		2	1	124	35	3.54	16	12	1.33
			2	125	31	4.03	15	9	1.67
			3	126	28	4.50	12	9	1.33
			4	123	28	4.39	13	9	1.44
			5	112	31	3.61	13	9	1.44
			6	117	32	3.66	15	9	1.67
			7	124	31	4.00	13	9	1.44
			8	118	26	4.54	12	8	1.50

	7	1	1	97	21	4.62	13	9	1.44
	835mm		2	96	20	4.80	10	8	1.25
	11		3	95	19	5.00	10	9	1.11
			4	104	19	5.47	10	9	1.11
			5	87	19	4.58	10	9	1.11
			6	96	20	4.80	10	8	1.25
			7	98	19	5.16	10	8	1.25
			8	98	18	5.44	9	7	1.29
		2	1	105	23	4.57	12	8	1.50
			2	103	23	4.48	12	9	1.33
			3	102	21	4.86	12	9	1.33
			4	102	21	4.86	11	8	1.38
			5	98	20	4.90	12	9	1.33
			6	99	20	4.95	11	8	1.38
			7	100	21	4.76	11	9	1.22
			8	104	20	5.20	10	8	1.25
	8	1	1	112	22	5.09	16	10	1.60
	740mm		2	118	23	5.13	15	12	1.25
	10		3	118	21	5.62	12	9	1.33
	LYMale		4	116	20	5.80	13	9	1.44
			5	111	20	5.55	13	8	1.63
			6	113	20	5.65	13	8	1.63
			7	115	20	5.75	13	9	1.44
			8	118	20	5.90	14	8	1.75
		2	1	102	20	5.10	12	8	1.50
			2	105	19	5.53	13	9	1.44
			3	111	19	5.84	13	9	1.44
			4	109	19	5.74	13	9	1.44
			5	91	20	4.55	15	9	1.67
			6	116	19	6.11	14	8	1.75
			7	110	18	6.11	15	7	2.14
			8	107	17	6.29	13	8	1.63
	9	1	1	108	29	3.72	13	10	1.30
	610mm		2	111	29	3.83	14	9	1.56
	8		3	106	28	3.79	16	9	1.78
			4	106	27	3.93	14	9	1.56
			5	102	30	3.40	15	10	1.50
			6	111	30	3.70	14	9	1.56
			7	110	27	4.07	13	10	1.30
			8	110	27	4.07	13	9	1.44
		2	1	101	28	3.61	15	8	1.88
			2	102	27	3.78	14	9	1.56
			3	103	28	3.68	15	10	1.50
			4	106	26	4.08	14	9	1.56
			5	105	23	4.57	13	9	1.44
			6	100	27	3.70	14	9	1.56
			7	98	26	3.77	14	9	1.56
			8	99	26	3.81	14	10	1.40
	10	1	1	153	35	4.37	22	16	1.38

	820mm		2	153	38	4.03	21	15	1.40
	8		3	149	32	4.66	18	15	1.20
	TYmale		4	151	30	5.03	19	11	1.73
			5	154	33	4.67	17	13	1.31
			6	162	32	5.06	20	14	1.43
			7	162	31	5.23	18	12	1.50
			8	162	31	5.23	17	12	1.42
		2	1	153	28	5.46	18	12	1.50
			2	154	27	5.70	16	11	1.45
			3	142	25	5.68	16	11	1.45
			4	144	30	4.80	18	12	1.50
			5	146	27	5.41	16	11	1.45
			6	150	28	5.36	16	11	1.45
			7	139	25	5.56	13	13	1.00
			8	140	26	5.38	14	12	1.17
5	1	1	1	83.5	29.5	2.83	15	13	1.15
Starke			2	85	36.5	2.33	16	12	1.33
			3	91	29.5	3.08	15	9	1.67
			4	87.5	27.5	3.18	12	11	1.09
			5	78	29	2.69	10	10	1.00
			6	91	31.5	2.89	15	11	1.36
			7	92	33.5	2.75	19	17	1.12
			8	89.5	28.5	3.14	15	14	1.07

2.2 Number of pinnae on the largest leaf on 101 putative *Bowenia spectabilis* at Tinaroo and nominate *B. spectabilis* at Tarzali, and 65 plants of *B. serrulata*.

leaf number	number of pinnae for B. Tinaroo	number of pinnae for B. spectabilis	number of pinnae for B. serrulata
1	12.00	9.00	12.00
2	9.00	8.00	11.00
3	9.00	8.00	12.00
4	11.00	8.00	11.00
5	6.00	10.00	9.00
6	9.00	8.00	13.00
7	10.00	11.00	5.00
8	10.00	9.00	9.00
9	9.00	8.00	14.00
10	10.00	8.00	10.00
11	11.00	10.00	6.00
12	9.00	8.00	12.00
13	10.00	9.00	5.00
14	9.00	8.00	10.00
15	10.00	8.00	10.00
16	6.00	7.00	14.00
17	6.00	10.00	10.00
18	8.00	8.00	11.00

19	7.00	8.00	10.00
20	8.00	11.00	11.00
21	12.00	8.00	12.00
22	8.00	9.00	14.00
23	7.00	8.00	15.00
24	10.00	8.00	10.00
25	8.00	9.00	12.00
26	10.00	7.00	13.00
27	10.00	9.00	12.00
28	10.00	9.00	15.00
29	9.00	7.00	14.00
30	7.00	6.00	10.00
31	9.00	10.00	10.00
32	9.00	7.00	15.00
33	12.00	7.00	11.00
34	6.00	7.00	12.00
35	6.00	6.00	10.00
36	9.00	10.00	12.00
37	7.00	9.00	10.00
38	10.00	8.00	12.00
39	9.00	7.00	12.00
40	8.00	6.00	13.00
41	9.00	7.00	12.00
42	9.00	7.00	9.00
43	9.00	7.00	12.00
44	11.00	6.00	10.00
45	10.00	8.00	12.00
46	8.00	6.00	10.00
47	10.00	8.00	11.00
48	9.00	6.00	12.00
49	11.00	6.00	9.00
50	7.00	9.00	15.00
51	10.00	9.00	10.00
52	8.00	8.00	10.00
53	7.00	7.00	12.00
54	11.00	7.00	11.00
55	10.00	8.00	12.00
56	9.00	8.00	12.00
57	9.00	9.00	14.00
58	12.00	7.00	12.00
59	10.00	8.00	12.00
60	9.00	10.00	12.00
61	9.00	9.00	13.00
62	8.00	10.00	11.00
63	9.00	8.00	8.00
64	10.00	8.00	11.00
65	7.00	9.00	10.00
66	7.00	8.00	
67	9.00	7.00	

68	7.00	6.00	
69	15.00	8.00	
70	9.00	9.00	
71	8.00	11.00	
72	8.00	7.00	
73	11.00	9.00	
74	8.00	8.00	
75	10.00	7.00	
76	10.00	7.00	
77	12.00	9.00	
78	7.00	7.00	
79	7.00	7.00	
80	8.00	9.00	
81	8.00	8.00	
82	10.00	7.00	
83	10.00	7.00	
84	8.00	11.00	
85	10.00	11.00	
86	12.00	8.00	
87	7.00	11.00	
88	9.00	9.00	
89	10.00	8.00	
90	10.00	6.00	
91	10.00	8.00	
92	9.00	11.00	
93	8.00	5.00	
94	7.00	7.00	
95	10.00	10.00	
96	8.00	7.00	
97	9.00	10.00	
98	8.00	9.00	
99	9.00	8.00	
100	10.00	15.00	
101	9.00	11.00	

2.3 Summary of morphometric data from pinnules from samples collected from each population of *Bowenia*.

Population	mean length (mm)	SE	mean width (mm)	SE	l:w ratio	mean adaxial (#)	SE	mean abaxial (#)	SE	ad:ab ratio	n
Byfield 'shade'	99.48	1.41	24.23	0.46	4.11:1	20.44	0.33	16.49	0.287	1.24:1	10x16
Byfield 'sun'	86.1	1.23	25.5	0.58	3.38:1	26.16	0.38	21	0.34	1.25:1	5x10
Tarzali	107.77	1.67	23.78	0.27	4.53:1	14.36	0.16	9	0.15	1.61:1	10x16
Tinaroo	115.35	1.40	27.35	0.39	4.22:1	14.43	0.17	10.22	0.18	1.41:1	10x16
Kuranda	105.58	1.12	30.63	0.41	3.46:1	16.89	0.27	13.61	0.20	1.24:1	10x16
Starke	87.19	1.6928*	30.69	1.0604*	2.84:1	14.63	0.9437*	12.13	0.8952*	1.21:1	1x8
McIlwraith Range	n.d.	-	n.d.	-	n.d.	n.d.	-	n.d.	-	n.d.	n.d.

* sample number too small to yield a meaningful statistic; n.d. = no data

2.4 Summary of mean surface area (mm^2) of pinnules from samples collected from each population of *Bowenia* and mean annual rainfall (mm), mean annual temperature (°C) and mean minimum temperature (°C) at each site.

Population	surface area (mm^2)	mean annual rainfall (mm)	mean annual temperature (°C)	mean minimum temperature (°C)
Byfield 'shade'	241.7	1745	22.3	12.2
Byfield 'sun'	236.6	1745	22.3	12.2
Tarzali	246.9	3988	24.1	14
Tinaroo	293.7	1749	24.3	14.5
Kuranda	314.7	2088	24.5	16.3
Starke*	n.d.			
McIlwraith* Range	n.d.			

* sample number too small to yield a meaningful statistic; n.d. = no data.

Table 2.2 Results of a one-way ANOVA of the number of pinnae on the largest leaves of three populations of *Bowenia*.

Source	DF	SS	MS	F ratio	P
Between populations	2	366.84	183.42	62.871	<0.001
Within populations	264	770.20	2.92		
Total	267	267			

Table 2.2.a Results of a Tukey Test comparison of the mean number of pinnae on the largest leaves of three populations of *Bowenia*.

Population	N	1	2	3
B. spectabilis Tarzali	101	8.24		
B. spectabilis Tinaroo	101		9.04	
B. serrulata	65			11.25
Sig.		(1.00)	(1.00)	(1.00)

Table 2.4a Results of a one-way ANOVA of the mean length of pinnules in four populations of *Bowenia* (*N* = 160).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20599.367	3	6866.456	21.502	<0.001
Within Groups	203097.930	636	319.336		
Total	223697.296	639			

Table 2.4b Results of a Tukey Test comparison of the mean length of pinnules in four populations of *Bowenia* (*N* = 160).

SITE	N	1	2	3
Byfield	160	99.4875		
Kuranda	160		105.5844	
Tarzali	160		107.7688	
Tinaroo	160			115.3500
Sig.		1.000	.694	1.000

Table 2.5a Results of a one-way ANOVA of the mean width of pinnules in four populations of *Bowenia* (*N* = 160).

	Sum of Squares	df	Mean Square	F	Sig.
Between Sites	4858.760	3	1619.587	66.222	<0.001
Within Sites	15554.575	636	24.457		
Total	20413.335	639			

Table 2.5b Results of a Tukey Test comparison of the mean width of pinnules in four populations of *Bowenia* (*N* = 160).

SITE	N	1	2	3
Tarzali	160	23.7813		
Byfield	160	24.2250		
Tinaroo	160		27.3500	
Kuranda	160			30.6325
Sig.		.853	1.000	1.000

Table 2.6a Results of a one-way ANOVA of length to width ratio of pinnules in four populations of *Bowenia*. (*N* = 160).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102.781	3	34.260	57.919	<0.001
Within Groups	376.203	636	.592		
Total	478.984	639			

Table 2.6b Results of a Tukey Test comparison of the length to width ratios of pinnules in four populations of *Bowenia* (*N* = 160).

SITE	N	1	2	3
Kuranda	160	3.5013		
Byfield	160		4.2232	
Tinaroo	160		4.3091	
Tarzali	160			4.5871
Sig.		1.000	.751	1.000

Table 2.8a Results of a one-way ANOVA of the mean number of adaxial serrations on pinnules in four populations of *Bowenia*.

	Sum of Squares	df	Mean Square	F	Sig.
Between Sites	3924.930	3	1308.310	138.163	<0.001
Within Sites	6022.506	636	9.469		
Total	9947.436	639			

Table 2.8b Results of a Tukey Test comparison of the mean number of adaxial serrations on pinnules in four populations of *Bowenia.*

SITE	N	1	2	3
Tarzali	160	14.3563		
Tinaroo	160	14.4313		
Kuranda	160		16.8938	
Byfield	160			20.4375
Sig.		.996	1.000	1.000

Table 2.9a Results of a one-way ANOVA of the mean number of abaxial serrations on pinnules in four populations of *Bowenia*.

	Sum of Squares	df	Mean Square	F	Sig.
Between Sites	5521.905	3	1840.635	286.674	<0.001
Within Sites	4083.531	636	6.421		
Total	9605.436	639			

Table 2.9b Results of a Tukey Test comparison of the mean number of abaxial serrations on pinnules in four populations of *Bowenia.*

SITE	N	1	2	3	4
Tarzali	160	9.0000			
Tinaroo	160		10.2188		
Kuranda	160			13.6063	
Byfield	160				16.4938
Sig.		1.000	1.000	1.000	1.000

Table 2.10a Results of a one-Way ANOVA of the ratio of number of ad- and abaxial serrations on pinnules in four populations of *Bowenia*.

	Sum of Squares	df	Mean Square	F	Sig.
Between Sites	15.769	3	5.256	119.500	<0.001
Within Sites	27.975	636	4.399		
Total	43.744	639			

Table 2.10b Results of a Tukey Test comparison of the ratio of number of ad- and abaxial serrations on pinnules in four populations of *Bowenia.*

SITE	N	1	2	3
Kuranda	160	1.2484		
Byfield	160	1.2607		
Tinaroo	160		1.4216	
Tarzali	160			1.6366
Sig.		.953	1.000	1.000

Data from Chapter 3 Reproduction Biology

Table 3.2 Production of cones by (a) *Bowenia serrulata* in SEVT at Byfield, Central Queensland (7 years), (b) putative *B. spectabilis* in SNVF at Tinaroo, North Queensland (3 years) and (c) nominate *B. spectabilis* in CMVF at Tarzali, North Queensland (3 years) - (data are not available for all years).

year	Byfield	male	female	Tinaroo			Tarzali		
1991		35	4						
1992		10	1.25						
1993		10	2.7		male	female		male	female
1994		15.46	1.36		7	3	1994	50	5
1995		15	3		no data	no data	1995	no	no data
1996		14	2		no data	no data	1996	no data	no data
1997		no data	no data		no data	no data	1997	no data	no data
1998		8.5	2		5	0.5	1998	1.8	0.18
1999		no data	no data		13.2	0.8	1999	3	3

Data from Chapter 6 Phylogenetic analysis, Biogeography and Systematics

Table 6.5 Apomorphies in the strict consensus tree of the analysis of twelve characters in six populations of *Bowenia* and a *Stangeria eriopus* outgroup.

Branch	Character	Steps	CI	change
node 10 – *B. serrulata*	1	1	1.000	0 → 2
	2	1	1.000	3 → 2
	3	1	1.000	5 →1
	4	1	1.000	5 → 0
	5	1	1.000	5 → 0
	6	1	1.000	5 →1
	7	1	1.000	5 → 2
	8	1	1.000	5 → 3
	9	1	1.000	5 → 0
	10	1	1.000	0 →1
	12	1	1.000	0 ⇒ 1
node 10 – node 9	1	1	1.000	0 →1
	2	1	1.000	3 →1
	3	1	1.000	5 →1
	4	1	1.000	5 →1
	5	1	1.000	5 →1
	6	1	1.000	5 → 0
	7	1	1.000	5 → 0
	8	1	1.000	5 → 0
	9	1	1.000	5 → 0
	10	1	1.000	0 → 2
	11	1	1.000	0 ⇒ 1
node 9 – node 8	2	1	1.000	1 ⇒ 0
	3	1	1.000	1 ⇒ 0
	6	1	1.000	0 → 2
	9	1	1.000	0 → 2
node 9 – *B. spectabilis* Tinaroo	4	1	1.000	1 ⇒ 2
	5	1	1.000	1 ⇒ 2
	6	1	1.000	0 → 1
	8	1	1.000	0 → 1
	9	1	1.000	0 → 1
node 9 – *B. spectabilis* Kuranda	7	1	1.000	0 ⇒ 1
	8	1	1.000	0 → 2

→ = single change, ⇒ = multiple change

Appendix II

Publications resulting from this study

Papers

Wilson, G.W. (1993a) Initial observations of coning phenology and frequency and the pollination biology of *Bowenia serrulata* (W. Bull) Chamberlain. *Encephalartos* 26: 13-18.

_____ (1993b) The relationship between *Cycas ophiolitica* K.D. Hill Cycadaceae), the butterfly *Theclinesthes onycha* (Lycaenidae), the beetle *Lilioceris nigripes* (Coleoptera: Chrysomelidae) and the ant *Iridomyrmex purpureus*. 1991 Symposium Series. UCQPGSA, Rockhampton.

_____ (1996) Variations in the foliage of *Bowenia serrulata*. *Encephalartos* 45: 21-23.

_____ (2001) Focus on *Bowenia serrulata* (W. Bull) Chamberlain. *Encephalartos* 65: 19-23.

_____ (2002a) Focus on *Bowenia spectabilis* Hook. ex Hook. f. *Encephalartos* 70: 10-14.

_____ (2002b) Insect Pollination in the Cycad Genus *Bowenia* Hook. ex. Hook. f. *Biotropica* 34(3): 438-441.

Kokubugata, G., Kondo, K., **Wilson, G.W.**, Randall, L.M., Schnas, A and Morris, D.K. (2000) Comparison of karyotype and rDNA-distribution in somatic chromosomes of *Bowenia* species (Stangeriaceae, Cycadales). *Australian Systematic Botany* 13(1): 15-20.

_____, Hill, K.D., **Wilson, G.W.**, Kondo, K. and Randall, L.M. (2001) A comparison of chromosome number and karyotype in somatic chromosomes of Stangeriaceae (Cycadales). *Edinburgh Journal of Botany* 58(3): 475-481.

Poster papers

Wilson, G.W. (2002) Insect Pollination in the rainforest cycad *Bowenia.* Ecology 2002, Annual Conference of The Ecological Society of Australia, Cairns, Australia.

Kokubugata, G., Kondo. K., **Wilson, G.W** and Randall, L.M. (1999) Comparison of karyotype and rDNA-Distribution in Somatic Chromosomes of *Bowenia* species (Stangeriaceae, Cycadales). XVI International Botanical Congress, St Louis, Missouri.[1]

Technical Reports

Wilson, G.W. (1995) Invertebrate pollination vectors, herbivores and defenders of the rainforest cycads *Bowenia spectabilis* and *B. 'Tinaroo'.* Report to the Wet Tropics Management Authority, Cairns, Australia.

[1] copy not available

Wilson, G.W. (1993a) Initial observations of coning phenology and frequency and the pollination biology of *Bowenia serrulata* (W. Bull) Chamberlain. *Encephalartos* 26: 13-18.

Wilson, G.W. (1993b) The relationship between *Cycas ophiolitica* K.D. Hill Cycadaceae), the butterfly *Theclinesthes onycha* (Lycaenidae), the beetle *Lilioceris nigripes* (Coleoptera: Chrysomelidae) and the ant *Iridomyrmex purpureus*. 1991 Symposium Series. UCQPGSA, Rockhampton.

Wilson, G.W. (1996) Variations in the foliage of *Bowenia serrulata*. *Encephalartos* 45: 21-23.

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