The reproductive biology
of four *Banksia* L.f. species
with contrasting life histories

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part fulfilment for the degree of
Doctor of Philosophy

by

Adrian Renshaw

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Statement of Authentication

Except where otherwise stated, all work presented in this thesis is the original work of the author and has not been presented for an award at this or any other University.

January 2005

Adrian Renshaw
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Abstract

In this thesis aspects of the reproductive biology of four *Banksia* species that display in various combinations all the life histories currently recognised in the genus were compared. Species like banksia, from fire prone regions of the world are typically characterised by life histories (traits and organs) that in reference to fire response allow them to be classified as seeders or sprouters. Plants killed by fire and that rely on seed for regeneration are called seeders and those that have the capacity to survive fire and produce vegetative sprouts are called sprouters. The species are *Banksia ericifolia* (a nonlignotuberous serotinous shrub and a seeder), *Banksia integrifolia* (a nonserotinous, nonlignotuberous tree, and a sprouter), *Banksia oblongifolia* (a lignotuberous serotinous shrub, a sprouter) and *Banksia serrata* (a lignotuberous serotinous tree, and also a sprouter). It is generally hypothesised that a different suit of traits might be associated with seeders and sprouters, particularly those traits associated with reproduction (eg Cowling & Lamont, 1998; Bell, 2001). The different aspects of the life histories and reproductive biology of these *Banksia* species was evaluated against this general hypothesis. The study was conducted in the Sydney Region of Australia, which was arbitrarily divided into North, Central and Southern Provinces for sampling across species.

The symmetry of the *Banksia* unit inflorescence (paired flowers and three bracts), phyllotaxy of the inflorescence (multiple unit inflorescences) and leaves was described. *Banksia* flowers are arranged in pairs with the ventral sutures of the flowers in a pair facing each other and lying at approximately $90^\circ$ to the inflorescence axis. A consequence of this arrangement is that flowers show handedness (left and right
flowers) and due to the somewhat asymmetric development of the ovary, fruits also reflect the chirality of flowers (both left and right flowers and fruits are distinguishable).

The unit inflorescences may occur in whorls or spirals (these are the primary and the least conspicuous) arrangements. The unit inflorescence and associated indumentum forms a quadrilateral, primarily as a result of the floral bracts being displaced from their theoretical position. This displaced condition of the floral bracts in *Banksia* presented an enigma for phylogenetic relationships in the Proteaceae (Johnson & Briggs, 1975). However the displacement of the floral bracts is shown to facilitate dense packaging. The result is the *Banksia* inflorescence, a highly condensed regular repeating array of unit inflorescences.

Secondary line patterns, orthostichies (vertical rows of flowers) and m and n parastichies (left and right spirals of flowers) are described. These secondary line patterns, notably the orthostichies are the most conspicuous. The relationship between the primary and secondary line patterns was described and a set of construction rules that apply to the architecture of the *Banksia* inflorescence were developed. Primary arrangements and their chirality as well as the number of, and relationship between m and n parastichies can all be inferred from the most conspicuous line patterns, the orthostichies or vertical rows of flowers.

Variation between the four study species in inflorescence phyllotaxy was reported and the possible biological/ecological implications considered. The seeder *B. ericifolia* differed from the three sprouter species in inflorescence phyllotaxy. Leaf phyllotaxy in contrast to inflorescence phyllotaxy was highly variable in all four species. However, it
was the least variable in *B. ericifolia* and *B. serrata* which may be characterised by specific arrangements. The implications of the arrangements are discussed in reference to plant architecture and reproduction.

The brood size (mean seed set fruit$^{-1}$), mean percentage fruit set and mean percentage cone or infructescence set were determined for the four species. These three estimates of fecundity and reproductive output were combined to give an overall assessment of reproductive output. The nonserotinous *B. integrifolia* (sprouter) had the highest overall reproductive output. This was primarily a consequence of high percentage cone set and a large brood size, otherwise the seeder *B. ericifolia* had the highest percentage fruit set. *Banksia ericifolia* however had the smallest brood size. This paradox was considered both in terms of the possible evolution history of the genus and estimates of fecundity and reproductive output generally. Several hypotheses for the low fruit to flower ratio were also considered in reference to the life histories of these species and their reproductive outputs. One possible explanation for the low fruit to flower ratios in *Banksia* is that there is insufficient room on an infructescence for a high percentage of fruits to develop. A ratio of unit inflorescence size to fruit size was calculated for the four species and this provides a relative assessment of the amount of space that is available for fruit set. The ratio is similar for the shrub species (*B. ericifolia* & *B. oblongifolia*) while percentage fruit set in *B. ericifolia* was twice that of the sprouter *B. oblongifolia*. However fruit shape differed in *B. ericifolia* and possibly accounts for the increased percentage fruit set.

A possible evolutionary pathway that might enable plants to bias the provisioning of resources within reproductive structures is via pre-existing patterns. The symmetry of
the unit inflorescence (left and right flowers) did not provide a template upon which natural selection has biased it provisioning of resources in the serotinous species. It may have provided a template in the nonserotinous \textit{B. integrifolia} which I interpret as a conservative bet-hedging strategy as both left and right flowers set fruit however more right fruits are set. The temporal separation of ovules within ovaries also provided a template upon which natural selection has biased the setting of more seeds by the lower ovule in the two shrub species that also only set one seed in each fruit. The possible biological and ecological implications of fecundity, reproductive output and patterns in seed set are discussed.

Aspects of the second part of the seeder sprouter dichotomy (the sprouter part) are also investigated, firstly at the level of the leaf axil. Epicormic shoots in eucalypts have been shown to be associated with accessory buds (additional buds in a leaf axil). Of the two \textit{Banksia} trees reported to sprout via epicormic shoots only \textit{B. serrata} was found to have accessory buds. A single accessory bud was present above the leaf petiole and below the axillary bud in all axils examined. In a pruning experiment (removal of apical meristems and branches) designed to force sprouting from accessory buds or meristems, only \textit{B. serrata} produced accessory shoots. Other axillary bud arrangements are noted and the results are discussed in relation to the species response to fire. One population of \textit{B. serrata} produced branches from accessory buds spontaneously, that is, without pruning treatments. These branches also developed inflorescences. This branching arrangement was considered unique to the 23 classical architectural models proposed by Hallé and Oldeman (1970).
Lignotubers are organs that also enable sprouters to regain biomass after fire. A unique variation to this axiom is presented in a case study of *B. ericifolia* (a seeder). In the southern most part of its range *B. ericifolia* displays transient lignotuber formation. That is, the lignotuber is generally only present during the early part of its life cycle. This transience is reported elsewhere (eg eucalypts and banksias) however in all these cases the species are considered sprouters. The lignotuber in *B. ericifolia* does not confer fire tolerance. If the lignotuber is not induced to persist by waterlogged conditions it ultimately disappears merging imperceptibly with the stem. At seasonally waterlogged sites where roots appeared to have died the lignotuber enables new root growth.

It has been generally hypothesised that other traits are likely to be associated with seeders and sprouters (eg Cowling & Lamont, 1998; Bell, 2000). However, these traits were not always clearly apparent from my studies. It is argued one reason for the anomalies in the expected associated traits is a result of a tendency to assume a evolutionary history that is based on fire as being the primary selection pressure. The process of extrapolation from a modern classification system of fire response to expected associated traits that have an ancient and perhaps diverse origin is likely to produce anomalies. The traits that might seem to be logically associated with seeders and sprouters such as higher reproductive output, the occurrence of a lignotuber and the role of serotiny are not unequivocally readily assigned. I suggest that the lack of congruence is due to a generally unrecognised evolutionary history that predates the regular occurrence of fire and thus biases the interpretation of associated traits so often invoked to account for the seeder sprouter strategies.
Chapter 1 – General Introduction

1.1. The family Proteaceae L.f.

All Proteaceae are perennial evergreens, usually woody trees or shrubs. Leaves are polymorphic even on the same branchlet, and arrangement is also variable (George, 1984). The family name comes from the type genus *Protea* L.f. after Proteus, a god given to changing form at will (Rourke, 1982).

With 80 genera and 1769 species the Proteaceae are concentrated in the Southern Hemisphere (George, 1998). The greatest diversity is found in south-western Africa and south-western Australia (Cowling & Lamont, 1998). Australia has the greatest generic diversity (46 genera and 1093 species ie c. 61.7% of the world total) as well as representatives of all subfamilies (Douglas, 1995; George, 1998). Seven subfamilies are recognised (Johnson and Briggs 1975; Weston, 1995; Douglas and Hyland, 1995). Grevilleoideae is the largest and the most widely distributed of all subfamilies. They are united by the arrangement of flowers into pairs subtended by a common bract. Banksieae, one of seven grevilleoid tribes, is characterised by elongate trichomes and biporate pollen. Tribe Banksieae consists of two subtribes, Musgraveinae (*Musgravea* F. Muell and *Austromuellera* C. T. White), endemic in rainforest in north-eastern Queensland and Banksiinae (*Banksia* and *Dryandra* L.f.). Banksiinae is characterised by a dense cone like inflorescence and a one or two layered dissepiment formed from the outer layers of the integument (Johnson and Briggs, 1975).
The Proteaceae are found in the greatest abundance (up to 19 species per 100 m² plot) on deep sands and lateritic rock over leached clays in south-western Australia (Lamont, Collins & Cowling, 1985). Few Proteaceae are found on heavy soils derived from granite or shale and are absent from saline and permanently wet situations (George, 1984). The family is primarily associated with oligotrophic soils and not Mediterranean climates as Johnson (1998) and Johnson and Briggs (1975) so often adamantly pointed out.

Douglas (1995) broadly identified two schools of thought on the affinities of the Proteaceae with other angiosperms. One school considers the family to be highly derived, having evolved from an ancestor with two perianth series, with reduction of the corolla to nectaries and a single carpel. The second school holds that the apocarpous nature of the gynoecium reflects an ancient angiosperm lineage. Gene sequencing supports the second school and the basal position of the Proteaceae relative to other angiosperms suggests a very early or ancient lineage (Douglas, 1995). The family is of Gondwana origin and must date back to the mid-Cretaceous (Johnson, 1998).

Johnson and Briggs (1975) considered the early Proteaceae to be trees (or sizeable woody plants) growing in closed rainforests in association with conifers and other early angiosperms. Stebbins (in Johnson & Briggs, 1975) has argued that the shrubby habit probably proceeded the arborescent habit in many families including the Proteaceae. White (1998) pointed out that the Gondwana forests were not closed forests analogous in structure to modern rainforests. They were ‘Paleoflora mixta forests’ growing under warm wet polar-ice-free climates with open but stratified canopies. Closed forest canopies would not have been able to exist due to limited light from a low-angle sun
and dark polar winters. As the continent drifted and dried the sclerophyll vegetation became dominant and more modern ecosystems ultimately evolved.

George (1981) also believed *Banksia* to have evolved under tropical or subtropical conditions before the emergence of the Nullarbor Plain. More recently Greenwood, Haines and Steart (2001) reported seed cones of *Banksia* in Neogene sediments near Marree and Woomera in South Australia indicating the presence of *Banksia* in central Australia in the mid tertiary. However *Banksia* seed cones are known from the Eocene (50 million years) (White, 1998), well before the onset of aridity.

The incidence of fire from lighting accompanied the increase of aridity in Australia. Fire might have been common in Australia 20 million years ago accelerating the change in forest composition (Florence, 1994). However, increased charcoal deposition profiles of Lake George has suggested an increased fire frequency two million years ago (Singh, Kershaw & Clark, 1981). The frequency of fire increased again with the coming of the Aboriginal (40,000 years ago\(^1\)) and again with the coming of European man 200 years ago (Attiwill, 1994).

1.2. The genus *Banksia* L. f.

*Banksia serrata* is the lectotype species of the genus and was one the first banksias collected from Botany Bay by Banks and Solander in 1770. The genus was named in honour of Banks by Carl Linnaeus fil. in 1782 (Salkin, 1981). *Banksia serrata* may also

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\(^1\) Dates for the arrival of Aboriginal man in Australia vary although Kohen (1995) suggests 50,000 or longer.
have seniority over all Australian flora as the first Australian plant to be cultivated outside Australia (Nelson, 1983).

Taxonomy and authorities for all *Banksia* follow George (1999), although there is some disagreement in the infragenic classification (see George, 1998, 1999; Theile & Ladiges, 1996; Mast, 1998). George’s (1999) classification of *Banksia* recognises two subgenera, three sections and 13 series.

Of the 76 tree, shrub and prostrate species of *Banksia* all but *Banksia dentata* are endemic to Australia with 60 of these endemic to south-west Western Australia. *Banksia dentata* extends across tropical Australia into Papua New Guinea, Irian Jaya and the Aru Islands. Fifteen species are endemic to eastern Australia with only *Banksia ornata* extending westward into South Australia (George, 1999).

In eastern Australia the genus displays considerable variation in geographical range. For example, *Banksia serrata* extends from northern Tasmania along the mainland coast to Queensland and may be locally common. In stark contrast *Banksia plagiocarpa* is restricted to Hinchinbrook Island and the adjacent mainland of Queensland (George, 1999).

Banksias exhibit extremes in growth form, but they are all woody evergreen plants (George, 1984). The tallest *Banksia* specimens recorded may have been *B. integrifolia* var. *compar* which grow to 30 m, with a diameter at breast height of almost one metre (Taylor and Hopper, 1991). This contrasts with the species of the series *Prostratae* in which the branches are prostrate on or below the soil (George, 1981).
The typical *Banksia* leaf is flat with entire or dentate, flat or slightly recurved, margins. Each flower is tubular at the base and enclosed by the floral bracts. The perianth tube consists of four segments which are often referred to as tepals. The apex of each segment is enlarged and is referred to as the perianth limb. Within each limb, an anther on a short filament is enclosed. The pistil is wiry and may be straight or bowed (George 1984).

The inflorescence is complex, flowers being arranged in pairs, in association with, and subtended by, a common bract and two smaller floral bracts around a central woody axis. Inflorescence development is typically acropetal (flowers opening from the base and proceeding up along the inflorescence) except for *Banksia orophila*, *Banksia quercifolia* and the section *Oncostylis* (*B. ericifolia* in this study) in which it is basipetal (George, 1981).

With the exception of the series *Tetragonae* and *Quercinae* the style elongates before the flower opens. This tension causes the style to bow where upon it is exserted through a split in the perianth. The difference in the colour of the perianth and style often provides a vivid contrast as the flowers open. The style continues to elongate (and thus bow) while the apex of the style is held in the perianth limb. Anthesis results when the limb opens releasing the style, where at the apex the pollen has been deposited on what is referred to as the pollen presenter. The pollen may completely cover the pollen presenter or the apex may be left bare. At the apex of the pollen presenter is the stigmatic groove. The stigma is located inside the groove. At the time of pollen presentation the stigmatic groove is closed. Many Proteaceae are also protandrous (Ramsey & Vaughton, 1991; Matthew, Gardner & Sedgley, 1999). When the stigmatic
groove is open pollen may be pressed into the groove, which may result in fertilisation (George, 1981; Sedgley, Sierp, Wallwork, Fuss, & Thiele, 1993).

*Banksia* fruits are hard woody follicles and an inflorescence that has produced fruits is referred to as an infructescence or cone. The follicle has two valves and may contain two winged seeds. The seeds are separated by a double false dissepiment formed from their outer integuments (Johnson and Briggs, 1975). This structure is referred to as the separator (George, 1981). Follicles may take up to two years to develop, and then open spontaneously at ambient temperatures, or remain closed until burnt (George, 1984), a condition referred to as serotiny.

### 1.3. Serotiny

Serotiny may be defined as the retention of mature seed in the fruit or cones on plants. Bradyspory has also been used to describe the same phenomena (e.g., Pate and Beard 1984; Whelan, De Jong & Von der Burg, 1998; Price & Morgan, 2003). Le Maitre (1985), Lamont (1991) and Lamont, Le Maitre, Cowling and Enright (1991) have argued in favour of the term serotiny over bradyspory. Definitions of serotiny generally incorporate a time factor. Some definitions are implicit. For example, seed retention should be sufficiently long such that an overlap of seeds from successive crops are simultaneously retained (Le Maitre, 1985) or retained for a prolonged period (Cowling and Lamont 1985a). Others are more explicit, for example, one to 30 years or more (Lamont, *et al.* 1991) or retained for more than one year (Enright, Lamont & Marsula, 1996). Serotiny is common among the taxa that occupy Australia’s sclerophyll vegetation where the heat of recurrent bushfires releases seed on mass from the woody fruits (Lamont *et al.* 1991).
George (1981) classified *Banksia* as having three classes of follicle dehiscence;

1. opening spontaneously when mature (nonserotinous),

2. normally remaining closed until burnt (but sometimes a few will open spontaneously), and

3. some opening spontaneously and some remaining closed until burnt.

The last two classes are serotinous and suggest degrees of serotiny. Intra-specific variation in the degree of serotiny for three *Banksia* species along a climatic gradient has been reported by Cowling and Lamont (1985b). Serotiny was greatest in the scrub heath at the xeric end of the gradient and almost absent in the low woodland at the mesic end. Whelan *et al* (1998) found variation in serotiny among *B. serrata* populations. More follicles were found to be opened at coastal sites than at inland heath sites. In low intensity fire some follicles may remain closed particularly those in the higher canopy of arborescent species (Bradstock, 1985; pers. obs.). Seed is also released from serotinous banksias when the plant dies, or from follicles on branches that die or from cones that have fallen to the ground (Whelan *et al*, 1998; per. obs.).

Lamont *et al* (1991) introduced the term pyriscence to describe seed release stimulated by fire and also reported banksias to be the only angiosperms with obligate pyriscence. Obligate pyriscence apparently meaning seeds of some species are ‘usually’ only released by fire, and thus corresponds to class 2 (above) as described by George (1981).
1.4. Lignotubers

Lignotubers, burls, root crowns, root stocks, xylopodia (Brazil), souches (France), toucas (Portugal), geoxylic suffrutes (Africa) (James, 1984) and caudex (Gardner, 1957) are all terms that have been applied to enlarged, sprouting woody structures. The two most commonly used terms are lignotuber and burl, both referring to a swollen woody plant base from which new shoots emerge. James (1984) considered the term lignotuber should be retained for all such structures and burl, reserved for those swellings that arise from tissue injury. Canadell and Zedler (1995) have argued that the term lignotuber is scientific and should specifically apply to those species that have genetically determined swollen structures. Conversely they consider the general term burl be retained for those swollen woody structures whose origin may be questionable. This convention is followed here. Other authors (Lacey & Johnson, 1990; Graham, Wallwork & Sedgley, 1990; Mibus & Sedgley 2000) have argued two distinct organs, lignotubers and burls may be distinguishable (see Chapter 7 & 8).

Eucalypt lignotubers were the first to be described and have been the most extensively studied (Fletcher & Musson 1918; Kerr 1925; Carter 1929; Jacobs 1955; Chattaway 1958). Kerr (1925) coined the term lignotubers for woody stem tubers characteristic of many of the eucalypts and angophoras. In studies of the structure of the lignotuber in the cotyledonary and lower leaf nodes of *Eucalyptus*, Chattaway (1985) found lignotubers to develop only in those species that have concealed (accessory) meristems/buds present above and below the axillary buds of the cotyledonary and lower nodes. Accessory buds are additional buds in a leaf axil (see Chapter 6). Lignotubers have now been described in more than a dozen plant families from around
Most of the earlier reports on lignotubers recognised their regenerative role and acknowledged the function of storage and a repository for dormant buds (eg Kerr, 1925; Carter, 1929). This regenerative function typically characterises most definitions that have generally remained sufficiently broad so as to encompass all analogous structures. For example, Molinas and Verdaguer (1993) defined lignotubers as ‘storage organs with a supply of protected dormant buds, allowing for rapid regrowth after injury of aboveground organs’. Canadell and Zedler (1995) defined a lignotuber as ‘a woody swollen structure at the stem base’ and Cruz, P•rez and Monero (2003) as an organ evolved to increase carbohydrate storage and/or the number of dormant buds.

Carrodus and Blake (1970) found that the amount of starch (percentage of dry mass) in the lignotuber (Eucalyptus obliqua, a timber tree) was not significantly different from stems and roots. They concluded that the prime function of the lignotuber was probably related to the protection of large numbers of dormant buds. However, Bamber and Mullette (1978) concluded that there is a starch storage role performed by the lignotuber in E. gummifera, a mallee forming species. This was evident when the size of the lignotuber is taken into account. This view is in keeping with previous workers (Kerr, 1925; Carter, 1929; Jacobs, 1955; Chattaway, 1958) who also acknowledged the additional role of the lignotuber as a source of dormant buds. Bamber and Mullette's (1978) anatomical study concluded that the lignotuber and stem were significantly different in quantitative, rather than qualitative aspects, with lignotubers having twice the proportion of storage tissue than stem wood and consequently greater potential for
starch storage. The concomitant reduction in supportive tissue was considered to be compensated by the increase in diameter of the lignotubers.

Mullette and Bamber (1978) also found the lignotuber of *E. gummifera* to be an important storage organ. While there was no significant difference in nutrient content between the tissues of the lignotuber and other organs, the absolute nutrient contents when considering the biomass of the lignotuber becomes important relative to the biomass of the regrowth after the aerial parts have been destroyed. In addition they found the lignotuber of this mallee to have a fourfold greater concentration of starch than the coppice stems.

Dell, Jones and Wallace (1985) reported the lignotuber in Jarrah (*E. marginata*) to have almost twice the phosphorus concentration of the stems or roots. Nitrogen concentrations were similar for stems, roots and lignotubers. They suggested that the lignotuber was a storage organ for young plants before the development of a woody trunk.

In George’s (1981) descriptions of *Banksia* most species are described as being lignotuberous or nonlignotuberous. Interestingly, two of the study species (*B. integrifolia* and *B. serrata*) are not characterised as being lignotuberous or nonlignotuberous. Fuss and Segdley (2000) considered that there was some confusion as to the occurrence of a lignotuber in *B. serrata* and noted the conspicuous lack of characterisation in George’s (1981) definitive work. This topic is considered further in Chapters 6, 7 and 8.
1.5. Response to fire

Banksias (and other taxa from fire prone environments) are often divided into fire sensitive or fire tolerant forms. Fire tolerance may be conferred by a thick bark and/or a lignotuber from which the protected new shoots emerge. At times this division may be quite arbitrary, depending on the frequency and intensity of fires and the life stage of the plant (Gill, 1981; Bradstock & Myerscough, 1988). George (1981) considered fire tolerant arborescent Banksia species to have a bark 1-3 cm thick while fire sensitive species have bark less than 5 mm thick. Approximately 35% of the genus Banksia are fire tolerant sprouters (estimated from George, 1981).

Historically, the study of species responses to fire regimes has followed three approaches: 'classification', 'species replacement' and the 'demographic' approach. In keeping with fire sensitive and fire tolerant forms the classification approach recognises 'seeders' and 'sprouters' respectively (Gill, 1981). Seeders are sometimes referred to as 'nonsprouters' or 'reseeders' and sprouters (or 'resprouters') may also produce seedlings (hence facultative sprouters) (Gill & Bradstock, 1992; Bradstock & Bedward 1992).

In general sprouters may live for hundreds or thousands of years while seeders senesce or are killed by hot fires within 5-50 years (Lamont & Wiens, 2003). If fires are too frequent seeders may not reach maturity and may become locally extinct. In Banksia, George (1981) considered fire tolerance (sprouters) to be a primitive condition and seeders derived. In Theile’s (1993) cladistic analysis of Banksia sprouters and seeders appear together in the phyletic sequence with sprouters more common at the end of

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2 Additional classifications have also been used (eg. Bell, Hopkins and Pate, 1984) while seeder/sprouter are considered the crudest or broadest categories of the classification approach (Gill, 1981).
sequence. It may therefore be misleading to classify sprouting as primitive or derived in *Banksia* (Lamont & Markey, 1995).

### 1.6. Life history traits, strategies and schemes

Organisms use an array of traits in various combinations (strategies) so as to survive and reproduce (Southwood, 1988). Stearns (1992) considered the principal life history traits to be size at birth, growth patterns, size and age at maturity, reproductive outputs and investments and mortality schedules. Particular trade-offs would impact on these traits. Ultimately the differences between species may be viewed as the differences in the trade-offs and the selection pressures that act upon the trade-offs (Sibly, & Antonovics, 1992). The optimum trade-off will maximise fitness (Crawley, 1986).

Conversely, temporal variation in the environment can result in variation in a given genotype’s fitness between generations. A trade-off between the mean and variance of fitness, may, in some circumstances, produce phenotypes with reduced mean fitness but otherwise with a selective advantage (Philippi & Seger, 1989). This type of trade-off is referred to as bet-hedging, after gamblers who bet on several horses in the same race to increase their average winnings (Stearns, 1977; 1989). Bet-hedging may be conservative or diversified and many life history traits may have undergone selection for either conservative or diversified bet-hedging. Philippi and Seger (1989) provided the following distinction: conservative bet-hedging is encapsulated in the adage ‘a bird in the hand is worth two in the bush’, which may be contrasted with diversified bet-hedging, ‘don’t put all your eggs in one basket’.
By investing in resprouting (eg producing storage tissue or a dormant bud bank) sprouters make trade-offs. Likewise seeders make a trade-off by not investing in such traits but instead develop alternate traits/strategies to the same or similar environmental circumstances. For example, in comparison with congeneric seeders, sprouters tend to produce fewer seeds, have smaller seed banks and lower germination rates (Cowling & Lamont, 1987; Enright, Marsula, Lamont, & Wissel, 1998; Enright & Lamont, 1992). In a recent review Bell (2001) found there were no differences in seed mass, viability or germinability of seeds between seeders and sprouters. In a meta analysis Lamont and Wiens (2003) found that in 30 out of 33 case studies fecundity was lower in sprouters. Other generalisations include: seeder seedlings tend to grow faster; shrub morphology tends to be different because of the multiple stems arising from the resprouting lignotubers; sprouters tend to have large more penetrating root systems; seeders tend to produce more flowers and floral rewards, and sprouters may be more common in higher rainfall areas (Bell, 2001).

Seed production would have to be greater in seeders to compete with sprouters. Sprouters would therefore be at an advantage in environments where seed and seedling mortality is high (Cowling & Lamont, 1998). A variety of factors (drought, herbivory and fire) all contribute to seed/seedling mortality. Specht (1981) noted that the abundance of seeders in Australian shrublands was inversely proportional to precipitation because of the lower density of sprouters in the more mesic environments. Seeders, presumably because of competition, could not get a foot-hold in these sites. Conversely in the more xeric sites seeders and sprouters tended to occur together. In South-western Australia Lamont and Markey (1995) found sprouter banksias to be more common in higher rainfall areas and where the spread of rainfall was also higher.
Sprouters were also more widespread than seeders suggesting that sprouters may have broader environmental tolerances.

Cowling & Lamont (1998) posed the question why give up space and also run the risk of recruitment failure, that is, why be a seeder? As an explanation for the predominance of seeders over sprouters in the Californian chaparral, Wells (1969) suggested that as seeders have shorter generation times, each generation of seedlings is subject to selection pressures. The argument holds that seeders would be more prone to diversification than sprouters. Approximately 65% of banksias are seeders and Well’s (1969) argument could be invoked to explain the pattern of diversification in Banksia. Lamont and Markey (1995) considered that cladistics provided an opportunity to test the prediction that seeder lineages are more likely to undergo rapid turnover (in their terms, speciation and extinction) over evolutionary time than sprouters. Why there should be greater turnover of seeders was not explained, however, as they pointed out the differential diversification of seeder/sprouter clades is ambiguous in Banksia (see Thiele, 1993).

Lower fecundity in sprouters might be a result of their higher genetic load. Lamont and Wiens (2003) considered that accumulating deleterious somatic mutations in association with self-incompatibility might best explain the observed fecundity in the seeder/sprouter dichotomy. They also argued that beneficial somatic mutations would favour ecotypic differentiation and speciation in sprouters and this may have prevented them from becoming extinct via competition and speciation of the more fecund seeders.
Midgley (1996) hypothesised that sprouters (particularly lignotuberous taxa) would be shorter in statue than seeders. In reference to polymorphic taxa that exhibit lignotuberous and non-lignotuberous growth forms (eg varieties of *B. spinulosa* may be lignotuberous or non-lignotuberous) such predictions can easily be explained on the basis of allocation of resources and the multi-stemmed habit. That is, plant height will be reduced when resources are directed into lignotuber development and multiple stems. Without providing detailed analysis he further hypothesised that the tallest species within a genus or clade (regardless of whether a lignotuber existed or not) will be a seeder. Data for Australian species were based on the genus *Banksia* and interpretation of the data is confusing. For example, mean seeder height was 4.56m (*n* = 45) while that of the lignotuberous and epicormic sprouters was 2.25m (*n* = 21) and 10m (*n* = 11) respectively. Average height of the lignotuberous and epicormic sprouters (4.91m) is greater than the seeders and the epicormic sprouters is more than twice that of the seeder height. However data presented for some of the South African Proteaceae, *Olea capensis* and *Euphorboia triangularis* support the hypothesis of seeders being taller than sprouters.

Several ecological strategy schemes have been proposed and more recent reviews are provided by Southwood (1998), Westoby (1999), and Westoby, Falster, Moles, Vesk and Wright (2002). The longest standing scheme has been r- and K-selection (MacArthur & Wilson, 1967; Pianka 1970). A major criticism of the r-K concept is that it is not possible to classify all the life histories strategies on a single axis. Perhaps the most comprehensive extension to the r-K scheme has been the triangular CSR- selection (Grime, 1977). Further expansion of the r-K and CSR schemes include the
quadrangular habitat template (Southwood, 1977; Greenslade, 1982) and its modifications (eg Taylor, Aarssen & Loetle, 1990).

While r-selection initially referred to selection for high population growth in uncrowded populations and K-selection for selection in competitive ability in crowded populations the definitions have broadened over the years. Some authors (eg Boyce, 1984) have argued that strict interpretation of the model as it was originally formulated is necessary for it to be useful. Gadgil and Solbrig (1972) considered that the r-K model was only meaningful in a comparative sense. They concluded that the most important distinction was that r-strategists devoted a greater proportion of available resources to reproduction than K-strategists.

Stearns (1977) considered the different suites of traits associated with r-selection are early age of maturity, large numbers of offspring, semelparity, no parental care and large reproductive effort. In K-selection the traits are delayed (sexual) maturity, small numbers of offspring, iteroparity, parental care and small reproductive effort. Brewer (1994) summarised traits favoured by r-selection as perennial, early maturity, many small young and a short life, and K-selection as annual, late maturity with fewer larger young and a long life. The dichotomy of seeder/sprouter was considered comparable to semelparity (one reproductive episode lifetime\(^{-1}\)) and iteroparity (multiple reproductive episodes lifetime\(^{-1}\)) respectively (Le Maitre, 1992). Parry (1981) recognised four different frequently used nonexclusive meanings applied to r and K selection and also noted the scheme’s lack of recognition of life history constraints imposed by seasonality, habitat stability, severe stress and other factors. Crawley (1986) noted that the r-K dichotomy was very crude because fitness could ultimately be influenced in
different ways by population density. Bell, Hopkins and Pate (1984) placed six
different fire regeneration strategies recognised for kwongan\(^3\) habitats along the r-K
continuum. K-selected were the long-lived sprouters and r-selected were the
monocarpic ephemerals. The other strategies (eg obligate seeders and facultative
seeder-sprouters) could only be assigned arbitrarily to intermediate positions along the
continuum. Within any one regeneration strategy species varied considerably in
morphological and functional characters, many of which have special relevance to
response to fire. Bell et al (1984) noted that precise evaluation of the biology of species
was required if their position on the r-K continuum was to be established. The
dichotomy of seeder/sprouter within fire response is mirrored to a degree by the r-K
continuum of environmental stress. Seeders representing r-selected and sprouters K-
selected species.

Grime’s CSR scheme was proposed as being more pertinent to plants than r and K-
selection. Grime (1977, 1981) recognised stress (reduced photosynthesis) and
disturbance (reduced plant biomass) as the two fundamental factors that limited the
amount of plant material in any given habitat. A 2 X 2 contingency table (creating 4
habitat categories) based on low and high intensities of stress and disturbance formed
the basis for the evolution of three primary strategies (represented by the corners of a
triangle). In low stress, low disturbance habitats competitors (C) evolved; in low stress,
high disturbance ruderals (R) evolved; in high stress, low disturbance stress tolerators
(S) evolved; and in both high stress and high disturbance no viable strategy could
evolve. A major distinction between the CSR trichotomy and the r-K dictomy is the
recognition of stress-tolerance (S). One problem with the CSR scheme is that stress for

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\(^3\) Kwongan is sclerophyll shrubland to 1 m high typically found on sandplains in south-western Australia
one particular species may represent optimal conditions for another species and that ‘stress’ is not measurable (Crawley, 1986) and is often confused with both response and stimuli (Harper, 1982). Grime (1977, 1981) also recognised secondary, intermediate or hybrid strategies based on the primary strategies such as C-R, C-S, S-R and the trihybrid C-S-R strategy.

Grime’s (1981) scheme was developed in the northern hemisphere and very little attention was given to severe stress and disturbance as exemplified by bush fires in Australia. For example, in considering regeneration via persistent seed banks, Grime noted, ‘a rather unusual example, seed stored in closed pine cones’ are released after fire and the plant dies. In the Northern hemisphere serotiny is confined to only three conifer families while in the Southern Hemisphere it is found in the Cupressaceae and six families of angiosperms (Lamont et al 1991) and is common within the fire prone vegetation formations of Australia. Molau (1993) also found that CSR (as well as r-K and the habitat template) did not apply to tundra plants.

Some events, for example shortage of water (drought) may result in both stress (reduced photosynthesis) and disturbance (reduced biomass) and thus the two fundamental factors recognised by Grime may be the extremes of a single continuum (in this case water availability). If this is correct than the contingency table used to support the argument for the evolution of three primary strategies may be confounded.

The purpose of the above discussion is in part acknowledgment, and not an exhaustive evaluation, of schemes for the purpose of classifying the study species. A greater understanding of the biology, the identification of patterns and traits, those factors that
combine to form strategies shaped by selectional forces of the environment and habitat are of primary interest. The schemes considered above may be to broad or crude to clearly characterise between very closely related species. However, r and K-selection is similar to the continuum represented within the classification of fire response (seeder/sprouter) and those traits associated with r and K-selection may also apply to seeders and sprouters. That is, if a contrast in life history traits is expected between seeders and sprouters particularly those traits relating to sexual reproduction then those traits associated with r and K-selection may also be associated with seeders and sprouters. For example seeders are expected to have greater fecundity than sprouters, likewise high fecundity is a trait associated with r-selected species and reduced fecundity a K-selected trait.

1.7. The study species

The genus Banksia L.f. and other members of the enigmatic family, the Proteaceae have attracted considerable scientific and horticultural attention over recent decades. Despite this, George (1998) considered that aspects of their morphology and biology such as life form, inflorescence structure, developmental anatomy and seed production were not well known or understood. The ecology of the woody-fruited serotinous species has dominated research and this, as Lamont and Groom (1998) suggested, provides great scope for comparative biological studies. This is a comparative study of four species that investigates aspects of floral anatomy, inflorescence architecture, seed and fruit set and vegetative regeneration. The four study species are Banksia ericifolia L.f., Banksia integrifolia L.f., Banksia oblongifolia Cav. and Banksia serrata L.f. Photographs of all four species are provided by George (1984), Holliday and Watton (1990), Wrigley and
Fagg (1991), Blomberry and Maloney (1992) and watercolour illustrations are provided by Rosser and George (2001).

The dichotomy of seeder/sprouter has commonly formed the basis for comparative and demographic studies investigating responses to fire and varying fire regimes. For many of these comparative studies congeneric serotinous seeders and sprouters have been the obvious choice (eg Bradstock, 1985; Zammit, 1986). However, this has not reflected a third group in *Banksia*, that is, the non-serotinous species (in this study *B. integrifolia*). Approximately seven percent of banksias are non-serotinous (five species, estimated from George, 1981). Two of these, *B. integrifolia* and *B. marginata*, the only non-serotinous species on the east coast of Australia, are also the most common occurring banksias (Taylor & Hopper, 1991).

One consideration in choosing the study species was availability (they are all common within the Sydney Region, the study area, see Chapter 2). Another was uniformity in variation, or in other words significant variation should be formally recognised. In Georges’s (1981) classical taxonomic revision of *Banksia* three species are recognised to be in need of further study (*B. integrifolia*, *Banksia marginata* and *Banksia spinulosa*). Thiele (1993) researched the variation in *B. integrifolia* complex (discussed below) but as yet the extent of variation in the other two species has not been investigated and this thus precluded them as possible study species. Additionally, with the exception of the nonserotinous *B. integrifolia* they have been among the most extensively studied species on the east coast of Australia (eg Bradstock, 1985; Zammit, 1986) so some aspects of their biology and ecology have been described. Other eastern Australian species occur outside the Sydney Region or are less common (eg
B. plagiocarpa, Banksia canei, Banksia saxicola, Banksia paludosa) and that precluded them as possible study species.

All eastern Australian banksias belong to the subgenus Banksia, having c. 200 to c. 5000 flowers inflorescence\(^{-1}\), distinguished from the only other subgenus Isostylis with c. 100 flowers inflorescence\(^{-1}\) and comprising three species all from Western Australia. Eastern Australian species belong to two sections and three series (George, 1981, 1999). The four study species span this infragenic classification and are briefly described below:

*Banksia* L.f. subgenus *Banksia* (all eastern Australian species)

*Banksia* section *Oncoctylis* Benth.

*Banksia* series *Spicigerae* A. S. George

B. *ericifolia* L.f.

*Banksia* section *Banksia*

*Banksia* series *Salicinae* Meisn.

B. *integriifolia* L.f.

B. *oblongifolia* Cav.

*Banksia* series *Banksia* A. S. George

B. *serrata* L.f.
Section **Oncostylis**

In Section *Oncostylis* the pistil is hooked below the apex, and inflorescence development in eastern Australia (series *Spicigerae*) is basipetal (anthesis proceeds from the top down the inflorescence). The follicles do not split from the stylar point (George, 1981).

**Series Spicigerae** A. S. George

The series contains two species in eastern Australia (*B. ericifolia* and *B. spinulosa*).

**B. ericifolia** L.f.

Two subspecies are recognised as occurring in disjunct locations, both endemic to N.S.W. Both are serotinous shrubs growing to 6 m and are without lignotubers. The extent of follicle dehiscence (serotiny) for the four study species as reported by George (1981) is presented in Table 1.1. Follicles usually opening with fire (George, 1981, 1999). *Banksia ericifolia* is killed by a hot fire and regeneration is from seed. The response of the four study species to fire, the presence or absence of a lignotuber and a description of the bark thickness is given in Table 1.2. Seeds are produced four to six years after fire (Zammit & Westoby, 1987) but may be as long as eight years on shallow soils (Bradstock & O’Connell, 1988). The specific name refers to the heath-like foliage (from the genus *Erica* commonly referred to as heath) (Wrigley & Fagg, 1991). This study was limited to the subspecies *ericifolia*.

**B. ericifolia** L.f. subsp. *ericifolia*

Subspecies *ericifolia* is found between Avoca Beach and Lake Conjola, occasionally extending inland notably west of Sydney, the Budawang Range and Morton National
Chapter 1

Park (Taylor & Hopper, 1991) (Figure 1.1). Inflorescences typically containing 500-2500 (Zammit & Westoby, 1987) golden-yellow to orange red flowers (George, 1981).

Table 1.1. Degree of serotiny or follicle dehiscence for the four study species as reported by George (1981).

<table>
<thead>
<tr>
<th>Species</th>
<th>degree of serotiny</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>remain closed till burnt (see class 2, Section 1.3) George (1981) also states that some will open spontaneously.</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>nonserotinous, all open spontaneously when mature</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>some open spontaneously some require fire</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>some open spontaneously some require fire</td>
</tr>
</tbody>
</table>

Table 1.2. The response of the study species to fire, the occurrence of a lignotuber and a description of the bark (after George, 1981,1999).

<table>
<thead>
<tr>
<th>Species</th>
<th>bark and response to fire</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>without lignotuber, bark 4-5 mm thick, killed by fire regenerates from seed.</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>bark up to 2 cm thick, fire tolerant, regenerates by epicormic shoots from the trunk.</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>regenerates from a lignotuber, above ground stems are killed by fire.</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>bark up to 3 cm thick, fire tolerant, regenerates by epicormic shoots from the trunk</td>
</tr>
</tbody>
</table>
Figure 1.1. Distribution of the four study species in Australia. *Banksia serrata* is the only study species whose range extends onto Tasmania (after George, 1999).

Section *Banksia*

Section *Banksia* have straight pistils below the apex and in eastern Australia development of the inflorescence is acropetal. The follicles of some species leave a lateral beak after opening. This is the largest section with nine series and 50 species. In eastern Australia this section is represented by two series, *Salicinae* and *Banksia* and 13 species (George 1999).
**Banksia** series *Salicinae* Meisn.

Series *Salicinae* contain 11 species and, with the exception of *B. dentata*, is confined to eastern Australia. Follicles are generally small and smooth and without a lateral beak after opening. In keeping with this character, the seed wing is said to be simple and without a lateral lobe. The species of this series are highly variable with the exception of *B. dentata* followed then by *B. robur* Cav. (George, 1981).

**B. integrifolia** L.f.

Three subspecies are currently recognised. Trees to 25m high and fire tolerant (see discussion on response to fire) (George, 1981). It is common along the east coast and into the mountain ranges. Follicles open when mature or usually within a year (non-serotinous, Table 1.1). This species, with the possible exception of *B. spinulosa* Sm., has the broadest latitudinal, altitudinal and ecological amplitude in the genus (Thiele & Ladiges, 1994). George (1981) considered that it gave the "impression that it is actively speciating to fill the many ecological niches throughout its range", and recognised three species and three varieties. Thiele and Ladiges (1994) showed var. *compar* to be polymorphic and comprising two forms. They recognised three subspecies to which var. *aquilonia* was phenetically distinct. George (1999) gave var. *aquilonia* species distinction and retained Thiele and Ladiges (1994) names and combinations for the subspecies of *B. integrifolia*. The specific name, from the Latin *integer* for entire, refers to the entire margins of the mature leaves (Wriggley & Fagg, 1991). This study is limited to the subspecies *integrifolia*. 
B. integrifolia L.f. subsp. integrifolia

Distribution is eastern, south eastern from the western side of Port Phillip Bay in Victoria along the coast to Wide Bay in Queensland and Fraser Island (Figure 1.1). This subspecies is usually found within 2 km of the coast, occurring inland, usually along inlets and estuaries and associated with consolidated coastal sand dunes as a component of woodland (Taylor and Hopper 1991; George, 1981, 1999). Weiss (1984) estimated that 28% of mature plants regenerated after fire. Taylor and Hopper (1991) reported it to resprout from a lignotuber and/or epicormic buds, but also noted that some plants were killed by fire and relied on regeneration from seed. George (1981, 1999) did not describe the presence or absence of a lignotuber in B. integrifolia while Thiele (1993) considered it to be nonlignotuberous. Banksia integrifolia may produce root suckers if disturbed or the roots are uncovered (George, 1981), while Taylor and Hopper (1991) noted that root suckers were found up to 4m from the parent plant.

There is generally less information on the biology and ecology of this nonserotinous species (in comparison to the serotinous study species) particularly response to fire, due no doubt to the less fire prone habitats that B. integrifolia often occupies. Flowers are cream and appear pale yellow after the flowers have opened.

B. oblongifolia Cav.

Banksia oblongifolia is a multi-stemmed lignotuberous shrub to 3m high. Zammit and Westoby (1987a) referred to the multiple stems that arose from the lignotuber as ramets and the lignotuber as the genet. This convention is followed here (but see Chapter 8). The above ground stems (ramets) are killed by a hot fire and the plant resprouts from its lignotuber (genet) (Table, 1.2). Follicles may open spontaneously but the majority remain closed until burnt (serotinous) (Table, 1.1). It is found mostly near the coast,
from Ulladulla N.S.W. to Bundaberg in Queensland (George, 1981, 1999) (Figure 1.1). It is also occasionally found inland for example, near Bell in the Blue Mountains west of Sydney (Taylor & Hopper, 1991). Conran and Clifford (1987) recognised two varieties but George (1981, 1999) and Thiele (1993) considered it to be a single variable taxon. Seeds are produced on regrowth two to three years after fire and inflorescences typically contain 500-2500 (Zammit and Westoby, 1987a) blueish-green (pre-anthesis) to yellow (post-anthesis) flowers (Wrigley & Fagg, 1991).

Series **Banksia** A. S. George

This series contains three species in eastern Australia, all with large follicles that are laterally beaked after opening and thus the seed wing has a short secondary wing or lobe (George, 1981).

**B. serrata** L.f.

A shrub or tree, typically single stemmed to 16m high. The presence of a lignotuber in *B. serrata* has been questioned (eg Theile, 1985; Fuss & Segdley 2000) or stated not to occur (eg Wrigley & Fagg, 1991). As with *B. integrifolia* George (1981,1999) did not describe the presence or absence of a lignotuber in *B. serrata*. Taylor and Hopper (1991) reported that it resprouted from a lignotuber and/or epicormic buds. Bradstock (1985) and Bradstock & Myerscough (1988) considered *B. serrata* to be lignotuberous. Some follicles may open spontaneously but most remain closed until burnt (Table, 1.1) (George, 1981). Flowering occurs about two years after a fire (Bradstock & Myerscough, 1988). *Banksia serrata* is common and generally found along the coast from Wilsons Promontory in Victoria (also occurring west of Burnie in Tasmania) to Fraser Island off the Queensland coast (Figure 1.1)(George, 1999). The specific name
comes from the Latin *serratus*, for serrate in reference to the leaf margins (Wrigley & Fagg, 1991). The inflorescence is broad with grey-green flowers (pre-anthesis) and appearing yellow-green (post-anthesis) (Wrigley & Fagg, 1991).

### 1.8. The thesis

The genus *Banksia* is composed of trees and shrubs, that may be lignotuberous or nonlignotuberous, serotinous or nonserotinous and that respond to perturbations, such as fire and drought by sprouting from a lignotuber and/or epicormic buds as well as root suckering or they die. All these characteristics (ie all the life history attributes currently recognised in the genus) are represented in various combinations in these four study species (Section 1.7, Table 1.3).

<table>
<thead>
<tr>
<th>Species</th>
<th>habit</th>
<th>lignotuber</th>
<th>serotinous</th>
<th>Seeder/sprouter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>Ss</td>
<td>NO</td>
<td>YES</td>
<td>seeder</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>T</td>
<td>YES/NO?</td>
<td>NO</td>
<td>seeder/sprouter?</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>Sm</td>
<td>YES</td>
<td>YES</td>
<td>sprouter</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>T</td>
<td>YES/NO?</td>
<td>YES</td>
<td>sprouter</td>
</tr>
</tbody>
</table>

*Banksia ericifolia* is a serotinous fire sensitive shrub (a seeder). *Banksia integrifolia* is a nonserotinous fire tolerant (to a degree) tree resprouting from epicormic shoots. *Banksia oblongifolia* is a serotinous fire sensitive shrub (stems are sensitive) with a lignotuber (a sprouter). *Banksia serrata* is a serotinous fire tolerant tree that may have a lignotuber and resprouts from epicormic shoots (a sprouter). Additionally the study
species span the infragenic classification of George (1999) (as represented in eastern Australia).

Without a doubt, the most conspicuous characteristic of the genus is the complex, sometimes enormous inflorescence, which George (1981, 1998) and others considered was in need of further study. The floral symmetry and orientation of the individual flowers as well as the arrangement of flowers on the massive and unique inflorescence is poorly understood. In Chapter 3 I investigate floral symmetry and arrangement in the four study species. The theory of spiral phyllotaxy is used to investigate the highest order of symmetry that is, the arrangement of paired flowers on the inflorescence. The phyllotaxy of the vegetative shoot is also investigated and the results are considered in terms of the life history attributes (tree/shrub, serotinous/nonserotinous etc) currently recognised. Different traits might be expected to occur in association with seeders and sprouters (Cowling and Lamont, 1998) and the differences in phyllotaxy are also considered in reference to those traits that might be hypothesised to occur in association with seeders or sprouters. In all other investigations (Chapters 3 – 7, see below) the results are also considered in reference to those life history traits currently recognised and those traits that might be hypothesised to occur with either seeders or sprouters.

_Banksia_ and other members of the Proteaceae produce thousands of flowers but relatively very few fruits. Several investigations into the fecundity or reproductive output have been made in _Banksia_ however fecundity in this genus can be investigated at different levels and these have not always been considered. In Chapter 4 I assess the different levels of fecundity and combine them to provide an overall assessment of reproductive output. The overall reductive output and the different levels of fecundity
of the four species is compared specifically in relation to their life histories and generally in relation to those traits that might be hypothesized to occur in seeders or sprouters.

Previous investigations into the very low fruit to flower ratios in *Banksia* have proceeded without an understanding of the structure of the inflorescence, albeit not always necessary. In Chapter 5 I investigate the possible partitioning of resources within flowers as possible explanations for the low fruit to flower ratio observed. That is, are the asymmetries and floral arrangements in *Banksia*, templates, upon which natural selection has partitioned the allocation of resources? The seeder *B. ericifolia* might be expected to have evolved distinct patterns from the sprouters.

In this study I also investigate the biological aspects represented (in name) by both parts of the dichotomy seeder/sprouter. Vegetative reproduction (the sprouter part to the dichotomy) is a vague phenomenon. Plants may sprout from many different structures and positions due to a variety of anatomical, morphological configurations and constraints. Accessory buds (additional buds in leaf axils) give rise to epicormic resprouting in eucalypts. In this chapter I asked if these *Banksia* species have accessory buds and also is resprouting reliant on accessory buds? Of interest was why are particular anatomical arrangements found in leaf axils and do they differ due to their life history strategies?

The type and extent of the stimuli subjected to a plant are major factors in eliciting the different resprouting responses. The occurrence of a lignotuber in *B. serrata* has been questioned. The occurrence of a lignotuber in *B. integrifolia* has also been questioned
and as a nonserotinous species that occupies relatively less fire prone habitats than the serotinous species its classification as either a seeder or sprouter might also be questioned. Although not forming the basis of a specific investigation (by chapter) I asked if *B. integrifolia* and *B. serrata* are lignotuberous?

Chapter 7 is a case study that investigates reports of resprouting in *B. ericifolia* in the southern most part of the species range. A newly described association of being lignotuberous and fire sensitive requires that the axiom - ‘lignotuberous species are resprouters’ be reconsidered and by default the seeder/sprouter dichotomy may no longer be valid.

In a broader context, if a contrast in life history traits is to be expected between seeders and sprouters how well does the comparative biology investigated in this thesis fit with this dichotomy that has so often been invoked to characterise the species of this genus. As r and K-selection provides the most similar scheme upon which the seeder/sprouter dichotomy may be compared I return to it in Chapter 8 when making a final assessment on the comparative biology of the four *Banksia* species. In this chapter I consider if the classification schemes (seeder/sprouter; r & K-selection) and their associated traits clearly characterise these species.
Chapter 2. The study area and general methodology

2.1. Study area: - the Sydney Region

The study area fell within the confines of the Sydney Basin, \textit{(cf.}, Bembrick, Herbert, Scheibner & Stuntz, 1980), which has often been modified for published floras and referred to as the Sydney Region \textit{(eg.}, Beadle, Evans & Carolin, 1986; Blombery & Maloney, 1992). The northern limit (or collection site) was Spencer near Wisemans Ferry (inland on the Hawkesbury River) and the southern limit was Lake Conjola (south of Jervis Bay on the far south coast). The most inland collection site was Kings Tableland west of Sydney near Wentworth Falls (Table 2.1).

Previous plant studies have typically been limited to a single site or population \textit{(eg.}, Zammit, 1986, 1988; Bradstock 1985, 1990). This study sought to increase the spatial scale so as to encompass potential novel variation. For each investigation, with the exception of Chapter 7 (a case study), the Sydney Region was stratified into three Provinces species\(^1\) (North, Central and Southern Provinces\(^4\)). All collection sites are given in Figure 2.1 and Table 2.1. In each Chapter a list of specific collection sites is provided.

\(^4\) The term Province, as used here, is subordinate to Region and does not refer to any specific or formally recognised structural subdivision or biogeographical area within the Sydney Region. It is used in a relative sense to distinguish between geographical areas or populations within each specific study. Provinces are distinguished relatively on a latitudinal basis, although geographically they may be further separated on a longitudinal basis.
Figure 2.1. Collection sites (NP = National Park) (map adapted from Microsoft, 1997).
<table>
<thead>
<tr>
<th>Name of collection site</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spencer 1 and 2</td>
<td>33° 24′ S 151° 00′ E</td>
</tr>
<tr>
<td>Marramarra NP 1 &amp; 2</td>
<td>33° 26′ S 150° 01′ E</td>
</tr>
<tr>
<td>Patonga</td>
<td>33° 34′ S 151° 15′ E</td>
</tr>
<tr>
<td>West Head</td>
<td>33° 36′ S 151° 18′ E</td>
</tr>
<tr>
<td>Bobbin Head</td>
<td>33° 35′ S 151° 17′ E</td>
</tr>
<tr>
<td>Ku-ring-gai Chase NP 1</td>
<td>33° 37′ S 151° 16′ E</td>
</tr>
<tr>
<td>Ku-ring-gai Chase NP 2</td>
<td>33° 38′ S 151° 18′ E</td>
</tr>
<tr>
<td>Agnes Banks 1</td>
<td>33° 38′ S 150° 43′ E</td>
</tr>
<tr>
<td>Agnes Banks 2</td>
<td>33° 39′ S 150° 43′ E</td>
</tr>
<tr>
<td>Castlereagah</td>
<td>33° 39′ S 150° 44′ E</td>
</tr>
<tr>
<td>Kings Tableland</td>
<td>33° 43′ S 150° 20′ E</td>
</tr>
<tr>
<td>Linden 1 &amp; 2</td>
<td>33° 43 S 150° 30′ E</td>
</tr>
<tr>
<td>Bulls Camp</td>
<td>33° 43′ S 150° 30′ E</td>
</tr>
<tr>
<td>Hepburn Ave</td>
<td>33° 44′ S 150° 30′ E</td>
</tr>
<tr>
<td>Red Bluff</td>
<td>34° 08′ S 151° 07′ E</td>
</tr>
<tr>
<td>Watamolla Beach</td>
<td>34° 08′ S 151° 08′ E</td>
</tr>
<tr>
<td>Garie Beach 1 &amp; 2</td>
<td>34° 08′ S 151° 07′ E</td>
</tr>
<tr>
<td>Royal NP 1 &amp; 2</td>
<td>34° 09′ S 151° 06′ E</td>
</tr>
<tr>
<td>Mt Kiera 1</td>
<td>34° 18′ S 150° 44′ E</td>
</tr>
<tr>
<td>Mt Kiera 2</td>
<td>34° 19′ S 150° 45′ E</td>
</tr>
<tr>
<td>Kiama</td>
<td>34° 42′ S 150° 51′ E</td>
</tr>
<tr>
<td>Seven Mile Beach 1 &amp; 2</td>
<td>34° 48′ S 150° 47′ E</td>
</tr>
</tbody>
</table>
2.2. The range of the study species within the Sydney Region

Two of the four study species (*B. ericifolia* & *B. oblongifolia*) have a restricted distribution within the Sydney Region (Figure 2.2).

![Diagram](image)

**Figure 2.2.** Relative range of the four study species within the Sydney Region. Only *B. integrifolia* and *B. serrata* extend over the entire study area.
Banksia oblongifolia extends southward as far as about Ulladulla but is less common beyond Wollongong. Banksia ericifolia extends as far south as Jervis Bay. The nonserotinous B. integrifolia is generally restricted to within a few kilometres of the coast while the serotinous species extend westward into the Blue Mountains, thereby having a broader altitudinal and longitudinal range. One consequence of the restricted distributions is that, the designation of Provinces does not coincide across species. For example a population of B. ericifolia designated as a Central Province may be adjacent to a population of B. oblongifolia that has been designated as a Southern Province. The three serotinous species are often sympatric (eg at ecotones of heath and woodland formation or on rocky more xeric sites) while B. integrifolia is often found in association with B. serrata in woodland/forest formations nearer the coast.

2.3. General procedure for the collection of samples
Multistage or nested sampling (Krebs, 1999) was used in this study. Populations nested within the North, Central and Southern Province of each species were chosen on a random basis (from a minimum of two potential populations). For each species, each population sampled was separated by at least 40km, but sometimes sites were more than 100km apart. At each collection site, two random line transects (using a compass bearing) were used to obtain samples. Banksia ericifolia and B. oblongifolia (shrubs) were sampled at a minimum of 10m intervals along transects. The larger B. integrifolia and B. serrata (trees) were sampled at a minimum of 15m intervals along transects. In the case of B. integrifolia, due to the possible clonal nature of stands (see Chapter 8), only one ‘potential ramet’ was sampled when clumps occurred. Sampling from the tree species (B. integrifolia and B. serrata) was limited to, and facilitated by, the use of long
handled loppers to a maximum height of approximately 5 metres. Specific details are provided with each chapter.

2.3.1. Habit and vegetation structural formations

To obtain samples of individuals that were representative of the species generally, collection of plant material was restricted to the more common habit and habitats of the four species. The structural formations below follow Specht (1981b). *Banksia ericifolia* is commonly found as a dominant or codominant shrub in closed heath, although it does occur as an understorey shrub in woodlands and forest. Collections were always made from closed heath associations (often in association with *Callistemon, Hakea* and *Allocasuarina*) of 2m high or taller, with the exception of the *B. ericifolia* case study Chapter 7 (details are provided there).

*Banksia integrifolia* may occur as an ecotypic prostrate shrub (eg cultivar Austraflora ‘Roller Coaster’) but more generally occurs as a tree on coastal sand dunes and along streams and rivers. Shorter forms are often found on windswept headlands. Collections were always made from the taller tree forms that were either dominant, or part of the substratum in Eucalypt woodland and forests.

*Banksia oblongifolia* generally occurs as a small shrub as part of the understorey in woodlands and forests, along swamp margins or as a component of heath or shrublands. Collections were always made from heath and woodland formations.

*Banksia serrata* may occur as a prostrate shrub (eg cultivar Austraflora ‘Pygmy Possum’) but more generally occurs as a very small tree in association with closed
heath, a dominant or codominant tree in woodlands, or a tree (sometimes forming part of the substratum) in a forest. Collections were always made from tree specimens in *Banksia* or *Banksia/Eucalypust* woodlands or *Eucalyptus* forest formations.

### 2.3.2. Cohort sampling

The ability to estimate the age class (cohort) of infructescences on serotinous plants typically varies within a year. That is, when the youngest infructescences have only recently matured they are easily distinguished from the previous years cones and at least three groups can be clearly distinguished on a plant at that stage. These are the youngest cohort, the previous season’s cohort and these are distinguishable from all other older infructescences. This last group may span several years of infructescence production. Cohorts are distinguished on the basis of colouration of fruits, the colour and persistence of perianths and styles and the extent of weathering of the fruits. Distinguishing between *Banksia* cone cohorts using this type of methodology has been reported by other researchers (eg. Bradstock, 1985; Zammit, 1986).

Nonserotinous species release their seeds spontaneously when mature but the cones are maintained on the plants for many years (pers. obs.). Distinguishing between cohorts in these species (seedless cones) is more difficult, although an age structure is clearly visually apparent. While this study did not specifically sample cohorts, sampling was carried out only on populations that contained at least three cohorts. The significance of this is that the populations sampled had not recently been disturbed by fire, and thus, for example, provided some buffer against the possible shortfalls of sampling from one year’s post fire reproductive/vegetative effort. This same procedure applied when sampling for inflorescences and branch material.
In all instances, all laboratory and nursery work was carried out at the University of Western Sydney, Richmond campus (hereafter UWS).
Chapter 3. Floral arrangement and phyllotaxy in *Banksia*

3.1. Introduction

3.1.1. The *Banksia* floral structure

*Banksia* flowers are arranged in pairs and are subtended by two floral bracts and one larger common bract (cf. the unit inflorescence\(^5\)) (Figure 3.1). The paired flowers arise from a short shoot axillary meristem initiated in the axil of the common bract (Douglas & Tucker, 1996a). The typical *Banksia* floral structure (the inflorescence) is highly condensed, compound and composed of multiples of unit inflorescences arranged at right angles to and around a central woody axis (George, 1981). The inflorescence varies in size and shape from those of the subgenus *Isostylis* with a head-like inflorescence of up to 100 flowers to those of the subgenus *Banksia* with ovoid and cylindrical inflorescences containing 200-5000 flowers (George, 1999). This complex floral structure of multiple unit inflorescences may more correctly be referred to as a conflorescence (Johnson & Briggs, 1975) or synflorescence (George, 1981). The terms unit inflorescence, for the paired flowers and three bracts and inflorescence, for multiple unit inflorescences, and infructescence (cone) for the senescent inflorescence that has developed follicles will be used here. Regardless of terminology, the above description serves the purpose of highlighting the structural complexity of the *Banksia* floral and fruiting structure considered by Johnson and Briggs (1975) and George (1981) to be in need of further study.

\(^5\) Some authors have used the term uninflorescence (eg Douglas & Tucker, 1996a).
Figure 3.1. Schematic (and uncondensed) representation of a *Banksia* inflorescence (and unit inflorescences) showing the arrangement of flowers, floral and common bracts.

The *Banksia* inflorescence is not only unique in its outward appearance but its vasculature is unique within the Proteaceae. Strands branch off from a central vascular system and anastomose giving rise to a mesh-like cortical vascular system that connects the appendages (flowers and bracts). The appendages are interspersed with dense hairs (Venkato Rao, 1964).

In Troll’s (1964) typology of angiosperm inflorescences two main types are recognised; monotelic inflorescences which end in a terminal flower, and polytelic inflorescences which do not (Weberling, 1965, 1989). The apex of the polytelic inflorescence does not complete development and the flowers atrophy in the same way as does the end of the axis (Weberling, 1965). Briggs and Johnson (1979) considered inflorescences of all Proteaceae to be indeterminate (polytelic).
3.1.2. The *Banksia* gynoecium and floral orientation

Patterns of floral symmetry vary considerably in the Proteaceae, not only among taxa, but also within single flowers. In the unit inflorescence of the Grevilleoideae another level of symmetry operates, and at the inflorescence level (multiple unit inflorescences) an additional level of symmetry is observed (Johnson & Briggs, 1975; Douglas, 1995).

In 1960 Haber described the ovary of *B. integrifolia* as grooved and open on its ventral margin and containing two collateral ovules in a unilocular carpel (see Figure 3.2a). Her illustrations clearly show this arrangement in a single flower but they do not indicate the relative arrangement of ovules in the flowers of a unit inflorescence relative to the floral bracts or long axis of the inflorescence. She considered the flower to be actinomorphic (having radial symmetry) or nearly so, however, all descriptions that have followed have reported *Banksia* to be zygomorphic (having bilateral symmetry) (eg Johnson & Briggs, 1975; Douglas, 1999).

In some actinomorphic genera of the Grevilleoideae there is a degree of obliquity with respect to the inner whorls and the midline of the floral bract. In actinomorphic genera lacking floral bracts there may be varying degrees of obliquity between flowers. In contrast zygomorphic genera of Grevilleoideae show consistency in floral orientation (Johnson & Briggs, 1963). Even so, in *Banksia* the floral bracts are slightly displaced from their theoretical positions in the reduced panicles or pseudo-racemes and Johnson and Briggs (1963) considered there was some difficulty in determining the floral orientation, at least in regard to the floral bract. The paired flowers are said to be concavely skewed in relation to the common bract (Douglas & Tucker, 1996a).
Figure 3.2. Carpel orientations as referenced by the ventral suture (a-c, and e) and carpel cleft (d) as reported in *Banksia* (axis refers to the inflorescence axis).  a) single flower and floral bract with suture 90° to bract; b) extension of orientation in a) to the unit inflorescence and common bract; c) ventral sutures 180° to bract and inflorescence axis and suture facing away (also facing opposite direction); d) carpel clefts (not ventral sutures) at 45° to bract and e) similar to c) but with sutures facing each other. Other orientations are clearly possible but have not been reported for *Banksia*. 
Regardless of orientation of flowers relative to the floral bracts Johnson and Briggs (1963) stated that direction of curvature of the style in Grevilloid flowers is towards the suture of the ovary. This interpretation is also consistent with the floral diagrams presented by Johnson and Briggs (1963, 1975) (Figure 3.2a). This was disputed by Thiele (1993) who stated that in Banksia the direction of curvature was longitudinal with respect to the inflorescence axis or at 90° to the ventral suture of the ovary and that it appears to be unique in the Proteaceae. It is clearly longitudinal with respect to the long axis of the inflorescence (pers. obs.) and as stated by Thiele (1993), but curvature in respect of the ventral suture is not obvious. Thus the contrast in the statements on curvature are probably a result of differing interpretations on the orientation of the ventral suture of the ovary.

Discrepancy in carpel orientation is found elsewhere in the Banksia literature. For example, Venkato Rao (1964) diagrammed the floral structure for Banksieae showing the ventral suture to lie at 90° to the floral bract (Figure 3.2b). Elsewhere in the same report Venkato Rao stated that the ventral suture was oriented 180° to the floral bract and that the ventral sutures of the unit inflorescence faced opposite directions (either Figure 3.2c or 3.2e). Wrigley and Fagg (1989) presented a floral diagram of the Banksia unit inflorescence and showed the ventral suture to lie at 90° towards the floral bract (Figure 3.2b). This arrangement is the same as presented by Johnson and Briggs (1964) for a single flower and that illustrated for Banksieae by Venkato Rao (1964).

Douglas and Tucker (1996b) reported six orientations within the Grevilleoideae, based on the appearance of the carpel cleft in the early ontogeny of the flower. They described the carpel cleft orientation in Banksia as abaxial lateral (Figure 3.2). Their
work was primarily concerned with hypotheses of homology and they used early stages of flower development as they are considered to be more straightforward and easier to interpret than mature stages. Conversely it is more likely to be the mature stages that would best reflect the functionality and thus most likely to provide ecological insights into the symmetry of the unit inflorescence and/or inflorescence.

3.1.3. Phyllotaxy

Plants have symmetries that are basic to our systems of classification and to our aesthetic appreciation of them. Phyllotaxy (phyllon –leaf; taxis –order) refers to the arrangement or pattern of organs (historically leaves) on a plant axis. Some of the terms used to describe the different arrangement of leaves (based on the number of leaves node$^{-1}$) are given in Table 3.1. More than 80% of the extant plant species have spiral arrangements (Goodwin, 1994).

Phyllotactic symmetry is perhaps the most striking or ‘eye catching’ symmetry in plants (Erickson 1998). The spiral patterns made by the florets (and ultimately seeds) in the capitulum of a daisy or sunflower, the scales of a pinecone or the multiplefruit of a pineapple are the best known examples of phyllotactic symmetry. The two spirals that radiate in different directions from the centre of sunflowers are called parastichies. The number of parastichies in both directions decline towards the centre and ultimately forms a single spiral (Jean, 1994). Explanation of these patterns wrote Darwin (Darwin, 1897), could drive the sanest man mad (but not a madman sane, my wife.).

While the spiral phyllotaxtic patterns have been recognised for centuries the mechanisms underlying the initiation of the pattern remains unclear (Niklas, 1997; Klar, 1994).
Theories, concepts and models that attempt to explain the patterns come from botany, mathematics (Adler, 1998), crystallography (Mackay, 1998) and molecular genetics (Lima-de-Faria, 1998). The extensive use of crystallographic terminology, the multidisciplinary approach and morphogenetical parallelism to other fields of research has led Jean (1994) to suggest Primordial Pattern Morphogenesis (PPM, also an acronym for Pyramidal Pattern Modelling), as an alternate to phyllotaxy. Mechanico-chemical field theory (Williams, 1975) and general field theory of phyllotaxis (Williams & Brittain, 1984) have also been proposed for similar reasons.

Leonardo da Vinci (1452 –1519) apparently observed the regular spacing of leaves and considered the benefit to be that branches could grow without covering each other. Charles Bonnet (1720-1793) after Linnaeus (1707-1778) is generally credited with recognising and studying spiral leaf arrangement. Bonnet also theorised that the spiral arrangement maximised the separation of leaves and allowed air to circulate more freely. Airy’s (1873) functional explanation was that the common divergences permitted the most dense packaging of leaves in a bud. Wiesner (1875) argued that the spiral arrangement gave plants the best access to light.

The mathematical approach to phyllotaxy was possibly initiated by Kepler (1571-1630), who pointed out the significant occurrence of the number five and the numbers of the Fibonacci series in the arrangement of leaves. A truly mathematical theory of phyllotaxy lay with Schimper (1830) and Braun (1835) followed shortly thereafter by the Bravais brothers (1837).6

6 This brief historical account of phyllotaxy was obtained from the reviews by Alder (1974), Jean (1994) and Jean and Barabé (1998).
Physical or structural explanations for (spiral) pattern formation include Snow and Snow’s (1932) space-filling or first available space theory. It predicts the formation of primordia in the first space available that attains some minimum size and distance from the apical tip. This is, in some ways similar to Hofmeister’s axiom or rule which states that a leaf will arise in the largest gap between existing leaves and as far away from them as possible (Jean, 1994). But as noted by Jean (1994) in spiromonostichy, primordia arise close to each other along a single spiral. Alder’s (1974, 1977) model for pattern formation is based on physical contact pressure between primordium. Perhaps the most comprehensive physical explanation for pattern formation was presented by Green (1992, 1999) and was based on biological and chemical activity, considered in terms of physical parameters.

Chemical explanations for pattern formation typically involve diffusion fields of chemical inhibitors and promoters that direct the initiation of primordia (Jean & Barabé, 1998). Distinguished from the physical and chemical explanations of ‘how’ patterns are formed is Jean’s (1994) ‘global’ approach that asks ‘why’. Other than this recognition of the environment, evolutionary and ecological considerations of phyllotaxy have been comparatively lacking.
Table 3.1. Terms used to describe types of leaf arrangement (Jean, 1994: Bell, 1991)

Jugy (J) refers to the number of primordia initiated plastochron\(^{-1}\). A plastochron is the period of time between the commencement of two successive repetitive phenomena eg the initiation of leaf primordia (Fahn, 1990).

<table>
<thead>
<tr>
<th>leaves node(^{-1})</th>
<th>description and variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>one (alternate)</td>
<td>Monostichous: all leaves are on the same side of the stem.</td>
</tr>
<tr>
<td>J = 1</td>
<td>Distichous: leaves in two rows usually 180° apart.</td>
</tr>
<tr>
<td></td>
<td>If either arrangement is twisted the prefix spiro or helio is used as in spiromonostichous or spirodistichous.</td>
</tr>
<tr>
<td></td>
<td>Spiral: when more than two rows are seen from above.</td>
</tr>
<tr>
<td>two</td>
<td>Opposite: when 180° apart.</td>
</tr>
<tr>
<td>J = 2</td>
<td>Opposite decussate: paired and 90° to each other.</td>
</tr>
<tr>
<td></td>
<td>Spiral decussate: less than 90° to each other.</td>
</tr>
<tr>
<td></td>
<td>The prefix pseudo has also been used, (eg Gómez-Campo, 1974).</td>
</tr>
<tr>
<td>three or more</td>
<td>Whorled or verticillate and these may be alternating or superimposed.</td>
</tr>
<tr>
<td>J ≥ 3</td>
<td>Pseudowhorls: series of very short internodes usually separated by a very long internode giving the impression of being whorled.</td>
</tr>
<tr>
<td>other</td>
<td>Metamorphosis or change in phyllotaxy in a single shoot. Secondary shifts in orientation, adnation, dimorphism and orixate. Variation between plagiotrophic and orthotropic shoots within a plant. Sometimes undeterminable or confused (not to the plant but the morphologist, as Bell (1991) put it) phyllotaxy occurs.</td>
</tr>
</tbody>
</table>
The mathematical aspects of phyllotaxy start with the Fibonacci numbers and the Golden Section or Golden Mean (Adler, 1974; Jean 1994; Green, 1999). Any sequence \((a_n), a_1, a_2, a_3, \ldots a_n, \ldots\), is called a Fibonacci sequence if the following recurrence relation holds:

\[
a_{n+1} = a_n + a_{n-1}, \quad n>1.
\]

The first two terms of a sequence dictate the type of sequence. The sequence:

\[
F_n = 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, \ldots \ldots
\]

is often referred to as the Fibonacci (or main) sequence as it was the first Fibonacci sequence to receive major attention (Alder, 1974) and the most common sequence (≈ 92% of species examined) found to occur (Jean, 1994). It holds that each term in a sequence is the sum of its two immediate predecessors. Other phyllotactic sequences are found but are relatively rare (Jean, 1994). Examples of the more common of the rarer sequences are:

- the first accessory \(<1, 3, 4, 7, 11, 18, \ldots \ldots>\) (≈ 1.5%) or Lucas sequence
- the second accessory \(<1, 4, 5, 9, 14, 23, \ldots \ldots>\) (≈ 0.1%)
- the third accessory \(<1, 5, 6, 11, 17, 28, \ldots \ldots>\) (≈ 0.1%)
- bijugate main \(2<1, 2, 3, 5, 8, \ldots \ldots>\) (6%)
- bijugate first accessory \(2<1, 3, 4, 7, 11, 18, \ldots \ldots>\) etc
Zagorska-Marek (1985), Jean (1994) and Jean and Barabé (1998) provided lists of sequences found in plants, including the multijugate sequences not indicated above. The phyllotactic patterns generally found in plants are represented by alternate terms of the Fibonacci sequence in fractions:

\[
\frac{1}{2}, \frac{1}{3}, \frac{2}{5}, \frac{3}{8}, \frac{5}{13}, \ldots
\]

The phyllotactic fraction (PF) is a measure of the angle of divergence (d) between the points of insertion of successive leaves (Figure 3.3).

**Figure 3.3.** Leaf arrangement as viewed from above and from the side of a vegetative shoot demonstrating an example of the phylltactic fraction (PF = 1/3), divergence (d = 120°) and the genetic spiral. Note the genetic spiral could run in the opposite direction (as viewed from above) as there is no convention on how it is visualised.
Chapter 3

The genetic (generative, fundamental or ontogenetic) spiral (helix) is an imaginary line that connects primordia by the shortest path around a plant axis in the chronological order in which they were produced (Thomas, 1975; Thomas & Cannell, 1980; Jean, 1994) (see Figure 3.3).

Genetic spirals may run either clockwise (sometimes referred to as S helix) or anticlockwise (Z helix) (Zagorska-Marek 1985). Klar (2002) described the spirals of a sample of pine cones as dextral, righty and clockwise. Perhaps because of the multidisciplinary approach, there is no consistent system of nomenclature within the phyllotactic literature. Fan Xudong, Peng Ju Lin and Bursill (1988) provided a convention for the determination of chirality, although it does not appear to have been widely supported by other authors. A spiral that turns in a clockwise direction is often referred to as being a left handed spiral in reference to the direction of the curvature of the fingers when a fist is made with the left hand. An anticlockwise spiral for the same hand/fist reasoning is referred to as a righthanded spiral (Kappraff, 2001). A clockwise spiral visualised from above a stem and running down the stem is an anticlockwise spiral viewed from the other direction. The Fan Xudong et al (1988) convention suggested that in plants chirality should be determined by pointing the thumb in the direction of growth with primordia numbered from the oldest to the youngest. This convention is followed here.

Chirality is not always completely random. In Hibiscus furcartus, lateral branches tend to have the same phyllotactic handedness as their parent branch, while a transition always occurs in handedness from the vegetative to reproductive shoot (Tennakone, Dayatilaka & Ariyaratne, 1982). Zagorska-Marek (1985) found that a change in
handedness of the genetic spiral often accompanied a change in the pattern of parastichy in *Abies balsamea*. Within the Eucalypts, Davis and Mark (1981) found stem twisting to be unidirectional in most species examined by them. In the coconut Davis (1962, 1963) reported a significant difference in the direction of spiralling between the north and southern hemispheres.

The golden ratio (Greek tau) τ or (phi) φ an irrational number (=\((\sqrt{5}+1)/2\)) was a symbol of harmony for the Greeks because of its generation of aesthetic proportions in geometry, art and architecture. It was also known to the ancient Egyptians and Hindus (Kapproff, 2001). The ratio of consecutive Fibonacci numbers 2/1, 3/2, 5/3,….gets closer to the golden ratio (approximately 0.38197) as the numbers in the ratio become larger. Phyllotactic fractions in the Fibonacci sequence also converge on 137.5092° (≈ 360°/φ²).

The biological significance is that if successive leaves are formed at this angle 137.5092° (or have this divergence) no leaf will lie exactly above any other leaf (Niklas, 1997). Phyllotactic fractions from the Fibonacci sequence 5/13, 8/21 and 13/34 and above approach this angle. Most plants (>92% examined according to Jean, 1994) apparently conform to the Fibonacci sequence and thus divergences tend toward the Golden ratio, thereby minimising leaf overlap and presumably shading. Divergences are visualised (and calculated) from directly above a plant stem. Clearly not all plant stems grow directly upward and not all sunlight is received from directly above. Leaf shape, orientation (some leaves track the sun) and internode distances play major roles in the light harvesting abilities of plants (Niklas, 1997).
Another aspect of the biological significance of this pattern is the relationship of the golden ratio with a logarithmic spiral and close packaging. A logarithmic spiral always has the same shape, regardless of its size (extended inwards or outwards it retains its shape). The clockwise and anticlockwise parastichies of the capitulum of a sunflower form logarithmic spirals and, therefore, the spaces (where seed are formed) between the intersecting spirals are of the same shape but not the same size. Sunflower seeds vary in size but not shape. This same relationship is found in the close packaging of the multiple fruit of a pineapple (Stevens, 1974; Bell, 1991). Ridley (1982) noted that packaging efficiency does not provide an explanation for Fibonacci phyllotaxy and that it should be regarded as a consequence and not a cause.

Jugy or jugacy (J) refers to the number of primordia that arise at each node. For example, when one leaf occurs per node the term unijugate (J = 1) applies and the plant has one genetic spiral. In bijugate plants (J = 2) two primordia arise per node and two genetic spirals can be visualised. The term multijugate applies to whorled systems (J>2, trijugate, tetrajugate etc). The plastochron is the period of time between successive initiations of leaves (organs) (Fahn, 1990). The plastochron ratio (R) is a measure for the increasing distance of leaves (nodes) from the shoot apex. The plastochron and the plastochron ratio often decrease in the transition of a shoot from the vegetative to the flowering state (Rutishauser, 1998).

A parastichy is any phyllotactic spiral visualised on a plant. A genetic spiral is in this sense a parastichy. The term parastichy comes from the Greek meaning side row, which distinguishes it from the genetic spiral or main row. Parastichies which are obvious to the naked eye are called contact parastichies. A parastichy pair (denoted m & n) refers
to a family of parastichies that run in different directions. The parastichy pair can form a variety of relationships all of which are discussed by Jean (1994). Of the contact parastichies the visible and opposed parastichy pair is perhaps the most familiar and includes the parastichies of the capitulum of the sunflower, the fruits of a pineapple or the scales of a pinecone. A typical sunflower will have 34 parastichies running in one direction with a family of 55 running in the other direction. These numbers belong to the Fibonacci sequence \(<\ldots 8, 13, 21, 34, 55, \ldots \)\>. Smaller and larger sunflowers show smaller and larger numbers of parastichies from the Fibonacci sequence (eg \(m, n\) may be 13, 21 or 89, 144). The changing value of \(m\) and \(n\) as parastichies move out across the surface of the capitulum of a sunflower is referred to as rising phyllotaxis. An orthostichy is a straight line or a common longitudinal line obtained by joining appendages that are approximately superimposed relative to either the plant axis or apical tip (Bell, 1991; Jean, 1994). For example, a line drawn from leaf numbered zero (0) to leaf numbered three (3) in Figure 3.3 represents an orthostichy.

3.1.4. Arrangement of the unit inflorescences on the *Banksia* inflorescence

Venkato Rao (1964) described the paired flowers of *Banksia* to be arranged in spiral or vertical series. Blake (1971) described the paired flowers of *B. ericifolia* (as well as *B. spinulosa* and *B. collina* (B. spinulosa var. collina) to be arranged in ‘about 15 slightly spiral vertical series’. Blake (1971) also reported a similar arrangement for *B. integrifolia* with 16-20 vertical series, *B. aemula* and *B. serrata* with 24-30 vertical series and *B. oblongifolia* with about 21-25 series. Blake (1971) gave no other description or explanation and the sample appears to be limited to specimens in a private garden as it is the only source mentioned.
George (1981) reported the unit inflorescences to be arranged on the central woody axis in not one but three line patterns, vertical and both dextral and sinistral spirals (Figure 3.1). In a study of floral initiation and development in *B. coccinea* and *B. menziesii*, Fuss and Sedgley (1990) found the unit inflorescences to be arranged in 13 genetic spirals and that the arrangement gave the appearance of forming vertical rows on the long axis. No other description or elaboration of the arrangement is given, although PF’s are given for *B. coccinea* (27.7°) and *B. menziesii* (14.6°). They also reported that the phyllotaxy of the vegetative shoot was 135° for *B. coccinea* and 138.5° for *B. menziesii*. A methodology of how they arrived at these PF values is not given in either case. Walker and Whelan (1991) described a sample of *B. ericifolia* and *B. spinulosa* inflorescences as consisting of 30-80 floral whorls, each of which contain 13-14 and 7-8 pairs of flowers respectively. Atwell, Kriedmann and Turnbull (1999) described the phyllotaxy of the *B. integrifolia* inflorescence as flowers forming vertical arrays. Lloyd, Ayre and Whelan (2002) described a typical *B. ericifolia* inflorescence to comprise ‘12 parallel columns of between 25-40 pairs of flowers arranged around a central rachis’.

The variation in the above examples of descriptions of banksia inflorescences highlights a deficiency in the current understanding of the structure. However, what is known tells us that it is unique, not only within the Proteaceae but also within angiosperms generally. Cowling and Lamont (1998) hypothesised that different traits might be expected to occur in association with seeders and sprouters especially traits associated with reproduction. A better understanding of the *Banksia* inflorescence would enable us to assess if differences do exist between seeder and sprouter and if other life history correlations exist.
Patterns are initiated in the primordia of the stem apex, whereas patterns of mature structures may be of secondary origin. In this chapter I sought to explore the evolutionary and ecological aspects of the phyllotaxy and thus the functions of the mature organs rather than the mechanisms that initiated the patterns.

In any phyllotactic study the first procedure is pattern recognition (Jean, 1994). The above reports (descriptions of *Banksia* inflorescences) generate several questions.

- How many line patterns are there in banksias?
- What is the relationship between spirals and rows in the *Banksia* inflorescence?
- How can there be odd numbers of vertical rows and odd numbers of paired flowers whorl$^{-1}$ as described by Blake (1971) and Walker and Whelan (1991) when adjacent vertical rows (around a cylinder) have alternating unit inflorescences as depicted by George (1981) and others (see Figure 3.1)?
- What variation, if any, exits inter- and intra-species and how do they compare?
- Do the arrangements correlate with other characteristics, either phylogenetic or ecological?
- Can the arrangements be described in terms of orthostichies, parastichies, and do they follow Fibonacci type series, Lucas or some other pattern?
- What are the phyllotactic fractions of the leaves of the four study species and how do they compare?
- What is the phyllotactic arrangement of the inflorescences of the four study species and how do they compare?
- Is there any relationship between the phyllotaxy of the vegetative shoot and the floral shoot?
What evolutionary and/or ecological consequences can be drawn from the arrangements and does the inflorescence phyllotaxy shed any light on the unique floral structure of *Banksia*?

Johnson and Briggs (1963) considered that in *Banksia* the floral bracts are slightly displaced from their theoretical positions and there was some difficulty in determining the floral orientation at least in regard to floral bract (Section 3.1.2). In this Chapter I also asked the following questions:

- What is the orientation of the ventral suture in *Banksia* flowers?
- What is the orientation of the ventral sutures in the unit inflorescence (paired flowers), and what are the possible consequences of the orientations?
- Can the obliquity of the floral bracts be explained at the level of the inflorescence rather than viewing the bracts at the level of the unit inflorescence?
- What might be the significance of the mesh-like cortical vascular system of *Banksia* that Venkato Rao (1964) considered as unique?
3.2. Methods

3.2.1. The *Banksia* gynoecium and floral orientation

Over a period of two flowering seasons (2000-2001) 1200 unit inflorescences were collected and dissected *in vivo* (attached to the inflorescence central axis). Ten unit inflorescences were sampled from along the length of two inflorescences from 5 trees Province\(^1\) \((n = 100)\) for the four *Banksia* study species (*B. ericifolia*, *B. integrifolia*, *B. oblongifolia*, *B. serrata*). Collection sites within each Province are given in Table 3.2. The sample of inflorescences was limited to those that had commenced anthesis. The sample thus contained flowers at various stages of development (pre and post anthesis). Dissection *in vivo* maintained the floral orientation. Slightly oblique transverse cuts were made through unit inflorescences at the level of the ovules and the cut surface viewed under a dissecting microscope (Olympus SZH X200, variable mag. up to 200X). The ‘sectioning’ was similar to the action of whittling. Staining with toluidine blue and slight drying (evaporation at ambient temperature) of the cut surface increased visualisation (particularly in mature ovaries) due to shrinkage and separation of the floral parts. The orientation of the ventral sutures of both flowers of a unit inflorescence were then scored.

3.2.2. The phyllotaxy of leaves

An initial investigation of leaf phyllotaxy was made to establish the general arrangement of leaves and to develop a suitable methodology to score phyllotactic fractions (PF’s).
During 2002/2003, five (relatively straight, non-twisted) sections of stems with leaves still intact were randomly sampled from each of five plant Province$^{-1}$ species$^{-1}$. Collection sites within each Province are given in Table 3.2. The length of the samples varied between species because of the considerable difference in internode lengths. Leaves were removed to facilitate counting (using petiole scars) and the arrangement scored (where possible) as a PF. Phyllotactic fractions were recorded as consistent only if they were recognisable over three consecutive counts branch$^{-1}$. That is, starting at leaf zero (see Figure 3.3) the number of leaves and turns of the genetic spiral were scored until orthostichy. Leaf one was then used as leaf zero to check the PF, and this was repeated again by the same process. The level of consistency (out of five branches plant$^{-1}$) was recorded and converted into a percentage. Phyllotactic fractions were recorded as inconsistent if they did not meet the criterion of consistency. Notes on the arrangements (including frequency of inconsistent PF’s) were also made.

### 3.2.3. Phyllotaxy of the inflorescence

An initial extensive pilot investigation of inflorescences was made to ascertain what patterns or characteristics should, or could, be scored.

During the flowering seasons of 2002-2003 three inflorescences were randomly sampled from each of five plant species$^{-1}$ Province$^{-1}$ (see Table 3.2 for collection sites within each Province). The number and direction of line patterns (made by flowers) were counted inflorescence$^{-1}$. The total number of unit inflorescences along the vertical rows were also scored. Three counts were scored when the initiation of the flowers on an inflorescence were not consistent to the base. The height of the inflorescence from where flowers were initiated to where they persisted at the apex was measured using
vernier callipers. Three measurements of height were also made when the initiation of the flowers on an inflorescence were not consistent at the base. The diameters of the central axis, including the floral and common bract, was also measured. If the central axis was irregular two measurements were made; one in the central portion of the bottom half and one in the central portion of the top half of the inflorescence.

Table 3.2. Location of collection sites within each Province for each species and investigation. NP = National Park. For location map and grid reference see Chapter 2.

Investigation: The *Banksia* gynoeicum

<table>
<thead>
<tr>
<th>Species</th>
<th>North Province</th>
<th>Central Province</th>
<th>South Province</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>Ku-ring-gai Chase NP1</td>
<td>Bulls Camp</td>
<td>Mt Kiera 1</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>Garie Beach 1</td>
<td>Seven Mile Beach 2</td>
<td>Lake Conjola</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>Ku-ring-gai Chase NP2</td>
<td>Agnes Banks 2</td>
<td>Red Bluff</td>
</tr>
<tr>
<td>B. serrata</td>
<td>Agnes Banks</td>
<td>Kings Tableland</td>
<td>Mt Kiera 2</td>
</tr>
</tbody>
</table>

Investigation: Phyllotaxy of leaves and inflorescence

<table>
<thead>
<tr>
<th>Species</th>
<th>North Province</th>
<th>Central Province</th>
<th>South Province</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>Ku-ring-gai Chase NP1</td>
<td>Royal NP</td>
<td>Hyman Beach</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>Spencer 1</td>
<td>Watamolla Beach</td>
<td>Kiama</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>Ku-ring-gai Chase NP1</td>
<td>Agnes Banks 2</td>
<td>Royal NP1</td>
</tr>
<tr>
<td>B. serrata</td>
<td>Ku-ring-gai Chase NP2</td>
<td>Royal NP 2</td>
<td>Mt Kiera 2</td>
</tr>
</tbody>
</table>

Average heights of unit inflorescences was calculated by dividing the height of the inflorescence by the number of unit inflorescences occurring along the length of the inflorescence. Average widths of unit inflorescences were calculated by dividing the
average circumference (estimated from diameter measurements) by the number of vertical rows of unit inflorescences. Notes on the arrangements of the unit inflorescences were also made. Removal of flowers was often necessary to establish the line patterns and use of cotton thread facilitated in distinguishing between whorled and spiral arrangements. Arrangements were scored as being whorled only, whorled and spiral if the arrangements changed along an inflorescence and spiral only. In those inflorescences that changed from whorled to spiral arrangements along an inflorescence it was noted which arrangement was the more predominant (i.e. mostly whorled or mostly spiral).
3.3. Results

3.3.1. The Banksia gynoecium and floral orientation.

With the exception of one flower each from *B. integrifolia* and *B. serrata*, all flowers were orientated such that their ventral sutures were at 90° to the inflorescence axis and approximately 180° to the common bract (Table 3.3, Figure 3.4 & 3.5a). In the case of the two exceptions the orientation of the ventral sutures were similar (moved through 45° or more and thus approaching 180° to the inflorescence axis) (Table 3.3, Figure 3.5b). In all dissections the ventral sutures of the flowers of the unit inflorescence faced each other and thus were oriented in opposite directions (Figure 3.4).

<table>
<thead>
<tr>
<th>Species</th>
<th>North Province</th>
<th>Central Province</th>
<th>South Province</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>99/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>100/100</td>
<td>99/100</td>
<td>100/100</td>
</tr>
</tbody>
</table>
Figure 3.4. Photographs of hand cut oblique transverse surfaces of unit inflorescences. a) *B. integrifolia* showing several immature flowers and bracts, with parts of a unit inflorescence indicated by the arrows, and in b) an enlargement of a single flower from a) showing the lateral orientation of ovules. In c) mature ovary of *B. oblongifolia* stained with toluidine blue. Two arrows indicate the two ovules laterally attached to one side of an asymmetrical ovary. Although transversely dissected the ventral suture is distinguishable as a slightly thicker section of ovary wall (heavier stain section) opposite the midline of the two ovules (indicated by the right hand arrow in c).
3.3.2. Leaf phyllotaxy

There was no regularly consistent leaf PF’s scored for any species (across all plants and Provinces). Inconsistent PF’s (of a variety of different divergences) were common in all species. Different PF’s within plants, and in a few cases within branches were also encountered. A 100% (across all five branches) consistent PF of 2/5 was found in 20% of B. serrata plants (Figure 3.6). The level of consistency of a 2/5 PF otherwise varied in B. serrata, with 40% of plants having no consistent PFs. In total (pooled across Provinces) 34.6% of B. serrata branches showed a consistent PF of 2/5.

The genetic spiral travelled in either a clockwise or anticlockwise direction. There was no significant difference in chirality for any species when data were pooled across Provinces (Figure 3.7) ($\chi^2_{0.05, 1} = 1.5$, B. ericifolia; $\chi^2_{0.05, 1} = 0.006$, B. integrifolia; and $\chi^2_{0.05, 1} = 0.06$ for both B. oblongifolia and B. serrata). Descriptions of leaf arrangements are given in Table 3.4.

**Figure 3.5.** Floral diagram showing a) the orientation of the ovules and flowers of the unit inflorescence as found in this study, and b) orientation as a result of rotation of a single flower found in only 0.0008% of the flower sample.
Figure 3.6. Percentage of branches \((n = 5)\) within \(B. \text{serrata}\) plants \((n =5)\) for three Provinces that showed a consistent phyllopectic fraction of \(2/5\) \((d = 144^\circ)\). Overall 34.6% of branches had a consistent PF of \(2/5\).

Figure 3.7. Percentage occurrence of clockwise genetic spirals on branches in the four study species across the three Provinces \((n = 75\) for each species). There was no significant difference between the occurrences of clockwise or anticlockwise spirals when data were pooled across Provinces for species.
Table 3.4. Leaf phyllotaxy in the four study species (PF = phyllotactic fraction).

<table>
<thead>
<tr>
<th>Province</th>
<th>B. ericifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Arrangement was spiral but divergences varied within plants. Orthostichy was not recognisable with PF’s approaching 1/5 in all cases.</td>
</tr>
<tr>
<td>Central</td>
<td>Arrangement varied within plants. Forty percent of plants had sections having no recognisable PF’s to a PF that was close to 1/5. Sixty percent were similar but with sections best described as pseudospiral decussate with PF’s close to 2[1/5] (Figure 3.8 a, b).</td>
</tr>
<tr>
<td>South</td>
<td>Arrangement was spiral but varied within plants. Orthostichy was not recognisable with PF’s approaching 1/5. One plant had an arrangement that may best be described as spiral decussate (Figure 3.8 a, b).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Province</th>
<th>B. integrifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Leaves generally arranged in alternate pseudowhorls (never superimposed) of 5 and 6 with internode length changing within and between pseudowhorls. Repeated pseudowhorls of 5 or 6 often showed PF’s of 2/10 and 2/12 respectively but consistent PF’s were not scored.</td>
</tr>
<tr>
<td>Central</td>
<td>Alternate pseudowhorls of 7 interspersed with different spiral arrangement were consistent within one specimen while alternate pseudowhorls of 5 and 6 were otherwise common. Whorls were never superimposed and often separated by spirals of varying internode length. Repeated pseudowhorls of 5 or 6 often showed PF’s of 2/10 and 2/12 respectively. Occasionally within pseudowhorls either drastic changes in the pitch of the genetic spiral (only just above the horizontal plane) or changes in jugacy (from 1 to 2) occurred (Figure 3.8 c, d). Where spirals were persistent on a section of branch inconsistent PF’s of 3/8 and 4/13 were recorded.</td>
</tr>
</tbody>
</table>
Branches with pseudowhorls of 5, 6 and 7 leaves with internode lengths varying within and between the pseudowhorls were common. Pseudowhorls were often separated by spiral sections. One plant with pseudowhorls of 7 leaves had an inconsistent PF of 2/14.

**Province** | **B. oblongifolia**
--- | ---
**North** | An inconsistent PF of 3/8 was the most common PF recognisable. Occasionally leaves appeared almost opposite before changing back to spiral arrangement.
**Central** | Predominantly spiral, sometimes changing to opposite (or decussate) or nearly so before reverting to spiral. Occasionally forming pseudowhorls of 3 and 4 leaves. Many sections did not show orthostichy and thus no PF. Those sections with orthostichy displayed 3/5, 3/8, 3/6, 3/7, 3/10, 4/11 and 5/11. The most frequent of these were 3/8 (Fibonacci) and 4/11 (Lucas).
**South** | An inconsistent PF of 3/6 was the most common PF recognisable. Pseudowhorls of three were common. Inconsistent PF’s included 3/5 and 3/8. Leaves tended to be crowded so as to be oriented toward the outside edge of the plant.

**Province** | **B. serrata**
--- | ---
**North** | One plant (20% of the sample) showed a 100% consistent PF of 2/5. In another plant 1 branch had a consistent PF of 2/5. Inconsistent PF’s of 2/5 were recorded but in general PFs were closer to 2/6 while generally orthostichy could not be established.
**Central** | Forty percent of the plants showed a 100% consistent PF of 2/5. Another 20% showed sections consistent with a PF of 2/5 but also had sections with no recognisable PF’s. Twenty percent had PF’s closer to 2/6 but also with
section with no recognisable PF’s.

**South** Three of five branches within two plants and one branch in another scored a consistent PF of 2/5. The remainder of the sample had either inconsistent PFs of 2/5 or PFs that approached 2/5.

Generally (across Provinces) *B. ericifolia* appeared to develop leaves randomly that is without regard to local organisation or architecture.

There was a strong tendency of leaves to be arranged in reference to adjacent structures in *B. integrifolia* (across Provinces). For example, (most notable in lateral branches) when a resting bud would proliferate (in a whorl of 5 or 6) and one shoot differentiated into an inflorescence, it would typically occur in the upper-most shoot, such that any branches would originate below the inflorescence. These branches typically produced an initial long internode, placing leaves well away from the inflorescence. Additionally the first leaves produced tended to be oriented away from the inflorescences as well. Within plants, leaves tended to be positioned to the outside of branches and, if positioned to the inside, they tended to be smaller.

In *B. oblongifolia* the most consistent feature of the arrangement was a strong tendency of leaves to be oriented away from adjacent branches.

*Banksia serrata* generally produced leaves seemingly wherever it could, that is leaves tended to be crowded without regard to local organisation or architecture.
Figure 3.8. Variation in (and interpretation of) spiral phyllotaxy in stems unrolled into a plane. In a) and b) the same arrangement of petiole scars interpreted as bijugate (two genetic spirals) and unijugate (one genetic spiral) respectively. In a) if ‘paired’ scars were clearly separated (in the vertical plane) the arrangement would be interpreted as pseudospiral decussate. Paired scars are not always clearly distinguishable as occurring at different levels on the stem. In c) by following the nearest neighbour (scar) a genetic spiral (that does not circumnavigate the stem) is drawn. In d) by joining scars of successively higher nodes the genetic spiral circumnavigates the stem.
3.3.3. The *Banksia* inflorescence

Four main line patterns (Figure 3.9, see also 3.10) could be visualised in all *Banksia* inflorescences. Primary arrangements were either spiral (Figure 3.9a, c) or alternate whorls (Figure 3.9b). When unit inflorescences were arranged in alternate whorls (horizontal rows Figure 3.9 b, 3.11, 3.12) vertical rows formed orthostichies aligned 180° to the central axis. Diagonal rows, formed by the alternation of unit inflorescences in the orthostichies, gave rise to conspicuous visible contact families of parastichies whose values m and n varied in direct relationship with the orthostichies (Figures 3.9, 3.11, 3.12). Whorled arrangements always corresponded with even numbers of orthostichies and, in such instances parastichies were always equal (m/n = 1).

When not arranged in alternate whorls genetic spirals formed low pitch screws (less than 90° to the central axis) of unit inflorescences that ran in either clockwise or anticlockwise directions (Figure 3.9 a, b). The pitch of the genetic spiral varied from those that were obvious to the naked eye to those that could only be visualised by use of cotton thread (see Figure 3.13a, b). Spiral arrangements resulted in a twisting or displacement of the orthostichies to the left or right, resulting in odd or even numbers of orthostichies and parastichies. Figure 3.14 and 3.15 illustrate how odd numbers of orthostichies, with alternating unit inflorescences, can be arranged on and around a cylinder. The twisting of the orthostichy would increase proportionally and in an opposing relationship with the pitch of the genetic spiral. The direction of the twist in the orthostichy was always opposite to the direction of the genetic spiral (Figure 3.9 a, c). That is, a genetic spiral that went to the right (clockwise) would orientate the orthostichies to the left (anticlockwise). This relationship extended to the parastichies. An orthostichy twisted to the left would have a concomitant increase in the pitch of the
n parastichies and visa versa. The sum of the parastichies \( m + n \) again always totalled to the number of orthostichies.

![Diagram of parastichies and orthostichies]

Figure 3.9. The four main line patterns, horizontal or nearly so, vertical or nearly so and left and right spirals found in Banksia (paired flowers shown only and inflorescence rolled into a plane). The variations (a and c) are based on spiral arrangements of different chirality and (b) on a whorled arrangement. Note in a 3-dimensional cylinder a) and c) would result in twisting of the orthostichy (vertical row) around the cylinder, while in (b) unit inflorescences of each orthostichy would be superimposed.

Flowers were always absent at the base of inflorescences where often only common bracts occurred. The extent to which the apex of inflorescences bore flowers varied considerably across species but generally development was greatest in B. integrifolia (Figure 3.16).
Figure 3.10. Orthostichies appear by the raised common bracts of an immature *B. serrata* inflorescence in a) when sighting along the length of an inflorescence. In b) paired flowers have grown above the bracts and opposed parastichy pairs m and n and orthostichies are visible.
a reduction the diameter of the axis coincides with a reduction in
the number of rows, now 10 and the number of parastichies, now 5

repeating regular array of 12 alternating rows forms a non tapering
inflorescence with 6 m and n parastichies

12 alternating orthostichies that lie 180° to the long axis and the
horizontal rows lie at 90° and 6 parastichies run in both directions

Figure 3.11. Diagrammatic explanation of whorled arrangement of unit inflorescences
(represented by the quadrilaterals) with the sum of parastichies (m + n) totalling the
number of orthostichies. Row reduction (-2) maintains a whorled arrangement in the
top part of the inflorescence in this example, although the maintenance of whorled
arrangement was rare in cases of rising (changing) phyllotaxy.
Figure 3.12. Schematic representation (viewed from above) of a whorled arrangement at the meristematic apex of an inflorescence showing 16 orthostichies (straight lines radiating from the centre). Note common bracts or primordia of alternate whorls are superimposed (that is orthostichies are not twisted). Jugacy ($J = 8$) is maintained and one genetic spiral is initiated for each unit or primordia (8 genetic spirals). Parastichies ($m = n = 8$) are orthogonal. For convenience circles are used as the primordial units or common bracts, allowing the visualisation of genetic spirals, orthostichies and parastichies.
Figure 3.13. Genetic spirals of different jugacy and row reduction. In a) (B. ericifolia) bijugate (J = 2) two separate threads are needed to connect the unit inflorescences. The threads in a) travel under the common bracts. In b) (B. serrata) unijugate (J = 1) a single thread follows the arrangement. In c) reduction (dislocation) in B. serrata after five unit inflorescences high and concomitant change from whorled to spiral arrangement. In d) lines copy the orthostichies and parastichies from c) indicating reduction in orthostichy number (-1) and parastichy from n to (n-1) while m remains constant. Note all flowers have been removed.
Two dimensional model in (a) turned into three dimensional cylinder in (b). The number of orthostichies (11) is maintained but three rows (10, 11 and 1) combine to form a single n parastichy in (c).

Figure 3.14. Diagrammatic explanation for ‘odd’ number of alternating vertical rows (orthostichies) and parastichies as illustrated in Figure 3.14. (Read from top down a-c).
Figure 3.15. Schematic representation of a spiral arrangement (viewed from above) at the meristematic apex of an inflorescence showing 13 orthostichies (slightly twisted larger arrows radiating from the centre and numbered in a)). The arrangement is unijugate ($J = 1$) and the genetic spiral travels in an anticlockwise direction (that is from the oldest primordia towards the youngest primordia or apex). The spiral arrangement causes a twist (in the opposite direction) in the orthostichies. A concomitant reduction in the number of parastichies also results. In this instance an anticlockwise genetic spiral twists the orthostichy in a clockwise direction and increases the pitch of the $n$ parastichies (those going to the right or anticlockwise) resulting in $n$ ($n = 7$) becoming larger than $m$ ($m = 6$) (accommodating more primordia or ultimately unit inflorescences). Parastichies $m$ and $n$ from a) have been transposed to b).
a) *B. ericifolia*

b) *B. integrifolia*

c) *B. integrifolia*

d) *B. ericifolia*
Figure 3.16. In a) the base and apex of *B. ericifolia* shows the occurrence of involucral bract and an example an atrophied apex (polytelic inflorescence) respectively. In b) base of inflorescence of *B. integrifolia* showing involucral bracts and the occurrence of common and floral bracts without flowers at the base. Variations in the extent of development of the apex in c) and d). In c) flowers developed with bracts almost to the apex and the inflorescence appears almost monotelic (capped with five common bracts). In d) elements of the visually opposed parastichies are suggested in the arrangement of bracts that have capped the inflorescence.

In two out of 180 inflorescences sampled (across the four study species) a double genetic spiral or bijugate (*J* = 2) arrangement was scored (see Figures 3.13a, 3.17, 3.18). The bijugate arrangement was found in *B. ericifolia* and *B. integrifolia*. In this instance the relationship between the m and n parastichies changed such that they differed by two as opposed to one in unijugate arrangements. In bijugate arrangements the sum of the parastichies (m + n) still totalled to the number of orthostichies (see Figure 3.18).

Lines drawn through the common bracts show that the basic repeating pattern is a quadrilateral (Figure 3.17b).
Figure 3.17. Photograph of a section of *B. integrifolia* inflorescence a) with the flowers removed (flower position indicated). Lines (following the common bracts) have been drawn over the inflorescence coinciding with the orthostichies and horizontal rows (genetic spiral $J = 2$) and m and n parastichies. The horizontal rows are at angles less than $90^\circ$ to the central axis of the inflorescence, resulting in a low-pitched spiral that runs in an anticlockwise direction (towards the right) from the base of the inflorescence. In b) a magnified view of a section of a) shows the basic repeating unit (a quadrilateral) that is used to represent a unit inflorescence in Figures 3.11 and 3.14.
Figure 3.18. Schematic representation of a spiral arrangement (viewed from above) at the meristematic apex of an inflorescence showing 16 orthostichies. The arrangement is bijugate ($J = 2$) and has 13 twisted orthostichies (large arrows). In this instance the pitch of the genetic spiral(s) was so steep that a dislocation occurs and while the parastichies sum to the total number of orthostichies ($9 + 7 = 16$) they differ by a factor of two. The $m$ and $n$ parastichies have been transposed from a) separated and reduced in b).
The mean number of flowers inflorescence\(^{-1}\) varied between species (Figure 3.19). *Banksia serrata* had the most flowers (mean ±s.e. across Provinces 1988 ±189; 1577 ±58; 2048 ±192) followed by *B. oblongifolia* (1407 ±98; 1647 ±127; 1657 ±134); *B. ericifolia* (1007 ±87; 841 ±59; 1012 ±68) and *B. integrifolia* (968 ±74; 823 ±60; 899 ±76). Conversely *B. ericifolia* had the tallest inflorescence (mean ±s.e. across Provinces 15.3cm ±1.18; 15.9cm ±1.33; 14.7cm ±1.18). *Banksia serrata* had the second tallest inflorescence (11.55cm ±0.91; 10cm ±0.39; 11.69cm ±0.98) while *B. integrifolia* (8.21cm ±0.42; 8.48cm ±0.5; 8.71cm ±0.53) and *B. oblongifolia* (9.17cm ±0.66; 6.41cm ±0.62; 8.32cm ±0.76) were of similar height (Figure 3.20).

*Banksia ericifolia* had the largest unit inflorescence (height 0.43mm ±0.02; width 0.31mm ±0.01) followed by *B. serrata* (height 0.38mm ±0.004; width 0.22mm ±0.01). *Banksia integrifolia* (height 0.31mm ±0.02; width 0.21mm ±0.01) and *B. oblongifolia* (height 0.32mm ±0.01 width 0.18mm ±0.002) had similar size unit inflorescence but different shapes (Figure 3.21). The relative size of the circumference of the central inflorescence axis, including the common and floral bracts is indicated in Figure 3.21. *Banksia serrata* had the largest inflorescence axis diameter (7.23mm ±0.08) while the other three species were similar (*B. ericifolia* 4.16mm ±0.17; *B. integrifolia* 3.84mm ±0.13; *B. oblongifolia* 5.30mm ±0.07).

The number of orthostichies (and parastichies) varied among species and within species (Figures 3.22, 3.23, 3.24). *Banksia serrata* had the largest number of orthostichies and the biggest range (27-41), followed by *B. oblongifolia* (26-34) and then *B. integrifolia* (16-21) and *B. ericifolia* (11-16). The number of orthostichies also varied within
inflorescences with the exception of *B. ericifolia* where the number of orthostichies was maintained along the length of the inflorescence (Figure 3.24). The constancy of orthostichy number in *B. ericifolia* is reflected in the relatively non-tapering inflorescence shape. While in *B. ericifolia* there was some small reduction in the width of the inflorescence axis between the base to the top, this was due to a change in the size of the unit inflorescence while orthostichy number remained constant.

*Banksia serrata* had the largest range of orthostichies as well as demonstrating the largest changes in rising or changing phyllotaxy. In one inflorescence the sequence of numbers of orthostichies from the base of the inflorescence to the top was 40, 39, 38, 37, 36, 35 and 34. A whorled arrangement occurred at the base (40 orthostichies \( J = 20 \)), thereafter, a spiral arrangement was maintained. Typically inflorescences that were initially whorled often changed to spiral arrangements but not vice versa and the spiral arrangements were maintained to the apex of the inflorescences. Thus generally across species and Provinces the spiral arrangement was more common (Figure 3.25). The increased range in orthostichy number and rising phyllotaxy is reflected in the relatively broad, more strongly tapered, less symmetrical *B. serrata* inflorescences. An example of changing phyllotaxy or rising phyllotaxy is shown in Figure 3.13c and 3.13d for *B. serrata*. Changes in phyllotaxy was often accompanied by torsion of unit inflorescences in the immediate vicinity of the change. In *B. oblongifolia* orthostichy ranged from 26 to 34 vertical rows and in *B. integrifolia* 16 to 21. In terms of shape, the inflorescence of *B. oblongifolia* is not an evenly tapered cylinder and was more like *B. serrata* whereas *B. integrifolia* tended to be more evenly tapered and more like *B. ericifolia*. 
**Figure 3.19.** Mean (±s.e.) number of flowers inflorescence$^{-1}$ for the four study species across Provinces ($n = 15$).

**Figure 3.20.** Mean (±s.e.) height of flowers on an inflorescence (height of an inflorescence) across Provinces ($n = 15$). This value does not include the distance on the inflorescence at the base where only bracts (involucral and common) occur and at the apex where common and floral bracts may be present without flowers.
Figure 3.21. Height and width (cm) of unit inflorescence represented by the midpoint of the circles for the three Provinces of the Sydney Region ($n = 15$). The circumference of each circle indicates the relative circumference of each inflorescence axis measured at the surface of the common bracts.

Figure 3.22. Pooled (North, Central and Southern Province) range of orthostichy number (vertical rows of unit inflorescences) for the four study species$^{-1}$ ($n = 15$).
Figure 3.23. Mean (±s.e.) number of orthostichies in the four species across Provinces

\( (n = 15) \). Orthostichy number did not change within inflorescences in \textit{B. ericifolia}.

Figure 3.24. Frequency of changes in orthostichy number within inflorescences across

Provinces \( (n = 15) \). Orthostichy number was always consistent within \textit{B. ericifolia}. 

Figure 3.25. Frequency of whorled (alternative was spiral) arrangement of unit inflorescences across Provinces ($n = 15$). Data included those inflorescences that changed from whorled to spiral arrangements. Those inflorescences were scored as being predominantly whorled or spiral.
3.4. Discussion

3.4.1. Floral arrangement

The sutures of mature Banksia fruits are typically approximately 90° to the central axis. Occasionally some may have their sutures oriented almost 180° to the inflorescence axis (per. obs.). This suggests that the orientation of the ventral sutures of the ovaries was also 180° (as suggested by Johnson & Briggs, 1975; Wrigley & Fagg, 1989). The results of the in situ hand cut sections of the unit inflorescences clearly showed that the orientation of the ventral suture of the ovary is 90° to the inflorescence axis. The ventral sutures of the unit inflorescences also clearly face each other. This orientation resulted in handedness in the unit inflorescence producing left and right flowers. No morphological distinction between the left and right flowers was evident and thus the flowers are mirror images. Implications of this type of symmetry in the unit inflorescence may best be explained at a later stage in the life cycle such as in the development of fruit and seed (this forms part of the investigation in Chapter 5). Explanation for the obliqueness of the floral bracts (Johnson & Briggs, 1975) and the unique mesh-like cortical vascular system (Venkato Rao, 1964) are discussed below.

3.4.2. Leaf phyllotaxy

Leaf phyllotaxy was complex, always spiral (see below), highly variable (plastic) and different among the four Banksia species. The phyllotaxy of the leaves was in stark contrast to that of the inflorescences (highly ordered, whorled and/or spiral) and a relationship between leaf phyllotaxy and inflorescence phyllotaxy was not established. Independent branch and inflorescence samples were scored for phyllotaxy and a direct
relationship of the transition between the vegetative and reproductive stage was, therefore, not investigated. As the phyllotaxy of leaves was sometimes ‘random’ it is not likely that a pattern or clear relationship would have been established. Clearly the metamorphosis or transition is characterised by a drastic change (decrease) in the plastochron and the plastochron ratio. The difference in phyllotaxy may best be explained by the different roles or functions of the organs (harvesting light and sexual reproduction). These findings are in contrast to the reported leaf phyllotaxy of *B. coccinea* (PF 3/8, d = 135°) and *B. menziesii* (PF 5/13, d = 135.5°) (Fuss & Sedgley, 1990). My findings generate questions not only about the utility of PF’s, but also about the possible strategies employed in the production of leaves.

Phyllotactic fractions of the main Fibonacci sequence <…2, 3, 5, 8, 13, 21, 34….> occur in more than 92% of the PFs reported from around the world (Jean, 1994). Jean (1994) however does not state how many plants actually have recognisable PFs and in those that do, how consistent the PFs are. That is, 92% is a high proportion but if the total sample is small, this is a small number of plants. A methodology for scoring PF’s is absent from the literature. For example, it appears that no minimum frequency of occurrence within a plant or species is required before a PF may be assigned. In this study, five branches were sampled within each plant and rigorous criteria applied (see Section 3.2.2). *Banksia serrata* had a consistent PF of 2/5 (d = 144°) in only 34% of cases. Assigning a PF of 2/5 for *B. serrata* without acknowledging the extent of the variation would mask the true picture. Leaf phyllotaxy was highly polymorphic in the four *Banksia* species studied. Perhaps the true PF of *B. serrata* is 2/5 and all other arrangements are environmentally cued polymorphisms. This would appear to be the case for *B. serrata* as arrangements had either consistent or inconsistent PF’s of 2/5 or
arrangements that approached it in most cases (Table 3.4). This scenario was similar for
B. ericifolia with a PF that most often approached 1/5 (d = 72°). Banksia integrifolia
and B. oblongifolia were more variable in leaf phyllotaxy.

In juvenile stages (before extensive branching) and competitive shading, such as in the
post-fire environment, consistent PFs in leaf arrangement (that approach the Golden
angle and reduce leaf overlap and shading) may confer advantage. Consistent PFs may,
like juvenile foliage, appear in the early stages of the life cycle. Conversely
B. oblongifolia from the Central Province displayed a range of inconsistent PF’s from
different sequences. For example there was the main Fibonacci (3/8), the first accessory
or Lucas (3/7 and 4/11), the third accessory (5/11) and other PF’s that do not coincide
with alternate numbers of recognised sequences (eg 3/6 and 3/10)\(^7\). Additionally there
were arrangements that could not be assigned PFs because orthostichy was not achieved
or could not be clearly established. This scenario was similar for B. integrifolia. Habit,
anatomical and architectural constraints, habitat, community structure and climate
(particularly micro aspects) are all likely to impact on leaf arrangements. It is,
therefore, not surprising that within species ‘ecotypic’ PF’s are found, as well as
variation in PFs within individuals and branches.

Additionally it is the larger numbers from the Fibonacci sequence whose fractions more
closely approach the golden angle or ratio. However the smaller numbers of the
sequence(s) are the ones that are typically reported for leaves. It becomes increasingly

\[\text{To obtain a Fibonacci sequence take any two numbers, sum them to obtain the third term and repeat the}
\text{process to obtain a sequence. The fraction obtain using consecutive numbers of that sequence and any}
\text{other Fibonacci sequence always converge on the Golden ratio.}\]
likely that as branches increase in length and are able to accommodate more leaves (eg 34) and more genetic spirals (eg 13, $PF = 13/34$, $d = 137.6^\circ \approx 360^\circ/\phi^2$) that the phyllotaxy will be impacted upon. If these *Banksia* species did have PFs from larger Fibonacci numbers, branching, twisting and secondary growth may mask the orthostichies. In the mature stage (with secondary thickening, twisting and branching) the existence of phylotactic fractions from Fibonacci type sequences becomes inconsequential (in terms of reducing leaf overlap). As a plant ages, branches show more lateral growth and shading becomes complex (self-shade and that from neighbours). Theoretically at least in the juvenile and young stages of growth (eg before branching) reducing leaf overlap with PF’s of Fibonacci type sequences may confer an advantage. But as noted by Niklas (1997) not all sunlight is received from directly above. However when the sun is positioned above there is less shading from adjacent neighbours and a PF that reduces self-shade (leaf overlap) will confer an advantage.

Two distinct observations on the general arrangement of leaves were made\(^8\). *Banksia ericifolia* and *B. serrata* both have a high leaf density and they tended to produce leaves without regard to local architecture while the species with lower leaf densities, *B. integrifolia* and *B. oblongifolia* showed a clearer trend in leaf arrangement. *Banksia ericifolia* and *B. serrata* appear to employ a lottery or conservative bet-hedging strategy and this strategy would be consistent with a regular PF. That is, the regular reproduction of leaves along a branch may be predetermined because it does not matter initially where they occur. Under this scenario, energy is put into the production of

\(^8\) These observations were made on mature whole plant specimens and not sections of branches as used in determining PFs.
leaves, so as to potentially increase photosynthesis. It is a lottery in that the plant cannot predetermine the amount of sunlight a particular leaf will receive and is thus reliant on chance. It is a bet-hedging strategy because there is a trade-off between mean fitness (in this case photosynthate produced leaf\(^{-1}\)) and variance (increased leaf density will increase self-shade). If \textit{B. integrifolia} and \textit{B. oblongifolia} exercise some control over leaf arrangement then it is less likely that a regular PF will be identified. The general observation on leaf arrangements concurs with the observed phyllotaxy of the four species. \textit{Banksia ericifolia} (PF 1/5) and \textit{B. serrata} (PF 2/5) could be characterised by a more regularly occurring PF while \textit{B. integrifolia} and \textit{B. oblongifolia} could not.

Several points can be drawn from the above discussion. Firstly if a lottery or bet-hedging strategy is in operation then the occurrence of Fibonacci type sequences (in reference to reduced leaf overlap) is inconsequential. Any regularly repeating arrangement would achieve this outcome. Nevertheless the lottery strategists (\textit{B. ericifolia} and \textit{B. serrata}) are characterised by PFs from the Fibonacci Main and second accessory sequence.

Secondly \textit{B. ericifolia} and \textit{B. serrata} are similar in that each is typically single stemmed or more importantly only capable of lateral spread via stem branching (axillary buds). \textit{Banksia integrifolia} and \textit{B. oblongifolia} are both capable of lateral spread via stem branching and additionally by either root sprouts or a spreading lignotuber respectively. The production of axillary buds for lateral spread and growth may be considered more important for \textit{B. ericifolia} and \textit{B. serrata} than it is for \textit{B. integrifolia} and \textit{B. oblongifolia}.
Concomitant with an increase in axillary buds is an increase in leaves, which have costs and thus must have potential benefits for the argument of a lottery strategy to be logical. As far as the role of increased and regular production of leaves is concerned, *B. serrata* stores large reserves of food in an early and fast developing lignotuber. *Banksia ericifolia* is a nonlignotuberous shrub with a precarious life span and has very rapid early growth and would require high levels of photosynthate to sustain growth. Additionally as an obligate seeder it sets a larger canopy of stored seed than does the lignotuberous shrub *B. oblongifolia* (Zammit & Westoby, 1987a). Conversely *B. oblongifolia* is long lived and would spend relatively more of its life in some understorey shade. *Banksia oblongifolia* often occurs as an understorey shrub in forest and woodlands while *B. ericifolia* is more often found as a dominant shrub in heathland formations. The benefits of high leaf density will be less in a shaded habitat. *Banksia integrifolia* is nonlignotuberous tree resprouter that typically occupies habitats that are not regularly consumed by fire or stressed by prolonged droughts. Large amounts of photosynthate are not required for lignotuber storage, frequent resprouting or serotinous cone production.

A potential hypothesis is that, plants that can only spread via lateral branching (axillary meristems) (typically single stemmed trees and shrubs) will have a higher leaf density (and thus axillary bud density) than congeneric species that can spread via roots, lignotubers or rhizomes etc. Furthermore if a high nodal density is under strict genetic control then the production of leaves with a PF (regular production) is more likely to occur. That is the plastochrone and the plastochrone ratio are less likely to show variation due to gene environment interactions. If this last point is correct then the hypothesis might be extrapolated to include phyllotaxy, eg plants that can only spread
via lateral branching are more likely to be characterised by a PF than congeneric species that can spread via roots, lignotubers, rhizomes etc.

3.4.3. Inflorescence phyllotaxy

Four related line patterns appear in the *Banksia* inflorescence. The line patterns readily appear because the regular repeating array of unit inflorescences are highly condensed. Whorled and/or spiral primary arrangements of unit inflorescences form horizontal rows. Orthostichies and parastichies are secondary line patterns and a direct relationship exists between all the line patterns. These relationships allow for the formation of construction rules and provides a new concise methodology to describe and compare *Banksia* inflorescences. Often the most conspicuous line patterns are the orthostichies (vertical rows) and as such the following rules/relationships may be discerned from the orthostichies.

1. If orthostichies are straight (and in total are an even number) the primary arrangement is whorled; if twisted, the primary arrangement is spiral.
2. The total number of orthostichies is always equal to the sum of the parastichies \( m + n \) (in whorled and spiral arrangements).
3. When unit inflorescences are whorled the number of orthostichies is always equal to twice the jugacy \( 2 \times J \) (because the whorls are alternate not superimposed), and the number of \( m \) and \( n \) parastichies are equivalent and also equal to \( J \) \( (J = m = n) \).
4. When unit inflorescences are spirally arranged the direction of the twist in orthostichies is opposite to the direction of the genetic spiral (eg orthostichies...
twisted to the right indicate a clockwise (left handed) genetic spiral and *visa versa*.

5. When unit inflorescences are spirally arranged the difference in m and n parastichies is equal to J. When J = 1 (m-n = 1 = J) and when J = 2 (rare) (m – n = 2 = J).

6. When unit inflorescences are spirally arranged the number of m parastichies will be greater than n when the orthostichies are twisted to the right and *visa versa*.

Fuss and Sedgley (1990) reported 13 genetic spirals to occur in inflorescences of both *B. coccinea* and *B. menziesii* with PFs of (27.7°; 360°/13) and (14.6°; 360°/?) respectively. In keeping with the above rules and phyllotaxy literature, 13 genetic spirals also means there are 13 m and n parastichies (26 orthostichies) and thus indicates a whorl arrangement. Fuss and Sedgleys’s (1990) observations were drawn from investigations of floral initiation and perhaps secondary shift in orientation lead them to conclude different PFs for the two species. It was common in *B. serrata*, for example, to have a whorled arrangement and then a switch to a spiral arrangement along the inflorescence. Within such inflorescences PFs would change as the diameter and the number of unit inflorescences around the central axis changes. When calculating a PF for an inflorescence with rising phyllotaxy, an average may give an unusual divergent angle and this may account for the different divergences reported by Fuss and Sedgley (1990). All other reports and descriptions of *Banksia* inflorescences (eg Venkato Rao, 1964; Blake, 1971; George, 1981) are consistent with the above construction rules.
Divergences that approach the golden ratio have, theoretically at least, important consequences for the arrangement of leaves (photosynthesis) but not for Banksia flowers. The second fundamental consideration of the occurrence of Fibonacci sequence and the Golden ratio in phyllotaxy is its relationship with a logarithmic spiral and regular packaging (Stevens 1974; Bell, 1991). The significance of consistent phyllotaxy and regular arrangement of unit inflorescences in these banksias does seem to be best explained by issues of close packing. Packing efficiency though does not provide an explanation for Fibonacci phyllotaxy and it should be regarded as a consequence and not a cause (Ridley, 1982).

The unique cortical vascular mesh of Banksia (Venkato Rao, 1964) may allow for secondary shifts in orientation of unit inflorescences. The highly compacted and condensed arrangement of subunits and unit inflorescences on an expanding central axis is likely to produce torsion and the cortical mesh allows for any readjustments of orientation. Banksia serrata, for example, shows considerable variation in inflorescence shape with the central axis occasionally bending strongly causing compression and expansion of the unit inflorescences at the inside and outside of the bend respectively. The vasculature of the inflorescence would have to be sufficiently elastic to accommodate such reorientation. Nevertheless a unique vascular system would be required for a unique arrangement and the mesh simply reflects this. It is ‘mesh like’ because traces creating gaps would give off to each unit inflorescence from a continuous vascular cylinder. Occasionally a skeleton of the mesh can be seen in deteriorating and desiccated inflorescences as the vascular tissue is more resistant to rot, and thus collapse, than the organs and tissue that it supplies. A similar mesh like vascular system might also occur in other members of the Proteaceae with dense
arrangements of flowers (eg *Isopogon, Petrophile* and *Telopea*) but these have not been investigated.

Johnson and Briggs (1975) found the obliqueness of floral bracts in *Banksia* to be distinctive in the Proteaceae and considered that it presented problems for the determination of floral orientation. Although composed of five subunits (three bracts & two flowers) the basic shape of the unit inflorescence in these *Banksia* species was a quadrilateral. Five sided shapes cannot produce regular repeating arrays (Stevens, 1974). Lines drawn tangentially to the subunits around a unit inflorescence would produce a pentagon. A single line tangent to the two flowers, two lines tangent to each flower and floral bract and two lines tangent to each floral and the single common bract results in a pentagon. In Figure 3.14 lines representing parastichies were drawn such that they dissected the common bracts (common subunit). The resulting quadrilateral instead of a five-sided subunit is due in part to the extensive indumentum associated within and between the unit inflorescences. Additionally and perhaps of greater significance, is that, if the floral bracts were not displaced from their theoretical positions a five sided shape is even more likely to have resulted. Thus the floral bract orientation in *Banksia* increases the packing efficiency of flowers on an inflorescence.

The obliqueness of the floral bracts and the significance of the arrangement to close packing is perhaps best illustrated in Figure 3.13c where the flowers have been removed. Within the Proteaceae, *Banksia* have the most condensed inflorescences. The dense indumentum appears to act as padding, assisting in the formation of a highly condensed and compact inflorescence. The indumentum may also assist in providing support to the flowers (see discussion below). For example, the paired flowers of
Grevillea are exposed and foraging pouts by birds often causes flowers to break and fall away (pers. obs.). In Banksia inflorescences flowers remained attached even after heavy foraging pouts by a range of animals, including small mammals.

It is not clear if the floral bracts have literally been displaced from their theoretical positions in evolutionary time or if they are only displaced due to contact pressure in the developing inflorescence. The later is open to experimentation. Neighbouring unit inflorescences could be removed to see if the lack of contact pressure changes the orientation of the floral bracts.

Carpenter (1978) considered that the hooked style of B. ericifolia was an adaptation to mammal pollination and that the troughs formed by the vertical rows of flowers channelled excess nectar to the ground for the attraction of mammals. The troughs are clearly the spaces between the orthostichies, which are conspicuous because of the size of the common bract and the consistency of orthostichy number in B. ericifolia. Banksia ericifolia had the largest (width x height) unit inflorescence and was the only study species to maintain constancy in orthostichy number within inflorescences (Figures 3.21, 3.24). This finding does give some credence to Carpenter’s hypothesis for the channelling of excess nectar via the troughs. Conversely in the straight styled species (B. integrifolia, B. oblongifolia and B. serrata) the lack of troughs could be interpreted as to impede the flow of excess nectar, thereby keeping the reward where the pollen and stigmas are. This could be tested.

Close packing also means that nectar produced by individual flowers is simply not lost to the environment but runs around other unit inflorescences generating a synergy that
makes the flowers more attractive than they would be individually. This is more than simple pollinator attractiveness via the visual cue of massed flowers and can be considered an additional benefit of the arrangements. Acropetal initiation types such as *B. ericifolia* may have some additional advantage in this synergy. The excess nectar flowing down, over and around lower flowers (excess nectar does not always travel down the trough, per. obs.) is likely to increase foraging activity, triggering pollen presentation and pollen removal thereby increasing stigma receptivity and potential pollination. It appears that close packing also ensures that if large pollinators are attracted, the flowers are protected. That is, the flowers have the increased stability conferred by the adjacent adpressed bracts.

Inflorescences are constrained from growing too large and there are also developmental constraints on the minimum size because of the size of the unit inflorescence and their arrangement around a cylindrical central axis. Unless the dimensions of the unit inflorescence can be reduced there is a minimum number of orthostichies that must circumnavigate the central axis and form a dense structure. On this basis the greatest size variation in the *Banksia* should be found in the height of the inflorescence. Given also that orthostichy is relatively stable (absolute in *B. ericifolia*) inflorescence height becomes the main dimension that a plant may potentially manipulate. Polytelic inflorescences may have evolved to confer a degree of plasticity and opportunity. From initiation *Banksia* inflorescence development may take up to 12 months (Fuss & Sedgley, 1990). Having initiated the inflorescence a plant is then likely to experience changes in the availability of resources (eg water) during this time. If more resources become available the polytelic inflorescence may develop further producing more flowers. Similarly if resources are limiting a polytelic inflorescence may cease
development of apical flowers developing a shorter inflorescence with fewer flowers and subsequent potential fruits to support. Often in very dry habitats, and at times during drought, larger sections of the apex of inflorescences do not complete development (per. obs.). Conversely in wetter habitats B. integrifolia inflorescences essentially complete development to the tip of the apex and appear almost as monotelic (see Figure 3.16).

The classical rising phyllotaxy of visible opposed parastichies in the capitulum of the sunflower often appears at smaller scale at the apex of an atrophying Banksia inflorescences (Figure 3.10, 3.16). In terms of x, y and z coordinates, the rising phyllotaxy of banksias stops when the unit inflorescences move into, and along the z axis, that is, along the length of the central axis. In the sunflower the size of the capitulum is measured along the x and y axis, and the broader the capitulum the greater the m and n parastichies. The Banksia inflorescence is like a sunflower, with logarithmic spirals (parastichies) briefly forming in its polytelic apex (or in the x and y plane), but it is different from the sunflower by its formation of Archimedean spirals (parastichies) down the long axis or z plane where the mature flowers form. As a result the orthostichies (vertical rows) become the conspicuous line patterns that are so often reported in Banksia.

If a seeder is expected to expend more resources on sexual reproduction (Cowling & Lamont, 1998) then traits associated with higher fecundity (eg floral display) might also be different. Banksia ericifolia differed from the other three resprouting species by maintaining a regular inflorescence phyllotaxy. A consequence of a stable (non-rising) phyllotaxy is the clear formation of vertical rows (orthostichies) of flowers.
Additionally, the much larger unit inflorescence (in particular the common bract) of *B. ericifolia* sets the orthostichies further apart making them even more conspicuous. In addition to these characteristics *B. ericifolia* has the tallest inflorescence which further emphasises the orthostichies. Furthermore while *B. ericifolia* had the tallest inflorescence it produced approximately half the number of flowers that *B. serrata* produced on an inflorescence. Thus it might be concluded that on a per flower basis *B. ericifolia* has a bigger display. Anthesis is also acropetal, the styles hooked and flower colour is orange/red, whereas the sprouters all displayed a variable inflorescence phyllotaxy, anthesis is basipetal, have straight styles, and flower colour is essentially pale yellow. All Proteaceae are polytellic and inflorescence height is the most plastic or the least constrained dimension which *B. ericifolia* appears to have made the most of. If colour is not as important to some potential pollinators then pattern (vertical stripes) clearly otherwise distinguish *B. ericifolia* inflorescences among the bushes and trees. On the basis of my results one further line of inquiry might investigate correlations between stable inflorescence phyllotaxy and other floral traits in other species (eg hooked styles, acropetal initiation as opposed to basipetal, flower colour, etc). As inflorescence phyllotaxy has not been described before in *Bankstia* this information in conjunction with infragenic classification may shed more light on the evolution of this unique genus.
Chapter 4. Infructescence, follicle and seed set in four species of *Banksia* L.f.

4.1. Introduction

Cavanilles (1800) was perhaps the first to note the excessive production of *Banksia* flowers and very few fruit. Low fruit to flower ratios are common in the Proteaceae (Collins & Rebelo, 1987; Walker & Whelan, 1991) and plants generally (Stephenson, 1981).

4.1.1. Proximate and ultimate explanations for low fruit set

The proportion of flowers that develop into mature fruits varies from zero to 100 percent (Lloyd, 1980; Lloyd, Webb & Primack, 1980; Stephenson, 1981; Weins, 1984). The number of female flowers, and ovules within these, sets the upper limit for the number of possible fruits and seeds that can be set by an individual. This reproductive potential is then subject to variations in pollination, fertilisation, predation, environmental conditions and maternal care or available resources (Stephenson, 1981). Proximate and ultimate explanations for flower and fruit abortion in plants generally, and low fruit to flower ratios in the Proteaceae, have been reviewed by Stephenson (1981) and Ayre and Whelan (1989), respectively. Proximate (mechanistic) explanations are concerned with the how, of physiological or behavioural responses. Ultimate (functional) explanations are concerned with the why, or how natural selection may have favoured the evolution of those responses (Waddington, 1962). Proximate explanations (generally more readily testable)
include pollen limitation, pollen source (pre- and post-zygotic mortality), resource limitation and predation (or disease). Ultimate explanations include pollinator attractiveness, male success, optimal outcrossing, bet-hedging and predator avoidance (Ayre & Whelan, 1989).

4.1.2. Seed, fruit and cone set

Brood size may be defined as the number of seeds that are matured in a single fruit. In comparing brood size or brood reduction among species the critical parameter is seed to ovule ratio. Multiovulated species (>1 ovule ovary\(^{-1}\)) generally form three brood categories based on the number of seeds set fruit\(^{-1}\); 1) negatively skewed distribution species (seed:ovule >0.75), 2) positively skewed distribution species (seed:ovule <0.50), and 3) the more rarely attained normal distributions (sometimes bimodal distributions) species (Uma Shaanker, Ganeshaiah & Bawa, 1988).

The hypotheses offered to explain low fruit set are similar, and clearly related to those offered to explain low seed set. The difference is that, in general, far more ovules are produced than ovaries. Additionally ovule abortion (in multiovulated species) has relatively little impact on fruit production, whereas fruit abortion will impact on all fertilised ovules.

*Banksia* flowers have two ovules ovary\(^{-1}\) and the number of seeds found within a fruit may vary from one to two or no viable seeds at all (George, 1981). Seed number varies due to predation or assumed lack of fertilisation, ovule or seed abortion. Seed number fruit\(^{-1}\) in *Banksia* species has often been quoted, but details on the methodology (eg whether seed number was scored specifically on a fruit\(^{-1}\) or
cone\(^{-1}\) basis), sample size and data are lacking. For example, *Banksia ericifolia* was reported to usually contain one viable seed follicle\(^{-1}\) (more often than zero or two?) by George (1981) in what was primarily a taxonomic work. Later Zammit (1986) and Zammit and Westoby (1987b) reported that *B. ericifolia* and *B. oblongifolia* both contained one seed follicle\(^{-1}\) but details as to how they arrived at this conclusion are not given. Blake (1971) also reported *B. ericifolia* and *B. spinulosa* fruits to contain a single seed but again, data or the methodology were not provided. Bradstock (1985) made the ‘assumption’ of one seed fruit\(^{-1}\) for *B. ericifolia* and *B. serrata* while Vaughton (1988) specifically examined 5360 follicles of *B. spinulosa* and found only one or zero seeds fruit\(^{-1}\). Application of a rigorous technique such as used by Vaughton (1988) is necessary to obtain quantitative data on the broad size of the four study species.

Fruiting in *Banksia* is generally partitioned into the number of flowers that develop into fruits infructescence\(^{-1}\) (F:F, or percentage fruit set) and number of inflorescences that develop into infructescences (I:I or percentage cone or infructescence set). An infructescence (by definition) always has at least one fruit. Here I make a distinction between a cone and an infructescence. In contrast to an infructescence, a cone may be fertile or fecund (with fruit = fertile or fecund cone = FC) or barren (without fruit = barren cone = BC). Reported flower numbers inflorescence\(^{-1}\), mean fruit set infructescence\(^{-1}\), mean percentage fruit set, mean percent cone set, mean percentage seed set and breeding system of some banksias are summarised in Table 4.1.
Table 4.1. Mean number of flowers inflorescence\(^1\), number of fruits cone\(^1\), percentage fruit set, percentage conversion of inflorescence into infructescence (cone set), percentage seed set and breeding system in *Banksia*. In most instances original data was given for several years and these values have been averaged for this table.

<table>
<thead>
<tr>
<th>Species</th>
<th>mean # flowers</th>
<th>mean # fruits</th>
<th>% fruit set</th>
<th>% cones set</th>
<th>% seed set</th>
<th>outcrossers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. attenuata</em></td>
<td>1306, 1350., 2629</td>
<td>11.3</td>
<td>0.9, 0.5, 0.2</td>
<td>49, 89</td>
<td>6.1</td>
<td>obligate S*</td>
<td>WB; WRRT LB</td>
</tr>
<tr>
<td><em>B. baxteri</em></td>
<td>250, 273</td>
<td>2.2</td>
<td>0.8, &lt;1.0</td>
<td>87</td>
<td></td>
<td></td>
<td>WRRT; H</td>
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<tr>
<td><em>B. burdetti</em></td>
<td>972</td>
<td>8.2</td>
<td>0.8</td>
<td></td>
<td>4.6</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td><em>B. chamaephyton</em></td>
<td>1573</td>
<td>0.9</td>
<td>0.1</td>
<td>20</td>
<td></td>
<td></td>
<td>Br</td>
</tr>
<tr>
<td><em>B. coccinea</em></td>
<td>275</td>
<td>13.8</td>
<td>5.0</td>
<td>97</td>
<td></td>
<td></td>
<td>WRRT</td>
</tr>
<tr>
<td><em>B. elegans</em></td>
<td>531, 393</td>
<td>0.1, 1.8-2.8</td>
<td>&lt;0.1</td>
<td>5</td>
<td></td>
<td></td>
<td>Br, Lb</td>
</tr>
<tr>
<td><em>B. ericifolia</em></td>
<td>1593</td>
<td>47.8, 7.5, 42.5</td>
<td>3.0</td>
<td>42.9</td>
<td>3.1</td>
<td>facultative</td>
<td>PT, CW, GSW,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CWA</td>
</tr>
<tr>
<td><em>B. grandis</em></td>
<td>3750, 2709</td>
<td>92.4</td>
<td>3.4</td>
<td>36</td>
<td></td>
<td></td>
<td>WRRT, WB</td>
</tr>
<tr>
<td><em>B. ilicifolia</em></td>
<td>69</td>
<td>1.1</td>
<td>1.6</td>
<td>29</td>
<td></td>
<td></td>
<td>WB</td>
</tr>
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</table>

\(^1\) Inflorescence and cone counts are based on counts of mature inflorescences and cones respectively.
<table>
<thead>
<tr>
<th>Species</th>
<th>mean # flowers</th>
<th>mean # fruits</th>
<th>% fruit set</th>
<th>% cones set</th>
<th>% seed set</th>
<th>outcrossers</th>
<th>References</th>
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<tbody>
<tr>
<td><em>B. integrifolia</em></td>
<td>1020</td>
<td></td>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td>Mc</td>
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<td><em>B. leptophylla</em></td>
<td>350</td>
<td>25.2</td>
<td>7.2</td>
<td>72</td>
<td></td>
<td></td>
<td>CLP</td>
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<tr>
<td><em>B. littoralis</em></td>
<td>1410, 1039</td>
<td>98.0, 67.7</td>
<td>7.0, 6.5</td>
<td>61</td>
<td>47</td>
<td>facultative</td>
<td>LB, WB</td>
</tr>
<tr>
<td><em>B. menziesii</em></td>
<td>930, 1002, 1044</td>
<td>2.9, 3.9, 7.5</td>
<td>0.3, 0.4, 0.8</td>
<td>29, 38</td>
<td>6.7</td>
<td>obligate S*</td>
<td>LB, CLP, WB, RV</td>
</tr>
<tr>
<td><em>B. nutans</em></td>
<td>300</td>
<td>9.0</td>
<td>3</td>
<td>32</td>
<td></td>
<td></td>
<td>WRRT</td>
</tr>
<tr>
<td><em>B. occidentalis</em></td>
<td>1131</td>
<td>57.7</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td>H</td>
</tr>
<tr>
<td><em>B. paludosa</em></td>
<td></td>
<td>24.8, 39.1</td>
<td>24, 28.6</td>
<td></td>
<td></td>
<td></td>
<td>WG, CW</td>
</tr>
<tr>
<td><em>B. prionotes</em></td>
<td>1000, 1431</td>
<td>30.3, 32.2</td>
<td>3.0, 2.3</td>
<td>95CLP</td>
<td>3.1</td>
<td>obligate</td>
<td>CS, CLP</td>
</tr>
<tr>
<td><em>B. spinulosa</em></td>
<td>792,</td>
<td>80.7, 49.4, 65.4, 2.3, 0.92-22, 26.7, 6.4</td>
<td>55.8</td>
<td>1.77, 8.4</td>
<td>15.3, 30.5, 58</td>
<td>Mc, CW, WG, C, V1, V2</td>
<td></td>
</tr>
<tr>
<td><em>B. tricuspis</em></td>
<td>1200</td>
<td>24 (wt. mean)</td>
<td>2</td>
<td></td>
<td></td>
<td>facultative</td>
<td>Lv, vL</td>
</tr>
</tbody>
</table>

The mean number of fruit infructescence\(^1\) varies from about one in *Banksia chamaephyton* (Barrett, 1985) and two in *B. elegans* (prostrate, serotinous and lignotuberous) (Lamont & Barrett, 1988) to 98.0 in *B. littoralis* (nonlignotuberous and nonserotinous) (Lewis & Bell, 1981).

The mean percentage fruit set varies from 0.1% in *B. chamaephyton* (Barrett, 1985) to 7.2% in *B. leptophylla* (Cowling, Lamont & Pierce, 1987) and 8.4% in *B. spinulosa* (Vaughton, 1988). The mean percentage cone set varies from 5% in *B. elegans* (Barrett, 1985) to 97% in *B. coccinea* (Wooller, Russell, Renfree & Towers, 1983). Both *B. elegans* and *B. chamaephyton* are rare species. *Banksia elegans* is also the only reported asexually reproducing Banksia species from Western Australia\(^9\). Estimates of fruit to flower ratios may (or may not) include those inflorescences that do not set fruits (barren cones). Barren cones are not as

---

\(^9\) Some members of the Series *Prostratae* may be clonal (Lamont & Markey, 1995).
well supported or developed as fertile cones and consequently are not as persistent
within the plant canopy. Lamont and Barrett (1988) took into account the fate of all
ovules and reported the percentage fruit set in *B. elegans* to be 0.001%, the lowest
recorded for *Banksia*, although probably the most precise measure.

4.1.3. Breeding systems in *Banksia*

The breeding system in *Banksia* varies across species and subspecies (Table 4.1). It
is recognised that the dichotomy used (either facultative or obligate outcrossers)
masks the extent of compatibility. *Banksia ericifolia* and *B. spinulosa* for example,
were shown to be largely self-incompatible (Goldingay, Schibeci & Walker, 1991;
Carthew, Whelan & Ayre, 1996) but nevertheless facultative outcrossers. Some
estimates of the level of outcrossing in *Banksia* have been among the highest
reported in plants (Scott, 1980; Carthew, Ayre & Whelan, 1996), while significant
levels of selfing have been reported in *Banksia cuneata* (Coates & Sokolowski
(1992) and *Banksia brownii* (Sampson, Collins & Coates, 1994).

Experimental bagging of inflorescences (pollinator exclusion) as tests of autogamy
have shown *B. spinulosa* and *B. paludosa* not to be autogamous (Whelan &
Goldingay, 1986), while *B. paludosa* did set fruit by autogamy in another study and
found autogamous fruit set in *B. spinulosa* var. *neoanglica* although fruit and cone
set were significantly less than in controls. Some autogamous fruit set was also
recorded in *B. ericifolia* (Goldingay *et al*, 1991).
4.1.4. Pollination studies

Pollen (manipulation) studies have perhaps dominated research on low fruit set in *Banksia*. Artificial supplementation of pollen to *Banksia* flowers has provided mixed results. Pollen supplementation did not increase fruit set cone\(^{-1}\) in *B. spinulosa* but did increase cone set in *B. paludosa* (Whelan & Goldingay, 1986). Pollen limitation was not found to be a likely explanation for low fruit set in *B. ericifolia* but may have accounted for it in *B. spinulosa* and *B. paludosa* (Copland & Whelan, 1989).

Abortion is another method available to plants to regulate pollen or embryo quality and thus fruit set (Stephenson, 1981). Evidence of selective abortion in *B. spinulosa* var. *neoanglica* of self-fertilised embryos, in the presence of outcrossed embryos on the same infructescence was reported by Vaughton and Carthew (1993). In a recent review Wilcock and Neiland (2002) found that pollination failure may be a result of ‘too little, too much, too late, too mixed in composition or too poor in quality’. In general terms, the pollination environment is highly variable and pollination failure is due to a multitude of factors. Pollen limitation may be difficult to demonstrate even when it is occurring (Haig & Westoby, 1988; Diggle, 1995).

Estimates of the maximum numbers of fruits infructescence\(^{-1}\) and range in fruit size dimensions (length & width) of banksias has been given by George (1981). Within the study species the largest fruits are found in the serotinous tree species *B. serrata* and the smallest in the nonserotinous tree species *B. integrifolia* (Table 4.2). *Banksia serrata* was also reported to set considerably fewer fruit infructescence\(^{-1}\) while the other three study species set approximately similar maximum numbers of fruit (Table 4.2).
Table 4.2. Descriptions and estimates of maximum numbers of follicles in infructescence\(^{-1}\) for *B. ericifolia*, *B. integrifolia*, *B. oblongifolia* and *B. serrata* (after George, 1981).

*B. ericifolia* follicle length 13mm-22mm, width 4mm-13mm. Irregular in plan view, oblong, elliptic rhombic or trapeziform; upper surface often convex such that in T.S. it is mushroom shaped; follicles opening 4-8mm. Up to 80 follicles set cone\(^{-1}\).

*B. integrifolia* follicle length 7mm-15mm, width 3mm-6mm. Valves shallowly convex, opening 5-9mm but varying with the extent of fruit set. Up to 60 follicles set cone\(^{-1}\).

*B. oblongifolia* follicle length 10mm-18mm, width 3mm-7mm. Usually slightly turned upwards, valves depressed semi-circular, slightly convex. Valves opening 6-12 mm. Up to 80 follicles set cone\(^{-1}\).

*B. serrata* follicle length 25mm-35mm, width 15mm-22mm. Prominently exerted, elliptic in plan view opening 15-30mm wide. Up to 30 follicles set cone\(^{-1}\).

4.1.5. Resource limitation and predation

The availability (or lack) of resources is often cited as the explanation for low fruit set when pollen supplements do not increase fruit set (Ayre & Whelan, 1989). Fruit set in some *Banksia* garden specimens or banksias receiving run-off from residential development is often much higher than that of adjacent plants in their natural habitat (per. obs.). Lamont, Rees, Witkowski and Whitten (1994) considered increased fecundity in roadside plants of *B. hookeriana* was due to increased access to water and mineral nutrients. Insecticide treatments were shown to increase seed set in *B. ericifolia* (Zammit & Hood, 1986). Nutrient addition with insecticide treatments showed a significant increase in fruit set in *B. spinulosa* (7\%) compared to controls.
(2%) (Wallace & O’Dowd, 1989) and, as noted by Ayre and Whelan (1989), while some treatments have resulted in significant increases in fruit set, the fruit to flower ratios are still far from unity.

Explanations for low cone set (as opposed to fruit set) include, nutrient availability, pollen limitation, pollen quality, insect predation and their combinations (Whelan & Burbidge, 1980; Paton & Turner, 1985; Whelan & Goldingay, 1986; Zammit & Hood, 1986). Due to the size (cost) of an infructescence, a threshold number of pollinated flowers may have to be reached before infructescence development proceeds (Paton & Turner, 1985). Ayre and Whelan (1989) discussed other possible explanations that should be explored as explanations for the low fruit to flower ratios in the Proteaceae (some of those issues are considered in Chapter 5).

It has been hypothesised that traits associated with the seeder life history might also be different from sprouters particularly those traits relating to seed reproduction (eg Cowling & Lamont, 1998; Bell, 2001). Can the seeder/sprouter dichotomy (and associated traits) account for the differences in reproductive effort of the four species? In relation to this broader question of traits associated with seeders and sprouters several specific questions are considered.

➢ Does the frequency distributions of fruit numbers infructescence\(^1\) suggest a threshold level of fruit set before cone set?
➢ What is the brood size (seed:ovule) and can the brood size be related to fruit size or shape?

---

1. infructescence
What are the fruit to flower ratios (percentage fruit set), inflorescence to infructescence ratios (percentage cone set)?

What is the relationship between fruit size and the number or percentage fruit set?

What is the ratio of the area of the unit inflorescence to the area occupied by the fruit and can it account for any differences in the percentage fruit set?

What is the overall reproductive output taking into account seed, fruit, and cone set in the four Banksia study species and how do they compare?
4.2. Methods

4.2.1. Fruit set

Five infructescences were randomly sampled from each of five plants along two transects Province$^1$ species$^1$ in February and March 2000. Collection sites within each Province are given in Table 4.3. All plants sampled had at least three cohorts of cones (including the nonserotinous *B. integrifolia*) on the plants when sampled (Section 2.3). An infructescence was defined as an inflorescence that had set at least one fruit. All fruits visible to the naked eye were counted regardless of their size or state, ie open, closed or damaged (eg borer damage). Damaged infructescences that would not allow a reliable estimate of the height were omitted from the sample. The total number of follicles and height of each infructescence was scored (using vernier callipers).

**Table 4.3.** Collection sites for infructescences (fruit set) and estimates of cone set within each Province for each study species. For location map see Figure 2.1, Table 2.1.

<table>
<thead>
<tr>
<th>Province</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>Ku-ring-gai Chase NP1</td>
<td>Linden 1</td>
<td>Mt Kiera 1</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>Garie Beach 1</td>
<td>Seven Mile Beach 2</td>
<td>Lake Conjola</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>Bobbin Head</td>
<td>Castlereagh</td>
<td>Royal NP 2</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>Bobbin Head</td>
<td>Kings Tableland</td>
<td>Mt Kiera 2</td>
</tr>
</tbody>
</table>
Chapter 4

Regression equations derived from the data on inflorescence height and flower numbers (from Chapter 3) were used to estimate percent fruit set based on fruit counts and infructescence height (flower number). Percentage fruit set data were heteroscedastic even after (square root +1) transformation. Unplanned comparisons among pairs of means (pooled across Provinces) of percentage fruit set was made using the Games-Howell Method (Sokal & Rohlf, 1995). Frequency distributions for the lower range of fruit set (1-18 fruits cone$^{-1}$) and overall fruit set were made for comparison between species.

4.2.2. Cone set

At the same time as the above sampling, an independent sample of plants from the same general location (Table 4.3) were scored to estimate cone set. Five branches within five plants along two transects were sampled Province$^{-1}$ species$^{-1}$. Barren (old inflorescences without fruit) (BC) and fertile or fecund cones (FC) (infructescences) were scored on plants. With the exception of *B. oblongifolia* (smaller open shrub where whole plants were scored) scoring started at the tips of five branches and continued along the branch until at least three counts of FC’s were made. A schematic explanation of the method is illustrated in Figure 4.1. Data were analysed by one factor ANOVA.

4.2.3. Seed set

Two infructescences were sampled from each of five trees species$^{-1}$ along two transects Province$^{-1}$ in October and November 1998. Collection sites within each Province are given in Table 4.4. For *B. ericifolia, B. integrifolia* and *B. oblongifolia* only infructescences with 10 or more fruits per cone were collected. If necessary
additional infructescences were collected along the transect from the larger fruited
*B. serrata* to ensure a minimum sample of 100 fruits.

![Diagram](image)

**Figure 4.1.** Schematic illustration explaining the methods of scoring for cone set. Only plants that had set some fruit were sampled. Scoring always started from the growing tips of a branch (a, b, & c). A minimum of three counts either BC, FC or both types of cones were made along a branch (b). Scoring always continued (> 3 cones) along a branch to a bifurcation (branch fork) where often a reproductive shoot occurred and was scored) (a & c) then stopped. Once started, scoring continued
along the branch, but always included side branches before proceeding along the branch (c) in keeping with the above sampling rules. All plants sampled contained at least three cohorts of infructescences (see Chapter 2).

*Banksia integrifolia* (nonserotinous) was sampled by collecting infructescences that had started to shed seed or were clearly very close to shedding seed (valves were starting to split). The infructescences were taped or wrapped in aluminium alfoil such that opening fruits were not able to shed seeds. Holes punched in the aluminium alfoil allowed condensation to escape. Infructescences were given several days to dry, to facilitate dehiscence. Careful removal of tape and foil allowed seed to be scored as originating from individual fruits. Ten fruits cone\(^{-1}\) for *B. ericifolia* and *B. oblongifolia* were randomly selected (marked with paint or scored with wire cutters) and placed in an oven at 200\(^\circ\)C for approximately 20 minutes to open the fruits. Fruits of *B. serrata* were also opened by this method. Fruits were scored as containing two, one or zero viable seeds. Data were analysed by one factor ANOVA.

### Table 4.4. Collection sites to estimate seed set within each Province for each study species. For location map see Figure 2.1, Table 2.1.

<table>
<thead>
<tr>
<th>Investigation: seed set</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>Marramarra NP 1</td>
<td>Heburn Ave</td>
<td>Jervis Bay</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>Spencer 2</td>
<td>Watamolla Beach</td>
<td>Lake Conjola</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>Marramarra NP 2</td>
<td>Agnes Banks 2</td>
<td>Royal NP 2</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>Marramarra NP 1</td>
<td>Bulls Camp</td>
<td>Jervis Bay</td>
</tr>
</tbody>
</table>
### 4.2.4. Ovule to seed ratio (O:S)

Percentage cone set (CS) multiplied by percentage fruit set (FS) (CS X FS) provided an assessment of percentage fruit set over all inflorescences whether fecund or not. The value (CS X FS), multiplied by the proportion of ovules that set seed (mean number of seeds set fruit\(^{-1}\)/2) gave the seed to ovule ratio or percentage of ovules that developed into viable seeds.

### 4.2.5. Ratio of mean inflorescence area to fruit area (I:F)

Mean unit inflorescence height and width (Chapter 3, Figure 3.21) from each of the three Provinces for each study species were compared with the maximum fruit area (height x width) (Table 4.2, George, 1981) to obtain the unit inflorescence to fruit area ratio. The ratio provided an estimate of the relative amount (surface area) of fruit that can be accommodated for by each unit inflorescence. In other words it is a relative indication of how much fruit can fit on an infructescence, based on the size of the unit inflorescence. A range was provided by George (1981) so maxima were used, although minima would have given the same relative assessment. Clearly other factors such as fruit shape, chirality and packaging efficiency would further impact on the amount of fruit that could be accommodated on an infructescence.

### 4.2.6. Viable and non-viable seed

Seeds were deemed viable if endosperm was fully developed and non-viable if they lacked fully developed endosperm. To test the validity of this criteria, three replicates of six seeds of both viable and ‘non-viable’ seeds from three Provinces for each species were tested for germination. Seeds were placed on saturated filter paper
in petri dishes and assigned randomly to a position on a laboratory bench top. The petri dishes were regularly inspected and watered as necessary. Seeds were deemed viable if they germinated (ie if the radicle emerged from the seed coat). The test was concluded after 34 days.
4.3. Results

Regression of inflorescence height and flower number were significant at the level of Province and when pooled across Provinces (Table 4.5). Differences between Provinces were not significant. Equations from pooled data were used to estimate percentage fruit set infructescence\(^{-1}\) using the heights of the infructescences and the number fruit infructescence\(^{-1}\) (see Table 4.6).

The frequency histogram for the four study species over the lower part of the range of fruit numbers (1-18 fruits infructescence\(^{-1}\)) is given in Figure 4.2. The range 1–8 included the minimum class interval and approach the interval where each species scored consistently. *Banksia serrata* set fruit over the whole range, some infructescences setting only one fruit; *B. integrifolia* infructescence development commenced at three fruits infructescence\(^{-1}\); *B. oblongifolia* commenced at four fruits infructescence\(^{-1}\); and *B. ericifolia* at 10 fruits infructescence\(^{-1}\).

Frequency histograms of number of fruits infructescence\(^{-1}\) over the whole range are given separately for each study species in Figures 4.3 - 4.6. *Banksia ericifolia* (Figure 4.3) had the largest range (9 - 162), followed by *B. integrifolia* (Figure 4.4) (3 – 126), *B. oblongifolia* (Figure 4.5) (4 – 111) and *B. serrata* (Figure 4.6) (1-23). All frequency distributions were asymmetrical (right skewed), with skewness most pronounced in *B. ericifolia* (skewness = 1.332) followed by *B. integrifolia* (skewness = 0.781), *B. oblongifolia* (skewness = 0.732) and least skewed in *B. serrata* (skewness = 0.206) (Figures 4.3 – 4.6).
Table 4.5. Regression results of inflorescence height and flower numbers for each study species, Province and Provinces species$^{-1}$ pooled.

<table>
<thead>
<tr>
<th>Species (Province)</th>
<th>$r^2$</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em> (North)</td>
<td>0.94</td>
<td>14</td>
<td>217.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. ericifolia</em> (Central)</td>
<td>0.89</td>
<td>14</td>
<td>111.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. ericifolia</em> (South)</td>
<td>0.95</td>
<td>14</td>
<td>269.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.76</td>
<td>44</td>
<td>140.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. integrifolia</em> (North)</td>
<td>0.94</td>
<td>14</td>
<td>195.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. integrifolia</em> (Central)</td>
<td>0.97</td>
<td>14</td>
<td>561.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. integrifolia</em> (South)</td>
<td>0.94</td>
<td>14</td>
<td>190.9</td>
<td>&lt; 0.000001</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.91</td>
<td>44</td>
<td>416.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. oblongifolia</em> (North)</td>
<td>0.90</td>
<td>14</td>
<td>123.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. oblongifolia</em> (Central)</td>
<td>0.94</td>
<td>14</td>
<td>191.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. oblongifolia</em> (South)</td>
<td>0.95</td>
<td>14</td>
<td>256.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.91</td>
<td>44</td>
<td>428.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. serrata</em> (North)</td>
<td>0.87</td>
<td>14</td>
<td>89.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. serrata</em> (Central)</td>
<td>0.81</td>
<td>14</td>
<td>58.8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>B. serrata</em> (South)</td>
<td>0.98</td>
<td>14</td>
<td>815.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.92</td>
<td>44</td>
<td>467.0</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 4.6. Regression equations from data pooled across Provinces used to estimate the percentage fruit set on an infructescence (Figure 4.8).

<table>
<thead>
<tr>
<th>Species</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>$y = 52.8 \times x + 142.5$</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>$y = 91.2 \times x + 169.3$</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>$y = 237.6 \times x - 432.9$</td>
</tr>
<tr>
<td>B. serrata</td>
<td>$y = 196.7 \times x - 313.7$</td>
</tr>
</tbody>
</table>

Peakedness was most pronounced in *B. ericifolia* (kurtosis = 5.627), followed by *B. oblongifolia* (0.622) then *B. integrifolia* (-0.171) while the distribution was platykurtic in *B. serrata* (-0.645) (Figures 4.3 – 4.6).

Similar mean numbers of fruits were set species\(^{-1}\) infructescence\(^{-1}\) across Provinces. When pooled across Province there was a significant difference in the number of fruits that were set infructescence\(^{-1}\) for the four study species ($F_{3, 596} = 111.9, P < 0.0001$) (Figure 4.7). *Banksia ericifolia, B. integrifolia* and *B. oblongifolia* were similar with pooled means (±s.e.) of 49.4 (1.7), 45.8 (2.4) and 46.3 (1.8) respectively while *B. serrata* set a mean of 10.3 (0.4) fruits infructescence\(^{-1}\).
Figure 4.2. Frequency histogram of the number of fruits infructescence\(^{-1}\) for the range of 1-18 fruit infructescence\(^{-1}\) for the four study species. Minimum values were lowest with *B. serrata* (1) followed by *B. integrifolia* (3) then *B. oblongifolia* (8) and *B. ericifolia* (9).
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*B. ericifolia* skewness = 1.3323, kurtosis = 5.6274

![Frequency histogram for B. ericifolia](image1)

**Figure 4.3.** Frequency histogram (bin labels indicate upper limit of bin, interval = 10 fruits) of number of fruit infructescence^{-1} for *B. ericifolia* (range 9-162, mode = 37, n = 150).

*B. integrifolia* skewness = 0.781, kurtosis = -0.172

![Frequency histogram for B. integrifolia](image2)

**Figure 4.4.** Frequency histogram (bin labels indicate upper limit of bin, interval = 10 fruits) of the number of fruit infructescence^{-1} for *B. integrifolia* (range = 3-123, mode = 34, n = 150).
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*B. oblongifolia* skewness = 0.7327, kurtosis = 0.6227

![Frequency histogram for *B. oblongifolia*](image)

**Figure 4.5.** Frequency histogram (bin labels indicate upper limit of bin, interval = 10 fruits) of the number of fruit infructescence\(^{-1}\) for *B. oblongifolia* (range 4-115, mode = 41, \(n = 150\)).

*B. serrata* skewness = 0.2065, kurtosis = -0.645

![Frequency histogram for *B. serrata*](image)

**Figure 4.6.** Frequency histogram (bin labels indicate upper limit of bin, interval = two fruits) of the number of fruit infructescence\(^{-1}\) for *B. serrata* (range = 1-24, mode = 8, \(n = 150\)).
Figure 4.7. Mean (±s.e.) number of fruit infructescence$^{-1}$ for each study species and Province ($n = 150$ species$^{-1}$).

Figure 4.8. Mean (±s.e.) percent fruit set for each study species and Province ($n = 150$ species$^{-1}$).
Percentage fruit set data were heteroscedastic even after (square root +1) transformation (Cochrans’ $\chi^2_{149, 4, p=0.05} = 0.57$). Comparisons among pairs of means (pooled across Provinces) of percentage fruit set was made using the Games-Howell Method (Sokal & Rohlf, 1995) (Table 4.7).

Table 4.7. Results of comparisons among pairs of means (pooled across Provinces) of percentage fruit set using the Games-Howell Method (Sokal & Rohlf, 1995). Differences between means of the species (below the diagonal line) and corresponding MSD for an experimentwise 0.05 and 0.01 level of significance above the diagonal line. Mean differences greater than the MSD are indicated with an asterisk (0.05*, 0.01**). Harmonic interpolation of $\alpha$ (0.05, 0.01) after Rohlf and Sokal (1995).

<table>
<thead>
<tr>
<th>species</th>
<th>B. ericifolia</th>
<th>B. integrifolia</th>
<th>B. oblongifolia</th>
<th>B. serrata</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>0.9922*</td>
<td>0.6713*</td>
<td>0.6268*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2060**</td>
<td>0.8170**</td>
<td>0.7638**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8192*</td>
<td>0.7832*</td>
<td></td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>1.0222*</td>
<td>0.9974**</td>
<td>0.9544**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2672*</td>
<td></td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>3.3756**</td>
<td>2.3534**</td>
<td>0.3254**</td>
<td></td>
</tr>
<tr>
<td>B. serrata</td>
<td>5.2906**</td>
<td>4.2685**</td>
<td>1.9151**</td>
<td></td>
</tr>
</tbody>
</table>

There was a significant difference in the percentage fruit set among species. Banksia ericifolia had the highest percentage fruit set (5.8%), followed by B. integrifolia (4.8%), B. oblongifolia (2.4%) and then B. serrata (0.5%). The same trend in fruit set was
found for each species across Provinces (Figure 4.8). Percentage fruit set in *B. serrata* and *B. oblongifolia* was significantly different to each other and from the other study species ($\alpha = 0.01$). Percentage fruit set in *B. integrifolia* was significantly different to *B. ericifolia* at an experiment-wise level of 0.05 (Table 4.7).

The trend in mean heights of infructescences was similar across Provinces for each species (Figure 4.9). Pooled across Provinces the mean ($\pm$ s.e) infructescence heights for *B. ericifolia* were 13.5 (0.49) cm, *B. serrata* 12.1 (0.31) cm, *B. oblongifolia* 9.8(0.30) cm and *B. integrifolia* 8.3 (0.25) cm.

![Graph showing mean infructescence heights for each species and Province](image)

**Figure 4.9.** Mean ($\pm$ s.e.) height of infructescences for each study species and Province ($n = 150$ species$^{-1}$).

The percentage cone set was similar across Provinces for each species and was significant when branch data for plants were pooled ($F_{11, 108} = 10.7, P<0.001$) (Figure 4.10). *Banksia integrifolia* had the highest percentage cone set while the other three study species were more similar in the percentage cone set. Pooled across Provinces the
mean percent cone set was *B. integrifolia* (73.52%), *B. ericifolia* (58.06%), *B. oblongifolia* (54.00%) and *B. serrata* (50.40%).

![Bar graph showing mean percent cone set for each study species and Province](image)

**Figure 4.10.** Mean (±s.e.) percent cone set for each study species and Province (*n* = 150 species⁻¹).

There was a significant difference in the brood size among the four species (*F*₃, 2₄₆ = 204.6 *P* < 0.0001), with a consistent trend of only one viable seed set fruit⁻¹ in *B. ericifolia* and *B. oblongifolia* across transects and Provinces (Figure 4.11). *Banksia serrata* set consistently more seed fruit⁻¹ than *B. integrifolia* across transects and Provinces. The pooled across transects and Provinces mean (±s.e.) number of seeds set fruit⁻¹ for *B. serrata* was 1.71 (0.05) and *B. integrifolia* was 1.39 (0.05).

Comparison of fruit size (based on length X width dimensions Table 4.2) and mean percent fruit set for the four study species is given in Figure 4.12.
Figure 4.11. Mean number of seeds fruit\(^{-1}\) (±s.e.) for the four study species transect\(^{-1}\) (X2) Province\(^{-1}\) (n= 100, except B. serrata where n= 126, n= 164, n= 176, n= 182, n= 238, and n= 197 from left to right).

Figure 4.12. Mean log percentage fruit set and mean log maximum fruit size (area, length X width from George 1981, see Table 4.2) for the four study species.
While *B. serrata* with the largest fruits, set the least number of fruits infructescence\(^{-1}\) a clear association between fruit size and the number of fruit set is not as apparent within the other three study species (Figure 4.12) (for the four study species \(r^2 = 0.67, df = 3, F = 1.67\) n.s.). Fruit set in *B. ericifolia* was not proportional to the size of the fruits relative to the other three species. Regression of fruit size and the number of fruit set is significant within the other three study species (*B. integrifolia*, *B. oblongifolia* and *B. serrata*; \(r^2 = 0.85, df = 2, F = 2.74\)).

Mean percentage cone set (CS), fruit set (FS), seed set fruit\(^{-1}\) and the percentage of ovules that develop into seeds are presented in Table 4.8 (see also Figures 4.13, 4.14). *Banksia integrifolia* had the highest percentage seed set ovule\(^{-1}\) (2.45%) followed by *B. ericifolia* (1.68%), *B. oblongifolia* (0.65%) and then *B. serrata* (0.22%) i.e. about a ten-fold variation.

**Table 4.8.** The relative reproductive output for the four study species. Mean percent cone set = \%CS; mean percent fruit set = \%FS; mean number of seeds set fruit\(^{-1}\) = seed #; the proportion of ovules that set viable seed (seed #/2), percent fruit set taking percent cone set in account = CS X FS; the percentage of viable seeds that develop from ovules taking mean seed number fruit\(^{-1}\) and percentage fruit set (CS X FS) into account = \%S/O.

<table>
<thead>
<tr>
<th>Species</th>
<th>% CS</th>
<th>% FS</th>
<th>seed #</th>
<th>seed #/2</th>
<th>CS X FS%</th>
<th>%S/O</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>58.06</td>
<td>5.8</td>
<td>1</td>
<td>0.5</td>
<td>3.37</td>
<td>1.68</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>73.52</td>
<td>4.8</td>
<td>1.39</td>
<td>0.695</td>
<td>3.53</td>
<td>2.45</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>54.00</td>
<td>2.4</td>
<td>1</td>
<td>0.5</td>
<td>1.30</td>
<td>0.65</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>50.40</td>
<td>0.5</td>
<td>1.71</td>
<td>0.885</td>
<td>0.252</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Figure 4.13. Transverse (follicle) longitudinal sections through infructescences of a) *B. oblongifolia* and b) *B. ericifolia*. Both species only set one seed fruit\(^1\). In a) a single seed can be seen in an upper and lower position. In a) and b) the fruits are open and the valves are reflexed to the typical extent (allowing seed dispersal), note there is only sufficient room within each fruit for the development of a single seed. The illustration c) is to show the shape of the *B. ericifolia* fruit in transverse view.
Figure 4.14. Transverse longitudinal sections through infructescences of a) *B. serrata* and b) *B. integrifolia* (sectioned while green). Both species may set two seed fruit\(^1\). In a) two seeds can be seen in both fruits (note fruits are not open). In b) single and two seeded fruits can be seen. In both species there is sufficient space to accommodate two seed. The endocarp, mesocarp and pericarp are visible in cross section (stars of top fruits in a). Note seeds in b) are still hydrated.
The ratios of unit inflorescence area to fruit area (I:F) are given in Table 4.9. The data for unit inflorescences are the means from the three Provinces of each species (Chapter 3, see Figure 3.21) and in the calculation for fruit size the maximum length and width data were used from George (1981) (see Table 4.2). Banksia ericifolia and B. oblongifolia have essentially the same I:F, while B. integrifolia with the smallest fruits and smallest unit infructescence has the biggest I:F and B. serrata with the largest fruits has the smallest I:F.

**Table 4.9.** Ratio of unit inflorescence area to fruit area (I:F) (unit inflorescence size, Chapter 3 and maximum fruit dimensions after George (1981) (see Table 4.2).

<table>
<thead>
<tr>
<th>Species</th>
<th>length</th>
<th>width</th>
<th>area</th>
<th>width</th>
<th>height</th>
<th>area</th>
<th>area/area</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>22</td>
<td>13</td>
<td>286</td>
<td>0.29</td>
<td>0.45</td>
<td>1.305</td>
<td>4.56 x 10^{-3}</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>15</td>
<td>6</td>
<td>90</td>
<td>0.214</td>
<td>0.313</td>
<td>0.672</td>
<td>7.46 x 10^{-3}</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>18</td>
<td>7</td>
<td>126</td>
<td>0.177</td>
<td>0.325</td>
<td>0.578</td>
<td>4.59 x 10^{-3}</td>
</tr>
<tr>
<td>B. serrata</td>
<td>35</td>
<td>22</td>
<td>770</td>
<td>0.226</td>
<td>0.385</td>
<td>0.857</td>
<td>1.13 x 10^{-3}</td>
</tr>
</tbody>
</table>

The results of the seed germination tests are given in Table 4.10. All ‘non-viable’ seeds did not germinate. Both B. ericifolia and B. oblongifolia had 100% germination in viable seeds. Two ‘viable seeds’ did not germinate in B. serrata (2/54) and eight (8/54) (85.2%) in B. integrifolia (Table 4.8). After 34 days the seeds that had not germinated were covered in fungi and the test was concluded.
Table 4.10. Pooled results of germination of viable and non-viable seeds for the four study species. Data are for three replicates of six seeds species$^{-1}$ Province$^{-1}$.

<table>
<thead>
<tr>
<th>Species</th>
<th>Germination of viable ($n = 54$)</th>
<th>Germination of non-viable</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>85.2%</td>
<td>0%</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>96.3%</td>
<td>0%</td>
</tr>
</tbody>
</table>


4.4. Discussion

Due to the regular phyllotaxy and size of the Banksia unit inflorescences (Chapter 3) the strong association between inflorescence height and flower numbers was expected and were similar to the results obtained for B. grandis (Abbott, 1985), B. ericifolia (Paton & Turner, 1985) and B. spinulosa (Vaughton, 1988).

The relative reproductive effort (RE) ie inflorescences, cones (26.9%) and seeds (0.54%) comprised 31.3% of the above ground biomass in B. hookeriana (serotinous sp.). The relative allocation (RA = proportion of a specific nutrient divided by the proportion of total dry mass) was low for cones as compared to seeds but the total allocation of nutrients was much higher (because of the mass) for cones as compared to seeds (Witkowski & Lamont, 1996). Two considerations can be drawn from these findings. Firstly my results support the hypothesis for a threshold number of fruit to be initiated before cone set. The fewer the fruits the greater relative cost of ‘housing’ the seed(s) in fruits on a cone. Secondly, serotinous species have larger, thicker and more dense fruits than the nonserotinous species and the infructescences (as opposed to the fruits) are also larger and more developed (woody) in serotinous species. Serotinous species show considerable woody development of bracts adjacent to fruits (see Figure 4.13, 4.14). Cone set and fruit set would have a higher cost in serotinous than nonserotinous species.

The nurturing of offspring (seed/fruit maturation) is far longer in serotinous species as well as having a higher cost. In B. integrifolia anthesis to fruit maturation may occur in four months, while in the serotinous species it may take a year (pers. obs.). In the
animal Kingdom this is akin to the comparison of the gestation period of a human and an elephant. In plants, the consequences of increasing the maturation period are increased risk of abortion and loss of offspring to insect and bird predation, fungal decay, drought, fire and changes in resource status. So serotiny has a higher cost and an associated higher risk due to the increased time to maturation. Serotiny in *Banksia* does not require a continued resource supply, that is, the vascular supply to the infructescence is cut off by resins after fruit maturation (Wardrop, 1983). The return for the higher risks and costs associated with serotiny, are seen to be paid in seed protection, both in the absence of fire (eg from herbivory) and during fire (insulation from heat). Additionally, seeds of serotinous species are released on mass into a post fire environment that has increased available nutrients and light as well as reduced competition due to death of seeders.

Paton and Turner (1985) found a bimodal distribution in the frequency of inflorescences that set fruit in *B. ericifolia*. Inflorescences set either no fruits (barren cones) or had 11 or more fruit infructescence$^{-1}$. The class interval of 1-10 fruits infructescence$^{-1}$ was zero in their study while in my study a single infructescence set nine fruits. It might have been predicted that the obligate seeder would not have a threshold level of fruit set (and thus seed set) for infructescence set, but the data suggested the opposite (*B. ericifolia* had the highest minimal fruit set level (9) Figure 4.2).

The platykurtic frequency distribution of *B. serrata* (Figure 4.6) suggested the opposite to a range threshold, that is, any number (within any upper structural limitations) will do. The setting of one fruit infructescence$^{-1}$ in *B. serrata* appears sufficient reproductive reward for the cost involved (Figure 4.2). Although *B. integrifolia* had a ‘threshold’ of three fruits (Figure 4.2), in other samples *B. integrifolia* was observed to
set only one fruit infructescence\(^{-1}\) (per. obs.) (see discussion below on multifactorial traits). Both *B. serrata* and *B. integrifolia* set more than one seed fruit\(^{-1}\). Conversely the two serotinous species with the more leptokurtic distribution (*B. ericifolia* and *B. oblongifolia*), and lowest mean viable seed set of 1.0 fruit\(^{-1}\) have the highest minimal fruit set infructescence\(^{-1}\). A plausible hypothesis is that the critical factor in establishing a threshold level of fruit set for cone set is single seededness.

Goldingay *et al.* (1991) tested the idea that the ‘high’ cost of an infructescence may require that a threshold number of pollinated flowers be reached before infructescence development. Reduction in the number of flowers on *B. ericifolia* inflorescences (to 10-20, 30-40, 50-100) did not alter the likelihood of infructescence development. All flowers were open (not bagged), and hand cross pollinated, but data on numbers of fruit set infructescence\(^{-1}\) within the treatments were not given. A pollination event may not necessarily result in fertilisation or fruit development. A threshold of successful fertilisation events and/or fruit set may therefore be of more relevance in terms of whether a plant should initiate an infructescence or not. If a threshold level of successful fertilisation events is required, undetected early embryo/fruit abortion may mask the threshold level in the mature infructescence. The extent of early abortion (before visible fruit growth takes place) in *Banksia* is unknown.

The data support the hypothesis that cone set will be greater in nonserotinous species. *Banksia integrifolia* (nonserotinous) with a relatively small cone (ie, seed size, fruit size, fruit wall or valve size and relative lack of development of bracts) had a significantly greater cone set (73.52\%) than the serotinous species (Figure 4.10). Cone set in the three serotinous species was similar (58.06, 54.0 & 50.4\%). In *B. integrifolia*, once an infructescence is initiated the cost of producing additional seeds and fruits is
relatively less when compared to serotinous species. The serotinous species show considerable enlargement of the bracts (woody development) adjacent to fruits and the same relationship would not apply to *B. integrifolia* (see Figure 4.13, 4.14).

The foraging behaviour of nectar seekers and the temporal opening of flowers along an inflorescence increases the likelihood of more than one or a few fruits being set infructescence$^{-1}$. Thus, if a threshold of fruit initiation did exist, it is possibly achieved in a single or a few pollinator visitations. As a result the threshold (minimum number of fruits that must be set for infructescence development) may be masked.

An alternative consideration to the concept of a threshold is that it is a multifactorial trait (multiple genes) conferring a predisposition or liability to a threshold that is regulated by various environmental stimuli. Threshold traits could be discrete (and species-specific) or continuously operating over some minimal range (and still be species-specific). A minimal range threshold seems more plausible than a discrete cutoff. It is unlikely that there is a mechanism that could ‘count’, and do so consistently across the range of micro-/macro-habitats, and be maintained in the presence of variation in pollination events that a species experiences. In contrast, a range as opposed to a discrete cutoff confers choice or a level of plasticity in a variable environment. The physiological basis of such a strategy could be conceived along similar lines to the pollen/embryo/endosperm hormone mediated development of the ovary/fruit. That is, a threshold level of hormone may be required for infructescence development. Alternatively and/or additionally a threshold level in the sink demand may be required for continued source supply. If some type of threshold is in operation it clearly operates in a system of diminishing resources. With increasing fruit/cone set there is decreasing resource supply on a seasonal basis. The threshold may be reduced
or cease to operate due to diminishing resources. If this were the case then it would be impossible to establish a threshold level based on a sample from plants whose early and late set infructescences were indistinguishable, although this could be tested by scoring inflorescences and subsequent infructescences across the season.

The estimate of brood size was based on a sample that contained at least one viable seed fruit\(^1\) and therefore the estimate is slightly higher than if nonviable seeded fruits were taken into account. The significantly higher seed set fruit\(^1\) of \(B. \text{serrata}\) is not surprising considering the cost of producing a very large fruit compared to the other species investigated. Conversely the smallest ‘cheapest’ fruits of the four study species were those of \(B. \text{integripolia}\) but it had the second largest brood size. Both these species had either a negatively skewed (seed:ovule >0.75 sensu Uma Shaanker \textit{et al} 1988, section 4.1.2) distribution or one that approached it (seed:ovule >0.75, 1.71/2 = 0.85 \(B. \text{serrata}\) and 1.31/2 = 0.65 \(B. \text{integripolia}\)). The fixed (always) single seeded fruits of \(B. \text{ericifolia}\) and \(B. \text{oblongifolia}\) gave a brood size that is equal to 0.5 and thus is not characterised by any of the brood size categories suggested to occur in multiovulated fruits by Uma Shaanker \textit{et al} (1988). They considered brood size with a normal or bimodal distribution to be rare. A brood size without a distribution must be even more unusual. Alternatively taking into account nonviable seeded fruits both \(B. \text{ericifolia}\) and \(B. \text{oblongifolia}\) had positively skewed distributions (seed:ovule, 1/2= 0.5, adjusted for nonviabales < 0.5). In terms of fruit costs we may account for a larger brood size on the basis of the high cost of housing (efficient use of resources within the constraint of large woody fruits in \(B. \text{serrata}\)). Alternatively it might be argued that because housing is cheaper in \(B. \text{integripolia}\) a large brood size suggests opportunistic or efficient use of resources. The difference is based on the evolutionary constraints (fruit size) and how resources are utilised. In \(B. \text{serrata}\) with relative enormous fruits, the costs are very
high and to improve the cost benefit ratio a large brood size is required. In
*B. integrifolia* the relatively very low cost of housing frees up resources for producing
more offspring. For *B. integrifolia* this applies to all components of the reproductive
output (seed, fruit and infructescences). George (1981) considered serotiny to be
primitive and nonserotiny derived. The trade-off in the evolution from serotiny to
nonserotiny is thus fewer big seed/fruit/infructescences for greater numbers of smaller
seed/fruit/infructescences. The other nonserotinous species (*B. marginata*) has the
smallest fruits of any *Banksia* species on the east coast of Australia.

Theory would predict that the obligate seeder (*B. ericifolia*) would have a negatively
skewed distribution (high seed:ovule ratio) while the resprouters might tend to have
lower seed:ovule ratios. The lack of variation in brood size (1 seed fruit⁻¹) suggests that
some strong selectional forces have operated in the past (and may still be in operation).

Single seededness, is (in addition to very low fruit set in *Banksia*) a mystery that seems
to defy a suite of suitably appropriate explanations. Nevertheless some possibilities are
considered below.

A similarity between the single seeded species (*B. ericifolia, B. oblongifolia*) is that
they are both shrubs and the above ground parts of the plants are totally consumed in a
hot fire. In a very hot fire, some fruits and infructescences are also consumed by the
fire (Zammit, 1986; pers. obs.). Infructescence in the canopy of tree species, typically
avoid the higher fire temperatures that are generally found closer to the ground
(Bradstock, Gill, Hastings and Moore, 1994). Additionally there is often a high level of
predation of fruits and seeds in *Banksia* (Scott, 1982; Zammit, 1986). Single
seededness may be a trade-off in fecundity for increased protection. If some fruits are
consumed by fire then a smaller proportion of seed is lost than if they set more than one
seed fruit\(^1\). Similarly if fruits are predated, the losses to the plant and rewards to the predator are reduced, but at an increased cost to the plant (lower fecundity) – the trade-off. These trade-offs are not seen in the tree species whose infructescences are often positioned well away from the most intense heat of a fire, but they are subject to predation, be it by a more neoteric suit of predators. Before the extinction of the megafauna predation must have been more intense for infructescences on shrubs than mature trees. Within these four study species the follicles are more exposed in the tree species (*B. integrifolia, B. serrata*) (see Figures 4.13, 4.14).

The descriptions of the fruits as given by George (1981) (Table 4.2) do not suggest any differences between species that may account for single seededness. In the transverse longitudinal sections of *Banksia* infructescences it is clear that in *B. integrifolia* and *B. serrata* there is sufficient room for two seeds and when only a single seed is formed a space for another seed still exists (Figure 4.14). This suggests that fruits do not mould or grow themselves around the current brood, but ovaries develop along predetermined lines. In the single seeded species there is only sufficient room for a single seed. In Figure 4.13 the fruits are open but if they were closed, on sectioning there would have been even less space around the seed. With inadequate space to fully develop two seeds, competition and abortion may result in single seededness. If this were the case it might be expected that considerable endosperm may have been laid down before the pressure of competition caused one seed to abort (this is not always the case, see Chapter 5). Alternatively aborted endosperm may be reabsorbed (this again appears not always the case, see Chapter 5).

Another possible explanation for single seededness is the reduction of sibs and increased genetic variation. This is based on the assumption that an initial pollination
event supplies the same parental pollen to both ovules. By nurturing one embryo from that union, resources are available to nurture other embryos from potentially different paternal donors. This strategy seems unlikely considering the chances of survival of both sibs to mature plants.

Alternatively, an initial higher ovule number may allow higher quality offspring to be identified and selectively matured or compete (Westoby & Rice, 1982; Bawa & Webb, 1984; Wiens, Calvin, Wilson, Davern, Frank & Seavey, 1987).

The capacity of selfing may confer an advantage to seeder species in comparison to sprouters that may otherwise tolerate lower fecundity due to self incompatibility (Carpenter & Recher, 1979). Sampson et al. (1994) considered this plausible specifically for the fire killed seeder *B. brownii* (highest levels of selfing reported in *Banksia*). Any association between breeding system and fire tolerance requires a further assessment of the breeding systems of other banksias (Sampson et al. 1994). *Banksia ericifolia* is essentially an outcrosser and clearly the relationship of selfing and seeder advantage does not hold. Alternatively it may be that seeders are more plastic, demonstrating preferential outcrossing, but with an increased capacity for selfing (as opposed to sprouters) in the absence of outcross pollen. Such plasticity seems contradictory (selfing) when compared to the possible explanation for single seededness above (the reduction of sibs and increased genetic variation).

The maximum number of fruits infructescence$^{-1}$ recorded here are double that reported by George (1981) for *B. ericifolia* (162 versus 80) and *B. integrifolia* (126 versus 60), approximately one and half times for *B. oblongifolia* (115 versus 80) but more similar for *B. serrata* (24 versus 30). The results for the mean number of fruit infructescence$^{-1}$
formed two general groups (see Figure 4.7, *B. ericifolia, B. integrifolia* and
*B. oblongifolia* were similar 46-49, and *B. serrata* 10 fruits infructescence$^{-1}$) and at first
this pattern appears to reflect the difference in fruit size. The larger the fruits, the fewer
of them that can be accommodated on an infructescence. In the case of *B. serrata* this is
clearly the case. The relationship between fruit size and fruit number is otherwise not
consistent across the four species. There is as much variation in fruit size within the
*B. ericifolia, B. integrifolia* and *B. oblongifolia* group (Table 4.2) as there is between the
two ‘artificial’ groupings that appear in Figure 4.7. *Banksia serrata* with the largest
fruits, had the smallest fruit set while *B. ericifolia*, with the second largest fruits, had the
highest fruit set (Figure 4.12). Comparisons of percentage fruit set and fruit size are
otherwise more proportional when comparing *B. serrata, B. oblongifolia* and
*B. integrifolia*. Due to the dependence on seed for survival it would be predicted that
there would be higher seed set in *B. ericifolia*. *Banksia ericifolia* has the largest unit
inflorescence of the four study species (Figure 3.21) and may physically be able to
accommodate more fruit on an infructescence. The ratio of mean unit inflorescence
area to fruit area (Table 4.9) is essentially the same in *B. ericifolia* as it is in
*B. oblongifolia*. The fruit area dimensions provided under-estimates across species
because maximum values were used, but the fruit shapes, in terms of surface area,
approximate ovals, not rectangles, and are thus over-estimates. Clearly factor(s), other
than the physical accommodation of fruits on an infructescence, are at work as
percentage fruit set was twice that of *B. oblongifolia* in *B. ericifolia*. However fruit
shape in *B. ericifolia* is distinctly different from that of the three sprouter species (see
Chapter 8).

The trend for mean percent fruit set (Figure 4.8), while similar across Provinces for
species, showed a different pattern to that of number of fruit set infructescence$^{-1}$ (Figure
As expected *B. serrata*, with the largest fruits had the lowest fruit set. Interestingly the other three species also differed significantly from each other in the percentage fruit set. This is an effect of unit inflorescence size and infructescence height (or total flower number) as well as fruit number. As theory predicts for the reasons outlined above, the obligate seeder (*B. ericifolia*) had the highest percentage fruit set.

Clearly percentage fruit set does not reflect total seed numbers. Total seed numbers is the product of percentage cone set, fruit set and seed set as well as the total number of cones held within the canopy. Estimates of the canopy stored seed banks were not made for all the study species (exception was *B. oblongifolia*, Section 4.2.2). Total cones held within the canopy is clearly related to time since fire. In stands unburnt for 10-15 years, *B. ericifolia* plants were found to have two and a half more seed bearing cones than the co-occurring *B. oblongifolia* plants (Zammit, 1986). Initially *B. oblongifolia* (sprouter) produces more cones after a fire but with increasing time since fire *B. ericifolia* (seeder) ultimately produce more cones plant$^{-1}$. While the seeder/sprouter dichotomy can be invoked to account for the initial difference (lignotuberous regrowth versus seed) the ultimate difference, reliance on seed for survival, only explains why it is different, not how it is achieved. The difference in phyllotaxy and habit are attributes that enable *B. ericifolia* to ultimately store more seeds in its canopy (see Chapter 8).

The reproductive output was greatest in *B. integrifolia* (Table 4.8). The cost of serotiny was earlier considered to end at maturation when resin sealed off the vascular system to the infructescence. Serotiny may have an indirect maintenance cost. The weight bearing properties of stem/branches are likely to receive readjustment during the
interfire period as the weight of the seed bank increases. This may be a small cost but an additional cost to serotiny over nonserotiny and would contribute towards accounting for the higher seed to ovule ratio or reproductive output in *B. integrifolia* (more available resources).

As *B. integrifolia* often occurs in less fire-prone habitats its offspring face a greater challenge, in finding and becoming established in suitable microsites. Additionally germination rates appear to be less than the serotinous species (Table 4.10). Nonserotiny selects for high reproductive output. *Banksia integrifolia* may also have a longer period of fire sensitivity within its life cycle (nonlignotuberous and must develop a thick bark), and in this way sits intermediate between the seeder and sprouter strategies in terms of reliance on seed. Conversely it is capable of asexual reproduction (a variation on reprouting strategy, see Chapter 8).

A clear distinction between the fruit set in serotinous and nonserotinous species may have been expected, but was not reflected in the results. *Banksia ericifolia*, perhaps the most strongly serotinous species (see Table 1.1), had a fruit set more similar to the nonserotinous *B. integrifolia* than it did with the other serotinous species.

The low costs of nonserotiny enable *B. integrifolia* to have the highest reproductive output of the four study species. Within the serotinous species the obligate seeder, *B. ericifolia*, has a reproductive output twice that of the resprouter species. Within the resprouters *B. oblongifolia*, with the smaller fruits/seeds and habit that predisposes seeds to greater potential death from the heat of a fire, has a greater reproductive output than the large fruited arborescent *B. serrata*.
Chapter 5. Does floral symmetry provide a template for patterns in fruit and seed set in *Banksia*?

5.1. Introduction

Biological patterns are the result of nonrandom events that have evolved through the action of natural selection on biological variation. If ‘the causes and consequences of variation are central to studies in evolutionary ecology’ (Willson, Michaels, Bertin, Benner, Rice, Lee & Hartgerink, 1990), then patterns (variation under tight genetic control) should be at the forefront of such studies. Patterns in themselves may be a direct consequence of natural selection (eg mimicry or camouflage) or an indirect consequence of natural selection due to developmental constraints (eg. organ size). Differential growth and/or development may also hide, or illuminate, patterns where they exist (eg allometry or seed set within fruits respectively). Additionally, natural selection may have to juggle (metaphorically speaking) between closely related ontogenetic events such as flowering and fruiting. Pattern formation in one particular stage of the life cycle is linked, and thus constrained by, earlier patterns formed in that life cycle. If pre-pattern formation is a constraint then it may also be considered a template upon which selection may operate.

5.1.1. Non-random patterns of seed and fruit set

Non-random patterns of seed set within fruits has been reported across a wide range of taxa. Seed set within legumes and other species with linearly arranged ovules have been studied extensively. In general terms four patterns are recognised. Seed set may
be more likely to occur in the basal, distal, middle or at other distinct positions within fruits. While there is a trend of increased likelihood of seed set towards the distal end in legumes, other positional effects have been reported (O’Donnell & Bawa, 1993). For example, Gutiérrez, Menéndez and Obeso, (1996), found an increased likelihood of maturation of ovules in the central position in *Ulex europaeus* but in the stylar position in *Ulex gallii*. Horovitz, Meiri, and Beiles (1976) found ovules in even numbered positions in *Medicago sativa* were more likely to set seed than ovules in odd numbered positions. Evidence for the nonrandom setting of seeds with fruits has also been demonstrated in *Cassia fasciculata* (Lee & Bazzaz, 1986), mustard, radish (Namai & Ohsawa, 1992) and cauliflower (Gurusamy, 1999).

Non-random patterns of fruit set has been commonly observed in those plants whose flowers are arranged in inflorescences (Diggle, 1995; Medrano, Guitián & Guitián, 2000). Vaughton (1993), for example, demonstrated nonrandom patterns of fruit set in *B. spinulosa*. More fruit were set in the basal and middle sections of an infructescence than in the apical section.

In a serotinous population of *B. marginata* Vaughton and Ramsey (1998) found the number of seeds follicle\(^{-1}\) was dependent on follicle position within the infructescence. Follicles occupying a basal position tended to produce more two seeded follicles than those produced in apical positions. There was no significant difference in the weight of seeds from the one or two seeded follicles. A small, but significantly greater difference in the number of seeds produced from basal and apical ovules (94 versus 86%) within follicles was also reported but again seed mass did not differ due to position within follicles. Within the follicles of *B. spinulosa* Salkin and Hallam (1978) reported that usually only the bottom ovule developed into a seed (no data given).
Several nonexclusive hypotheses have been proposed to account for the observed nonrandom patterns of ovule and fruit set (Lovett Doust & Lovett Doust, 1988; Gutiérrez, Menéndez, & Obeso, 1996). Typically hypotheses are based on some type of resource limitation and/or chemical mediated dominance hierarchy. Ganeshaiah and Uma Shaanker (1994) have argued that distinct patterns of seed and fruit abortion cannot be accounted for on the basis of resource limitation per se. Additionally the chemically mediated dominance hierarchy hypothesis assumes a hierarchy already exists between developing sinks. Why and how such a hierarchy is generated is not clear. An alternative hypothesis or model to account for patterns in fruit and seed set is self organisation (Ganeshaiah & Uma Shaanker, 1994; Uma Shaanker, Ganeshaiah & Krishnamurthy, 1995). The model starts with a set of identical sinks (i.e. sink drawing ability is equal). Ovules that receive more resources (due to temporal differences in fertilisation) are autocatalytically favoured and thus dominant to others at a rate defined by the sink drawing ability of the ovules of that species. Another explanation for patterns, and low seed and fruit set is that ovules and ovaries are not functional.

5.1.2. Androgyny and andromonoecy

Johnson and Briggs (1963) reported that the flowers of some species of Banksia lacked ovules while appearing perfect. No other description was given, and the question remained, was there an ovary but it was empty, or did the flowers lack female form altogether? In a later report Johnson and Briggs (1975) considered a low percentage of fertilisation may be advantageous and concluded that some Banksia and other members of the Proteaceae are andromonoecous (male and hermaphrodite flowers on the same plant) with many flowers having abortive ovules. Again a description was not given of
the state of the aborted ovules or the methodology used in making the judgement. Collins and Rebelo (1987) reported ovule abortion in *Dryandra* and *Protea* and, like Johnson and Briggs (1963, 1975), did not provide a methodology (i.e. pre- or post-fertilisation observations, etc). Walker and Whelan (1991) recognised that if ovules were present, but aborted, then the term andromonoecy (male and hermaphrodite flowers) does not necessarily apply.

Some confusion in this earlier literature appears to stem from the terms used to describe the various types of sexual dimorphisms. Walker and Whelan (1991) considered androgyny (male and hermaphrodites on the same inflorescence) to be the appropriate term for these earlier reports in *Banksia*. They removed and examined c. 30000 *B. spinulosa* and c. 20000 *B. ericifolia* ovaries. With only a few minor exceptions they did not find ovules that appeared dysfunctional or any male flowers, in contrast to the report by Keighery (1980). Investigations of *B. integrifolia* by Mudie (1982) were also consistent with the findings of Walker and Whelan (1991). They concluded that andromonoecy and androgyny could not explain low fruit set, at least in the populations they examined and that further investigations were warranted.

Darwin (1884) recognised the production of excess flowers in plants and in reference to males, females and hermaphrodites gave more consideration to gynodioecy (females and hermaphrodites) over androdioecy\textsuperscript{10} (males and hermaphrodites). Reported cases of true functional androdioecy in animals and plants have only appeared relatively recently and such instances are considered to be extremely rare as Darwin must have presumed (Pannell, 2002).

\textsuperscript{10} Andromonoecy and androgyny are categories within androdioecy.
5.1.3. Left and right flowers and fruits

Left and right flowers are distinguishable in the unit inflorescence (established in Chapter 3). The asymmetry of the ovary is reflected in the mature fruit with pronounced development of the anterior side of the ovary (see Figure 3.4c). Thus fruits can be distinguished as left and right. In many banksias when the follicle opens, a split occurs at the stylar point leaving a W-shaped tear (Wardrop, 1983) or beak (George 1981). Distinguishing between the left and right fruits is relatively simple in the large fruited *B. serrata* because of the size of the W-shaped tear or beak. The presence/absence of a beak is diagnostic (Chapter 1). Follicles within the series Spicigerae (*B. ericifolia*) and Salicinae (*B. integrifolia* and *B. oblongifolia*) do not split leaving a beak (George, 1981). Left and right fruits are distinguishable in these species by the position of the seed, and/or curvature of the anterior side of the valve, which is also reflected in the curvature of the separator (intersemenal plate) and the seed wing.

Two of the above investigations (Mudie, 1982; Walker & Whelan, 1991) covered two of the current study species viz *B. ericifolia* and *B. integrifolia*. While the sample sizes for *B. ericifolia* (above) were enormous they were restricted to 10 specimens from a single population and the authors concluded further examinations were warranted.

The *Banksia* inflorescence is a highly ordered and condensed reproductive structure. The pattern of unit inflorescences varies only slightly within specific developmental phyllotactic constraints (Chapter 3). Does this highly ordered structure provide a template for pattern formation in the later stages of the life cycle? At the higher order (inflorescence pattern) of genetic spirals, orthostichies and parastichies there is no obvious pattern in respect to fruit production on an infructescence (per. obs.). That is,
fruits are not specifically produced in long rows (orthostichies) in left and right spirals (m and n parastichies) or in genetic spirals and whorls. There are however, other possible patterns (via the bias partitioning of resources within reproductive structures) that may have evolved. It might be expected that if such patterns have evolved they might also be different in the four Banksia species, a possible reflection of their life histories. In this context I asked the following more specific questions of the four study species;

- do these Banksia species have male flowers (lack ovaries/ovules)?
- Do all ovaries appear functional (similar to each other) both externally and internally?
- Do all ovaries contain two ovules?
- Are the left and right flowers of the unit inflorescence equally fecund, that is, do they set the same number of fruits?
- Within the flowers are ovules (upper and lower) equally fecund, do they set the same number of seeds?
- Do those species that set one seed fruit\(^1\) (B. ericifolia and B. oblongifolia, Chapter 4) show signs of endosperm development in the non-viable seeds?
- Is Banksia pollen geotropic and therefore account for any observed patterns in seed set within single fruits?
5.2. Methods

5.2.1. Examination of ovaries and ovules

The same sample used to investigate the floral orientation (Section 3.3.1) was used to examine the appearance of ovaries and ovules. In brief, after inspecting flowers, slightly oblique transverse cuts were made through unit inflorescences at the level of the ovules and the cut surface was viewed under a dissecting microscope (variable magnification, up to 200X). Left and right ovaries and upper and lower ovules were deemed functional if they appeared similar in size, shape and colouration. Areas of the inflorescence that appeared damaged (eg from borers) or where flowers had prematurely died and dried out were omitted from the sample. Both ovaries of a unit inflorescence were scored so that direct comparisons could be made between left and right flowers.

5.2.2. Embryo and endosperm development

*Banksia oblongifolia* and *B. serrata* inflorescences were collected from Agnes Banks (Agnes Banks 1, see Figure 2.1) in May 2000 to compare upper and lower ovule development. As no information was available on gametogenesis in *Banksia*, inflorescences of various ages (from when the flowers had just appeared above the bracts to post anthesis) were collected. Flowers were grouped into five categories on the basis of their development. Development was based on the length the flowers appeared above the bracts (10 & 20 mm above bracts) (categories 1 and 2) through to the bowing of the style prior to pollen presentation (3), after presentation (4), and older flowers whose pollen had been removed (5). Ten flowers (with ovaries) were prepared from each stage/category. The technique of Langer and Koul (1982) was followed. It
consisted of fixation (12:6:1 alcohol, formaldehyde and acetic acid for 4 days), washing
with distilled water and hydrolysis (NHCl, 60°C for 2.5hrs), staining (2% acetocarmine
for 1 hr) and gentle squashing of ovaries (with the cover slip) to release stained ovules
directly onto the microscope slide. A single ovary was squashed on each microscope
slide to release ovules so that they could be directly compared under the microscope.
Slides were examined under a light microscope (magnification up to 400X).

The non-viable sample of seeds used to investigate the presence of endosperm, was
obtained from the collection of fruits/infructescences used to estimate the proportion of
upper/lower ovules that set seed (Section 5.2.4). A random sample of 150 non-viable
seeds (Chapter 4) of *B. ericifolia* and *B. oblongifolia* were scored as either having or not
having some endosperm development. Simple visual observations of the presence or
absence of white tissue (presumably endosperm) were made with the naked eye.

5.2.3. Fruit set by left and right flowers

Two infructescences were sampled from five trees species⁻¹ transect⁻¹ (2) Province⁻¹ in
October and November 1998 from a North, Central and Southern Province of the
Sydney region (Table 5.1). For *B. ericifolia*, *B. integrifolia* and *B. oblongifolia* only
infructescences with 10 or more fruits cone⁻¹ were collected. To ensure a minimum
sample of 100 fruits, additional infructescences were collected along the transect (when
necessary) from the larger fruited *B. serrata*. Ten fruits per cone for *B. ericifolia* and
*B. oblongifolia* were randomly selected (marked with paint or scored with wire cutters)
and placed with the *B. serrata* sample in an oven at 200°C for approximately 20 minutes
to open fruits. Marked fruits (all fruits in *B. serrata*) were scored as having originated
from either a left or right flower on the basis of a w-shaped tear, and/or the curvature of
values and the seed impression in the valves of the fruit. The data were converted to a mean percent for *B. serrata*. Data were analysed via replicated G test (Sokal & Rohlf, 1995).

5.2.4. Seed set by upper and lower ovule

Independent collections using the same sample size and a similar procedure as above were used to estimate seed set by the upper and lower ovule within a single ovary of a flower. *Banksia integrifolia* (nonserotinous) was sampled by collecting infructescences that had started to shed seed or were clearly very close to shedding (valves were starting to split). These infructescence were taped or wrapped in aluminium foil such that opening fruits would not be able to shed seeds. Holes punched in the aluminium foil allowed condensation to escape. Careful removal or cutting of tape and foil allowed seed to be extracted and scored as originating from either the upper or lower ovule. All fruits on ten or more infructescences (so that the sample size was at least 100 fruits) were similarly scored for *B. serrata*.

Extracted seed from each infructescence was laid along a bench, maintaining the relationship of upper and lower origins. Ten seed pairs were randomly scored from each infructescence. Seeds were deemed viable if the endosperm was fully developed, and non-viable if they lacked fully developed endosperm. The criteria of viable and non-viable seed was checked with germination tests and the results supported the visual method of determination (see Chapter 4).
5.2.5. The collection sites

The location of collections for the investigations of upper/lower and left/right fruit and seed set are given in Table 5.1. Random independent collections of infructescences for tests of unity in left and right fruit set and upper and lower seed set were made.

Table 5.1. Collection sites within each Province for each species. NP = National Park.

For location map and grid reference see Figure 2.1, Table 2.1.

<table>
<thead>
<tr>
<th>Investigation: left/right fruit set, upper/lower seed set</th>
<th>Province</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>Marramarra NP 1</td>
<td>Heburn Ave</td>
<td>Jervis Bay</td>
<td></td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>Spencer 2</td>
<td>Watamolla Beach</td>
<td>Lake Conjola</td>
<td></td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>Marramarra NP 2</td>
<td>Agnes Banks 2</td>
<td>Royal NP 2</td>
<td></td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>Marramarra NP 1</td>
<td>Bulls Camp</td>
<td>Jervis Bay</td>
<td></td>
</tr>
</tbody>
</table>

5.2.6. Pollen tube geotropism

*Banksia oblongifolia* inflorescences (for investigation of pollen geotropism) were obtained from plants at Agnes Banks. Pollen from freshly opened flowers of *B. oblongifolia* was smeared onto agar plates (10% sucrose) by running pollen presenters over the agar surface. Twelve plates were prepared and assigned to four groups of three replicates each (Figure 5.1). The treatments varied by the orientation of the ‘pollinated’ agar surface. Plates were oriented such that the pollinated surface was facing up, down and sideways. The fourth treatment group was stood sideways and then turned through 90° after pollen tubes had grown an appreciable distance (approximately 2-3 cm). Prior to reorientation three pollen tubes that were growing in the same direction in each replicate were identified. Tubes were identified by lightly
scoring the agar surface with a needle in a line parallel to the direction of the tubes of interest. All plates were laid on and supported with pins against a polystyrene sheet.

**Figure 5.1.** Illustration of orientations of pollen treatments used to investigate geotropism. Four treatments were applied; pollinated surface facing upwards, downwards, sideways and sideways but reoriented through 90° after germinated pollen had grown an appreciable distance (approximately 2-3 cm) across the agar.
Chapter 5

5.3. Results

5.3.1. Examination of ovaries and ovules

No male flowers (lacking ovary or ovules) were found in any of the Banksia species. All ovaries in all dissections appeared functionally similar. One ovule appeared crenated in one ovary of B. integrifolia. All ovules otherwise appeared functionally similar (Table 5.2).

Table 5.2. Results of hand cut oblique transverse sections of unit inflorescences for the determination of functional appearing ovaries and ovules. Results are pooled (except for North Province B. integrifolia) for 100 unit inflorescences (200 ovaries & 400 ovules) Province\(^{-1}\) species\(^{-1}\).

<table>
<thead>
<tr>
<th>Species/Province</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
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<tr>
<td>B. ericifolia</td>
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<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>399/400</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
<tr>
<td>B. serrata</td>
<td>100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
</tbody>
</table>

5.3.2. Gametogenesis

The squash techniques for the study of gametogenesis (Langer & Koul, 1982) did not produce any stained spreads in B. serrata. In B. oblongifolia three squashes from young flowers (20mm above the bracts) revealed the megaspore mother cell and the two nucleate embryo cell of both ovules from single ovaries, indicating gametogenesis had occurred in both ovules.
5.3.3. **Endosperm development in non-viable seeds**

‘Non-viable seeds’ of *B. ericifolia* rarely showed any sign of endosperm development (Figure 5.2), whereas *B. oblongifolia* show signs of endosperm development in nearly 100 percent of cases (Figure 5.2 and Table 5.3).

![Diagram of seeds](image)

**Figure 5.2.** Non-viable seeds (including a large seed wing) of *B. ericifolia* (top) and *B. oblongifolia* (bottom). No visible sign of endosperm development can be seen in *B. ericifolia* while some endosperm can be seen in *B. oblongifolia*.

**Table 5.3.** Results of assessment of signs of endosperm development in ‘non-viable seeds’ of *B. ericifolia* and *B. oblongifolia*. Data are scores for signs of endosperm in 150 ‘non-viable’ seeds Province$^1$.

<table>
<thead>
<tr>
<th>Species/Province</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>0/150</td>
<td>3/150</td>
<td>1/150</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>147/150</td>
<td>143/150</td>
<td>140/150</td>
</tr>
</tbody>
</table>
Banksia oblongifolia showed considerable variation in the extent of endosperm development from amounts just visible to the naked eye to amounts comparable to approximately 20% of that of a viable seed. While not specifically sampled and scored, non-viable seeds of B. serrata also showed signs of considerable endosperm development. Banksia integrifolia on the other hand show very little development of endosperm in non-viable seeds, and in this respect it was similar to B. ericifolia.

5.3.4. Fruit set by left and right flowers

Figures 5.3 - 5.6 shows the mean percent fruit set (pooled across transects) by the left and right flower for B. ericifolia, B. integrifolia, B. oblongifolia and B. serrata. Table 5.4 shows the results of replicated G tests (goodness of fit) (H\textsubscript{0} of 1:1, left:right) for proportion of flowers that set fruit. There was no significant difference between the proportion of left or right flowers that set fruit for all species and Provinces. For all species G\textsubscript{Total}, G\textsubscript{Pooled} and G\textsubscript{Heterogenity} were also not significant (p = 0.05).

Banksia integrifolia set significantly (alpha 0.1) more fruit (323/600) from the right flowers when data for Provinces were pooled (Table 5.4). Banksia ericifolia showed a consistent but not significant trend across Provinces of setting more fruit by the right flower than the left. Banksia oblongifolia and B. serrata did not show any consistent trends in left or right fruit set across Provinces. One proportion power analysis (Pass 6.0) showed a significant difference in left and right fruit set in B. integrifolia at the 0.05 alpha level. The proportion 323 (of 600) had an actual alpha of 0.047 and a power of 0.58085.
Figure 5.3. Mean (±s.e.) percent left and right fruit set infructescence$^{-1}$ for *Banksia ericifolia* across Provinces. Data from two transects Province$^{-1}$ were pooled. Ten fruits infructescence$^{-1}$ were scored (pooled $n = 20$ infructescences).

Figure 5.4. Mean (±s.e.) percent left and right fruit set infructescence$^{-1}$ for *Banksia integrifolia* across Provinces. Data from two transects Province$^{-1}$ were pooled. Ten fruits infructescence$^{-1}$ were scored (pooled $n = 20$ infructescences).
Figure 5.5. Mean (±s.e.) percent left and right fruit set infructescence\(^{-1}\) for *Banksia oblongifolia* across Provinces. Data from two transects Province\(^{-1}\) were pooled. Ten fruits infructescence\(^{-1}\) were scored (pooled \(n = 20\) infructescences).

Figure 5.6. Mean (±s.e.) percent left and right fruit set infructescence\(^{-1}\) for *Banksia serrata* across Provinces. Data from two transects Province\(^{-1}\) were pooled. All fruit were scored infructescence\(^{-1}\) in the case of *B. serrata.*
Table 5.4. Replicated G test for goodness of fit to 1:1 left and right fruit set in *B. ericifolia*, *B. integrifolia*, *B. oblongifolia* and *B. serrata* (*n*=100; 10 fruits cone\(^{-1}\) transect\(^{-1}\) except for *B. serrata* where all fruits were counted on 10 cones site\(^{-1}\); *p*< 0.1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Province</th>
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<th>right</th>
<th>n</th>
<th>df</th>
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<td></td>
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<td>0.2736 ns</td>
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</table>

| *B. integrifolia* | North T1 | 47   | 53    | 100 | 1   | 0.3602 ns |
|                   | North T2 | 48   | 52    | 100 | 1   | 0.1600 ns |
|                   | Central T1 | 46  | 54    | 100 | 1   | 0.6407 ns |
|                   | Central T2 | 48  | 52    | 100 | 1   | 0.1600 ns |
| * 0.1 * South T1  | 42   | 58    | 100  | 1   | 2.5710 ns |
| South T2          | 46   | 54    | 100  | 1   | 0.6407 ns |
| total             |        |       |       |     |    | 3.5718 ns |
| \(\Sigma\)       |        | 277  | 323   | 600 | pooled | 3.5301 * |
| heterogeneity     |        |      |       |     |    | 1.0026 ns |
Table 5.4 cont.

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<th>right</th>
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<td>1</td>
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<td>57</td>
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| *B. serrata*   | North T1 | 60   | 52    | 112| 1  | 0.5719 ns |
|                | North T2 | 51   | 55    | 106| 1  | 0.1510 ns |
|                | Central T1 | 60   | 49    | 109| 1  | 1.1119 ns |
|                | Central T2 | 52   | 64    | 116| 1  | 1.2436 ns |
|                | South T1  | 63   | 71    | 134| 1  | 0.4778 ns |
|                | South T2  | 48   | 56    | 104| 1  | 0.6160 ns |
| total         |          | 6    |       |    |    | 4.1724 ns |
| Σ             |          | 334  | 347   | 681|    | 0.2482 ns |
| heterogeneity |          |      |       |    |    | 3.9242 ns |
5.3.5. Seed set by upper and lower ovules

There was a significant difference in the number of seeds set by the upper and lower ovules of *B. ericifolia* and *B. oblongifolia* (Figures 5.7 & 5.9, Table 5.5). In contrast there was no significant difference between the percentage of seed set by either the upper or lower ovules in *B. integrifolia* and *B. serrata* (Figures 5.8 & 5.10, Table 5.4).

Heterogeneity was significant for *B. ericifolia* (Table 5.5). Unplanned test of homogeneity of replicates (transects) using the simultaneous test procedure (Sokal & Rohlf, 1995) showed two homogenous sets, Central Province, transect 1 and all other transects and Provinces. The magnitude in the difference between the proportion of upper and lower seed set fruit⁻¹ resulted in the heterogeneity, not a difference in the trend itself.
Figure 5.7. Mean (±s.e.) percent upper and lower seed set fruit$^{-1}$ for *Banksia ericifolia* transects$^{-1}$ (X2) and Provinces$^{-1}$. Ten fruits infructescence$^{-1}$ were scored ($n=10$ infructescences).

Figure 5.8. Mean (±s.e.) percent upper and lower seed set fruit$^{-1}$ for *Banksia integrifolia* transects$^{-1}$ (X2) and Province$^{-1}$. Ten fruits infructescence$^{-1}$ were scored ($n=10$ infructescences).
Figure 5.9. Mean (±s.e.) percent upper and lower seed set fruit\(^{-1}\) for *Banksia oblongifolia* transects\(^{-1}\) (X2) and Province\(^{-1}\). Ten fruits infructescence\(^{-1}\) were scored \((n = 10\) infructescences\).
Table 5.5. Replicated G test for goodness of fit to 1:1 upper and lower seed set in the four study species (n=100; 10 fruits cone^{-1} transect^{-1} (T1 & T2) except for *B. serrata* where all fruits were counted on 10 cones site^{-1}). Data for *B. integrifolia* and *B. serrata* have been converted to a percent (*p < 0.05, ** 0.01, *** 0.005, **** 0.001).

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*B. integrifolia* data is percent

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*B. serrata* data is percent

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5.3.6. Pollen geotropism

*Banksia oblongifolia* pollen started to germinate readily on all the agar plates within 24 hrs. No evidence for negative or positive geotropism was found. On the upward facing treatment, pollen germinated and grew up off the plates as well as horizontally across the agar surface in all directions. In the downward treatment, pollen grew down off the agar surface as well as horizontally across the agar surface in all directions. In the sideways treatment pollen also grew out off the surface as well as across the agar surface in all directions. In the sideways and reorientated treatment, pollen tubes also grew out off the surface as well as across the agar surface in all directions. In the reorientation treatment those pollen tubes that were identified did not show reorientation as a result of turning the plates through 90°. Those tubes tended to continued their growth in the same direction although some changed direction, but not consistent with a geotropic response. The only pollen to show reorientation were the longer pollen tubes (>3 cm) that grew out of the surface.
5.4. Discussion

The four species of *Banksia* investigated did not have male flowers or essentially dysfunctional female parts (visual inspection of section ovaries *in situ*) (Table 5.2). The squash techniques for gametogenesis also supported this result and all flowers were hermaphroditic and presumably equally functional as found by Walker and Whelan (1991).

The squash technique of Langer and Koul (1982) was tried on *B. oblongifolia*, a species that sets only one seed fruit\(^1\) (Chapter 4). If only one ovule was functional, staining of nuclear bodies in both ovules of an ovary might not have been achieved. The technique was also tried on *B. serrata*, a species that more often sets two seeds (Chapter 4). If *B. serrata* consistently showed the development of two embryo sacs ovary\(^1\) and *B. oblongifolia* one embryo sac ovary\(^1\) (all with nuclear bodies) then it could have been concluded that single seededness in *B. oblongifolia* was a result of forming a single functional embryo sac. Ovule abortion could have then explained how single seededness occurred in *B. oblongifolia*.

Embryo sac development appeared in squashes from flowers at an early stage of development, well before anthesis. In developing a methodology, ovule abortion at various stages of ovule development should be considered.

The small but consistent amount of endosperm development in non-viable seeds of *B. oblongifolia* (Figure 5.2, Table 5.3) suggested that single seededness is a result of seed abortion and not ovule abortion. This is consistent with the development of two
embryo sacs found to occur in *B. oblongifolia*. The comparative lack of any endosperm development in non-viable *B. ericifolia* seeds could be a result of ovule or seed abortion (at least before any apparent endosperm has been laid down). Alternatively *B. ericifolia* may absorb endosperm of aborted seeds and redistribute the saved resources. Conversely *B. oblongifolia* has a lignotuber (storage organ), sets fewer infructescences plant$^{-1}$ (Chapter 4; Zammit, 1986) and the lost resources (endosperm) to aborted seeds may not be as critical for the species survival. If dysfunctional ovaries/ovules were encountered in some banksias, they might be expected to be found in the respouter species, as opposed to obligate seeders that rely on their regeneration solely from seed.

Walker and Whelan (1991) considered that if andromonoecy or androgyny occurred in the Proteaceae, it would not be widespread. The findings here support their hypothesis. If male, or dysfunctional females flowers occurred in banksias it is likely that they would occur in a regular pattern: either left or right flower, or in one family of parastichies, or in alternating orthostichies, occurring every nth primordia, rather then occurring randomly. That is, their occurrence would be under tight genetic control. In terms of fruit set, the left and right flowers were equally functional in the serotinous species (Figures 5.3 – 5.6, Table 5.4) but *B. integrifolia* showed a strong and significant trend of setting more fruit by the right flower (Figure 5.4). The result (if not a type I error) indicates that some partitioning of resources has occurred in *B. integrifolia*. As nonserotiny is considered a derived trait (George, 1981) it is also likely that during its evolution other changes may have also occurred. For example, the reduction in vascular tissue that supplies left flowers or reduction of resources to right ovaries would give an advantage to right flowers and ovaries while maintaining some function in the left. As stigmas of the flowers of a unit inflorescence are close together and pollinators are relatively large, foraging is likely to result in pollination of both flowers. Both flowers
cannot typically develop into fruits because of structural limitations (fruit size). Partitioning of resources within unit inflorescences is a bet hedging strategy that further reduces the cost of the reproductive output of *B. integrifolia*. The biased pattern of fruit set in *B. integrifolia* suggest androgyny (see below).

Under very dry weather conditions *Banksia* inflorescences may abort. While this was observed, it appears to be limited to the smaller inflorescence, well before the appearance of flowers above the bracts. *Banksia* inflorescences are large (take a long time to develop, up to 12 months, Fuss and Sedgley, 1990) and are resource costly structures (Witkowski & Lamont, 1996) and it appears that once initiated they reach a point of no return in their development once they achieve a certain size. Having a choice to pursue (or not pursue) sexual reproduction may reduce the costs and/or burden of sex organ production in an increasingly stressful environment, which may well have not been evident at the onset of inflorescence initiation. Additionally (having developed an inflorescence to past the point of no return) female function may give way to male only function with increasing drought/stress. Mature pollen grains are independent organs and stress induced late flower abortion has a greater probability of aborting ovules than pollen.

Given the degree of latitude in the functional definitions of andromonecy and androgyny (morphological hermaphrodites with dysfunctional or reduced gynoecia on the plant and inflorescence respectively) these terms could equally apply to bankssias on the basis of observed fruit and cone set. Likewise the observation that some plants that develop inflorescences, do not develop fruit, means that androdioecy could also apply. For example, Salkin and Hallam (1978) reported colonies of flowering but non-fruiting
plants of *B. marginata* (a root sprouter) and Carthew (1993) reported a group of flowering but nonfruiting *B. spinulosa* (see reference to root sprouting below).

The Bateman Principle (Bateman, 1948) holds that male fitness is limited by mating opportunities, whereas female fitness is more likely to be limited by resources for maternal roles (seed/fruit set). Selfing by hermaphrodites in an androdioecous species limits male mating opportunities while selfing hermaphrodites in gynodioecous species does not directly compromise the mating opportunities of females (Pannell, 2002). While the occurrence of androdioecy is very rare, its occurrence in some *Banksia* species seems reasonable, particularly in those species where males may persist via vegetative reproduction. One possible evolutionary path to dioecy from hermaphroditism is via andromonoecy (Ross, 1982). With the exception of *B. elegans* (Western Australian sp.) the breeding system of those *Banksia* species that vegetatively reproduce has not been investigated. Vegetative reproduction can, at least theoretically, confer a degree of plasticity or latitude in the selection pressures on sexuality. Vegetative reproduction via underground stems or stolons in *B. integrifolia* may be extensive (per. obs.). Relaxation of selection pressure, given the necessary higher fecundity of males if they were to persist in an androdioecious species, may be reduced via asexual reproduction.

The temporal separation of ovules within ovaries provided a template upon which natural selection has biased the setting of more seeds by the lower ovule in the two shrub species that set only one seed in each fruit (Table 5.5). While both species showed a highly significant setting of more seed by the lower ovule, the trend was stronger in *B. ericifolia* than *B. oblongifolia*. Ovules fertilised first may have a temporal
advantage in competing for resources (Lee & Bazzaz, 1986). However pollen was found not to be geotropic (but see discussion below).

Obturators are typically swellings of the placenta that grow towards the micropyle and form a hood or canopy over the nucleus that serves as a bridge for the pollen tube (Maheshwari, 1978). In *B. oblongifolia* Venkato Rao (1964) found that epidermal cells proliferated in the base of the loculus forming glandular hairs that extended towards the micropyle (fertilisation is porogamous) and these could possibly function as an obturator. Obturators were not reported in the ovaries of the other banksias studied by Venkato Rao (1964) (viz. *B. serrata, B. grandis* and *B. marginata*). These species differ from *B. oblongifolia* as they set more than one seed fruit\(^1\) (Chapter 4; Abbott, 1985, Vaughton & Ramsey, 1998). The obturator may account for the setting of more seed by the lower ovule than upper in *B. oblongifolia* and *B. ericifolia*. A temporal bias in fertilisation, due to the obturator, followed by a temporal advantage in competition for resources is a plausible explanation for the pattern of seed set. The base of the ovary is, in effect, the back of the ovary as the flowers are positioned at right angles to, and on, the vertical central axis of the inflorescence. Venkato Rao (1964) did not investigate *B. ericifolia* or other species that set only one seed fruit\(^1\) (eg *B. spinulosa*, Vaughton 1988). Further investigations as to the occurrence of a potential obturator (proliferation’s of epidermal cells in the base of the ovary) in single seeded species are warranted as it may help to explain single seededness.

The test for pollen geotropism revealed that pollen is neither negatively nor positively geotropic. The significant seed setting of the lower, over the upper ovule in *B. ericifolia* and *B. oblongifolia* cannot be attributed to a positive geotropism of pollen. However pollen tube growth is generally different *in vivo* to that found in cultures (Read, Clarke
& Bacic, 1993). Thus pollen may be positively geotropic over very short distances or under the right environmental conditions (such as within the ovary). As the mechanisms responsible for geotropisms are not completely understood (Hart, 1990) the role of geotropism, although unlikely, cannot be ruled out.

Chemotropism has generally been accepted to account for the directional growth of pollen (Hart, 1990). The topic has more recently been reviewed by Franklin-Tong (1999). Convincing evidence has been accumulated for the role of Ca\(^{2+}\) in the rate and direction of pollen tube growth. When reaching the bottom of the ovary, a complex guiding system must operate to direct pollen tubes towards the micropyle. The lower ovule may have a positional advantage if a Ca\(^{2+}\) gradient develops in the ovary of \textit{B. ericifolia} and \textit{B. oblongifolia}. Add to this the possible role of an obturator and a reasonable hypothesis starts to emerge to account for more setting of seed by the lower ovule. What remains a greater mystery is why only one seed is set fruit\(^{-1}\), particular in the obligate woody fruited seeder \textit{B. ericifolia} when sprouters such as \textit{B. serrata} may set two seeds.

Watson and Casper (1984) considered that patterns of seed set are influenced by patterns in vascular tissue that may represent a morphological constraint. Fruit shape (see Figures 4.13, 4.14) did not indicate any possible bias for the significantly greater setting of the lower ovule in seed. The orientation of fruits might provide one possible line of inquiry. George (1981) reported the fruits of \textit{B. oblongifolia} to be usually slightly upturned and this is apparent in Figure 4.13. The upturning of fruits is also apparent in \textit{B. ericifolia}, particularly more so than in \textit{B. integrifolia} and \textit{B. serrata}. In the upturning of fruits the vascular supply to the upper ovule may become compressed and resource supply to it reduced. This must happen early in the development of the
fruit/seed to account for the pattern of increased seed set by the lower ovule. As the development of anthesis (and thus fruit set) is acropetal in *B. ericifolia*, pressure from developing fruits and bracts might tend to force fruits downwards instead of upwards, but that is not how they are oriented. The reason for the orientation of fruits would have to be understood before its possible role in pattern formation is considered.

An alternative hypothesis is that seed abortion is under maternal control and plants preferentially abort seeds that have the lowest potential to succeed (Stephenson, 1981). For example Lee and Bazzaz (1986) considered that seeds occupying the distal end of fruits in the ballistically dispersed seeds of *Cassia fasciculata* have an advantage by being dispersed further than those at the base. Plants that selectively abort seeds at the base would have a higher mean dispersal distance and presumably a higher mean fitness. No dispersal advantage can be attributed to the lower seed position over the upper in *Banksia*.

Fixed rates of abortion, in particular cases that result in single seededness have been reported in a variety of legumes (Bawa & Webb, 1984) and other taxa (Casper & Wiens, 1981). While reducing seed number can enhance fruit dispersal (less weight) it may also reduce sibling competition at the site where seedlings become established. Again no dispersal advantage can be concluded in *Banksia*.

Another possible explanation for the setting of more seeds by the lower ovule is that the lower position confers some protection from the heat of bush fires. Intense heat rapidly ruptures follicles creating a small gap between the follicles allowing more heat to enter the fruits. Seeds in a lower position may have better germination percentages and rates of germination. Time of germination relative to neighbours may be a critical factor in a
plant’s survival (Ross & Harper 1972). Seeds slow to germinate may be at greater risk of predation and fungal decay. In a pilot trial I mechanically removed seeds (cut them out of fruits with a saw) from *B. oblongifolia* and *B. serrata* and compared their germination rates and percentages with those seeds extracted by heat (190°C for 0.5 hours). A marked difference between species response was noted. Heating greatly slowed the germination rate and reduced the germination percentage in *B. serrata*, but there was virtually no difference between treatments (either the rate or percentage germination) in *B. oblongifolia*. The follicles of *B. oblongifolia* are not as exposed (above the bracts) as they are in *B. serrata* (see Figures 4.13, 4.14) and this may confer some insulation. Follicles of *B. oblongifolia* (a shrub) experience greater temperatures from bush fires than those of the arborescent *B. serrata*. It is proposed that the setting of more seed in the lower position in the singleseeded shrubs is an adaptation to heat stress.
Chapter 6. Accessory buds and resprouting in *Banksia* Lf.

6.1. Introduction

6.1.1. Axillary and accessory buds

Branches in seed plants commonly appear to originate in the axils of leaves, and hence have been referred to as axillary buds. Initially they arise exogenously on the stem but become displaced closer to the leaf base due to growth readjustments (Esau, 1965). The protection conferred by the axillary position was considered one reason for the universal adoption of this arrangement in flowering plants (McLean & Ivimey-Cook, 1962). For the few exceptions to this arrangement see Daviero, Gomez and Philippe (2001).

Additional or supernumerary (usually the less prominent) buds in a leaf axil are referred to as accessory buds and these may be collateral or in series. Accessory buds may develop into vegetative shoots, reproductive shoots, spines, tendrils or a combination of these (Bell, 1991).

When several shoots develop from a single leaf axil they represent either a condensed branching system with reduced internode elongation, referred to as proliferation, or are the result of activity of several accessory buds. In some instances, it may be difficult to distinguish between the two situations. With proliferation each shoot or bud would be subtended by a leaf and accessory buds are all subtended by the same leaf (Bell, 1991). Most of the information on accessory buds has come from studies of eucalypts, particularly in reference to lignotuber formation, epicormic shoots and resprouting.
6.1.2. Studies in the genus *Eucalyptus*

Accessory buds of eucalypts have been referred to as concealed, proventitious, dormant and epicormic (Cremer, 1972). In the branches they are found below the axillary bud and above the petiole. This nested location seems to be a protected position (Chattaway, 1958). Graham, Wallwork and Sedgley (1998) considered that the term naked bud for the Eucalypt axillary is used to distinguish it from the ‘protected’ accessory bud. The term naked originally referred to the lack of cataphylls or bud scales on axillary buds, a term coined by Carey (1930) in comparison to the buds of deciduous trees of temperate latitudes of the Northern Hemisphere. Carey (1930) identified three classes of leaf buds in woody perennials of NSW: scaly, intermediate and naked buds. All banksias (including *B. ericifolia, B. integrifolia* and *B. serrata*) examined by Carey were classed as intermediate. The concept that the nested position confers protection to the accessory buds (Chattaway, 1958; Graham *et al*, 1998) raises a question. The axil is formed by the leaf and stem. Both the larger axillary and smaller accessory bud are located in the axil with the accessory bud basal or abaxial to the axillary. When leaf abscission occurs the smaller accessory could be considered more exposed than if it were adaxial to the axillary bud. Why is this arrangement found and not *visa versa*?

In eucalypts accessory buds or meristematic regions may be recognisable between the axillary and leaf petiole and these buds may develop into shoots once the axillaries have been removed. The new shoots have a relatively short initial internode with intermediate size and sub-opposite leaves (Jacobs, 1955).
Jacobs (1955) described Eucalypt accessory buds and showed that the origin of epicormic shoots (cf. shoots that arise on the trunk, Blake, 1983; Shigo, 1989) was associated with a trace that originated at the leaf axil. Epicormic shoots are thus different from lignotuberous shoots, although in practise it may be difficult to distinguish where the lignotuber ends and the trunk begins in some species.

In eucalypts the number and arrangement of accessory buds typically changes from one organ to another. In studies of the structure of the lignotuber in the cotyledonary and lower leaf nodes of *Eucalyptus* sp. Chattaway (1958) found lignotubers developed only in those species that have accessory buds present above and below the axillary buds. She also noted that in the leaf nodes of older stems the accessory buds occur only below the axillary shoots and above the leaf petiole.

### 6.1.3. Studies in conifers and other plant groups

In contrast to flowering plants, most conifers lack bud-forming structures and have blank leaf axils (Burrows, 1987). However, meristems occur in the leaf axils of several species of both *Agathis* and *Araucaria* (Fink, 1983; Burrows, 1986, 1987, 1990). The apparently blank leaf axils of these Araucariaceae species contained persistent meristems, without vascular connections to the central vascular cylinder, or bud-like organisation. The meristems are exogenous in origin but become buried in the stem by localized phellogen (Burrows, 1986). However, when released from apical dominance (by pruning apical meristems) the meristems developed into buds (Burrows, 1989). The recently described Wollomi pine (*Wollemia nobilis*, Araucariaceae) also possess the unusual leaf axil anatomy typical of those other members of the family so far investigated (Burrows, 1999).
More than half of the 36 tree species sampled from the canopy surface of a wet tropical lowland forest in Africa were found to have accessory buds. These buds always formed a vertical series. All buds examined were considered protected, by either scale leaves, stipules or the protection conferred by the subtending leaf base (Bell, Bell and Dines, 1999). A similar result was obtained from a survey in a South American tropical rainforest (Bell & Bell, 1998). The maintenance of a crown branch system is dependent on an adequate supply of meristems and these are often present as well protected accessory buds. The authors suggested a relationship between the size of the canopy bud bank with the vulnerability of the buds to the relatively dry microclimate and herbivory in the canopy. In this context a comparison can be made between the canopy of a tropical rainforest and dry shrub vegetation (Bell et al, 1999).

6.1.4. Experimental defoliation

Plant species respond differently to defoliation and their tolerance has been related to how rapidly new growth is initiated (Bilbrough & Richards, 1993). Inherent relative growth rate has been suggested to account for a plant’s capacity to regrow lost foliage (Gulmon & Mooney, 1986). In a pruning experiment on six species of semi-arid land shrubs Wandera, Richards and Mueller (1992) found five species to exactly compensate for lost tissue and one species (sagebrush), with the highest relative growth rate, died following clipping treatments (all plants/species were four years old). Compensatory growth capacity and inherent growth rates were not correlated. Compensatory growth was considered to be a response to high resource availability and activatable axillary buds.
In mountain birch a tree by treatment interaction was found in response to pruning (Senn & Haukioja, 1994). An increase in response was also found to occur by increasing intensities of pruning treatments, indicating the importance of the apical meristems for the regulation of resources. Conversely Honkanen, Haukioja and Suomelas (1994) found different responses to different defoliation treatment in Scots pine. Pruning needles, as opposed to debudding, resulted in either growth stimulation or growth suppression effects while debudding had a positive effect on growth.

6.1.5. Accessory buds in Banksia

Accessory buds have been reported in the cotyledonary nodes of Banksia menziesii, but absent from B. serrata (Mibus & Sedgley, 2000). These authors considered that there was confusion in the literature as to the occurrence of a lignotuber in B. serrata citing George (1981), Bradstock and Myerscough (1988) and Theile (1993). Curiously Bradstock and Myerscough’s (1988) paper concerned aspects of the ontogeny of the lignotuber in B. serrata. In contrast, Thiele (1993) explicitly questioned the occurrence of a lignotuber in B. serrata. George’s (1981) conspicuous lack of affirmation does suggest some confusion as to the occurrence of a lignotuber in the species. For example, when describing B. oblongifolia he clearly stated that it was lignotuberous and that B. ericifolia was nonlignotuberous, but that B. serrata simply resprouts from epicormic shoots. He also does not describe the presence or absence of a lignotuber in B. integrifolia and, as with B. serrata, simply stated that it resprouts from epicormic shoots. Thiele (1993) on the other hand reported B. integrifolia to be nonlignotuberous. While acknowledging Debenham’s (1970) definition of a lignotuber (any conspicuous woody swelling bearing dormant buds) Mibus and Sedgley (2000) explained the prolific basal sprouting observed in B. serrata as possibly due to the trilacunar leaf node.
structure and reduced internodal distances. That is, the trilacunar node of *B. serrata* gives rise to three lateral buds at each node, and with reduced internode distances at the base of the plant, the observed prolific basal sprouting can be explained. These observations were provided as the basis of the conclusion that *B. serrata* may be nonlignotuberous. The conspicuous swelling of the base of *B. serrata* is not explained or accounted for. This interpretation sits well with their suggestion that the occurrence of accessory buds in the cotyledonary axils may be a prerequisite for lignotuber formation in *Banksia* (the correlation initially reported by Chattaway 1958 in eucalypts; see also Chapter 7). For the purposes of prediction and discussion, accessory bud arrangement in eucalypts can be considered as a model for *Banksia* (Table 6.1).

Table 6.1. Summary of predicted arrangement of buds at 1) the cotyledonary nodes and 2) at nodes higher on the stem for four *Banksia* species using the arrangement found in *Eucalyptus* as a model. (*cotyledonary node examined by Mibus & Sedgley 2000 and found not to have accessory buds*).

<table>
<thead>
<tr>
<th>Predictions</th>
<th><em>B. ericifolia</em></th>
<th><em>B. integrifolia</em></th>
<th><em>B. oblongifolia</em></th>
<th><em>B. serrata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. More than one accessory bud at a cotyledonary node</td>
<td>NO</td>
<td>YES/NO (?)</td>
<td>YES</td>
<td>YES* (?)</td>
</tr>
<tr>
<td>2. One accessory bud at each leaf node higher on the stem</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

That is, in those species that develop lignotubers there will be a double set of accessory buds in the cotyledonary nodes (as suggested by Mibus & Sedgley 2000) and in those species that resprout via epicormic shoots there will be a single accessory bud at each
node above the cotyledonary node. Additionally it may be predicted that in those species that are nonlignotuberous and that do not resprout, all nodes will be devoid of accessory buds or meristematic areas. Thus a different set of predictions can be made for each Banksia species (Table 6.1).

The species with stems that are killed by fire are predicted to not have accessory buds (viz. B. ericifolia and B. oblongifolia). The species that may resprout via epicormic shoots are predicted to have at least one accessory bud per leaf axil (viz. B. integrifolia and B. serrata (Table 6.1, prediction 2). Why the arrangement of accessory buds should be different at the cotyledonary node and at nodes higher on the plant is also of interest. To test these predictions and to establish some anatomical basis to the sprouting and nonspouting or seeder response in Banksia I asked;

- do these four Banksia study species have accessory buds at leaf nodes above the cotyledonary node? and
- if these species do not appear to have accessory buds can accessory shoots be induced by the removal (pruning) of apical meristems?
- What advantage, if any, is conferred by having only one accessory bud when two would increase a node’s potential to reprout?
- What advantage is conferred to a basal (below the axillary but above the leaf petiole) position of the accessory bud when greater protection may be conferred in an adaxial position (above the axillary particularly after leaf abscission)? and
- can the trilacunar node of B. serrata account for the prolific basal resprouting in B. serrata as opposed to it having a lignotuber and can it also account for the reports of lignotubers in B. integrifolia?
6.2. Methods

6.2.1. Examination of nodes from field collections for accessory buds

Branches (1-2 years old with leaves still intact) were randomly collected in early February 1998 from a North, Central and Southern Province within the Sydney Region (see Table 6.2, Figure 2.1, Table 2.1). One branch with at least six nodes was randomly collected from each of three plants along two transects at each location. These were stored in plastic bags in the laboratory refrigerator and examined within three weeks of collection. The leaf axils were examined for accessory buds using a dissecting microscope (Olympus SZH X200, variable mag. up to 200X). Longitudinal hand cut sections through at least three nodes, using an independent sample from the same locations and using the same experimental approach were also made. The sections were stained with toludine blue and viewed under a dissecting microscope (Olympus SZH up to X200).

6.2.2. Pruning experiments

A random, independent collection of infructescences was made along two transects at the same time as the above branch sampling took place. For the serotinous species, five plants were sampled along each transect (B. ericifolia & B. oblongifolia) and two infructescences (three for the larger fruited B. serrata) were collected from each plant. Four infructescences were collected from each B. integrifolia as some infructescences had begun to shed seeds. Seeds were extracted from the cones of serotinous species after rupturing the follicles by heating in an oven at 200°C for 15 minutes. Seeds from infructescences and transect were pooled for each Province.
Table 6.2. Location of collection sites for examination of nodes for accessory buds and infructescences for seed to grow plants for the pruning experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>North Province</th>
<th>Central Province</th>
<th>South Province</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. ericifolia</td>
<td>Marramarra NP 2</td>
<td>Heburn Ave</td>
<td>Currarong</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>Spencer 1 &amp; 2</td>
<td>Watamolla Beach</td>
<td>Lake Conjola</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>Marramarra NP 2</td>
<td>Agnes Banks 2</td>
<td>Red Bluff</td>
</tr>
<tr>
<td>B. serrata</td>
<td>Marramarra NP 1</td>
<td>Bulls Camp</td>
<td>Currarong</td>
</tr>
</tbody>
</table>

A random sample of 20 viable seeds (viable criteria see Chapter 4) transect$^{-1}$ Province$^{-1}$ species$^{-1}$ were germinated directly into tubes with a potting mixture of 1:1 crushed quartz and sphagnum peat moss (Yates Fine Grade). Seedlings were raised and repotted on two occasions (at week 32, 150mm then week 88, 250mm pots). Watering of plants was automated except at each potting stage when pots were hand watered until runoff. Plants from transects were pooled and a random sample of four replicates was made. A detailed account of the procedure is given in Table 6.3.

Prior to the commencement of the experiment data were collected on plant height, stem and lignotuber width, total number and order of branches, and average number of nodes for B. ericifolia. These data, in part, provided a description of the experimental plants as well as providing data for a case study (Chapter 7).
When the plants were 102 weeks old the experiment commenced. The three experimental treatments were

1. controls, (no removal of apical meristems)
2. removal of all apical meristems (once only), and
3. removal of all apical meristem and removal of all axillaries that subsequently grew.

The number and location of accessory buds prior to commencement of the first (experimental week 1) and second treatment (experimental week 44) and at the completion of the experiment (experimental week 99) were scored. General observations were also made on vegetative buds and shoots of all plants.

In some pruning experiments some species have died after the first pruning (Wandera et al, 1992) and in *B. oblongifolia* lignotubers did not survive a complete removal of stems on the fourth successive pruning treatment (Zammit, 1988). As no prior information on the survival of these four species to severe pruning was available the plants were allowed to grow for approximately two years. Pilot trials indicated that at that age plants were of sufficient size to respond to the first and second treatments.

For treatments two and three *B. ericifolia* plants were pruned to 60 cm high to reduce the number of nodes (to an average of 400 plant$^{-1}$) that were to be treated and scored. Additionally all branches less than 5 cm long were pruned to below the first node to further reduce the total number of nodes and complex branching system that would otherwise develop.
Table 6.3. Detailed account of (and notes on) the procedure used to raise seedlings of the four study *Banksia* species for the pruning experiment. The week number in the right hand column of the table below indicates the age of the plants, starting with the week when seeds were first imbibed. The number in square brackets [ ] indicates the experimental week from the start of the first pruning treatment.

<table>
<thead>
<tr>
<th>Date</th>
<th>Procedure</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-2-98</td>
<td>A total of 480 seeds were planted (20 seeds x 2 transects x 3 locations x 4 species). Each seed was planted directly into a 50mm square tube. Seeds were pressed into the potting mix so that approximately 50% of the seed body was below the surface. Tubes were placed on trays in a hot house with bottom heat and misting for four days.</td>
<td>1</td>
</tr>
<tr>
<td>23-2-98</td>
<td>Imbibed seeds and seedlings were removed from the hot house and placed in a propagation house with overhead watering.</td>
<td>1</td>
</tr>
<tr>
<td>11-6-98</td>
<td>All plants were moved to open air benches with automated overhead watering.</td>
<td>18</td>
</tr>
<tr>
<td>11-9-98</td>
<td>A random sample of 263 plants were potted up into 150mm pots. Any plants with damaged or broken stems were omitted from the sample.</td>
<td>32</td>
</tr>
<tr>
<td>3-10-99</td>
<td>A random sub-set of 184 plants were potted up into 260mm pots from the 150mm pots. Again plants with damaged or broken stems were omitted from the sample.</td>
<td>88</td>
</tr>
<tr>
<td>16-12-99</td>
<td>Four plants were randomly assigned to three experimental treatments (control, primary apical removed once only, and all apicals/branches removed on two successive occasions) labelled</td>
<td>98</td>
</tr>
</tbody>
</table>
and returned to the open benches using complete randomisation. Micro sprinklers were added to the watering system just above the level of the pots to increase the coverage and concentrate water below the foliage. Plants were given time to settle (2 weeks) before treatments commenced.

5-1-00 To obtain a general description of the experimental plants, plant height (cm) and stem/lignotuber diameter (cm) were determined. Stem diameter for *B. ericifolia*, *B. integrifolia* and *B. serrata* was measured at approximately 1 cm above soil level. Due to the contrasting morphology of the *B. oblongifolia* lignotuber, two diameters were measured. The lignotuber diameter at soil level was measured and the stem diameter immediately above the lignotuber.

12-1-00 Experimental treatments were first applied to plants (denoted as week [I] in right hand column). Each plant was visually inspected and scored for the number of accessory shoots. Prior to application of the first pruning treatment the number of *B. ericifolia* nodes that may have to be scored was reduced by pruning the plants to 60cm high in the first pruning treatment (except controls). In addition branches were reduced to a maximum of 3 and these were pruned to 10 cm long. A preliminary investigation indicated that average internode length in *B. ericifolia* was approximately 0.14cm giving an average of 429 nodes (and possible axillary and accessory shoots) over a 60cm length of stem. Nodes on any branches increased this
number by up to 50% for plants with 3 X 10cm branches (see Figure 6.1).

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-1-00</td>
<td>Using the branch material pruned from the <em>B. ericifolia</em> plants, the average number of nodes cm(^{-1}) was calculated for each Province. [2]</td>
</tr>
<tr>
<td></td>
<td>Five random 10cm long branch sections had their leaves removed and nodes counted from four randomly selected plants Province(^{-1}) (see Chapter 7).</td>
</tr>
<tr>
<td>16-10-00</td>
<td>The second pruning treatment was applied to the third treatment group of plants. All shoots that had arisen since the first pruning treatment were removed and counted. At this time all plants were again scored for the presence of accessory shoots. [41]</td>
</tr>
<tr>
<td>17-10-00</td>
<td></td>
</tr>
<tr>
<td>18-10-00</td>
<td></td>
</tr>
<tr>
<td>8-11-00</td>
<td>Each plant was supplied with an average of 10.22g (± 0.13g, n=10) of Osmocote® for native gardens spread about the top of each pot. This amount had been applied to non-experimental plants previously without producing any visually harmful effect on growth and was far less than the recommended rate. Fertiliser was applied at this stage to help maintain vigorous growth as plants were not going to be repotted. [44]</td>
</tr>
<tr>
<td>19-11-01</td>
<td>All plants were scored for the presence of accessory shoots. [99]</td>
</tr>
</tbody>
</table>
Figure 6.1. In a) *B. ericifolia* before application of first pruning treatment and in b) the same plant after the first pruning treatment. In c) plants randomly assigned to a nursery bench (ruler in c) is 1m long). In d) an example of *B. integrifolia* plant prior to first pruning treatment.
With secondary thickening, proliferation and lignotuber development it is not possible to reliably distinguish from accessory shoots and those that are a result of proliferation. As a result all shoots to a height of 5 cm from the base of the plants were removed in treatments two and three.

6.2.3. Examination of petioles for trilacunar nodes

After completion of the pruning experiment six leaves were removed from three specimens of *B. oblongifolia* and *B. serrata* and petiole viewed under a dissecting microscope (variable mag. up top 200X). The anatomy of the two species was compared.
6.3. Results

6.3.1. Description of the experimental plants

*Banksia integrifolia* was slow to germinate/grow with only 8.3% of seeds appearing to have germinated and/or grown (ie. seeds raised above the soil surface and/or with split testa and/or with fully expanded and/or reflexed cotyledons) after 4 weeks. At the same time the three serotinous species had fully expanded and reflexed cotyledons and some had their first true leaves starting to expand. The germination percentage (using the above criteria) for the serotinous species was 92% for *B. ericifolia*, 86% for *B. oblongifolia* and 91% for *B. serrata*.

A summary of the heights of the experimental plants just prior to the commencement of the pruning experiment (102 weeks old) are presented in Figure 6.2. *Banksia ericifolia* was the tallest species with a pooled (across Province) mean (± s.e.) of 139.5 (7.1) cm, followed by *B. integrifolia* 138.1 (4.9) cm, *B. serrata* 118.5 (2.8) cm and *B. oblongifolia* 93.3 (3.7) cm.

A summary of the diameters of the lignotubers and stems of the experimental plants at 102 weeks of age are presented in Figure 6.3. Pooled across Provinces *B. ericifolia* and *B. integrifolia* had similar sized stem diameters while *Banksia integrifolia* did not develop a conspicuous swelling at the base (ie it was nonlignotuberous). *Banksia serrata* did develop a conspicuous swelling at its base (ie it was lignotuberous). The morphology of the *B. oblongifolia* and *B. serrata* lignotubers was distinctly different (see Figures 6.4, 6.5).
**Figure 6.2.** Mean (±s.e.) height (cm) of experimental plants just prior to commencement of experimental treatments (plant age 102 weeks) \((n = 4)\).

**Figure 6.3.** Mean (±s.e) diameter (cm) of stems at 1 cm above ground level of experimental plants at age 102 weeks pre-treatments. Two columns appear for *B. oblongifolia*, the largest diameter is the diameter of the lignotuber taken at ground level the other, the stem diameter immediately above the lignotuber \((n = 4)\).
Figure 6.4. Bud proliferation in *B. oblongifolia*. The blocked arrows show that bud proliferation occurs well above the cotyledonary node. c) is an enlargement of b) and indicates the extent of proliferation at a single node. Proliferation occur on stem nodes as well (bottom right of the figure). At the base of the plant proliferation is so extensive that the distinction between nodes is lost. Plant age, 145 weeks.
Figure 6.5. *Banksia serrata* lignotuber morphology showing extensive folds in the tissue due to restriction brought about by petiole scars in a rapidly expanding lignotuber. There is no visible bud proliferation as seen in *B. oblongifolia* (compare Figure 6.4). Plant age is 200 weeks.
6.3.2. Examination of nodes from field collections for accessory buds

Examination of nodes (both visual Table 6.4 and hand-cut sections Table 6.5) from branch material collected in the field showed that only *B. serrata* had accessory buds. At all *B. serrata* nodes examined a single accessory bud was present. The accessory bud was always in series lying above the leaf petiole and below the axillary bud (Figure 6.6, 6.7).

6.3.3. Pruning experiment to force the development of accessory shoots

*Banksia serrata* was the only species that developed shoots or branches from accessory buds (Tables 6.5) ie. it confirms the finding from hand sectioning. There was a significant difference between locations (*F*$_8$, 35 = 8.29 *P* < 0.0001) with plants from the Central location producing the greatest number of branches from accessory buds (Tables 6.6 and Figures 6.7, 6.8, 6.9). In the control and treatment one groups, only plants from the Central Province developed branches from their accessory buds. In treatment two, plants of *B. serrata* from all locations developed branches from accessory buds but again more so from the Central Province than the North or South.
Table 6.4. Results of examination of nodes for accessory buds for *B. ericifolia*, *B. integrifolia*, *B. oblongifolia* and *B. serrata*. The results for each tree are for the total number of accessory buds that occurred at 6 nodes.

<table>
<thead>
<tr>
<th>Province of Sydney region Replication</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
<td>Rep 2</td>
<td>Rep 1</td>
</tr>
<tr>
<td>B. ericifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. serrata</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 6.5. Results of examination of hand sectioned nodes for accessory buds for *B. ericifolia*, *B. integrifolia*, *B. oblongifolia* and *B. serrata*. The results for each tree are for the total number of accessory buds that occurred at 3 nodes.

<table>
<thead>
<tr>
<th>Province of Sydney region Replication</th>
<th>North</th>
<th>Central</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rep 1</td>
<td>Rep 2</td>
<td>Rep 1</td>
</tr>
<tr>
<td>B. ericifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. integrifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. oblongifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. serrata</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 6.6. The single accessory bud of *B. serrata* (in series) showing petiole scar and the bases of the petioles of the axillary bud. Note the axillary bud has developed and the petioles are visible. Carey (1930) noted that in *B. serrata* occasionally the two outer leaves developed while the rest of the bud remained dormant. The dense indumentum can be seen to cover all surfaces.

The accessory shoot in *B. serrata* was in all respects similar to axillary shoots except for initial growth. While axillary branches are sometimes plagiotropic (growth is toward the horizontal) but often reorientate to become orthotropic (vertical) accessory shoots often displayed marked tropism orienting downwards away from the axillary shoot. Accessory shoots also develop terminal inflorescences but usually at a greater distance along the shoot (see Figure 6.10).
Table 6.6. Summary results of pruning experiment. Data are for the total number of accessory shoots on four replicate plants Province\(^1\) treatment\(^1\). (C, T1 and T2 are control, treatment 1 and treatment 2 respectively).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>North Province</th>
<th>Central Province</th>
<th>South Province</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td><em>B. ericifolia</em></td>
<td>0</td>
<td>0</td>
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<tr>
<td><em>B. integrifolia</em></td>
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<tr>
<td><em>B. oblongifolia</em></td>
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<tr>
<td><em>B. serrata</em></td>
<td>0</td>
<td>0</td>
<td>21</td>
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</tbody>
</table>

6.3.4. Additional observations

The response to the second pruning in treatment two was different for each species. After pruning of axillary branches in *B. ericifolia* the stumps continued to swell/enlarge (Figure 6.10). Towards the completion of the experiment the main stems started to die back from the top (where the first pruning treatment occurred) in several specimens and regrowth tended to be strongest from shoots that arose lower on the main stem. In *B. oblongifolia* pruning always tended to increase sprouting from the lignotuber or lower parts of the main stem. Axillary stumps did not appear to continue to grow in *B. oblongifolia* as they did in *B. ericifolia*. *Banksia integrifolia* was more similar to *B. ericifolia* as axillary stumps tended to continue to expand. This was particularly noticeable as it increased the pronounced swelling that otherwise sometimes appeared at a pseudowhorl (Figure 6.12).
Figure 6.7. Mean cumulative number ($n = 4; \pm s.e.$) of accessory shoots scored for $B. serrata$. Experimental week 1 was prior to application of the first pruning treatment, experimental week 41 was at the time of application of the second pruning treatment, and experimental week 99 was at completion of the experiment. Data are arranged as control (C), treatment 1 (T1) and treatment 2 (T2) for North, Central and Southern Province.
Figure 6.8. Accessory shoot from a node on *B. serrata* after the axillary had been removed. c) is an enlargement of b).
Figure 6.9. In a) above an accessory shoot has arisen from a node below an axillary branch on the main stem of *B. serrata*. The plant in a) was a control from the Central Province. In b) the large arrow at the top of the figure indicates the first pruning treatment scar in *B. serrata* (this plant was from the Northern Province). Arrows indicate in b) where two axillary shoots have been removed (second pruning treatment in treatment two), and at both nodes accessory shoots have arisen.
Figure 6.10. In a) *B. ericifolia* after second pruning treatment with swollen axillary stumps and scars. In b) *B. oblongifolia* with axillary stumps and no resprouting at nodes. In c) a terminal inflorescence on an accessory shoot that resprouted after the axillary was removed in *B. serrata*.
The pattern of occurrence of axillary buds or nodes in *B. integrifolia* varied across plants. Sometimes axillary buds (second order)\(^\text{11}\) were very low on the first order axillary branches of the main stem (Figure 6.11). In other plants (regardless of treatment) first order axillary branches were completely devoid of second order axillary buds up to 8 cm along the stem (Figure 6.12). Often second order and higher order branches in *B. integrifolia* developed cataphylls (scale leaves) with reduced internode distances very low on the stem (Figure 6.13). Cataphylls were more common in *B. integrifolia* and *B. oblongifolia* than in *B. serrata* and in comparison rare, in *B. ericifolia*. When cataphylls were present plants were dimorphic displaying to various degrees, a heteroblastic series from cataphylls to leaf.

The anatomy of the leaf nodes of *B. oblongifolia* and *B. serrata* were similar and trilacunar as described by Howard (1979). The anatomy of the nodes of *B integrifolia* and *B. ericifolia* were not examined.

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\(^{11}\) The convention used here for branch order is the primary shoot or main stem and branches arising from axillary buds from the main stem are first order branches. Branches that arise from axillary buds on the first order branches are second order and so on.
Figure 6.11. In a) above axillary buds of *B. integrifolia* are present very low on the base of an axillary shoot. In b) (*B. integrifolia*) the first and second pruning treatments can be seen. In b) second order axillaries have been pruned as well but accessory buds did not shoot.
Figure 6.12. The blocked arrows in a) show four of the typical five branches that arise at each node (pseudowhorl) in *B. integrifolia*. c) is an enlargement of b). In this figure there are no buds present on the lower part of the axillary shoots. This is in stark contrast to the specimen of Figure 6.11. In c) petiole scars and lenticels can be seen but axillary buds are otherwise lacking.
Figure 6.13. Cataphylls on three young third order axillary shoots of *B. integrifolia*. The blocked arrows indicate only three cataphylls but there are several on each shoot some of which occur very near the junction of the branch. Cataphylls were common on *B. integrifolia* and *B. oblongifolia* compared to *B. serrata* and even less common on *B. ericifolia*. 
6.4. Discussion

6.4.1. Examination of nodes and the pruning experiment

Examination of nodes from field collections showed that only *B. serrata* has accessory buds. The single accessory bud is in series and positioned between the leaf petiole and the axillary bud (Figure 6.6), an arrangement similar to that reported in eucalypts by Chattaway (1958). The pruning experiment indicated that *B. ericifolia*, *B. integrifolia* and *B. oblongifolia* do not have meristematic areas present in the axils of their leaves as has been found in some eucalypts (Jacobs, 1955) and gymnosperms (Fink, 1983; Burrows 1986, 1987). With the exception of the lack of accessory buds in *B. integrifolia* these results support the set of predictions based on the response of these species to fire (prediction 2 Table 6.1).

It was predicted that *B. integrifolia* would have accessory buds because it is reported to resprout via epicormic shoots after fire (George, 1981; Weiss, 1984). Resprouting in *B. integrifolia* must be due to dormant axillary buds that may also undergo proliferation and/or adventitious buds. Epicormic means ‘upon the trunk’ and the term does not reflect an anatomical basis, although in eucalypts epicormic appears to be associated with accessory buds (Jacobs, 1955). Bud (meristems) and vascular system survival are both necessary for a plant’s persistence. Thick bark and/or a subterranean location can protect both buds and vascular systems from the heat of a fire. *Banksia integrifolia* has both (thick bark and root suckers) and with less frequent fires (due to habitat), has less reliance on a bud bank for survival compared to those resprouting species of the more fire prone habitats. *Banksia serrata*, for example, does not root sucker and because of
its habitat is required to resprout more frequently if it is to persist. Weiss (1984) found that only 28% of *B. integrifolia* adults resprouted after fire (October wild fire and December control fire). No other information or data were provided by Wiess. The 82% that did not resprout may have lacked a sufficiently thick bark and/or may have had a depleted bud bank. A high fire frequency could deplete the dormant bud bank in *B. integrifolia*. The lower (adjacent to the main stem) second order branches were often devoid of buds in *B. integrifolia* (Figure 6.12).

Clearly the first line of defense for a plant’s above ground stems in a hot fire is a thick bark, for if the xylem is destroyed, all buds above such a point are doomed. The prediction for *B. ericifolia* was based on the assumption or premise that accessory buds would be obsolete if the bark of the species is insufficient to withstand a hot fire. The costs associated with accessory bud production can be considered to outweigh the benefits for a ‘thin’ barked species living in a fire prone environment. This argument also follows for the thin bark stems of *B. oblongifolia*.

Of the three serotinous species, *B. serrata* is the only tree species and recorded resprouter via epicormic buds as opposed to resprouting from the lignotuber. The results for *B. serrata* were predicted from the eucalypt model (Table 6.1 prediction 2). The highest temperatures in a bush fire are close to the ground (Bradstock *et al* 1994). In *B. serrata* a thick bark protects the xylem for a dormant bud bank, much of which is held well above the ground in mature plants away from the hottest parts of a fire. In keeping with the economic analogy, the costs of accessory buds to a ‘thick’ barked species living in a fire prone environment can be considered as value adding. Its value
is soon eroded in the absence of an extensive bud bank. In the absence of proliferation a single accessory bud node\(^{-1}\) increases the bud bank of a species by a factor of two.

*Banksia serrata* (≤ 3 cm) has thicker bark than *B. integrifolia* (≤ 2 cm) (George, 1981; see Table 1.2). George (1981) considered a fire tolerant stem was achieved by bark greater than 5 mm, but there are degrees of sensitivity. Hare (1965) found plant cells could not survive temperatures above 60°C and that the duration of a heat pulse that is required to kill the vascular cambium is proportional to the square of the bark thickness. Only small differences in bark thickness result in large differences in fire resistance (Bond & van Wilgen, 1996).

While the examination of field collections showed all nodes on *B. serrata* to have a single accessory bud the pruning experiment indicated significant variation in behaviour of accessory buds between locations and treatments. In both control and treatment one only plants from the Central Province of the Sydney Region produced branches/shoots from accessory buds (Figure 6.8). The genotype of the Central Province may inherently tend to develop branches/shoots from accessory buds, and/or they may be fast to respond to treatments. Given more time the plants from the North and Southern Province may have developed shoots/branches from accessory buds. The collection site (Bulls Camp) for the Central Province and Marramarra National Park (Northern Province) were more similar than the collection site of the Southern Province (Currarong) (Table 6.2). The North and Central Province collection sites were xeric, with a rocky substrate and of a higher altitude than the Southern Province (more mesic, deep sands and adjacent to the coast). Habitat differences therefore cannot account for the result. The nursery grown plants were watered regularly and showed very vigorous
growth compared to plants growing in xeric habitats (pers. obs.). Perhaps the difference in nursery conditions compared to Provinces were greatest for the Central Province and the plants responded differently for that reason.

Often plants at the extremes of their range show considerable variation (Maynard Smith, Burian, Kauffman, Alberch, Campbell, Goodwin, Lande, Raup, and Wolpert, 1985). The North and Southern Provinces were not limits to the range of *B. serrata* (only limits in this study) and therefore the argument that variation occurs in the extreme of species ranges cannot be invoked to account for the result.

### 6.4.2. The position of accessory buds

McLean and Ivimey-Cook, (1962) considered that the protection conferred by the axillary position for buds was one reason for the universal adoption of the arrangement in flowering plants. It follows that this protected position (axillary) applies to accessory buds as well. Bell *et al* (1999) considered a protection role for the subtending leaf of accessory buds. Graham *et al* (1998) considered the accessory buds of eucalypts were even more protected than the ‘naked’ axillary bud. The position of these buds (in nonlignotuberous tissue) so far reported for woody species (eucalypts Chattaway, 1958; this study for *B. serrata*) have indicated that the accessory buds are located beneath the axillary bud and above the leaf\(^{12}\). Bell *et al* (1999) and Bell and Bell (1998) did not report the relative position of accessory buds to the axillary in those tropical species

\(^{12}\) It should be noted all buds in an axil are axillary and if more than one bud is present they are all also accessory (A. Bell pers. com.). The convention I apply here is that the axillary is the large bud in an axil while the smaller is the accessory (as used by Chattaway, 1958 and suggested by Bell, 1999).
they surveyed. Initially this nested position seems ‘protected’ but as leaves are readily abscised, the accessory bud would be more exposed than if it were located above the axillary. In woody plant species the accessory buds may ultimately become buried within the tissues of the tree. When released from apical dominance they grow out through the phellogen. If accessory buds were located above the axillary and if the axillary had developed into a branch, the accessory bud may have to travel further keeping up with radial expansion of the trunk (and the branch) before breaking through the phellogen. It appears the basal position of accessory bud confers an advantage when axillaries develop into branches as their passage is ultimately less likely to be impeded. Accessory buds travelling laterally with an increasing trunk girth have an easier passage than those that would have to travel in a tangential path across the tissues of a branch (see Figure 6.14).

In Figure 6.14 the length of the trace from its origin (below the axillary) is only approximately half the distance of that in b) where the accessory bud was located above the axillary bud. In this hypothetical example the accessory buds are placed equidistance (d in Figure 6.14) above and below the axillary bud. If either passage were possible, advantage would be conferred to an accessory bud/trace that has less distance to travel.
distance travel by trace or accessory bud if accessory bud is located below axillary bud

distance travel by trace or accessory bud if accessory bud was located above axillary bud

**Figure 6.14.** Hypothetical longitudinal sections through identical branches (a and b) illustrating the difference in distance that an accessory bud or trace would have to travel in order to produce epicormic shoots. In a) and b) the accessory bud is indicated by a blocked oval and arrow, with the trace indicated by the horizontal heavy dotted line leading to the shoot. The position of the original axillary bud that developed into the branch is indicated by the blocked rectangle at the intersection of the primary axis and branch axis (dotted lines).

While Figure 6.14 illustrates the difference in distance that an accessory bud would have to travel it does not illustrate the more significant anatomical restrictions bought
about by the development of an axillary into a branch (as in Figure 6.15 b). The development of an axillary into a branch would restrict the subsequent passage and development of an accessory bud or trace if it were located above the axillary position. It is difficult to imagine a scenario where an accessory bud could transverse the various tissues (bark and vascular) to form an epicormic shoot on the axillary branch. On this basis I predict that in all woody plants that have accessory buds the accessory will be adaxial to the axillary and abaxial to the leaf petiole. This prediction (and the logic) does not apply to those nodes that are incorporated into a lignotuber because of the different morphology, ontogeny and function of that organ. If there is not a clear distinction between two buds in an axil (eg by size) then the prediction also holds that the bud in the upper location of the axil will always develop into a branch before the lower ‘accessory’ bud.

Bell (1991) considered all young plants of a species conform to a blueprint of branch architecture, the genetic ground plan of Hallé and Oldeman (1970), who proposed 23 models (each named after botanists). More recently Hill (1997) described the architecture of the Wollemi pine to be unique to the 23 architectural models and interpreted the branching and coppicing habits as adaptive reiterations. However Tomlinson (1983) pointed out that due to a continuum of architectural possibilities some species may be intermediate to some models. The classical architectural models do not describe the branching as found to occur in the Central Province specimens of *B. serrata*. Some individuals (in the control group) of *B. serrata* naturally developed both axillary and accessory shoots at a single node.
The trilacunar nature of *B. serrata* was considered by Mibus and Sedgley (2000) to account, in part, for the prolific basal resprouting as opposed to *B. serrata* having a lignotuber. They hypothesised that the trilacunar node in *B. serrata* gave rise to three lateral buds node$^{-1}$. There was no resprouting other than from a single axillary bud in the pruning experiment of *B. oblongifolia* and the nodal anatomy was similar to *B. serrata* which only produced a single axillary and accessory shoot/branch node$^{-1}$. The trilacunar nodes described by Howard (1979) for *B. serrata* refer to the gaps that appear when three traces leave the central vascular cylinder and enter the petiole. Unilacunar, trilacunar and multilacunar nodal types are the most common. Sinnott (1914) reported only one nodal type for each plant family and Howard (1979) found no significant variation even when leaf ecotypes (sun/shade leaves etc) were surveyed within species. The trilacunar nature is characteristic of *B. oblongifolia* and *B. serrata* (and *Banksia* and Proteaceae if Sinnott’s 1914 contention is correct) and therefore does not provide specific evidence to suggest that the prolific basal resprouting in *B. serrata* is something other than lignotuberous.

Opportunistic observations in the field and observations on nursery raised stock over four years, corroborate that *B. integrifolia* is nonlignotuberous and *B. serrata* is lignotuberous.

Some authors (eg Lacey & Johnson, 1990; Graham, Wallwork & Sedgley, 1990; Mibus & Sedgley 2000) have argued that two distinct organs, lignotubers and burls may be distinguishable. The distinguishing criteria (between burls and lignotubers) is based on an anatomical correlation that I believe was originally misinterpreted as a cause (see below).
In studies of the structure of the lignotuber in the cotyledonary and lower leaf nodes of *Eucalyptus* Chattaway (1958) found lignotubers to develop only in those species that have concealed (accessory) meristems/buds present above and below the axillary buds of the cotyledonary and lower nodes. Chattaway (1958) also found that a small number of nonlignotuberous species also had the double set of accessory buds at the cotyledonary node. Chattaway (1958) concluded that some other factor must inhibit lignotuber development when the double set of accessory buds is present. This conclusion is interesting as it implies lignotuber formation is reliant on the double accessory bud arrangement. The correlation (all lignotuberous species have a double set of accessory buds) appears to have been interpreted as cause. Carr, Carr and Jahnke (1982) reported *Eucalyptus lehmannii* a lignotuberous species, to lack the upper and lower accessory meristems reported by Chattaway (1958) to be necessary for lignotuber formation. More recently Graham, Wallwork and Sedgley (1998) argued that in *Eucalyptus cinerea* at least, the double set of accessory buds was necessary for lignotuber initiation.

The arrangement of the accessory meristems/buds (at the cotyledonary and lower leaf nodes) in lignotuber initiation and occurrence has taken on, as it appears important consequences in defining which species may develop lignotubers. Mibus and Sedgley (2000) have implied that lignotuberous species may be identified by the presence or absence of a double set of accessory buds at the cotyledonary nodes. Lacey and Johnson (1990) defined the lignotuber as 'a woody organ arising from accessory meristems in the axils of cotyledons or leaves near the base of a stem and having a proliferation of dormant buds’. They defined a basal burl as, 'a woody organ formed by
the uniform expansion of the base of a stem due to increased cambial activity and having a proliferation of dormant buds’.

It is as if Lacey and Johnson (1990) have invoked a type of taxonomic ‘rule of priority’ and define lignotubers on the basis of a correlation (yet to be established relationship) of accessory bud/meristems arrangement in *Eucalyptus*. If the rule of priority is to be invoked then perhaps Kerr’s (1925) initial simple definition ‘a woody tuber’, should be upheld. Most definitions of lignotubers do not incorporate a prerequisite anatomical arrangement (ie upper/lower accessory meristems or buds) (eg Debenham 1970; Beadle, Evans, Carolin & Tindale, 1986; Recher, Lunney & Dunn, 1992; Augee & Fox, 2000 as well as those already mentioned in Chapter1). Furthermore, Carter and Paton 1985 found that lignotubers did not form from *Eucalyptus* cuttings at normal potential sites (nodes 1-3 above the cotyledonary node) if the cuttings were prepared without the cotyledonary node. It was interpreted that an inductive stimulus for lignotuber formation originates at the cotyledonary node. Molinas and Verdaguer (1993) also considered that the cotyledonary node participated in lignotuber formation in the cork oak. In an attempt to establish if the same phenomena occurred in *Banksia* I took cuttings and made air layerings of three banksias. Cuttings (without hormone) and air layering (wounding & wrapping with sphagnum & black plastic) of *B. integrifolia* (cuttings only) *B. oblongifolia* and *B. serrata* (both treatments) were made from stem sections well away from the cotyledonary node. All cuttings (*n = approximately 200*) while setting some roots failed to survive. All air layering (*n = 30*) attempts in the field also failed to set roots.
On the basis of the above discussion and Section 1.4 I define a lignotuber as any conspicuous woody swelling at the base (root/hypocotyl/stem interface) of a plant from which new roots and/or shoots (or modified roots or shoots) may emerge. Lignotubers may be transient, completely disappearing (merging imperceptibly with the stem, nonconspicuous), vestigial or subterranean. Vestigial in that some visual evidence remains of its position and biologically, in that it is no longer likely to have utility. Alternatively lignotubers may be intuitively (unless exposed) conspicuous throughout the life of a plant because of a multistemmed habit. Lignotubers like other organs have an ontogeny with biological age states and varying functional capacity.
Chapter 7. The lignotuberous seeder – transient lignotuber formation in the life cycle of *Banksia ericifolia* L.f.

7.1. Introduction

The phrase ‘the lignotuberous seeder’ appears contradictory because seeders are fire sensitive (killed) and lignotuberous species are not, or are generally considered fire tolerant (resprouters or sprouters, see Chapter 1).

Kerr (1925) first published the term lignotuber in a study of eucalypt seedlings. The gall-like\textsuperscript{13} swellings on the seedling stems were classed as tubers because of their morphology and physiology but they became woody (ligno) when adult. She considered lignotubers to be ‘merely embryonic storage organs developed in order to enable the seedlings to survive temporary unfavourable conditions’. The unfavourable conditions included drought and herbivory, but clearly not fire as a thick protective bark has not developed in a seedling. The current interpretation of the term lignotuber is primarily based on the function of the mature structure in fire prone environments (a resprouting organ, see Section 1.4). In nearly all descriptions of banksias (George 1981, 1999) some reference to their status as being lignotuberous or not, and their response to fire is given, often in reference to their lignotuberous status (see Table 1.2). For example

\textsuperscript{13} Fletcher and Musson (1918) considered the conspicuous swellings of eucalypt seedlings (lignotubers) to be shoot bearing galls or tumors of pathogenic origin, but otherwise correctly noted, that they only occurred in certain species.
Shrubs and/or small tree without lignotuber, killed by fire and regenerates from seed.

Shrub with lignotuber, fire tolerant and sprouting from the lignotuber.

Theile (1993) also reported that ‘approximately half the species of *Banksia* have lignotubers, from which the plants resprout after fire’. In general, across taxa, lignotuberous species are considered to be fire resistant (e.g., Recher, Lunney and Dunn, 1992; White 1997) as it is the only described condition thus far.

At times, the division between fire sensitivity and fire tolerance may be quite arbitrary and depends on the frequency and intensity of fires and the life stage of the plant (Gill, 1981; Bradstock & Myerscough, 1988). For example, some lignotubers of the eucalypt alpine species ‘rest’ in the lower layers of the matted snowgrass (Jacobs, 1955). In normal years alpine fires run over the upper part of the ground vegetation that is moist near the soil surface. In dry years, very hot fires have killed off lignotuberous species (Jacobs, 1955). Bradstock and Myerscough (1988) studied lignotuber (diameter) and age (size class, d.b.h.=1.5m) at which young juveniles of *B. serrata* first become fire resistant. Regardless of fire intensity small stems of *B. serrata* (<1.0 cm d.b.h.) died, whereas stems >2.0 cm d.b.h. were tolerant of low intensity fires. The smallest lignotuber size tolerant of high intensity fires was found to be 5 cm, although this was assumed to be the minimum size capable of tolerating high intensity fires (the sample range being 5-60 cm). In an experimental treatment the smallest lignotuber diameter found to be tolerant to low intensity fire was 4 cm.
There is a lignotuber ontogeny, although developmental stages have not been clearly identified for any species. There are juvenile lignotuberous structures (e.g., distinct gall-like swellings at nodes of *Eucalyptus* seedlings as first described by Kerr (1925) and fire sensitive) as opposed to mature lignotubers (fused and enlarged as occur in the adult eucalypt and these are fire tolerant). There is also considerable variation in the juvenile and adults forms (Kerr, 1925; Jacobs 1955; James, 1984). *Eucalyptus pilularis* for example, does not form the characteristic gall-like lignotubers of other eucalypt species. The base of the stem and part of the hypocotyl uniformly enlarges but it may also ultimately develop into a spreading or plate-like lignotuber (Lacey & Johnson, 1990).

Some species are lignotuberous in the earlier stages of their life cycle but the lignotuber eventually is masked by the development of a main stem or trunk (e.g., *B. serrata*, Bradstock, 1988; pers. obs.; tree forms of *E. gummifera* Mullette, 1978). That is, a swelling (a tuber) is no longer apparent. This I refer to as transient lignotuber formation. In jarrah (*Eucalyptus marginata*) the lignotuber phase may last for 60 years before forming a single stemmed tree (Dell, Jones & Wallace, 1985). Other variations also occur. *Eucalyptus gummifera*, (a lignotuberous species,) for example, is dimorphic, being either a tree without a recognisable lignotuber (transient) or a multistemmed mallee¹⁴ (Mullette, 1978). When the stem and bark were sufficiently developed to protect epicormic buds Mullette (1978) considered that the lignotuber stops developing in the tree habit. Conversely Lacey (1983) found tree forms of both *Eucalyptus*

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¹⁴ Mallee is the European derivative of the Aboriginal term ‘mali’ for those eucalypts whose roots held obtainable quantities of free water. The term mallee applies to plants with multi-stemmed habit and woodlands dominated by such plants. Some eucalypts may develop as ‘bull’ malles with 1-3 stems to 10m in height or as ‘whipstick’ malles with multiple stems 1-3m high (Noble 1982).
botryoides and E. gummifera to maintain lignotubers (or at least the potential to form lignotubers) as mallees could arise from the tree forms in these species if the main stem was destroyed. Other species are lignotuberous throughout their life (eg B. oblongifolia, Zammit, 1986).

In all these lignotuberous species fire tolerance is achieved either through developing a stem with thick bark and/or via the continued development of a lignotuber. Thus lignotuberous species become fire tolerant regardless of their final habit, a tree or multistemmed plant such as the mallee. Here then is another aspect that again highlights the apparent contradiction in the phase ‘the lignotuberous seeder’. Lignotuberous species (so far reported) even though displaying fire sensitivity at an early stage in their life cycle ultimately become fire tolerant adults regardless of their final habit. A unique variation to this axiom is presented here.

7.1.1. Banksia ericifolia

Banksia ericifolia is a non-lignotuberous, serotinous shrub or small tree that is killed by fire and regenerates from seed (George, 1981). Two geographically disjunct subspecies are recognised: subsp. macrantha occurs along the north coast and subsp. ericifolia, which inhabits the central and southern coast of N.S.W. Banksia ericifolia subsp. macrantha differs from subsp. ericifolia in having more crowded, narrower leaves, a longer perianth and pistil and the seedling leaves have fewer teeth along the margin (George, 1999; Table 7.1).

In Banksia the first 8-10 seedling leaves are usually different to the adult leaves. The first two seedling leaves are opposite (and the most distinctive) with a transition to adult
forms and a scattered, spiral or alternate phyllotaxy. In *B. ericifolia* the transition from an opposite phyllotaxy to spiral and crowded is delayed for several nodes (George, 1981; Thiele, 1993). George (1981) cautioned that his description of seedlings was based on a small sample and that more work was necessary to establish the full extent of the variation.

**Table 7.1.** The distinguishing characteristics of the subspecies of *Banksia ericifolia*: subsp. *ericifolia* and subsp. *macrantha* (George, 1999).

<table>
<thead>
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<th>subsp. <em>ericifolia</em></th>
<th>subsp. <em>macrantha</em></th>
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<tr>
<td>perianth length</td>
<td>19-22 mm</td>
<td>26-28 mm</td>
</tr>
<tr>
<td>pistil length</td>
<td>30 – 35 mm</td>
<td>46 –48 mm</td>
</tr>
<tr>
<td>leaf density</td>
<td>less crowded</td>
<td>more crowded</td>
</tr>
<tr>
<td>teeth per seedling leaf</td>
<td>2-6 margin$^{-1}$</td>
<td>1 rarely 2 margin$^{-1}$</td>
</tr>
</tbody>
</table>

*Banksia ericifolia* subsp. *ericifolia* was reported to be resprouting at Beecroft Peninsula (D. Keith per. com. 1997, Figure 7.1). These plants were near the southern most limits of the species range. Reports of *B. ericifolia* resprouting from lignotubers following fire are recorded in The Banksia Atlas (Taylor & Hopper, 1991), although no location or description of the plants was given.

George (1981) considered that significant differences in the morphology of flowers and/or fruits were necessary for species distinction. *Banksia cunninghamii* Sieber ex Reichb and *Banksia collina* R. Br. were reduced to varieties of *B. spinulosa* by George in 1981 for this reason. Four varieties of *Banksia spinulosa* are currently formally recognised (George, 1988). Varietal distinction is given to *Banksia spinulosa* var.
cunninghamii because it is non-lignotuberous, the other three varieties are lignotuberous. Banksia ericifolia and B. spinulosa are closely related species belonging to the series Spicigerae and several presumed hybrids have been reported (Table 7.2). Interestingly George (1981) noted that no intermediates between B. spinulosa var. cunninghamii and var. spinulosa (nonlignotuberous X lignotuberous) have been reported, even though they occur as mixed populations. Hybrids between B. ericifolia subsp. ericifolia and B. spinulosa var. spinulosa have been reported (lignotuberous X nonlignotuberous). The possible link between hybridisation and the observed variation (resprouting B. ericifolia) will be discussed.


<table>
<thead>
<tr>
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<th>X</th>
<th>Banksia sp. var.</th>
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</thead>
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<td>2</td>
</tr>
<tr>
<td>lignotuberous</td>
<td>lignotuberous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. spinulosa var. spinulosa</td>
<td>X</td>
<td>B. ericifolia subsp. ericifolia</td>
<td>2 and 3</td>
</tr>
<tr>
<td>lignotuberous</td>
<td>nonlignotuberous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. spinulosa var. cunninghamii</td>
<td>X</td>
<td>B. ericifolia subsp. ericifolia</td>
<td>1 and 2</td>
</tr>
<tr>
<td>nonlignotuberous</td>
<td>nonlignotuberous</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Most reports of presumed natural Banksia hybrids are of isolated individual plants with very little information given other than noting some type of morphological intermediacy (Renshaw, 1992).
Thiele (1993) noted that George’s (1981) species concept and treatment of *Banksia* was classical and topological with biological, genetic and ecological considerations not taken into account. He cited as an example, the reduction of *B. collina* and *B. cunninghamii* to varieties while at the same time noting that hybridisation between *B. spinulosa* var *spinulosa* and var *cunninghamii* has not been reported, even in mixed populations. *Banksia spinulosa*, var. *neoanglica* and var. *cunninghamii* are distinguished by the presence or absence of a lignotuber, otherwise flowers and fruits are the same (George, 1999).

The major life history traits in *Banksia* and other taxa such as serotiny are generally considered discrete. For example, well-circumscribed taxa are either serotinous or nonserotinous although variation within the categories is recognised (see Section 1.3). *Banksia ericifolia* was chosen as it represented the classical seeder. This life history distinguished it from the other three study species. As a seeder it is killed by fire and regeneration is from seed. Other distinct traits are expected to be associated with the seeder life history, particular traits associated with reproduction (Cowling & Lamont, 1998; Bell, 2001). A lignotuber is not an organ that would be expected to be associated with the seeder life history, however there have been anecdotal reports of sprouting by *B. ericifolia*, notably in the southern part of its range. The questions asked in this chapter were;

- does resprouting occur in *B. ericifolia* in the southern limits of its range?

And if reproduting did occur

- were the *B. ericifolia* plants lignotuberous, or did the resprouting result from dormant buds on the trunk and thus represent epicormic shoots or were both processes involved?
Are the resprouting plants in the southern limit of the species range, morphologically different to that described for the subspecies or species? Particularly, was leaf or node density, and thus axillary bud, or meristem density different as this is directly related to resprouting potential? and

- can hybridisation account for any variation in the southern limit of *B. ericifolia* range?
7.2. Methods

7.2.1. A survey of Beecroft Peninsula and the surrounding local area

Beecroft Peninsula and the adjacent Jervis Bay local area were surveyed for evidence of resprouting by *B. ericifolia*. The approximate local area that was surveyed on several occasions (1997, 1999, 2002) is indicated by the oval in Figure 7.1.

![Figure 7.1](image-url)  
**Figure 7.1.** General location of reported resprouting *B. ericifolia*, and an approximate indication of the ‘local area’ that was surveyed for evidence of resprouting.

7.2.2. The diameters of stems

The diameter of the stems of *B. ericifolia* from three Provinces (North, Central and South = Currarong) of the Sydney Region were compared. The comparisons were
based on a sample of seeds collected and seedlings grown under identical conditions. The plants from which these data were obtained are those described in Chapter 6. A detailed account of the procedure used to raise the plants is given in Table 6.2. Diameters of the stem at 1 cm above the soil level were made using vernier callipers when the plants were 102 and 200 weeks old. The data were analysed by a one factor ANOVA.

7.2.3. The density of nodes

Five random branch sections were selected from each of six plants grown under identical conditions (the same cohort of plants mentioned above Section 7.2.2 and described Table 6.2) were scored from each of three Provinces (North, Central and South) of the Sydney region. To estimate the density of nodes, branch material was cut into 10cm lengths, the leaves were removed and petiole scars mark and scored. The data were analysed by one factor ANOVA.

7.2.4. Capacity to resprout

The potential capacity to resprout was determined by calculating the potential average number of nodes or meristems that could resprout after removal of all apical meristems and any branches (see Chapter 6). The number of meristems that did resprout were scored 98 weeks after the first treatment (removal of all apical meristems) and converted to a percentage. The data were analysed by one factor ANOVA.
7.2.5. **Floral morphology**

To investigate possible differences in floral morphology of the Southern Province a random collection of flowers was made along two transects at Curraong and Jervis Bay (Southern Province) (Figure 2.1 and Table 2.1). Pistils and perianths were independent (sampled from different inflorescences) and measured to the nearest mm. The flowers collected were desiccated (old flowers are held on the central woody axis of the inflorescence for months after flowering) and were subsequently rehydrated in warm water and detergent mixture overnight before being measured. These data were compared to that described for the species by George (1981).

7.2.6. **The number of teeth seedling leaf\(^{-1}\) margin**

Two infructescences were randomly collected from five plants at six locations over the range of *B. ericifolia* subsp. *ericifolia* (Ku-ring-gai Chase National Park 1, Marramarra National Park 1, Linden 2, Mt. Kiera 2, Jervis Bay and Beecroft Penninsula see Table 2.1 and Table 2.1). The last two sites were in the southern most part of the subspecies range, adjacent to where resprouting was reported to have occurred. The other sites in the Sydney Region were sampled as the extent of seedling leaf variation had been questioned by George (1981).

Seed was extracted by heating as described in Chapter 6 and a random sample of 30 seeds site\(^{-1}\) were planted into tubes using the same procedure as outlined in Chapter 6. At 11 weeks the plants were scored for leaf arrangement and the number of teeth margin\(^{-1}\) for the first six leaves. As the data were heteroscedastic and square root transformation did not result in homoscedasticity, a non-parametric test for samples of unequal size was performed (Sokal & Rohlf, 1995).
7.3. Results

7.3.1. A survey of Beecroft Peninsula and the local area

The survey of the Beecroft Peninsula revealed that *B. ericifolia* had resprouted after slashing. The slashed and resprouting plants were found adjacent to low windswept (<1m) heathland vegetation (Figures 7.2). Subsequent enquires revealed that the navy had performed a one-off slash of the road-side vegetation in 1996 (C. Locke per. com). On the opposite side of the road where slashing had not occurred, similar species (*Banksia*, *Hakea* and *Casuarina*) formed closed-heathland and closed-scrub (to 2m). The road ran along an ecotone. The slashing resulted in cut, frayed and torn main stems and branches only a few centimetres above ground level (Figure 7.3). While the resprouting was prolific and occurred at the base of the plants, a lignotuber (or swollen region) could not be clearly distinguished from the stem (which had been severed) or root (Figure 7.3). Additionally, lateral branches that had escaped complete removal had also resprouted prolifically.

On low (0.5m) heathland formed on swampy or seasonally waterlogged ground, approximately 1 km from Beecroft Peninsula, *B. ericifolia* plants were found to have developed a lignotuber (Figure 7.4, 7.5). A single stem rose from the enlarged or swollen base and the base otherwise appeared devoid of dormant bud banks. The often partly decayed lignotubers (as shown in Figure 7.4, 7.5) appeared to be supported slightly above the ground by a decaying older primary stem/root and the new roots that had arisen from one side of the lignotuber.
Figure 7.2. a) Slashed roadside plants from open heathland vegetation on Beecroft Peninsula. b) Slashed *B. ericifolia* plant resprouting from the base and branches (large arrows indicate some slashed branches).
Figure 7.3. *Banksia ericifolia* root and stem section from Beecroft Peninsula showing prolific resprouting (red arrows) from bud proliferation after being slashed (yellow arrow indicating scar).
Figure 7.4. *Banksia ericifolia* a) showing whole plant with lignotuber and one infructescence; b) enlargement of longitudinal section of the base in a) showing the remains of the primary root, shoot and the regrowth of new roots from the lignotuber. The sections have been opened flat like a book.
Figure 7.5. Same plant base as in Figure 7.4 but here showing the reverse side of the section. The yellow arrows point to the decaying dead wood and the blue arrows indicate areas of extensive new root and tissue development.
Several burnt sites in the Jervis Bay area were visited in 1997, 2001 and 2002. All _B. ericifolia_ plants that had been burnt had subsequently died and regeneration was from seed.

### 7.3.2. The diameters of stems

Figure 7.6 shows the results of the diameters of the stem of the three Provinces of _B. ericifolia_ at a plant age of 102 weeks. Plants from the Northern and Central Province were similar with means of 1.6 cm (± 0.05.) and 1.5 cm (± 0.02) respectively, while those from the Southern Province were significantly greater with a mean of 2.6 cm (±0.0467, $F_{2, 51} = 145.5$, $P< 0.0001$). At 200 weeks of age (after treatments were applied see Chapter 6) a similar pattern across the Provinces in the stems diameter was still clearly apparent (Figure 7.7). Morphological differences in the stem also clearly distinguished the Southern Province from the North and Central Provinces (Figure 7.8). Figure 7.8a shows not only a wider stem base (lignotuber) but also a series of folds in the bark. Petiole scars form restriction sites in the bark and the rapidly expanding stem tuber forms bulges between the scars. Figure 7.8b shows a typical smaller and evenly tapered nonlignotuberous stem as found in the North and Central Province. Lenticels, conspicuous by the relatively lighter coloured complementary cells than the stem are more numerous in Figure 7.8. In Figure 7.8a the lenticals and corky complementary cells have elongated transversely as a result of lignotuberous growth (compare Figure 7.8b).
Figure 7.6. Mean (± s.e.) diameter of stem at 1 cm above soil level for the three Provinces of *B. ericifolia* (*n* = 18) from the Sydney Region. Plant age 102 weeks.

Figure 7.7. Mean (± s.e.) diameter of stem at 1 cm above soil level for the three Provinces of *B. ericifolia* (*n* = 16) from the Sydney Region. Plant age 200 weeks.
Figure 7.8. The difference in the diameters and morphology of the bases of the stems of *B. ericifolia* form a) Southern Province (Jervis Bay) and b) Northern Province (Marramarra National Park) grown from seed in pot trials. Plant age 102 weeks.
There was a general trend of reduced stem diameters across treatments (Figure 7.9).

**Figure 7.9.** Mean diameters (±s.e.) of *B. ericifolia* stems (1 cm above soil level) in the three treatment groups (1 = controls, 2 = apicals removed once only and 3 = apicals removed on two occasions, see Chapter 6) for the three Provinces (N = north, C = central, S = south). Plant age is 200 weeks and treatment group 3 had all apical meristems removed twice.

### 7.3.3. The density of nodes

Figure 7.10 shows the mean (± s.e.) number of nodes 10 cm⁻¹ of stem in *B. ericifolia* for the three Provinces of the Sydney Region. There was a significant difference in the density of nodes across the three Provinces \( (P<0.0001, F_{2,87} = 162.4114) \). The Central Province was the least dense with a mean of 55.8 (± 1.259 s.e.) nodes 10 cm⁻¹. The Southern Province was the most dense with a mean of 94.5 (± 1.809 s.e.) nodes 10 cm⁻¹.
Figure 7.10. Mean (± s.e.) number of nodes 10 cm\(^{-1}\) in *B. ericifolia* for three Provinces of the Sydney Region (*n* = 30).

### 7.3.4. Capacity to resprout

The total number of nodes plant\(^{-1}\) was calculated from the average number of nodes Province\(^{-1}\) and the total length of stem treatment plant\(^{-1}\) Province\(^{-1}\). The total number of resprouts 98 weeks after the initial removal of shoots were scored and are indicated in Figure 7.11. There was a significant difference in the total number of resprouts Province\(^{-1}\). The Southern Province (207.3 ± 30.98) produced approximately twice the number of resprouts as the North and Central Province (118.5 ± 14.11, and 92.25 ± 15.72 respectively).

This resprouting data were calculated as a percentage of total possible meristems that could respout and are presented in Figure 7.11 as a line. There was no significant difference in the percentage of resprouts between Provinces. The percentage of
meristems that resprouted was greatest in the Southern Province while the North and Central were more similar.

Figure 7.11. The total number of resprouts (bars) and the same data as a percentage (line) of the total possible meristems that could have resprouted, for *B. ericifolia* from a North, Central and Southern Province of the Sydney Region.

7.3.4. Floral morphology

The means (± s.e.) and range are given for both perianth and pistal lengths for the two transect and locations sampled in the Southern Province of *B. ericifolia* in Table 7.3. The means fall within that described for the subspecies by George (1999) but a 1 mm increase in the range for the pistal lengths was found across transects and locations.
Table 7.3. Mean (±s.e.) perianth and pistal length (mm) and range from two transects and two locations at the most Southern limits to *B. ericifolia* subsp. *ericifolia* range and the range as described for the subspecies by George (1999) (*n* = 45, T1 and T2 = transect 1 and 2 respectively).

<table>
<thead>
<tr>
<th>Location or source</th>
<th>Perianth lengths (mean ± s.e.)</th>
<th>Perianth range</th>
<th>Pistal lengths (mean ± s.e.)</th>
<th>Pistal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jervis Bay Rd (T1)</td>
<td>20.02 ± 0.18</td>
<td>18-22</td>
<td>34.20 ± 0.20</td>
<td>32-36</td>
</tr>
<tr>
<td>Jervis Bay Rd (T2)</td>
<td>20.69 ± 0.15</td>
<td>19-22</td>
<td>33.62 ± 0.30</td>
<td>30-36</td>
</tr>
<tr>
<td>Beecroft Peninsula (T1)</td>
<td>20.80 ± 0.09</td>
<td>20-22</td>
<td>34.83 ± 0.08</td>
<td>34-36</td>
</tr>
<tr>
<td>Beecroft Peninsula (T2)</td>
<td>20.47 ± 0.11</td>
<td>19-22</td>
<td>34.44 ± 0.12</td>
<td>33-36</td>
</tr>
<tr>
<td>Described (George, 1999)</td>
<td></td>
<td>19-22</td>
<td></td>
<td>30-35</td>
</tr>
</tbody>
</table>

7.3.5. The arrangement of, and number of teeth, seedling leaf

With the exception of seedlings from the Jervis Bay site all seedling leaves were arranged oppositely with the transition to spiral phyllotaxy varying along the stem but always above the third pair of leaves (Table 7.4). Fifty percent of seedlings from Jervis Bay had leaves arranged in either a pseudowhorl of three leaves or spirally arranged. At seven weeks of age seedlings from the Jervis Bay site had also branched from the cotyledonary node, whereas branching at any node had not occurred in plants from other sites.
Table 7.4. Seedling leaf arrangement of *B. ericifolia* from six locations across its range.

<table>
<thead>
<tr>
<th>Location</th>
<th>Phyllotaxy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ku-ring-gai Chase NP</td>
<td>opposite</td>
</tr>
<tr>
<td>2. Marramarra NP</td>
<td>opposite</td>
</tr>
<tr>
<td>3. Blue Mountains NP</td>
<td>opposite</td>
</tr>
<tr>
<td>4. Mt Kiera</td>
<td>opposite</td>
</tr>
<tr>
<td>5. Jervis Bay</td>
<td>opposite, whorled and spiral</td>
</tr>
<tr>
<td>6. Beecroft Peninsula</td>
<td>opposite</td>
</tr>
</tbody>
</table>

Estimation of the variance component showed 16.57% of the variation in number of teeth margin$^{-1}$ to be among sites and 83.43% within sites. The mean number of teeth found at Ku-ring-gai Chase (ranked 6$^{th}$, Table 7.5) differs from that found at all other locations. The mean number of teeth found at Jervis Bay (ranked 1$^{st}$) differs from all other locations except Marramarrna National Park (ranked 2$^{nd}$). The means of all other locations do not differ significantly.
Table 7.5. Unplanned comparisons among pairs of means using the Games-Howell method (Sokal & Rohlf, 1995) for mean number of teeth seedling leaf⁻¹ margin. Differences between means of ranked locations (below the diagonal line) and corresponding MSD for an experimentwise 0.05 level of significance above the diagonal line. Mean differences greater than MSD are indicated with an asterisk (*). Harmonic interpolation of $\alpha$ (0.05) after Rohlf and Sokal (1995).

<table>
<thead>
<tr>
<th>Ranked locations</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jervis Bay</td>
<td>0</td>
<td>0.7887</td>
<td>0.6646</td>
<td>0.7285</td>
<td>0.7261</td>
<td>0.6833</td>
</tr>
<tr>
<td>Marramarra</td>
<td>0.6103</td>
<td>0</td>
<td>0.6608</td>
<td>0.7250</td>
<td>0.7225</td>
<td>0.6796</td>
</tr>
<tr>
<td>Beecroft Peninsula</td>
<td>0.8603*</td>
<td>0.2500</td>
<td>0</td>
<td>0.5810</td>
<td>0.5780</td>
<td>0.5226</td>
</tr>
<tr>
<td>Mt Kiera</td>
<td>1.1728*</td>
<td>0.5625</td>
<td>0.3125</td>
<td>0</td>
<td>0.6508</td>
<td>0.6025</td>
</tr>
<tr>
<td>Blue Mountains</td>
<td>1.2889*</td>
<td>0.6786</td>
<td>0.4286</td>
<td>0.1161</td>
<td>0</td>
<td>0.5995</td>
</tr>
<tr>
<td>Ku-ring-gai Chase</td>
<td>2.0686*</td>
<td>1.4583*</td>
<td>1.2083*</td>
<td>0.8958*</td>
<td>0.7798*</td>
<td>0</td>
</tr>
</tbody>
</table>
7.4. Discussion

The survey of the Beecroft Peninsula and Jervis Bay area revealed *B. ericifolia* to be polymorphic, displaying considerable variation in habit, often over relatively short distances. Three adult morphotypes of *B. ericifolia* based on habit, habitat and response were identified at the southern limits of the species range;

1. a short (1m) prolific vegetative resprouter (as seen at the slashing site, Figure 7.3; 7.4),
2. a short (<1m) lignotuberous root sprouter from swampy ground (Figure 7.5; 7.6), and
3. a tall (>2m) seeder (killed by fire) typical of that generally described for the species (as seen at three burnt sites).

Resprouting *B. ericifolia* plants following a fire were not found, however, all burnt sites inspected contain only the typical morphotype (habit 3 above). The short root sprouters (Figure 7.4, 7.5) are clearly ligno- (woody) and tuberous (swollen at the base). These lignotuberous plants are not likely to survive a hot fire as the lignotubers are small and not subterranean (not insulated from the heat by the soil). The other two morphotypes were not lignotuberous (at least as adults). As all seedlings grown from the Southern Province developed lignotubers (Figure 7.8a) I conclude that these plants display an adaptive phenotypic plasticity, ie given a threshold disturbance (eg waterlogging) a lignotuberous habit develops in the adult plant.

Eucalypt lignotubers have been considered to confer selective advantage by resprouting after die-back brought about by water-logged conditions (Ladiges & Ashton, 1974). In
the absence of such disturbance lignotuber formation in *B. ericifolia* is transient and the stem and lignotuber ultimately merge imperceptibly together. Plasticity and dimorphism have also been observed in other lignotuberous taxa (Bamber & Mullette 1978; Canadell & Zedler 1995).

The comparison of the diameter of the stems of 102 week old *B. ericifolia* plants grown under identical conditions showed not only a significant quantitative difference between the Provinces but also a stark qualitative one (Figure 7.6, 7.7, 7.8, 7.9). The seed collected for the comparison of the three Provinces were from plants typical of that described for the species and were similar in all respects, including the vegetation formation from which they were sampled (Section 2.3.1). The diameter, and to some degree, the morphology of the stems of the Southern Province of *B. ericifolia* were more in keeping with that of 102-week-old lignotuberous *B. serrata* stems. *Banksia serrata* and *B. ericifolia* are typically single stemmed and lignotuber development/morphology appears similar (Figure 6.3, 6.5). Petiole scars form restriction sites and the excessive development of the phellogen results in folds of tissue not unlike excess fat in a mammal. The first constriction site starts with the cotyledonary node that typically circumscribes the stem, followed by the first and then succeeding nodes. With radial growth (lignotuber development), petiole scars stretch around the lignotuber giving the characteristic pattern of rolls of tissue. This type of lignotuber development is different to that displayed in *B. oblongifolia* where considerable bud proliferation forms masses of bud complexes at the base of stems (Figure 6.4).
Clearly lignotuber morphologies and ontogenies vary. Considering the significance of the organ a typology of lignotubers should be developed. For example, there are arborescent lignotuberous species and multistemmed shrub species. The genes for lignotuberousness may display full penetrance in some species (eg *B. oblongifolia* is always lignotuberous) or variable penetrance and expressivity in others (eg *E. gummifera*).

The parental types of the Southern Province from which the seeds were collected did not show any signs of lignotuber development or resprouting from the base after fire. The adult or mature stages are nonlignotuberous and the immature stages are lignotuberous. The Southern Province displays a significant alternation of form in the immature stage of its’ life cycle. This alternation or transience may not be obligatory and gene environment interactions may show an adaptive phenotypic plasticity as noted above. This type of alternation is displayed in other species (eg *B. serrata*, *E. gummifera*) but they typically attain fire tolerance (resprouters), either through the continued development of a lignotuber or fire-resistant stem. *Banksia ericifolia* in the southern limits of its range is unique in that it does not attain the resprouter status even though it is lignotuberous. The function of the lignotuber, as first described by Kerr (1925) as ‘an embryonic storage organs that develop so as a seedling may survive temporary unfavourable conditions’, now seems more appropriately applied to this variant of *B. ericifolia*, a lignotuberous seeder.

The increased density of nodes (Figure 7.11) means a greater number of meristems (axillary buds) unit area$^{-1}$ and thus greater resprouting potential. The prolific resprouting at the slashed site (Figure 7.2) initially gave the impression of lignotuberous
regrowth. The increased density of meristems can account for the prolific resprouting as opposed to lignotuberous regrowth. The Southern Province also had greater resprouting following pruning treatments (Figure 7.11). Resprouting is also reliant on resource supply as well as meristem potential. It is tempting to suggest that the increased supply for the increased resprouting of the Southern Province came from the lignotuber. Resprouters have a significantly greater amount of stored non-structural carbohydrate than non-resprouters (Pate, Froend, Bowen, Hansen & Kuo, 1990) and these concentrations have been shown to decrease following resprouting (Van der Heyden & Stock, 1995, Cruz et al 2003). The increased nodal density also means a greater number of leaves unit area$^{-1}$ and thus potential photosynthetic capacity. It seems reasonable to speculate that the increased photosynthetic potential (nodal density) of the Southern Province provides excess photosynthate for lignotuber development.

Leaf density was one of a few criteria that distinguished between the two subspecies of *B. ericifolia* (Table 7.1). Leaf density, and lignotuber formation distinguishes this Southern Province within *B. ericifolia*. Floral characteristics are otherwise consistent as described for the subspecies (Table 7.3). George (1999) described *B. ashbyi* and *B. violacea* as either lignotuberous or nonlignotuberous (ie both) and formal recognition is not given in either case. The lignotuberous and nonlignotuberous forms of *B. ashbyi* are geographically distinct, and Taylor and Hopper (1991) considered that varietal distinction may be warranted. The early (in the life cycle) transient nature of lignotuber formation in this Southern Province of *B. ericifolia* presents taxonomic problems distinct to that occurring in *B. violacea* and other taxa. If formal distinction was to be given to this lignotuberous variety of *B. ericifolia*, identification in the field may generally only be possible in post-fire periods when the lignotuber is present (ie in the
seedlings). The adult morphology (nonlignotuberous) presents the same taxonomic problems as found with sibling or cryptic species.

Hybridisation seems unlikely to account for this lignotuberous form of *B. ericifolia*. The most likely cross may have been with *B. spinulosa* var. *spinulosa*, a lignotuberous species with range and flowering times that overlap. Floral and leaf characteristics of the Southern Province of *B. ericifolia* were consistent with that described for the species suggesting gene flow has not occurred, at least in the recent past. Additionally the lignotuber of *B. spinulosa* is more like that of *B. oblongifolia*, (a multi-stemmed shrub). The lignotuber described here for *B. ericifolia* is otherwise more like the arborescent *B. serrata*.

Alternatively, hybridisation followed by introgression may have resulted in the formation of a distinct ecotype that displays briefly an atavistic character (the lignotuber) from an hybridisation event that occurred in long-distance past.
Chapter 8. Final Discussion

The seeder/sprouter dichotomy is a broad classification of species response to fire and its utility as such is widely accepted (eg Bradstock, 1991; Gill & Bradstock, 1992; Enright et al, 1996; Bell, 2001; Lamont, & Wiens, 2003). The heuristic value of the seeder/sprouter dichotomy is also clear. It has been hypothesised that other traits are likely to be associated with seeders and sprouters (eg Cowling & Lamont, 1998). However, traits that might have been expected to be associated with seeders or sprouters were not always clearly apparent from my studies. It is argued below that one reason for the anomalies in the expected associated traits is a result of a tendency to assume an evolutionary history that is based on fire as being the primary selection pressure. The process of extrapolation from a modern classification system of fire response to expected associated traits that have an ancient and perhaps diverse origin is likely to produce anomalies. A few of the traits pertinent to this point are discussed below.

Extensive field observations over the course of this study supported by nursery observations of specimens grown from seed to four years of age (Chapter 6) revealed *B. integrifolia* to be nonlignotuberous and *B. serrata* to be lignotuberous. I classed *B. integrifolia* as a sprouter (Table 8.1) not only because of its limited ability to sprout after fire (Wiess, 1984) but also because of its ability to produce mature root suckers (per. obs. and see below). These results have been entered in Table 8.1, which is an updated and modified version of Table 1.3 that presented the current knowledge of the four study species before this study. My conclusion as to the presence/absence of a lignotuber in *B. integrifolia* and *B. serrata* is based on a subjective determination of a swelling (the common criteria of a tuber).
On the basis of this subjective criteria (as well as comparative stem diameters between Provinces) I described transient lignotuber formation in the ‘classical seeder’ *B. ericifolia* from the southern most part of its range (Chapter 7). This phenomenon (transient lignotuber formation) may be more widespread within sprouters and seeders. Several points arise from these findings. Firstly it suggests that a typology of lignotubers should be developed considering the significance and widespread occurrence of the organ. Having developed a typology, objective criteria may then also be developed and applied. Confusion as to the presence/absence of a lignotuber within taxa is also then less likely to occur. Secondly transient lignotuber formation in *B. ericifolia* detracts from the axiom that lignotuberous species are sprouters (in reference to fire response). As a result some general hypotheses about seeders and sprouters may no longer apply or be logical. For example, Midgley (1996) and others (eg Kruger, Midgley & Cowling, 1997) have hypothesised that seeders will be taller than sprouters, particularly lignotuberous sprouters. There was no significant difference in plant height (after 102 weeks of growth) between the three Provinces of *B. ericifolia* (Chapter 6). There was however a significant difference between Provinces in leaf density (Chapter 7). The southern Province had the greatest leaf density. Thus transient lignotuber formation may not necessarily be a sink that results in reduced resources for plant height growth when an increased leaf density may provide a source for lignotuber development. Midgley’s hypothesis appears to be based on a concept of lignotubers that does not recognise transient formation and would probably only apply to the multi-stemmed lignotuberous habit. However Midgley hypothesised that the tallest species within a genus or clade (regardless of whether a lignotuber existed or not) will be a seeder. In *Banksia, B. integrifolia* (a sprouter) is the tallest recorded species (Taylor &
Hopper, 1991) and certainly within eastern Australia, *B. serrata* (sprouter) is the next tallest species (per. obs.).

Table 8.1. Updated Table 1.3. The four *Banksia* study species, habit (S = shrub with single stem (s) or multiple stems (m) T = tree), lignotuber, serotinous, seeder or sprouter strategy. Additional columns are parental care and ability to asexually reproduce. Changes to Table 1.3 are underlined.

<table>
<thead>
<tr>
<th>Species</th>
<th>habit</th>
<th>lignotuber</th>
<th>serotiny</th>
<th>seeder</th>
<th>parental care</th>
<th>asexual</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ericifolia</em></td>
<td>Ss</td>
<td>YES/NO?</td>
<td>YES</td>
<td>seeder</td>
<td>YES?</td>
<td>NO</td>
</tr>
<tr>
<td><em>B. integrifolia</em></td>
<td>T</td>
<td>NO</td>
<td>NO</td>
<td>sprouter</td>
<td>YES?</td>
<td>YES</td>
</tr>
<tr>
<td><em>B. oblongifolia</em></td>
<td>Sm</td>
<td>YES</td>
<td>YES</td>
<td>sprouter</td>
<td>YES?</td>
<td>YES/NO?</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>T</td>
<td>YES</td>
<td>YES</td>
<td>sprouter</td>
<td>YES?</td>
<td>NO</td>
</tr>
</tbody>
</table>

Height is a complex trait with several components and the cost benefits are currently not well understood (Westoby et al, 2002). Height is also a distinguishing characteristic of trees and shrubs. Hypotheses about traits/characteristics such as trees and shrubs that have arisen independently several times in evolutionary history are unlikely to be characterised by a hypothesis based on response to one, or a few similar types of stimuli.

If there was negative correlation between bark thickness and tree height (taller trees have thinner bark) then on the basis that a thick bark protects the vascular system, Midgely’s hypothesis might be supported. The correlation however does not always exist and therefore the hypothesis does not always apply across taxa and clades. For example it does not apply in *Banksia* (as noted above) but it does apply in eucalypts at the level of genus. *Eucalyptus regnans* is a seeder and the tallest flowering plant in the world. It has a relatively limited ability to control water use and is thus restricted in its
distribution to sites that are near optimum because of its water use (Pate & McComb, 1981). Midley’s hypothesis may not however apply to eucalypts at the infragenic level. The difference in eucalypts and banksias is to some extent biogeographical. Banksias typically occupy more xeric habitats (with higher fire frequency) as compared to eucalypts that are found in a broader range of habitats including those mesic environments with very low fire frequency. Xeric sites are less likely to support the tree habit than mesic sites. The almost contradictory hypothesis, that the tallest species of a fire prone vegetation will be a sprouter is probably correct for fire prone sites that can sustain the tree habit.

If a seeder is expected to expend more resources on sexual reproduction then traits associated with higher fecundity might also be different (Cowling & Lamont, 1998). For example, a difference in floral display (pollinator attractiveness) may be different in seeders when compared to sprouters. *Banksia ericifolia* differed from the other three resprouting species by maintaining a regular inflorescence phyllotaxy (Chapter 3). Other floral traits that distinguish *B. ericifolia* from the sprouter species in this study are hooked styles (diagnostic for Section Oncostylis within *Banksia*), flower colour and acropetal anthesis. *Banksia spinulosa* (Oncostylis) can also be distinguished from the three sprouter species on the basis of similar floral characteristic (hooked style, colour and acropetal initiation) however three of the four varieties of *B. spinulosa* are lignotuberous (sprouters). Thus associated traits may bear no relevance in terms of fire response. However a consequence of a stable (non-rising) phyllotaxy in *B. ericifolia* is the clear formation of vertical rows (orthostichies) of flowers. Additionally, the much larger unit inflorescence (in particular the common bract) of *B. ericifolia* sets the orthostichies further apart making them even more conspicuous. *Banksia ericifolia* has
the tallest inflorescence, which further emphasises the orthostichies. An additional notable different is that while *B. ericifolia* has the tallest inflorescence and as a result of a smaller diameter inflorescence axis and larger unit inflorescence size, it produces approximately half the mean number of flowers that *B. serrata* does on an inflorescence. All Proteaceae are polytellic and inflorescence height is the most plastic or the least constrained dimension which *B. ericifolia* appears to have made the most of.

If colour is not important to some potential pollinators then pattern (vertical stripes) clearly otherwise distinguish *B. ericifolia* inflorescences among the bushes and trees.

While the floral characteristics mentioned above provide a stark contrast between the seeder and sprouters, the theoretical expectations of the seeder having the greater fecundity were not born out in my study (Chapter 4). Traits associated with *r* selection (equivalent to seeders and *K*-selected traits equivalent to sprouters see Section 1.6) are early age of maturity, large numbers of offspring, semelparity, no parental care and large reproductive effort (Steams, 1977). *Banksia ericifolia* (a seeder) had a brood size of only 0.5 while the three other species (sprouters) had brood sizes of 0.5 or above. Why *B. ericifolia* should reduce its potential offspring number by 0.5 is paradoxical. It is only slightly less comprehensible that *B. oblongifolia* (sprouter) should reduce its potential offspring number in the same way. If the positional effect of setting the lower seed is shown to have some potential benefit in terms of insulation from the heat of a fire (as proposed in Chapter 5) this may explain the predominance of setting more lower ovules but not necessarily why it sets a single seed. Single seededness might best be explained as an adaptation to predation. For example, predation may have driven selection for single seededness and then fire selected for flowers/fruits that set seeds occupying the lower position in fruits.
Adaptations to large predators (eg megafauna) may have selected for thicker woodier fruits and it is more likely to have occurred before adaptation to fire because the megafauna were present long before the onset of aridity and the regular occurrence of fire (Chapter 1). If woody fruits in Banksia are adaptations to predation then they can now be considered preadaptations to fire. In Chapter 3 a possible explanation for the dense and tight packing arrangement of unit inflorescences (and wiry styles) was protection from heavy foraging bouts. The copious amount of energy rich nectar may well have attracted the now extinct megafauna whose browsing could also have effected pollination.

The evolution of serotiny need not have arisen once as a result of a single type of selection pressure (serotiny is generally considered to be a fire adaptation eg Lamont et al, 1991). The Gondwana forest were not ‘closed’, they had open but stratified canopies that grew under warm wet polar-ice-free climates (White, 1998). Serotiny may well have evolved in response to gaps in this forest type. Gaps in the canopy may have occurred via branch and/or plant death or tree-fall, as a result of old age, natural agents including very heavy browsing\textsuperscript{15}, or wind. Seed is released from serotinous banksias when the plant dies, or from follicles on branches that die or from cones that have fallen to the ground (Whelan et al, 1998; per. obs.). Sunlight, whether via a photochemical reaction and/or radiant heat is also sufficient to melt the resins that helps bind the two valves of the follicles in Banksia and death of the branch supporting the infructescence is not necessary for follicle rupture (per. obs.). Thus gaps in the canopy may allow

\textsuperscript{15} Browsing by megafauna such as the tree-fellers (Palorchestes) and the diprotodontids must have been very destructive to vegetation.
sufficient sunlight to stimulate seed release and also provide sunlight to seedlings that may have germinated in those gaps. Serotiny (as opposed to woody fruits themselves) most likely continued to evolve as a consequence of an increasing fire regime. Selection for fruits that required increasing stimuli to produce a dehiscence response has most likely led to the levels of serotiny that we see in the fire prone floras today. Selection for thicker valves (woodier follicles) in *Banksia* and other taxa may well have continued as a consequence of an increasing fire regime.

Serotiny is a significant phenomenon in our fire prone flora yet like the lignotuber a detailed knowledge of it seems lacking. For example, it is often assumed that seed is released in serotinous species only after desiccation of the follicle wall due to fruit, infructescence, branch and/or plant death (eg Lamont & Groom, 1998). Yet it is generally recognised that some serotinous fruits open spontaneously (even having been referred to as leaky) or some species are weakly serotionous. Spontaneity is the key term here and suggests that the mechanisms in operation, particular follicle opening are not well understood. Midgley (2000) considered that the costs of serotiny were mechanical support and maintenance of a vascular supply to the infructescences so as to keep them alive and prevent follicles from opening. He considered a consequence of maintaining a vascular supply to cones was a cumulative cost because of the continued loss of water over time via the cones. On the basis of this premise Midgley discussed the significance of variation in serotiny. Wardrop (1983) however found that in mature infructescences of *Banksia* the vascular supply is cut by secretion of resin into the vascular tissues. In all dissections of infructescences I made on the three serotinous study species there was no visually apparent living tissue within the woody
infructescence. Thus hypotheses about serotiny that are based on the premise that infructescences are living structures (e.g., Midgley, 2000) are possibly invalid.

Another trait associated with r-selection is lack of, or no parental care (Stearns, 1977). Serotiny can be considered a form of parental care. The only cost to the plant after completing the development of a ‘home’ for the seed is mechanical support to keep the home within the canopy. That is, seeds are held within the woody follicles providing protection from potential predators or the heat of fire. *Banksia ericifolia* was reported to be strongly serotinous (lots of parental care) whereas the other three species were either nonserotinous (no parental care) or serotiny was considered to be weaker (George, 1981; see Table 1.1) (less parental care). Single seededness in addition to serotiny may be considered even greater parental investment (or care). Parental care in terms of seeds is thus greatest in *B. ericifolia* (a seeder equivalent to an r strategist), a trait otherwise associated with K-selection by Stearns (1977). The two traits, large reproductive effort and no parental care, associated with r-selection are contradictory when serotiny is considered a form of parental care. Gadgil and Solbrig (1972) considered that the most important distinction was that r-strategists devoted a greater proportion of available resources to reproduction. In this context single seededness and a high level of serotiny in *B. ericifolia* is consistent with their contention. Thus a reduced brood size (lower fecundity) in the seeder *B. ericifolia* appears be a trade-off for greater parental care. In this instance a trade-off between the mean fitness (more than one seed follicle\(^{-1}\)) and variance (always one seed follicle\(^{-1}\)) provides an example of diversified bet-hedging (don’t put all your eggs in the one basket). It should be noted that Stearns’s (1977) list of traits are what might be expected to occur in animals. The difference noted here highlights a fundamental difference between plants and animals.
Parental care in plants can also occur via asexual reproduction. Three species, *Banksia elegans* (from Western Australia), *B. marginata* and *B. integrifolia* (from Eastern Australia) are known to root sucker\(^\text{16}\) (George, 1981; Lamont, 1988; Taylor & Hopper, 1991; K. Thiele pers. com.; per obs.). The only reported root suckers in eastern Australia are nonserotinous although some variants of the *B. marginata* complex may be serotinous (George 1999). *Banksia elegans* on the other hand, although serotinous, is essentially sterile (Lamont & Barret, 1988). Thus parental care in *Banksia* appears to be partitioned into either serotiny or asexual reproduction. A potential hypothesis, worth investigation in other genera is that nonserotiny may be correlated with asexual reproduction. I excavated the soil around small but mature (flowering and fruiting) *B. integrifolia* plants and found that root suckers can become mature. *Banksia integrifolia* has theoretically the potential to produce independent ramets via death and decay of the root attaching them to the ortet (the seed mother plant) (see below and Figure 8.1). In this way the ortet provides parental care (mothering) to young clones until they are sufficiently provisioned (and have their own root system) to be able to survive independently. Asexual reproduction is also theoretically possible in *B. oblongifolia* (see Figure 8.2) and thus this species could also be considered, theoretically at least, capable of providing parental care.

An ortet (a term used by foresters) is the original parent plant (arising from seed) from which a clone was derived and a ramet is an individual member of a clone (Sutton & Tinus, 1983; Hartmann & Kestor, 1983). Bell (1991) considered ramets to be

\(^{16}\) Members of the Series *Prostratae* may be clonal eg *B. goodii* is rhizomatous (Lamont & Markey, 1995).
independent daughter plants that were collectively referred to as a genet or clone. Thus root suckers, even if mature (flowering & fruiting), are not ramets according to Bell (1991) until they become detached from the parent plant. The term ramet has been applied to the aerial stem arising from the lignotuber of *B. oblongifolia*. In either case excavation is required to determine the status of the above ground stems.

No evidence of asexual reproduction was found in the case of *B. ericifolia, B. serrata* or *B. oblongifolia* and there was only an implied capability of asexual reproduction in *B. integrifolia* because of root suckers. Parental care (a characteristic of K strategists, and considered the sprouter equivalent in the seeder/sprouter dichotomy) is at least theoretical possible in two out of the three sprouters. Overall, if parental care is considered both in terms of serotiny and asexual reproduction the equivalence of r and K-selection to the seeder/sprouter classification of fire response does not hold.

The extent of vegetative reproduction in *B. integrifolia* needs to be examined. Mature (fruiting) suckers or ramets do occur (per. obs.). In some situations such as on windswept coastal headlands long leeward rows of *B. integrifolia* stands occur (C. Morris pers. com.). These may be giant clones whose rows have been formed under the influence of wind. Extensive clonal formation within the Proteaceae has been observed in *Lomatia tasmania*, a species that exists as a single clone 1.2 km long and has never been observed to set seed. In this case the lack of seed production is not a result of maleness but presumably as a result of being triploid (Lynch, Barnes, Cambecides & Vaillancourt, 1998).
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Figure 8.1. Root suckers in *B. integrifolia*. In a) the mature mother plant has given rise to five root suckers, one of which is mature (has fruit). Collectively the mother plant and the five root suckers are referred to as the genet. In b) asexual reproduction has resulted from the death and decay of sections of the root connecting the suckers that are now mature independent clones or ramets.
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Figure 8.2. Hypothetical asexual reproduction in *B. oblongifolia*. Lateral growth of the lignotuber in a) followed by death and decay of some sections of the lignotuber results in mature independent clones or ramets in b). The ortet is most likely to be the dominant ramet of a genet as it is the seed mother plant and thus older in calender age and most likely biological age state. Extensive asexual reproduction will make it impossible to distinguish the ortet (if still living) from older ramets. Calender age and biological age do not necessarily correlate. No distinguishing term is applied in the literature for non-independent and independent plants derived from a zygote (ie the seed mother or ortet). Application of the term ortet becomes somewhat difficult in the case of asexual reproduction in *B. oblongifolia* because the woody lignotuber is an integral (and indistinguishable) part of the ortet and the above grown stems are often consumed in a hot fire. As a result of fire the ortet stem is destroyed and the regrowth ramets are an even age class.
As noted earlier for brood size, the theoretical expectations of the seeder having the greater fecundity and the greater overall reproductive output was not born out in my assessment. *Banksia integrifolia* had the greatest overall reproductive output because of increased cone and seed set. *Banksia ericifolia* however did have the greatest percentage fruit set. Additionally by comparing unit inflorescence size with fruit size I showed that structural limitation cannot account for the increased fruit set in *B. ericifolia* as it had essentially the same I:F as *B. oblongifolia* (Table 4.9) but set more than twice the percentage of fruit infructescence\(^{-1}\) (Table 4.8). It was noted in Chapter 4 however that the I:F may not be a true reflection of the space available on an inflorescence for fruit set as it did not take into account fruit shape. As a result the I:F could have been biased and under estimated the space available on a *B. ericifolia* inflorescence for fruit set. Fruit shape in cross section in *B. ericifolia* is completely different to the other three study species (Figures 4.13, 4.14). The shape of the fruits is such that adjacent fruits can overlap to a degree by being either more or less exerted (see Figure 8.3). Exerted in this context refers to how far fruits protrude out from the central axis of the inflorescence. As a result of the unusual shape they tend to overlap which enables more fruit to be set unit area\(^{-1}\) of inflorescence. Nevertheless the overlapping of fruit is unlikely to account for the more than two-fold increase in percentage fruit set in *B. ericifolia* as compared to *B. oblongifolia*. So within the serotinous species the seeder has a higher reproductive output than the sprouters, an associated trait that would seem logical as its continued existence in fire prone habitats is via seed.
overlapping fruits  
\[
\begin{align*}
\text{B. ericifolia} & \quad \text{B. oblongifolia} \\
\end{align*}
\]

no overlap possible

Figure 8.3. Illustration (not to scale) of the two different types of fruits found in the four Banksia study species (see cross sections of fruits in Figure 4.13, 4.14). Fruit in B. ericifolia have a different shape to B. oblongifolia (as well as B. integrifolia & B. serrata) and as a result when fruits are exerted to different degrees on an infructescence the shape in B. ericifolia allows for greater overlap of fruits. In this way B. ericifolia reduces its’ I:F and may physically be able to accommodate more fruit on an inflorescence than B. oblongifolia.

However fruit shape also suggests an additional adaptation to herbivory in B. ericifolia. A consequence of this shape is an increased crowding of fruits particularly at the outward-facing surface of the infructescence. Fruits of B. ericifolia are often displaced, twisted and ultimately moulded together in the developing infructescence such that a continuous surface of dense woody fruits often forms for the greater part of an infructescence. By being moulded together the fruits have greater protection from potential herbivores than they would individually. That is, it is more difficult to obtain leverage on a fruit because the fruits are moulded together. Alternatively B. oblongifolia (shrub) fruit’s are the least exerted (most protected within the bracts) and the floral and common bracts enlarge, become indurated and pungent during
infructescence development (George, 1981; pers. obs.). Harvesting of cones by hand (snapping the cone from its branch) requires considerable pressure, sufficient to break the skin, sometimes drawing blood, but otherwise this is a relatively painless operation in *B. ericifolia*. Within the serotinous species *B. serrata* is a tree with very large thicker valved follicles that are more exerted than the shrub species. So the serotinous species show potential adaptations to herbivory in different ways.

The greater reproductive output of the seeder *B. ericifolia* operates at the level of the inflorescence/infructescence (percentage fruit set) but not at the level of the ovary/fruit (brood size or seed set) or plant (percentage cone set). Including cone set as part of the overall assessment of reproductive output introduces a bias because the nonserotinous *B. integrifolia* expends comparatively fewer resources in producing a cone compared to the serotinous species. In serotinous species a consequence of increased maturation period (of the infructescence) are increased risk of abortion and loss of offspring to insect and bird predation, fungal decay, drought, fire and changes in resource status. Serotiny therefore has a higher cost and an associated higher risk due to the increased time to maturation. How then should the reproductive output and fecundity be compared, only within serotinous species? Clearly percentages are better than absolute numbers because of issues such a size, but fecundity in the strict sense refers to the number of offspring (seeds) produced in a given period of time. Fecundity in *Banksia* in the strict sense should be estimated at the level of the brood (mean seed number fruit\(^{-1}\)). Comparisons of fecundity of the serotinous species based on mean numbers of seed infructescence\(^{-1}\) is not logical because the size of the inflorescence/infructescence varies considerably. However in assessing mean percentage fruit set infructescence\(^{-1}\) *B. ericifolia* may be at a slight advantage. *Banksia ericifolia* produces the tallest
inflorescence but has the smallest mean number of flowers inflorescence\(^{-1}\) (Figure 3.19) within the serotinous species. At the inflorescence level \textit{B. ericifolia} may have a greater amount of resources available flower\(^{-1}\) and is thus able to set a greater percentage of fruits.

One trend in the observed leaf phyllotaxy suggests two different strategies in response to the environment may be in operation. The basis of the observation is not related to leaf size but node/leaf density. \textit{Banksia ericifolia} has relatively small leaves (9-20 mm long, 0.75-1 mm wide, George, 1981) and a high nodal density (Chapter 7). The other three study species have leaves of more comparable size, although \textit{B. serrata} has the largest leaves (4-26 cm long, 1-4.5 cm wide) and they are more crowded (George, 1981; per. obs.). More crowded also means the highest nodal density of the three larger leafed study species. An increased nodal density can, and in respect of these species does, mean more branching and subsequently denser foliage. \textit{Banksia serrata} also has accessory buds that may produce shoots that further increases the density of its foliage (Chapter 6). \textit{Banksia ericifolia} and \textit{B. serrata} did not show a clear pattern of leaf arrangement or orientation of leaves with respect of other organs (leaves, branches, inflorescences) architecture. Production and arrangement of leaves in these two species appeared to be based on a lottery strategy, producing a large quantity of leaves, regardless of local or interspecific architectural considerations. \textit{Banksia integrifolia} and \textit{B. oblongifolia} on the other hand both showed interspecific orientation with respect to the plants’ architecture (Chapter 3). Both these species are theoretically at least capable of asexual reproduction.
Banksia ericifolia and B. serrata are also similar in that both are (generally) single stemmed. Banksia serrata is lignotuberous but the resprouting ability of the lignotuber is limited to the earlier part of its life, before it has developed a fire resistant trunk (Chapter 6). Banksia integrifolia (root sprouter) and B. oblongifolia (large spreading lignotuber) are similar in that both can be and are respectively multiple stemmed, and both have the potential, theoretically at least, to persist indefinitely via vegetative reproduction. Banksia serrata has been shown via carbon dating of its lignotuber to have a life span of approximately 100 years (Ross Bradstock, per. com). There are no data on the life span of the other three species, but it is clear that in fire prone environments B. ericifolia is killed, and therefore, its life span is the shortest and equivalent to the interfire period. However, in general, sprouters may live for hundreds or thousands of years while seeders may senesce or are killed by hot fires within 5-50 years (Lamont & Wiens, 2003). Lottery recruitment of leaves may favour shorter lived species in particularly the obligate seeder B. ericifolia. At their most vulnerable life stage, and when competition is the greatest in a rapidly changing post-fire environment, the lottery recruitment (maximised production) of leaves is more likely to confer advantage in the high light environment. While B. serrata is clearly a sprouter, it also reproduces via seeds but not asexually and thus can be considered to be advantaged, to some degree, by lottery recruitment of leaves for the same argument given for B. ericifolia above.

Banksia ericifolia and B. serrata have the highest leaf density and neither species reproduces asexually. Plant growth and lateral spread is limited to apical and axillary meristems. Conversely B. oblongifolia and B. integrifolia can grow laterally by the expansion of a lignotuber and root suckering. On this basis I propose the hypothesis that species limited to growth via apical and axillary meristems will have a higher leaf
density than those species that do not. Inherent in this hypothesis is the assumption that seeders will generally have a higher nodal density than sprouters. If nodes (meristems) are not used for plant growth they may be used for sexual reproduction.

Clearly percentage fruit set does not reflect total seed numbers held by a serotinous plant. Total seed number is the product of percentage cone set, fruit set and seed set as well as the total number of cones held within the canopy. Estimates of the canopy stored seed banks were not made for all the study species (exception was *B. oblongifolia*, Section 4.2.2). Total cones held within the canopy is clearly related to time since fire. In stands unburnt for 10-15 years, *B. ericifolia* plants were found to have two and a half more seed bearing cones than the co-occurring *B. oblongifolia* plants (Zammit, 1986). Initially *B. oblongifolia* (sprouter) produces more cones after a fire but with increasing time since fire *B. ericifolia* (seeder) ultimately produce more cones plant$^{-1}$. While the seeder/sprouter dichotomy can be invoked to account for the initial difference (lignotuberous regrowth versus seed) the ultimate difference, reliance on seed for survival, only explains why it is different, not how it is achieved. The difference in phyllotaxy (nodal density) and habit are attributes that enable *B. ericifolia* to ultimately store more seeds in its canopy.

Peters (1991) claimed that the continua of selection gradients (eg r & K-selection, CSR triangle) can rarely be used directly as they are operationally undefined and thus species cannot be unambiguously classed independently from the characteristics the classes are considered to have. Furthermore he considered the lists of characteristics of the strategies are often added to by logical interdependencies. This might be considered akin to hypothesising about traits that might be expected to be associated with seeders.
or sprouters. However utilisation of r and K-selection and their associated traits has provided a structure upon which some characteristics of the study species could be evaluated as far as the seeder/sprouter analogy would go. Clearly characterisation of the study species along the r and K continuum is not possible, not however because the biology of the species is deficient as was suggested by Bell et al. (1984; Chapter1) but partly because of the reason outlined above by Peters (the continua are operationally undefined). The use of logical interdependencies when associating traits for seeders and sprouters is clearly a reasonable and resourceful approach to understanding the biology and ecology of species that make up fire prone floras. However traits that may seem to be logically associated with seeders and sprouters, such as higher reproductive output, the occurrence of a lignotuber and the role of serotiny are not unequivocally readily assigned. I suggest the lack of congruence is due to a generally unrecognised evolutionary history that predates the regular occurrence of fire and thus biases the interpretation of associated traits so often invoked to account for the seeder sprouter strategies (eg Cowling & Lamont, 1998; Bell, 2000). There is, for example no reason for highly correlated traits, such as woody fruits and serotiny as found in the Proteaceae, to be considered to have evolved as a result of a single selection pressure. Likewise there is no reason for an organ, such as the lignotuber whose occurrence is highly correlated with species that occupy fire prone floras, to be considered to be an evolutionary adaptation to fire. The widespread occurrence and different morphologies of lignotubers clearly tells us that they have arisen several times. A better appreciation of the evolutionary history and thus the possible preadaptation status of traits and characteristics will help to account for the anomalies.
References


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