School of Environmental Biology

REPRODUCTIVE BIOLOGY AND MATING SYSTEM OF *BANKSIA TRICUSPIS* (PROTEACEAE)

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ABSTRACT

An understanding of the role of genetic and ecological factors that influence demographic change is paramount for the conservation of plant populations. These genetic and ecological factors often act in concert to influence reproductive success and thus the ability of a species to maintain its presence in the community. Paramount among the array of factors influencing a species' fitness are mating system considerations and their interaction with ecological processes. Both mating systems and ecological processes are influenced by intrinsic and extrinsic forces that may operate synergistically to constrain pollen transfer, gametic union and the provisioning of progeny. Plants respond to these constraining forces, which typically vacillate, through mechanisms that minimise random effects while maximising the quality and quantity of progeny.

Mating system values indicated that *B. tricuspis* is an obligate outbreeder. The extent to which populations of this *Banksia* indulged in mixed mating was associated with habitat disturbance and its impact on pollinators. A reduction in outcrossing in one disturbed population was ascribed to an increase in inbreeding promoted through pollinator/pollen limitation. Conversely, a reduction in the diversity of pollinator types which enhanced the opportunity for outbreeding was the favoured interpretation of elevated outcrossing in another disturbed population.

Disparity in outcrossing among inflorescences was driven by pollinator/pollen limitation considerations. Inconsistency among outcrossing values between inflorescences was ascribed to incongruity in floral attractiveness and sympatric assortative pollinator/pollen limitations. Augmentation of the floral display, as inferred through greater inflorescence size and presentation height, was positively associated with maternal fecundity and progeny fitness. This advance in zygote quality and quantity was attained despite greater attractive displays increasing the probability of geitonogamous and consanguineous matings. This ambivalent accomplishment indicated that attractiveness of the floral display in *B. tricuspis* enhanced the opportunity for sorting among possible mates, a proposition supported by the increased abandonment of zygotes with increasing display effort. The increases in maternal fecundity and progeny provisioning with increasing inflorescence size and height indicated that maternal resource considerations were not limiting reproductive success.

Attractiveness of the floral display was also credited with the moderation of pollinator/pollen limitations within an inflorescence. The apparent increase in floral display with progress of the 'advancing front' towards the peduncle significantly altered pollinator foraging behaviour and differentially enhanced the opportunity for pollen receipt and export, especially of pollen types which promoted outbreeding. Commensurate with this moderation in pollinator/pollen limitation was an increase in maternal fecundity and progeny provisioning, although selection among zygotes was tempered and outbreeding declined slightly. The availability of nutritional resources within an inflorescence and sink strength relationships are submitted as proximate causes for this moderation, which conforms with the notion of mate choice and the proposition that the genetic threshold determining selection alternates with resource provisions.

Scenarios propelled by selection among compatible mates which are commensurate with maternal resource considerations imply a high level of intrinsic control over reproductive success in *B. tricuspis*. Reproductive synchrony, continuity and predicability in fecundity and the compensatory repartitioning of resources following floral damage also signify a high level of intrinsic control. This control is required to mitigate extrinsic proximate constraints which impinge on reproductive success thereby synchronising maternal investment with resources and maximising fitness. The randomness of pollination was advanced as the proximate constraint affecting reproductive success in this species. This constraint was mitigated firstly by a mating system which sorted among compatible mates and tolerated deleterious matings, and secondly through an decrease in the opportunity for deleterious matings afforded by increases in the attractiveness of the floral display.

The production of 'surplus' flowers by *B. tricuspis* was proposed as the principal adaptive response to the forces constraining reproductive success. 'Surplus' flowers primarily served, at various levels of organisation with the species, to enhance attractiveness, thereby moderating the influence of pollinator/pollen limitations on plant fitness. Maternal fecundity and progeny fitness within and between inflorescences was augmented by increases in attractiveness while conversely, at the population level, decreases in attractiveness deleteriously impinged on fitness. 'Surplus' flowers also conferred other functional advantages in this species which operated synergistically with the enhancement to floral attractiveness. These advantages were primarily related to the opportunities that 'surplus' flowers conferred for selective abortion and bet hedging.

Caution is required in attributing 'surplus' flowers primarily to the mitigation of pollinator/pollen limitation considerations as other forces may represent stronger selective process. While the attractiveness of the floral display undoubtedly increases fitness, it also inherently increases the opportunity for floral damage by birds and insects as the discrimination among displays by damaging agents was driven by attractiveness cues. The impact of floral herbivores ranged from minimal for parrots and cockatoos to severe for insects, with the pattern of floral damage by both birds being determined by the pattern of insect floral herbivory. The predicability in floral damage between seasons and serial adjustment between successive reproductive development stages in *B. tricuspis*, as evident by compensatory responses in fecundity, moderates the influence of floral damage as a selective force influencing reproductive success.

The production of 'surplus' flowers in *B. tricuspis* has facilitated the development of intrinsic maternal processes which, in reply to genetic and ecological stimuli, operate to maximise fitness through mitigating the deleterious impacts on reproductive success of floral damage and pollinator/pollen limitations. These maternal processes strategically maximise the utilisation of maternal resources and allow progeny with low fitness expectations to be tolerated. Ultimately, these maternal processes and deterministic genetic and ecological stimuli promote reproductive assurance which contributes to population and species persistence through advances in fitness.

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CHAPTER 1

INTRODUCTION

1.1 General introduction

Protecting and maintaining populations of plants *in situ*, especially rare and endangered species, is the principal goal of conservation biology. Successful attainment of this goal will ensure maintenance of biodiversity at each level of biological organisation (Falk 1990) and facilitate ecosystem processes which permit the biota to function and respond to perturbations and other evolutionary forces (Risser 1993). Fulfilment of this goal will be influenced by the ability to identify and maintain or enhance the state of the most important criteria contributing to the current status of the species and interactions with its environment (Benson 1990, Pantone *et al.* 1995). The main criteria are: (1) the availability of suitable habitats for continued *in situ* persistence (Ehrlich 1988, Lesica 1992); (2) the role of diverse genetic and ecological factors which influence demographic change, especially vital rates (Caswell 1989, Pavlik 1994); and (3) the extent of genetic variation which reflects evolutionary history, influences population persistence (Huenneke 1991) and preserves evolutionary potential (Menges 1991, Fenster and Dudash 1994).

The first conservation criterion is readily discernible and can be assessed through the synchronous interrogation of specimen databases (held by herbaria or in literature on taxa distribution - eg Banksia atlas, Taylor and Hopper 1984) and an appraisal, with the aid of satellite imagery and geographical information systems, of habitat preservation, land tenure and reservation status (Whitson and Massey 1981, Given 1994). Conversely, criteria two and three are poorly documented for most species and, when known, their functional and adaptive significance are often difficult to comprehend (Pavlik 1987, Hopper *et al.* 1990, Hopper and Coates 1990, Holsinger and Gottlieb 1991).

For most species, rigorous scientific investigation and experimental assessment is required to obtain the fundamental demographic information necessary for the development of appropriate conservation strategies (Hopper *et al.* 1990, Schemske *et al.* 1994). Protocols to obtain the necessary demographic information and undertake such assessments have been developed (Cropper 1993, Pavlik 1994, Schemske *et al.* 1994). A comprehensive appreciation of the influence of criteria two and three on life history processes which distinguish the biology and demographics of a species is essential to the development of conservation strategies, although the role of genetic variation is probably more pertinent to *ex situ* conservation efforts, reintroductions and intensive remedial population management action (Hopper 1993, Fenster and Dudash 1994, Mistretta 1994).

A diverse array of genetic and ecological factors, which may operate in concert and interact (Barrett & Kohn 1991) affect a plant's vital rates - birth, growth and death (Caswell 1989). These vital rates are the components of a species' population demographics and affect life history processes which inherently influence persistence and fitness. These factors are primarily intrinsic, reflecting fundamental biological processes which constrain abundance and distribution (e.g. low fecundity, restrictive mating systems, inadequate dispersal mechanisms, low resilience to perturbations). Extrinsic factors may also affect these processes (e.g. predation, competition, inadequacies of pollinators) and can operate synergistically with intrinsic considerations to further constrain abundance and distribution. For example, indiscriminate pollinator behaviour may promote pollination which does not comply with the mating system requirements of a self-incompatible species.

Paramount among the array of genetic factors affecting population demographics is the mating system of a species and its interaction with the ecological processes which influence reproductive success (Clegg 1980, Davis *et al.* 1993, Hopper 1993, Weller 1994). The mating system of a species is an important microevoloutionary force which influences fecundity and fitness of individuals, their propagules and the resilience and persistence of populations. The mating system also affects evolutionary potential (Allard 1975, Brown *et al.* 1989), through its influence on genetic variation which reciprocally determines the pattern of mating and gene flow (Hamrick 1983, Waller 1993). Plants exhibit a diverse array of mating systems which vary from complete and obligate selfing to highly outcrossed systems (Brown 1990). In many instances these systems are not fixed, varying spatially and temporally at all levels of organisation within a species. This variation, described by the mixed mating model (Aide 1986, Barrett and Eckert 1990, Hamrick and Godt 1990), provides reproductive assurance and increases the opportunity for maximising fitness. The level of outcrossing of a species with a mixed mating system is indicative of different demographic process, life history strategies and functional relationships (Lande and Schemske 1985). The apparent reduction in fecundity and high quality progeny of a species which resprout after stress and disturbance compared with nonsprouters, which have abundant progeny with lower fitness expectations, exemplifies how adaptive forces may influence mating systems (Carpenter and Recher 1979, Zammit and Westoby 1987).

The fidelity of mixed mating and the extent to which a species outbreeds is determined by intrinsic attributes which govern gamete union and post-zygotic selection together with the extrinsic ecological circumstances of individual flowers, inflorescences, plants and populations (Huenneke 1991, James 1996, Sampson *et al.* 1996). Ecological factors, such as mode of reproduction, pollination mechanism, architectural complexity of flowering structures, phenology, seed dispersal and dormancy, population size and density, life cycle and successional stage, all influence mixed mating system parameters and affect reproductive success (Brown *et al.* 1989, Loveless and Hamrick 1984, Huenneke 1991). For many species, moderation of the spatial and temporal randomness of pollen transfer and pollination may be the principal force influencing a species' mating system (Brown *et al.* 1989). However, other selective forces, such as population size and density (Barrett and Kohn 1991, Ellstrand and Elam 1993, Oostermeijer *et al.* 1994), may operate simultaneously and may represent even stronger selective influences (Lande and Barrowclough 1987, Waller 1993).

Apart from their influence on genetic factors and mating systems, ecological factors also constrain reproductive success through various intrinsic and extrinsic processes, which are not necessarily mutually exclusive and can function synergistically (Haig and Westoby 1988, Stephenson 1992, Lawrence 1993). These ecological factors primarily constrain reproductive success through pollen and nutrient limitations (Ehrlén 1992, Vaughton and Ramsey 1995, Wuff 1995), although predation and pathogen limitation may also be deleterious (Janzen 1971, Travis 1992, Siemens 1994, Ehrlén 1995a, 1995b). Sexual selection theory predicts that plants should respond to such adaptive forces by maximising investment in functions which yield the greatest return per unit effort. The selective sequential adjustment of maternal resources between successive reproductive developmental stages is one such adaptive response (Lloyd 1980, Lloyd *et al.* 1980, Stephenson 1992). The matching of finite maternal resources with expenditure on reproduction at each developmental stage, promoted by selective sequential adjustment, increases the opportunity to maximise plant fitness.

Another adaptive response to the forces constraining reproductive success is the production of 'surplus' flowers (Stephenson 1981, Sutherland 1986). 'Surplus' flowers also permit the regulation of maternal resource commitments under varying stochastic influences thereby maximising the opportunity for reproductive success (Stephenson 1992). This adaptive response confers a considerable fitness advantage to plants, as evident by the proliferation of the phenomenon and analogous low fruit/seed flower ratios in numerous species (Sutherland and Delph 1984, Sutherland 1986a, Cohen and Dukas 1990). Ultimate explanations submitted to explain the advantage that 'surplus' flowers confer to reproductive success focus on: (1) the ability to capitalise on temporal and spatial variation in constraining factors during unpredictable periods - the bet hedging hypothesis (Ehrlén 1991, Guitián 1993); (2) the enhancement of male function where the benefit of increasing the potential for pollen donation surpasses expenditure on female function - the male fitness hypothesis (Campbell 1989, Walker and Whelan 1991); (3) increasing the attractiveness of the floral display to pollinators thereby reducing the potential for pollen/pollinator limitation - the attractiveness hypothesis (Vaughton and Ramsey 1995, Harder and Barrett 1996); (4) the selective abortion of zygotes with low fitness expectations - the selective abortion hypothesis (Kozlowski and Stearns 1989, Vaughton and Carthew 1993); and (5) a reduction in predispersal fruit/seed predation through a disproportionate decrease in herbivore foraging by limiting the amount of food available - the predation avoidance hypothesis (Scott 1992, Siemens *et al.* 1992).

As with identifying the ultimate forces influencing the mating system of plants, determination of the ultimate stimuli for 'surplus' flowers is difficult, because similar patterns of reproductive success are predicted by different hypotheses and functional relationships with varying adaptive forces (Ayre and Whelan 1989, Ehrlén 1991, Goldingay and Whelan 1993). Consequently, several possible adaptive functions for 'surplus' flowers have been identified in any one study (Guitián 1993, Lawrence 1993, Vaughton and Carthew 1993, Siemens 1994).

This thesis investigates the major attributes controlling reproductive success, including the mating system, of *Banksia tricuspis* and strives to provide functional explanations for the role of genetic and ecological factors in constraining population demographic and life history processes.

1.2 *Banksia tricuspis*

Banksia tricuspis Meissner (Proteaceae) is a rare species endemic to the Gairdner Range, 200 km north of Perth, Western Australia. The species is known from 19 030 individuals, representing 72 populations (van Leeuwen *et al.* 1990), which occur over a geographic range of 13 km predominantly within the Lesueur National Park (see Figure 2.1, page 22). *B. tricuspis* is listed in the Department of Conservation and Land Management's (CALM) Schedule of Declared Rare Flora (Hopper *et al.* 1990) and is considered to be a 'vulnerable' species (A. A. Burbidge, personal communication). CALM defines 'vulnerable' in Policy Statement No. 50 (1994) as a taxon that faces a high probability of extinction in the wild in the medium-term future and needs ongoing research and monitoring. This definition concurs with the meaning of 'vulnerable' as presented in the international recognised system of the International Union for the Conservation of Nature (Mace *et al.* 1992).

This species is likely to be removed from the Schedule of Declared Rare Flora following recommendations of the Western Australian Threatened Flora and Fauna Ranking Panel (K. J. Atkins, personal communication). The panel no longer considers the species to be vulnerable to extinction in the medium-term future. This recommendation was primarily promoted by the declaration of the Lesueur National Park in January 1992, the formulation and implementation of an approved management plan (CALM 1995) and retraction by CRA Exploration Pty Ltd of its Notice of Intent to develop an open-cut mine and coal-fired power station in the Lesueur area. Continued monitoring will ensure that ecological processes, management regimes and development interests do not propel this species towards a threatened status again. Potential forces and actions which may pose a threat include inappropriate fire management regimes (van Leewuen *et al.* 1990), changes in the abundance of cockatoos promoting discord in the biotic interaction between inflorescences, insects and cockatoos (Lamont and van Leeuwen 1988, Lamont 1994), the further encroachment of *Phytophthora* species (Hill 1990), and the alienation of coal reserves from the relatively secure land tenure afforded by the national park.

Previous studies have provided details on the taxonomy, demographics, pollination biology and fire ecology of this species (George 1981, van Leeuwen and Lamont 1986, Taylor and Hopper 1988, Lamont and van Leeuwen 1988, Hopper *et al.* 1990, van Leeuwen *et al.* 1990, Lamont 1992, Lamont 1994). These fields of investigation will not be examined further in this thesis, but existing knowledge has been integrated into the discussion and conclusion sections.

1.3 Thesis objectives

This thesis describes reproductive success and the mating system of *Banksia tricuspis* and attempts to identify genetic and ecological factors responsible for fitness variability. Overall, the aim is to assess the role of genetic and ecological factors in constraining fitness and provide functional explanations for their adaptive significance and impact on demographic and life history processes. These investigations were undertaken to provide an understanding of the role that genetic and ecological factors play in determining plant fitness and to clarify their importance in the persistence and perpetuation of *B. tricuspis* and other banksias.

The approach taken throughout this dissertation is one of descriptive interrogation and analysis of reproductive success patterns observed under natural conditions. Opportunities for experimental manipulation to test for causation were negligible given the constraints imposed on experimental design and replication by the high incidence of inflorescence predation (Lamont and van Leeuwen 1988), the long generation time of the species, difficulties associated with manipulation of the characteristically large inflorescences and the time limitations imposed on this investigation

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Chapter 2 assesses the mating system of this *Banksia* and attempts to furnish explanations for contrasting patterns identified between eight populations. Population size and disturbance history are cited as plausible explanations. In Chapter 3, fidelity between floral damaging animals and reproductive success is appraised. The deleterious impact of this constraint on fitness and the axiomatic importance of insects and floral displays are reaffirmed. Chapter 4 examines temporal variations in fitness over three successive years and uncovers reproductive synchrony, continuity and predictability in fecundity and a compensatory response to floral mortality. Stochasticity in maternal fecundity and progeny fitness within and between inflorescences/infructescences is considered in Chapters 5 and 6. Heterogeneity in resource provisioning and pollen/pollinator limitation, together with non-stringent mate choice, are promoted as explanations for the observed variation in fecundity and fitness. The relationship between inflorescence display and pollination foraging behaviour is examined in Chapter 7. Inflorescence attractiveness is found to exert a considerable deterministic influence on pollinators which may account for the stochasticity of fecundity and fitness reported in previous chapters.

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CHAPTER 2

THE MATING SYSTEM OF *BANKSIA TRICUSPIS*

2.1 Introduction

Plants exhibit a variety of mating systems, ranging from self-incompatible, obligate outcrossers to self-compatible with obligate self fertilisation (Innes and Hermanutz 1988). A quantitative determination of a species' mating system is paramount in understanding the dynamics of genetic change over time and between generations (Brown 1979, Clegg 1980) and is of primary importance in species' conservation studies (Brown 1978, Hopper and Coates 1990). The mating system fundamentally influences the distribution, transmission and pattern of genetic variation among and within populations and determines the rhythm and mode of evolutionary change between generations (Allard *et al.* 1975, Loveless and Hamrick 1984). In general, populations of inbreeders are expected to be less heterozygous and more highly differentiated from one another than populations of outbreeders (Levin 1978, Brown and Jain 1979, Schoen 1982).

Traditionally, mating systems have been inferred by analysis of floral morphology (Faegri and van der Pijl 1979, Schoen 1982), experimental crossings (Carpenter and Recher 1979, Goldingay and Whelan 1990), pollen-ovule ratios (Cruden 1977, Cruden *et al.* 1990) and observations on pollinator behaviour (Clegg 1980, Waddington 1983). Although reflecting the mating system, such approaches provide qualitative estimates only and are inadequate for accurately predicting the levels of outcrossing in open-pollinated plant populations (Brown *et al.* 1975, Hamrick 1982). The identification of polymorphic allozymes, through biochemical techniques and the development of statistical models and estimation procedures, has proven a powerful tool in providing quantitative estimates of plant mating system parameters (Brown *et al.* 1975, 1989, Brown 1978, 1979, Clegg 1980, Hamrick 1989). Such an approach has been used to determine the mating system of numerous plant species (Schemske and Lande 1985, Barrett and Eckert 1990), including members of the Australian genera *Eucalyptus* (Brown *et al.* 1975, Moran and Brown 1980, Hopper and Moran 1981, Sampson *et al.* 1989, Sampson *et al.* 1995), *Banksia* (Scott 1980, Carthew *et al.* 1988, Coates and Sokolowski 1992, Sampson *et al.* 1994, Vaughton and Carthew 1993) and *Acacia* (Coates 1988, Moran *et al.* 1989).

The mixed mating model (Fyfe and Bailey 1951) is the simplest and most frequently employed estimation procedure to determine mating system parameters (Brown and Allard 1970, Brown *et al.* 1985). This robust method divides mating into two components, on the grounds that a proportion of the progeny arises from mixed self-pollination and the remainder is the product of independent random mating with other plants in the population (Ritland 1983). This dichotomy is acceptable when the principal assumptions of this model are satisfied (Clegg *et al.* 1978, Brown and Allard 1970). These assumptions are that:

- mating events are the result of random outcrossing (with probability t) or self-pollination (with probability $s = 1 - t$);
- pollen pool allele frequencies are homogeneous among maternal parents;
- the rate of outcrossing is independent of maternal genotype;
- maternal parents exhibit identical mating systems; and
- selection does not intervene between fertilisation and the determination of progeny genotypes.

Violations of one or more of these assumptions frequently occurs in natural plant populations as the key assumptions of random mating are unlikely to be satisfied (Clegg 1980, Brown *et al.* 1989, Hamrick 1982). In particular, random mating is likely to involve biparental inbreeding (mating with sibs) as a result of mating between similar or adjacent plants. Violations of these assumptions may lead to significant disagreement in estimates of outcrossing for different allozymes and result in underestimates of the true outcrossing rate, especially if single locus estimation procedures have been employed (Ritland 1984, Bijlsma *et al.* 1986). Non-conformity with the assumptions of this model are especially serious in animal-pollinated species (Schoen and Clegg 1984), as matings are influenced by pollinator characteristics and will not be the result of random events.

Violations may also result from demographic, life history, genetic and ecological factors. The assumption of a homogeneous pollen pool over maternal parents is not justified when temporal or spatial heterogeneity in the distribution of pollen types occurs within a population (Schoen and Clegg 1984, Brown *et al.* 1989, Sampson *et al.* 1990). Ecological variables which influence these factors include local plant densities (Vasek and Harding 1976, Brown *et al.* 1978, Vaquero *et al.* 1989), subpopulation structuring (Ennos and Clegg 1982, Sampson *et al.* 1989), habitat dryness (Allard *et al.* 1977, Holtsford and Ellstrand 1992), variation in male and female fertility (Schoen and Stewart 1986, Brown *et al.* 1989), restricted pollen dispersal (Schmitt 1983), limited pollen carryover (Thomson and Plowright 1980), mode of pollination (Aide 1986), and variation in floral morphology (Holtsford and Ellstrand 1992).

In recent years, several novel approaches to the analysis of plant mating systems have been developed (Ritland 1984, 1989, Brown *et al.* 1985, Schoen 1988, Brown *et al.* 1989, Devlin *et al.* 1989). Among these is the 'effective selfing model' which computes estimates of the total amount of selfing, including both self fertilisation and mating with relatives in spatially structured populations (Ritland 1984, 1985). The assumptions upon which this model are based are:

- homogeneity of allele frequencies between mates;
- equality of gene frequencies among pollen and ovule generations;
- independence of mate selection from genotype; and
- open pollination.

This model is constrained by the assumption that a sample of maternal parents are a random sample of the total population gene pool. This model differs from the mixed mating model as it distinguishes between the effective selfing rates of inbred *vs* outbred parents. As a result, the mixed mating model gives biased estimates of effective selfing when mating among inbred relatives occurs (Ritland 1984).

Knowledge about the mating systems of *Banksia* and other proteaceous genera is limited (Ayre and Whelan 1989, Walker and Whelan 1991), despite a considerable volume of information on their reproductive biology (see reviews by Lamont *et al.* 1985, Collins and Rebelo 1987). Traditional methods of estimating mating systems for *Banksia* species have included controlled cross- and self- pollination trials

(Carpenter and Recher 1979, Paton and Turner 1985, Collins and Spice 1986, Goldingay and Whelan 1990, Vaughton and Ramsey 1991 Vaughton and Carthew 1993), estimations of pollen-ovule ratios (van Leeuwen and Lamont 1986, Lamont and Barrett 1988) and observations of pollinator foraging behaviour (Hopper 1980, Turner 1982, Wooller *et al.* 1983, Goldingay *et al.* 1987, Vaughton 1990, Carthew 1993, 1994). These qualitative studies have indicated considerable variation in the levels of outcrossing among species.

Biochemically based estimates of mating system parameters for *Banksia* have been determined for seven taxa. Scott (1980) estimated outcrossing rates in *Banksia attenuata* and *B. menziesii* which approached levels predicted for panmixia ($t = 1$). Carthew *et al.* (1988) and Vaughton and Carthew (1993) reported similar outcrossing rates in *B. paludosa* and two varieties of *B. spinulosa*. These estimates are among the highest reported for plant species (Schemske and Lande 1985). Subsequently, Coates and Sokolowski (1992) reported levels of outcrossing significantly less than unity ($s < 1$) in roadside populations of *B. cuneata*, while Sampson *et al.* (1994) obtained similar high levels of selfing in two undisturbed populations of *B. brownii*. These investigations concluded that environmental and genetic characteristics can significantly influence the mating systems of banksias.

This chapter investigates the mating system of *Banksia tricuspis*, as determined by the mixed mating and effective selfing models, for progeny resulting from open pollination events. Estimates are presented for the inbreeding coefficient or Wright's fixation index, outcrossing rate, effective selfing rate and various other measures used to determine plant mating system parameters. The study was designed to determine the mating system of *B. tricuspis* and identify the influencing environmental and genetic characteristics which affect the mating system of this species. I envisage this study assisting with the development of optimal strategies for the management and conservation of this species.

2.2 Materials and Methods

2.2.1 *Sampling*

Eight populations of *B. tricuspis*, which ranged in size from one to 4 150 plants, were selected from the 72 populations of the species for mating system analysis (Figure 2.1, Table 2.1). In P6, P8 and the three smallest populations, P1 P2 and P3, all mature plants (plants contributing to the pollen pool in the current (1987) flowering season) were sampled. In P4, P5 and P7, plants were sampled within 5 m wide transects. Individuals sampled within the transects were greater than 10 m apart and the transects were terminated after ten plants were encountered. Transects ranged in length from 90 to 570 m.

The maternal genotype of each sampled plant was determined from pollen samples collected in 1987, by removing the distal end of 10 recently anthesed pollen presenters from a single inflorescence per plant. Pollen samples were stored in liquid nitrogen until subsequent biochemical analysis. Seeds used to determine progeny arrays were obtained from one-year-old cones on sampled plants in 1988. Seeds were extracted using wet-dry cycles (Cowling and Lamont 1985) and stored in paper bags prior to biochemical analysis. The sampling design ensured that seeds and pollen samples were obtained from the same flowering event, thereby removing any bias as a result of any temporal difference in gene frequency within population pollen pools between years.

2.2.2 *Electrophoretic procedure*

A random sample of approximately 10 to 20 seeds (offspring) from each plant (family) was germinated on moistened filter paper at 15°C for 14 days (Lamont and van Leeuwen 1988). In cases where fewer seeds were available all were germinated. In P1, all seeds obtained from the one plant were assayed. The emerging radical and associated embryonic material was removed from the testa for assay. This material was homogenised in 120 μL of 100 mmol L^{-1} Tris HCl buffer, pH 7.5 containing 5% (w/v) polyvinylpyrrolidone, 10% sucrose (w/v), 0.17% EDTA (w/v), 0.02% ascorbic acid (w/v), 0.1% bovine serum, 0.05% NAD (w/v), 0.02% NADP (w/v) and 0.01% β

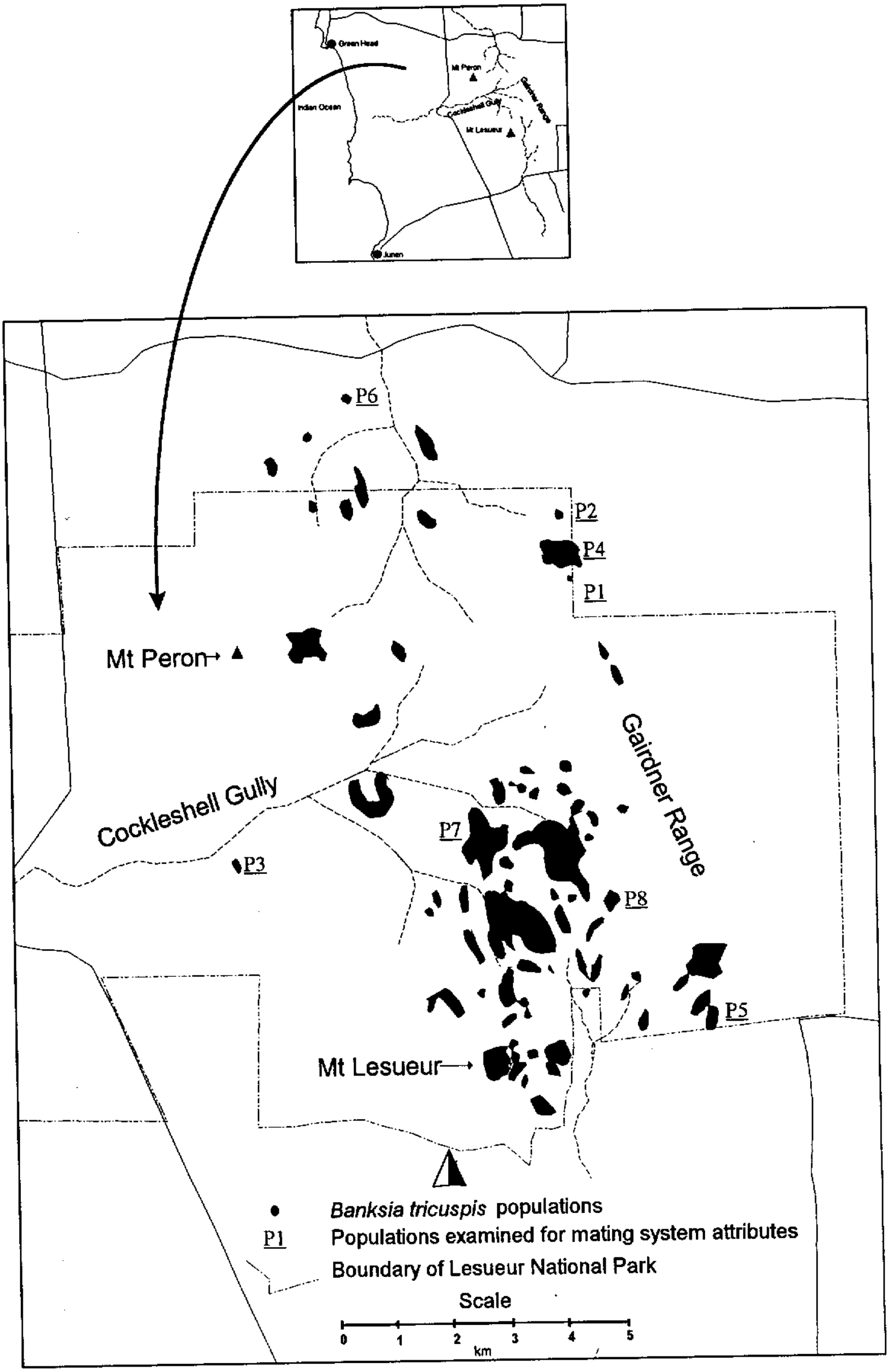

Figure 2.1 Distribution of *Banksia tricuspis* and the eight populations examined for mating system attributes.

-mercaptoethanol. Pollen samples were treated in the same manner with one replicate per plant, to confirm maternal genotype determination.

Electrophoresis was conducted using the Helena Laboratory cellulose acetate plate system (Coates 1988, Hebert and Beaton 1989) at 200 mV for 45 minutes using 80 mmol L^{-1} Tris, 1 mmol L^{-1} EDTA, 1 mmol L^{-1} $MgCl_2$ buffered to pH 8.2 with maleic acid. Isozyme activity was detected using the methods described by Coates (1988). These procedures are similar to those presented by Wendel and Weeden (1989) with slight modification for 1.75% agar overlays.

Samples were assayed for two enzyme systems: phosphoglucomutase (PGM, E.C. 5.4.2.2) and malate dehydrogenase (MDH, E.C. 1.1.1.37). These enzyme systems were selected because previous surveys (van Leeuwen, unpublished data) of allozyme variation on seventeen systems demonstrated that they were polymorphic and had suitable allele frequencies at the Pgm-1 and Mdh-2 locus for mating system estimations. Genetic interpretation of zymograms was inferred from segregation patterns of open-pollinated progeny arrays.

2.2.3 *Mating system estimates*

Maternal genotypes were obtained from direct interpretation of segregation patterns on zymograms. Where resolution was poor and maternal genotypes were unknown they were estimated from arrays of at least ten progeny per tree employing the method of Brown and Allard (1970) in the computer program developed by Ritland and Jain (1981). Allelic frequencies were calculated for the most common alleles of the diallelic Pgm-1 locus and the two most common alleles for the triallelic Mdh-2 locus. Variance of these estimates were computed using the formula of Brown and Weir (1983). Observed average heterozygosity values, together with those expected under Hardy-Weinberg equilibrium, were calculated for maternal and progeny arrays at each locus for each population. Arithmetic means and variance were determined.

Estimates of mating system parameters were based on the coefficient of inbreeding, or Wright's fixation index (F) (Wright 1969), the mixed mating model ($\hat{t}$) (Ritland and Jain 1981), the effective selfing model (E) (Ritland 1984) and the covariance of effective selfing with parental gene fixation (D) (Ritland 1986). Wright's fixation index highlights any deficiency of heterozygotes which result from mating between individuals more closely related than expected under Hardy-Weinberg equilibrium ($F = 0$). Values of F approaching zero indicate panmixia. Negative F values, indicating an excess of heterozygotes compared with Hardy-Weinberg proportions, may in part be associated with disassortative mating and selection favouring heterozygotes. Positive values, indicating a deficit of heterozygotes, may imply assortative mating and selfing. Maternal (F_m) and progeny (F_p) values of Wright's fixation index and variance were estimated at each locus for all plants using methodologies employed by Brown *et al.* (1975) and Phillips and Brown (1977) respectively. An arithmetic mean for the fixation indexes obtained for each locus were calculated for each population. The variance of the mean was calculated using the methodology of Brown and Weir (1983) where the variance is the sum of the separate locus variances divided by the total number of loci.

Mixed mating model estimations for outcrossing rate ($\hat{t}$) were made using the maximum likelihood procedures developed by Ritland and Jain (1981) and provided in the computer programs MULTI and VTP. Single-locus and multilocus estimates of the mixed mating model were obtained using this procedure. The multilocus procedure is considered to be superior, as it is based on the behaviour of all loci simultaneously, is more resistant to bias introduced by biparental inbreeding and is least affected by violations of the mixed mating model. Estimations using the procedure of Ritland and Jain (1981) were made on populations with greater than 20 families (sampled plants) as the method of calculating maternal parentage (Brown and Allard 1970) is biased towards assigning the most frequent genotype in the population to maternal parentage. Arithmetic and minimum variance means (El-Kassaby *et al.* 1987) of outcrossing rate over loci were calculated for single locus estimations in each population.

Single ($\hat{t}_s$) and multilocus ($\hat{t}_m$) mean estimates of outcrossing and their variance for the three smallest sample populations were determined using the procedures outlined by Ritland (1983) and Shaw *et al.* (1981), respectively. Both procedures require the maternal genotype to be known and are based on the number of novel alleles (outcrossed) present in the progeny of a homozygous parent. As noted by

Brown *et al.* (1989) and Ritland (1983), bias in the estimation of $\hat{t}$ occurs when these prediction procedures are employed because the progeny of heterozygous parents are ignored as matings are ambiguous.

The effective selfing model estimates the apparent amount of selfing at a single locus caused by either self-fertilisation or mating between genetically similar individuals (Ritland 1984, 1986). The effective selfing rate (E) is the mean proportion of effective selfing in the population calculated as a minimum variance average over all loci and may be defined by:

$$E = Fs^i + (1 - F)s^o$$

where F, s^i and s^o are the joint maximum likelihood estimates of the fixation index of the parents, the selfing rate of parents with inbred loci (fixed alleles) and the selfing rate of parents with outbred loci (alleles not fixed), respectively. When $F = 0$ or $s^i = s^o$ the effective selfing model is equivalent to the mixed mating model. E equals the self-fertilisation (s) rate when there is no mating with non-self relatives. Programs developed by Ritland (1984, 1985, 1986) were used to calculated the mean values for these estimates for populations with >20 plants as sufficient numbers of maternal genotypes are needed to avoid unreliable estimations. P1 to P3 were therefore neglected in this analysis. Variance estimations were obtained by differential approximation (Ritland 1986). The generational change in the fixation index (ΔF) from parent to progeny ($F_m - F_p$) was also calculated using the procedures of Ritland (1986).

The covariance of effective selfing with parental gene fixation (D) is a measure of the tendency for inbred individuals to effectively self, contrary to the population average. The inbred assortative selfing rate is defined as:

$$D = F(1 - F)(s^i - s^o).$$

When $D = 0$ the mixed mating and effective selfing models are mathematically identical, although mating between relatives may still be occurring. Positive D may be attributed to population structuring and mating between relatives (Ritland and Ganders 1987).

According to the mixed mating model (Brown *et al.* 1975, Clegg 1980), maternal plants are expected to receive a random sample of pollen from the pollen pool thereby inferring homogeneity in the proportion of heterozygous progeny among homozygous maternal plants at each locus. Therefore, homozygous maternal plants should, on average, show the same proportion of heterozygous progeny. Homogeneity in the pollen pool of maternal plants at each locus was examined using the *G* test statistic (Sokal and Rohlf 1995).

The multilocus procedures used to estimate outcrossing rate ($\hat{t}$) and the effective selfing rate (*E*) requires that alleles at each locus be assorting independently of those at other loci. Similarly, single loci estimates can only be considered independent if loci are not correlated and are therefore in linkage disequilibrium. To test for correlations the composite measure of linkage disequilibria (Δ_{AB}), together with chi-squared statistics, were calculated for pairwise combinations of the two most frequent loci in all populations. Estimates were calculated using the program LD86 as defined by Weir (1990). If Δ_{AB} is equal to zero loci are considered to be assorting independently.

Genetic homogeneity of maternal plants was also investigated to determine if the distribution of genotypes was random or spatially heterogeneous. Spatial heterogeneity, i.e. population substructuring, can severely bias outcrossing estimates in favour of selfing (Ennos and Clegg 1982). The distribution of the two most common genotypes at each locus along sampled transects in P4, P5 and P7 and along three randomly placed transects through P6 and P8 were examined using run tests (Sokal and Rohlf 1995). Spatial heterogeneity was not examined in the remaining populations as their small population size precluded statistical analysis. Run tests were also used to examine spatial heterogeneity in the ten nearest neighbour plants in P6 and P8, for three randomly selected plants within each population.

2.3 Results

Table 2.1 summarises population and sample statistics for the eight populations of *B. tricuspis* for which mating system parameters were determined. The number of plants sampled per population ranged from <1 per cent to the entire population (100%). Offspring sampled per family ranged from 10 to 94 per plant.

Table 2.1 Population and sample statistics for the eight *Banksia tricuspis* populations examined for their mating system attributes. (Offspring/family = seeds/plant.)

Population	Total number of plants	Number of plants sampled	Offspring/family
1	1	1	94.00
2	3	3	22.00
3	5	4	12.75
4	350	20	10.00
5	108	20	20.00
6	24	23	18.26
7	4150	30	10.00
8	89	78	10.00

Allelic frequencies estimates for the maternal and progeny gene pools are summarised in Table 2.2. No significant differences were detected in the gene pool frequency between maternal plants and their progeny. The dominant allele recorded for the diallelic locus *Pgm*-1 in the maternal gene pool of P2 and P3 was converse that recorded in the progeny gene pool. Similarly, in the triallelic locus *Mdh*-2, the frequency of the two most common alleles in the maternal gene pool of P7 and P8 differed from that recorded for the progeny gene pool. Estimated allelic frequencies for both loci in the maternal and progeny gene pools were heterogeneous between populations, with the exception of the *Pgm*-1 maternal gene pool.

H_O frequencies were generally lower than expected under Hardy-Weinberg equilibrium for both the maternal and progeny genotypes, indicating a deficit of heterozygotes (Table 2.3). Such deviations, although not significant, may imply departure from random mating. Failure to adhere to this trend was recorded in the maternal gene pool of P6 and the progeny gene pool of P1, P2 and P6 where H_O was in excess of H_e. In all but one population, the difference between H_O and H_e was not significant ($P < 0.05$). However, in P8 the H_e estimate was significantly greater than the H_O estimate. H_O values obtained for the maternal gene pool were, with the exception of P3 and P5, lower than those recorded in the progeny gene pool, although the differences were not significant.

Table 2.2 Estimated allele frequencies for maternal and progeny gene pools for the eight populations of *Banksia tricuspis* on which mating system studies were conducted. The first allele for the diallelic and first two alleles for the triallelic loci are given. Standard errors in parentheses.

	Locus				
	Pgm-1		*Mdh*-2		
Population	A1	A2	A1	A2	A3
Maternal					
1	1.00 (0.00)		1.00 (0.00)		
2		0.83 (0.02)	1.00 (0.00)		
3	0.62 (0.04)		0.62 (0.04)	0.38 (0.04)	
4	0.60 (0.03)		0.40 (0.05)	0.31 (0.04)	
5	0.85 (0.02)			0.28 (0.05)	0.55 (0.06)
6	0.57 (0.04)		0.80 (0.01)	0.11 (0.01)	
7	0.64 (0.03)		0.41 (0.07)		0.32 (0.06)
8	0.67 (0.04)		0.34 (0.05)		0.38 (0.05)
Progeny					
1	0.81 (3.12)		0.80 (3.15)	0.20 (3.15)	
2	0.63 (0.30)		0.82 (0.27)	0.18 (0.27)	
3		0.61 (0.14)	0.55 (0.12)	0.45 (0.12)	
4	0.55 (0.02)		0.35 (0.02)	0.37 (0.02)	
5	0.81 (0.02)			0.42 (0.06)	0.44 (0.06)
6	0.58 (0.02)		0.74 (0.01)	0.13 (0.01)	
7	0.60 (0.01)		0.35 (0.02)	0.37 (0.02)	
8	0.64 (0.00)			0.37 (0.01)	0.34 (0.01)

Estimates of the mean F statistics were higher for maternal plants in P4, P7 and P8 than in their progenies (Table 2.4). Conversely, in the remaining populations, progeny F values were higher than maternal values. These differences were only significant in P7 and P8 implying heterozygote excess in the maternal gene pool of these populations relative to their progeny. Negative values of F, indicating possible disassortative mating and selection favouring heterozygotes, were recorded in the maternal trees in P6 and progeny in P1, P2, P3, and P6. Mean values of F approaching panmixia ($F = 0$) were only obtained in P2 and P3 for maternal trees and P1 and P2 for progeny.

Table 2.3 Observed and estimated heterozygosity (± SE) frequencies for maternal plants and their progeny in eight populations of *Banksia tricuspis*.

(H_o = observed heterozygosity, H_e = expected heterozygosity.)

Population	H_o	H_e
Maternal		
1	0.00 (0.00)	0.00 (0.00)
2	0.17 (0.17)	0.17 (0.17)
3	0.50 (0.25)	0.54 (0.00)
4	0.50 (0.10)	0.58 (0.09)
5	0.32 (0.02)	0.43 (0.17)
6	0.48 (0.11)	0.43 (0.07)
7	0.41 (0.05)	0.57 (0.10)
8	0.46 (0.05)	0.58 (0.11)*
Progeny		
1	0.38 (0.01)	0.31 (0.01)
2	0.45 (0.08)	0.38 (0.08)
3	0.47 (0.12)	0.49 (0.01)
4	0.54 (0.03)	0.58 (0.08)
5	0.30 (0.00)	0.46 (0.15)
6	0.50 (0.01)	0.45 (0.04)
7	0.52 (0.04)	0.57 (0.09)
8	0.48 (0.03)	0.56 (0.10)*

Significance: * $P < 0.05$.

Single, minimum variance mean and multi locus estimates of outcrossing are presented in Table 2.5. Estimates of $\hat{t}_s$ per locus ranged from 0.460 to 1.176 and were significantly heterogeneous among loci within all populations except P1 and P6. Pooled over loci, minimum variance mean estimates of $\hat{t}_s$ ranged from 0.476 to 1.062 with a mean of 0.787. These minimum variance mean estimates were significantly less than unity ($t = 1$) in all populations, except P3 and P6. There were no significant differences recorded between either of the single locus estimates of $\hat{t}$ for each population. In all but P6, $\hat{t}_m$ was significantly less than unity, ranging from 0.707 to 1.022 with a mean of 0.811. Both $\hat{t}_s$ and $\hat{t}_m$ estimates were homogeneous among populations. Significant deviations between $\hat{t}_s$ and $\hat{t}_m$ estimates were only recorded in P1 and P2, a feature which may be attributed to the differences in mathematical procedures used to obtain the estimates. Both $\hat{t}_s$ and $\hat{t}_m$ estimates were homogeneous between populations. The $\hat{t}_m$ values obtained for P3 and P6, which

Table 2.4 **Values for Wright's fixation indices (± SE) at each of two loci for maternal plants (F_m) and their progeny (F_p) in *Banksia tricuspis*.** (* rejection of the null hypothesis that F equals zero ($P < 0.05$), ◆ significant difference between F_m and F_p ($P < 0.05$).)

Population	Locus				Mean	
	Pgm-1		*Mdh*-2			
	F_m	F_p	F_m	F_p	F_m	F_p
1	-	-0.22 (0.98)	-	-0.24 (0.93)	-	-0.23 (0.48)
2	0.00 (0.67)	-0.13 (0.31)	0.00 (0.00)	-0.21 (0.16)	0.00 (0.17)	-0.17 (0.12)
3	0.53 (0.21)	-0.66 (0.40)	-0.40 (0.31)	0.29 (0.20)	0.07 (0.13)	-0.18 (0.05)*
4	0.19 (0.09)	-0.02 (0.05)	0.11 (0.09)	0.15 (0.04)*	0.15 (0.05)*	0.07 (0.02)*
5	-0.15 (0.06)*	0.01 (0.05)	0.42 (0.06)*	0.51 (0.02)*	0.14 (0.03)*	0.26 (0.02)*
6	-0.18 (0.08)*	-0.05 (0.04)	-0.03 (0.09)	-0.16 (0.04)*	-0.09 (0.04)	-0.11 (0.02)*
7	0.24 (0.06)*	-0.00 (0.03)◆	0.31 (0.05)*	0.17 (0.03)*◆	0.27 (0.03)*	0.08 (0.02)*◆
8	0.10 (0.03)*	0.03 (0.01)*◆	0.24 (0.02)*	0.24 (0.01)*◆	0.17 (0.01)*	0.13 (0.01)*◆

Table 2.5 **Single and multilocus estimates (± SE) of outcrossing ($\hat{t}$) for eight populations of *Banksia tricuspis* based on the mixed mating model and calculated from maternal and progeny genotype arrays.** ($^{\oplus}$ single locus estimate calculated using the procedure of Ritland (1983) and multilocus estimate calculated following the procedure of Shaw *et al.* (1981); ♦ significantly less than panmixia ($t = 1$) ($P < 0.05$); * $P < 0.05$, ** $P < 0.01$ for G test of $\hat{t}_s$ over the two loci (df = 2 in all cases); ♣ significant difference between minimum variance mean and multilocus estimate of $\hat{t}$ ($P < 0.05$).)

Population	Locus		*G*	Minimum variance mean	Multilocus
	Pgm-1	*Mdh*-2			
1$^{\oplus}$	0.460 (0.004)♦	0.492 (0.004)♦	0.108	0.476 (0.045)♦	0.773 (0.007)♦ ♣
2$^{\oplus}$	0.846(0.009)♦	0.699 (0.013)♦	-3.953*	0.786 (0.073)♦	0.744 (0.006)♦ ♣
3$^{\oplus}$	0.844 (0.020)♦	0.898 (0.403)	10.314**	0.846 (0.138)	0.920 (0.011)♦
4	1.013 (0.096)	0.783 (0.065)♦	11.863**	0.876 (0.058)♦	0.840 (0.051)♦
5	0.980 (0.079)	0.702 (0.050)♦	17.250**	0.810 (0.047)♦	0.789 (0.042)♦
6	0.926 (0.068)	1.176 (0.057)♦	-1.397	1.062 (0.044)	1.022 (0.038)
7	0.895 (0.068)	0.613 (0.049)♦	17.967**	0.723 (0.046)♦	0.694 (0.041)♦
8	0.867 (0.048)♦	0.626 (0.049)♦	14.286**	0.721 (0.029)♦	0.708 (0.026)♦

were significantly different, were significantly greater than those recorded in the other populations examined. Small non-significant $\Delta\hat{t}$ values, the absolute difference between $\hat{t}_s$ and $\hat{t}_m$ which denotes the lower limit of biparental inbreeding (Ritland 1984), were recorded for all populations.

Values for E varied from 0.021 to 0.307 for the four populations examined and were similar in magnitude to estimates of selfing obtained from the mixed mating model ($s = 1 - t$) (Table 2.6). With the exception of P6, values were significantly different from zero, which is the condition that exists when there is no mating with self relatives. With the exclusion of P6 all other populations were homogeneous for E estimates. A significant decrease in ΔF, indicating changes in the rate of gene fixation between generations, was recorded in P4 only. With the exception of P5 the covariance of selfing with parental gene fixation (D) was negative in all populations (Table 2.6). D was not significantly different from zero in three of the five populations examined, the exceptions being P7 and P8.

Table 2.6 Mating system estimates (± SE) for five populations of *Banksia tricuspis* based on the effective selfing model. (E is the effective selfing rate, D is the covariance of effective selfing with gene fixation, ΔF is the change in F from parent to progeny and s is the selfing rate determined by $1 - \hat{t}_m$.)

Population	E	D	ΔF	s
4	0.222 (0.007)*	-0.073 (0.003)	-0.221 (0.006)*	0.160
5	0.217 (0.003)*	0.015 (0.001)	-0.059 (0.004)	0.211
6	0.021 (0.002)	0.000 (0.001)	0.059 (0.005)	-0.022
7	0.252 (0.004)*	-0.070 (0.004)*	-0.153 (0.006)	0.306
8	0.307 (0.002)*	-0.077 (0.001)*	-0.099.(0.002)	0.292

* Significantly different from zero ($P < 0.05$).

The estimates of composite linkage disequilibrium (Δ_{AB}) are given in Table 2.7. Generally, with the exception of P4, these estimates indicate little association between the two loci implying that single locus estimates of outcrossing and effective selfing are likely to be independent.

The possibility of heterogeneity in the pollen pool of each population was tested by examining heterogeneity in the proportions of heterozygous progeny among maternal homozygous plants for each locus (Brown *et al.* 1975). In general, *G* test results showed a lack of heterogeneity in the proportion of heterozygous progeny produced by homozygous adults indicating that, in most instances, maternal plants received a random sample of pollen (Table 2.8). Family structuring within most populations was negligible based on the homogeneous distribution of alleles (Table 2.9). The exception was P6 where significant levels of spatial heterogeneity were recorded along the transects and among nearest neighbours (Table 2.10).

Table 2.7 Estimates of composite linkage disequilibrium (Δ_{AB}) (± SE) between allozyme loci *Pgm*-1/*Mdh*-2 in the progeny of maternal plants from eight populations of *Banksia tricuspis*.

Population	Δ_{AB}
1	0.000 (0.001)
2	0.001 (0.003)
3	-0.023 (0.007)
4	0.006 (0.002)*
5	0.001 (0.002)
6	0.004 (0.002)
7	0.008 (0.004)
8	0.004 (0.003)

* significantly different from 0 ($P < 0.05$).

Table 2.8 *G* tests for heterogeneity in proportions of heterozygous progeny from homozygous maternal genotypes. (* rejection of the null hypothesis that the proportion of progeny from homozygous maternal plants was homogeneous among families ($P < 0.01$).)

Population	Locus			
	Pgm-1		*Mdh*-2	
	G	df	*G*	df
1	-	-	-	-
2	2.29	2	-	-
3	0.31	2	-	-
4	0.03	12	16.56	9
5	80.70*	15	-	-
6	5.64	7	102.29*	15
7	2.11	19	1.71	15
8	10.24	48	10.47	26

Table 2.9 Run test results on the level of spatial heterogeneity within five populations of *Banksia tricuspis* based on an analysis of the distribution of genotypes among the ten plants sampled per transect. (Standard errors in parenthesis, r = number of runs, μ_r = mean of the r statistic, * rejection of the null hypothesis that the two most common genotypes are randomly distributed along transects ($P < 0.05$).)

Population	Locus			
	Pgm-1		*Mdh*-2	
	r	μ_r	r	μ_r
4	5	4.75 (1.21)	4	3.67 (0.94)
	5	4.43 (1.18)	4	4.00 (0.92)
5	4	4.11 (0.91)	5	4.43 (1.18)
	5	5.20 (1.20)	5	3.86 (0.94)*
6	4	3.40 (0.91)*	4	2.50 (0.50)*
	3	2.60 (0.49)*	3	2.60 (0.49)*
	3	2.50 (0.50)*	4	3.00 (0.82)*
7	4	4.11 (0.91)	4	2.75 (0.43)*
	4	3.86 (0.94)	4	3.86 (0.94)
	5	5.00 (1.22)	4	3.67 (0.94)
8	4	4.00 (0.92)	5	5.00 (1.22)
	5	4.20 (0.88)*	6	5.44 (1.38)
	4	3.86 (0.94)	5	5.00 (1.22)

Table 2.10 Run test results on the level of spatial heterogeneity within two populations of *Banksia tricuspis* based on an analysis of the distribution of genotypes among the ten nearest neighbours. (Standard errors in parenthesis, r = number of runs, μ_r = mean of the r statistic, * rejection of the null hypothesis that the two most common genotypes are randomly distributed along transects ($P < 0.05$).)

Population	Locus			
	Pgm-1		*Mdh*-2	
	r	μ_r	r	μ_r
6	6	5.00 (1.22)*	6	4.43 (1.18)*
	6	4.11 (0.91)*	4	2.78 (0.42)*
	5	4.20 (0.88)*	2	1.00 (0.00)*
8	5	4.43 (1.18)	4	4.43 (1.18)
	6	5.44 (1.38)	5	4.43 (1.18)
	7	5.00 (1.31)*	5	5.00 (1.31)

2.4 Discussion

2.4.1 *The mating system*

Banksia tricuspis is a facultatively xenogamous species as determined by the mixed mating and effective selfing models. Estimates of $\hat{t}$ and E ranged from 0.694 to 1.022 and 0.021 to 0.307 respectively and concurs with mating system estimates obtained from pollen/ovule ratios and experimental pollination trials (van Leeuwen and Lamont 1986). Biparental inbreeding was recorded in all populations examined, although levels were not significant. Values for E could be mostly ascribed to geitonogamous mating - i.e. selfing. Differences in mating system estimates between the two models may be attributed, in most populations, to a F_m index which was significantly different from $F = 0$, the condition required for both estimation procedures to be mathematically identical.

The low $\Delta\hat{t}$ value reported for P6 corresponded with the highest estimate of $\hat{t}$, which approached unity. Together with an absence of D, these results imply that the E value obtained for this population could be attributed mostly to selfing. Further evidence supporting this case was provided by the absence of significant maternal gene fixation ($F_m = 0$) which implies notable levels of biparental inbreeding and may also indicate an absence of family structuring within populations. Non-significant differences between $\hat{t}_s$ and $\hat{t}_m$ also confirm the existence of a homogeneous pollen pool supporting the notion of no family structuring. Clearly, an anomaly exists in the mating system estimates for P6, as $\hat{t}_s$ which exceeds unity is suggestive of disassortative mating (Brown *et al.* 1985) and family structuring was evident in run tests (Table 2.10), which under normal conditions would promote biparental inbreeding.

It is suggested that the mating system anomaly detected in P6 has evolved as a result of modified pollinator assemblages and therefore changes in the amount and type of pollen received by flowers. P6 is a small population of 24 plants located in a 0.2 ha heathland remnant in otherwise cleared agricultural land. With the exception of a few insects, the only pollen vectors observed foraging on inflorescences of *B. tricuspis* in this remnant population were transient honeyeaters (Meliphagidae).

The foraging behaviour of honeyeaters, which predominantly move between inflorescences on different plants in this population (van Leeuwen, unpublished data), promoted xenogamous pollination events. Despite a trapping effort of 150 nights at P6, no mammalian pollen vectors were observed. The foraging behaviour of honey possums, *Tarsipes rostratus*, would most likely promote geitonogamous pollination events and biparental inbreeding in this *Banksia*, as they predominantly forage among inflorescences on the same plant and/or between near neighbours (van Leeuwen, unpublished data). It was suggested that the small heathland remnant at P6 no longer supported a honey possum population with the subsequent loss of this pollen vector increasing $\hat{t}$ levels, compared to undisturbed populations with intact pollinator assemblages. This suggestion supports the hypothesis that bird pollination promotes high levels of outcrossing in the Australian flora (Paton and Ford 1977, Hopper 1981) and concurs with Levin's (1981) proposition that differences in the foraging behaviour of floral visitors influences the mating system of plant populations.

The failure to detect high levels of biparental inbreeding in isolated populations, namely P2 and P3, when such populations are predisposed to mating events between selfed relatives, could be attributed to a lack of genetic structuring. However, such populations were of a size not significantly different from the maximum seed dispersal distances for the species which previously has been demonstrated to be 14 m (van Leeuwen and Lamont 1986). Therefore, the lack of genetic structuring may be discarded as the reason for failure to detect biparental inbreeding. Alternatively, selection against zygotes originating from consanguineous matings (matings with sibs) is proposed as a more acceptable explanation. Evidence, in the form of extremely high zygote abortions (Chapters 4, 5 and 6), supports a selection mechanism acting post-zygotically in this species. The existence of post-zygotic selection favouring heterozygotes has been reported in other species (Ledig 1986, Wein *et al.* 1987, Seavey and Bawa 1986, Waser 1993). If post-zygotic selection favours disassortative mating it may be responsible for the low levels of biparental mating recorded in all populations. Indeed, the level of seed abortion was homogeneous among populations and between individual populations and the pooled population estimate (Table 2.11).

Table 2.11 Statistics on percentage seed abortion within eight populations of *Banksia tricuspis* and significance of differences between means.

Population	$\bar{x}$	SE	t	df	P
1	30.00	-	-	-	-
2	31.63	7.23	1.0320	151	0.3037
3	41.97	2.69	0.1384	152	0.8901
4	38.44	2.51	-0.8750	168	0.3828
5	37.75	3.29	0.5081	168	0.6121
6	37.21	3.09	0.7113	170	0.4778
7	40.31	2.16	-0.3884	178	0.6981
8	39.96	1.80	-0.3023	198	0.7628
Pooled	41.23	1.04	-	-	-

In P6, where considerable family structuring exists, and in P2 and P3, where restricted gene dispersal is likely, it could be argued that values of $\hat{t}$ should be smaller due to the effects of consanguineous matings. However, maternal control over seed set in the form of a selection mechanism favouring heterozygotes, would bias estimates of $\hat{t}$ upwards, as appears to be the case here (see Chapters 5 and 6).

Spatial heterogeneity in $\hat{t}$ between populations appears to be a common phenomenon in plant species (Schoen 1982, Sampson *et al.* 1989, Coates and Sokolowski 1992, Sampson *et al.* 1995). It has been predicted that populations in equilibrium should exhibit predominantly selfing or outcrossing mating systems (Lande & Schemske 1985). However, Brown *et al.* (1989) noted that the multitude of ecological, demographic and genetic factors which influence a species' mating system, many of which vary between populations, may result in variable levels of outcrossing between populations. Spatial heterogeneity between *B. tricuspis* populations could have arisen as a result of differences in exposure to selection pressures induced by environmental perturbations which have resulted in localised genetic drift between populations. *B. tricuspis* is long-lived and such heterogeneity may also reflect differences in age class structure between populations and exposure of these cohort classes to different selective forces during their life cycle.

Further evidence on the effect of environmental perturbations, particularly habitat modification, on the mating system of this species were available from P7, the only sampled population razed by the 1985 fire in the Lesueur area. I postulate that the low $\hat{t}_m$ estimate recorded in this population was a consequence of habitat modification induced by burning. Inflorescence production is nonexistent during the first two post-burn years in *B. tricuspis* with the re-attainment of normal production taking up to five years (van Leeuwen, unpublished data). In P7 there was a significant decline in inflorescence production with only those plants which escaped the ravages of the 1985 fire producing inflorescences during the period of this investigation. Temporal changes in pollinator abundance as a consequence of fire-induced habitat modifications, have been documented in similar Proteaceae-dominated heathlands (Fox and McKay 1981, Bamford 1985). Similarly, a reduction in nectar availability conferred by a reduction in inflorescence production, can markedly alter pollinator assemblages, abundance and foraging behaviour (McFarland 1986, Pyke 1988). The low $\hat{t}_m$ value reported for this population could be attributed to a reduction in inflorescence production and changes in pollinator assemblage and abundance resulting in modified pollinator foraging behaviour. Combined, these changes may have promoted geitonogamous mating events, which is further supported by the presence of a statistically significant value of the covariance of selfing with fixation.

"Heterozygosity paradox" (Brown 1979), the tendency for outbreeding species to exhibit lower levels of heterozygosity than expected under panmixia, was recorded in most populations of *B. tricuspis*. This phenomenon has been recorded in a number of species including both herbs and trees (Brown 1979). Among the factors responsible for its manifestation are population structuring, limited gene flow, the Wahlund effect due to non-random spatial distribution of genotypes (Wahlund 1928), selection favouring homozygotes and biparental inbreeding. In the case of *B. tricuspis*, this paradox may be attributed to biparental inbreeding since family structuring within populations was negligible, gene flow was unrestricted in undisturbed populations and genotypes appeared to be randomly distributed. The existence of a post-zygotic selection mechanism in this species, favouring heterozygotes, appears to be a suitable explanation of the phenomenon. Significantly, positive F_m values, which indicated a deficit of heterozygotes in most populations and implies assortative mating, supports the existence of the heterozygote paradox phenomenon.

The obvious exception to this heterozygote deficit was P6 which recorded heterozygotes in excess of levels expected under panmixia. Clearly disassortative mating can be rejected as the main cause of this heterozygote excess as P6 exhibits considerable family structuring. The existence of a post-zygotic selection mechanism favouring heterozygotes appears the most plausible explanation. This conforms with earlier suggestions and is supported by $\hat{t}_s$ and $\hat{t}_m$ values greater than unity which imply a degree of selection favouring heterozygotes (Epperson and Allard 1984, Symth and Hamrick 1984).

The absence of heterozygote excess in maternal plants and the failure to observe significant differences between F_m and F_p values implies that heterozygote advantage (Clegg and Allard 1973, Farris and Mitton 1984) is not exhibited in the seedling to adult stage of the life cycle of this species. Seedling mortality in excess of 98% was recorded in this species over a five year period (van Leeuwen, unpublished data). The failure to record heterozygote excess in maternal plants can therefore be attributed to the extremely high mortality of seedlings, which results in the masking of any competitive advantage afforded to heterozygote genotypes. Low ΔF values also imply an absence of selection favouring heterozygotes in the post-germination stage of the life cycle.

The lack of a significant difference between F_m and F_p values, and hence of the rate of gene fixation between generations, contradicts the proposition that a post-zygotic selection mechanism favouring heterozygotes operates in this species. It is argued that this result is an artefact of the sampling strategy employed in relation to the stage in the life cycle when estimates of F_p were obtained. If F_p estimates were obtained from developing sporophytes rather than seeds, which represent sporophytes that have survived post-zygotic selection, I hypothesise that the difference between F_m and F_p values would have been significant. Supporting evidence for this hypothesis was found in the high number of aborted zygotes recorded in this species (Chapters 4, 5 and 6) together with the 100% germination rate of seeds (van Leewuen and Lamont 1986), which implies that selection against homozygotes has already occurred. However, discrimination against homozygotes is not complete as progeny originating from selfing and biparental inbreeding events survive, as indicated by the large positive F_m values in P4, P5, P7 and P8. The homogeneous distribution of allelic frequencies between maternal and progeny gene pools (Table 2.2) also support the notion that discrimination against homozygotes is not complete.

Reproductive assurance is another explanation for the persistence of progeny which have arisen from selfing and biparental inbreeding, despite *B. tricuspis* reputedly possessing a post-zygotic selection mechanism which favours heterozygotes. Reproductive assurance is conferred through a type of bet hedging where selection promotes the non-random abandonment of developing zygotes in response to genetic and ecological considerations. Those zygotes with low fitness expectations (homozygotes) are abandoned hierarchically, although the intensity and fidelity of selection varies in response to maternal resource considerations (James and Kennington 1993).

The term 'inbreeding avoidance' (K. Ritland, personal communication), where mild inbreeding is tolerated but strong inbreeding is suppressed, could also describe the mode of action of this post-zygotic selection mechanism. Indeed, negative values of D, as obtained in P4, P7 and P8, indicate that the level of selfing in outbred parents was at least as high as that of inbred parents.

2.4.2 *Conformity with model assumptions*

The mating system of *B. tricuspis* fails to conform with the assumptions of the mixed mating and effective selfing models. Compliance with the assumption that mating events are the consequence of random pollination was not achieved. Violations induced by small amounts of biparental inbreeding and $\hat{t}$ values lower than unity in most populations indicate some level of assortative mating. $H_o < H_e$ also indicates deviation from random mating, implying imperfection in the mating system.

The proposition of maternal control over seed set, in the form of post-zygotic selection which favours heterozygotes, similarly violated the assumptions of the model. Such selection may contribute to an overestimate of $\hat{t}$, especially for $\hat{t}_s$ values which are more susceptible to violation of the model's assumptions. Violations of this type therefore cause a significant underestimate of the levels of biparental inbreeding. This was clearly demonstrated in P2, P3 and P6 where estimates of $\hat{t}$ were higher that expected.

In contrast, the assumption of homogeneous gene frequencies among maternal trees appears to have been satisfied, as progeny gene frequencies did not depart significantly from those estimated for maternal trees. The result also indicates a lack of spatial structuring of similar genotypes within populations. Results of run tests generally supported an absence of family structuring. Small non-significant $\Delta\hat{t}$ values also imply homogeneity in the gene pool. A lack of family structuring within populations is characterised by adequate gene dispersal which indicates satisfactory pollen flow. The homogeneity of population gene pools was further confirmed by the general failure to detect significant differences in the proportion of heterozygous progeny produced by homozygous parents.

Circumstantial evidence suggesting compliance with the assumption that outcrossing rate is independent of maternal genotype was provided by a lack of differentiation between the proportion of heterozygote progeny produced by homozygous maternal parents (Table 2.8).

2.4.3 *Comparison with other* **Banksia** *species*

Estimates of $\hat{t}$ determined for the eight populations of *B. tricuspis* examined are among the lowest and highest reported for *Banksia* species.

The presence of a homogeneous gene pool among adult plants in *B. tricuspis*, which implied an absence of genetic structuring and unrestricted gene dispersal, has been reported in other *Banksia* species (Scott 1980, Carthew *et al.* 1988, Coates and Sokolowski 1992, Sampson *et al.* 1994). Pollen flow, which is primarily achieved by vertebrate vectors (Lamont *et al.* 1985, Collins and Rebelo 1987, Carthew 1993, 1994), can be extensive and unrestricted as a consequence of pollen carryover between plants during foraging bouts (Goldingay *et al.* 1987, Hopper 1980, Paton and Turner 1985, Whelan and Goldingay 1986). Evidence supporting extensive pollen flow was available from P1, a population comprising only one plant, which had a moderate $\hat{t}$ estimate and exhibited no heterogeneity in the proportion of heterozygous progeny produced by the homozygous parent. Similarly, the dispersal of the winged seeds of *Banksia* species can be extensive (Abbott 1985, van Leeuwen and Lamont 1986) further restricting the development of structuring within populations.

Values of $\hat{t}$ in *B. tricuspis* were heterogeneous among populations. This was attributed to differences in exposure of populations to selective pressures such as habitat-modifying environmental perturbations, like fire and land clearing. Similar variation in $\hat{t}$ reported between four populations of *B. cuneata* was attributed to differences in plant density, population structure, pollinator availability and pollinator activity (Coates & Sokolowski 1992). However, Carthew *et al.* (1988) documented homogeneous levels of $\hat{t}$ between two populations of *B. paludosa* despite differences in pollinator types and abundance, and habitat.

The fecundity advantage bestowed on non-sprouting *Banksia* species (Zammit and Westoby 1987, Cowling *et al.* 1990, Witkowski *et al.* 1991) has been attributed to the ability of such species to indulge in selfing (Carpenter and Recher 1979). This hypothesis denotes that resprouting species, which survive from one disturbance bout to another, are bestowed with sizeable levels of outcrossing through self-incompatibility systems. Data from the literature, which represents 9.8% of *Banksia* taxa, tentatively corroborate this proposition (Table 2.12). Three of the six resprouting species examined electrophoretically have $\hat{t}$ estimates implying obligate outcrossing, while $\hat{t}$ values were below unity in the two non-sprouting species, suggesting effective selfing. *B. tricuspis* fails to conform with these trends and the fecundity advantage assertion as it indulges in high levels of selfing for a resprouting species.

This feature of *B. tricuspis* may be attributed to a fecundity advantage which operates at the recruitment stage in the life cycle. It is proposed that selection, driven by extremely high seedling mortality and negligible recruitment to the adult stage, has induced a relaxation of the selection mechanism that favours heterozygotes. This results in an increase in seed production which in turn increases the opportunity for some cohorts to attain adulthood. A fecundity advantage favouring this species over other resprouters would need to exist to substantiate this proposal. Corroborating data in Table 2.13 show that *B. tricuspis* has among the highest level of reproductive success of several sympatric resprouting species in the northern kwongan. It is interesting to note that the low reproductive success and $\hat{t}$ values indicating panmixia in *B. attenuata* and *B. menziesii* authenticate the original proposal on the relationship between fire response, maternal fecundity and mating system (Carpenter and Recher 1979). Data for *B. tricuspis* offers little support for the original proposal.

Table 2.12 Estimates of the mean rate of $\hat{t}$ for non-sprouting and resprouting *Banksia* species determined by isozyme electrophoresis. (Estimates are mean single locus values for undisturbed populations. Values in parentheses indicate number of populations sampled.)

Species	$\hat{t}_s$ estimate	Reference
Nonsprouter		
B. brownii	0.69 ± 0.03 (2)	Sampson *et al.* (1994)
B. cuneata	0.94 ± 0.06 (1)	Coates and Sokolowski (1992)
Resprouter		
B. attenuata	1.10 ± 0.00 (1)	Scott (1980)
B. menziesii	1.04 ± 0.12 (1)	Scott (1980)
B. paludosa	0.80 ± 0.09 (1)	Carthew *et al.* (1988)
B. spinulosa var. *spinulosa*	1.03 ± 0.06 (1)	Carthew *et al.* (1988)
B. spinulosa var. *neoanglica*	0.86 ± 0.18 (1)	Vaughton and Carthew (1993)
B. tricuspis	0.83 ± 0.03 (3)	This thesis

Also of interest in Table 2.13 is evidence of a possible relationship between degree of serotiny (canopy seed storage) (Lamont 1991), maternal fecundity and mating system. The hypothesised relationship is that weakly serotinous species produce more seeds annually, many of which are probably derived from selfing events, than strongly serotinous species in order to maintain an equivalent sized seed bank. Highly serotinous species will produce considerably fewer seeds, probably derived entirely from outcrossing events, which contribute to a more static seed bank. Mating system estimates and maternal fecundity values for *B. tricuspis* and *B. attenuata*, species with opposing values for the degree of serotiny, substantiate the existence of such a relationship. Data for *B. menziesii* do not support the proposition.

The $\hat{t}_m$ estimate for P7 is among the lowest reported for *Banksia* species and is similar to that reported by Coates and Sokolowski (1992) for populations of *B. cuneata* suffering significant habitat modification. They suggested that habitat modification resulted in higher plant densities and reduced or altered pollinator activity thereby increasing the levels of biparental inbreeding and significantly reducing the level of outcrossing. In contrast, Sampson *et al.* (1994) recorded a low level of outcrossing in two undisturbed *B. brownii* populations which was attributed to the advantages of high fecundity in non-sprouting species. Conformity with the proposition that habitat modification has a deleterious effect on outcrossing in *Banksia* species (Coates and Sokolowski 1992), was not evident in all populations. The $\hat{t}_m$ and E values for P6, a highly disturbed population, were among the highest and lowest respectively reported for a *Banksia* species and clearly violate the expectations of this proposition. This violation was attributed to a suggested increase in xenogamous pollination events following a change in the pollinator assemblage. The ability of different pollinators to influence seed production has been reported previously in *B. tricuspis* (van Leeuwen and Lamont 1986) and also occurs in other *Banksia* species (Paton & Turner 1985, Whelan and Goldingay 1986, Goldingay *et al.* 1987, Ramsey 1988, Vaughton 1992, Carthew 1993, 1994).

Table 2.13 Fecundity and degree of serotiny of resprouting *Banksia* species in the northern kwongan. (Measures of fecundity are means for published data.)

Species	Measures of fecundity			Degree of serotiny *	Reference$^{\otimes}$
	Fruit set (%) ◆	Follicles infructescenece^{-1}	Seeds plant^{-1}		
B. attenuata	0.45	8.6	11.8	15.9	1,2,3,4,8,9
B. candolleana	0.03	1.90	10.1	300.0	8,9
B. elegans	0.004	0.16	0.2	>900.0	6,8
B. grossa	-	8.60	-	13.0	8
B. littoralis	5.80	73.90	>100.0	-	1,2,3
B. menziesii	0.49	5.69	0.8	11.9	1,2,3,4,7,8,9,10
B. tricuspis	1.93	25.30	53.8	9.0	5,8,11

⊗ 1 - Whelan and Burbidge (1980), 2 - Lewis and Bell (1981), 3 - Scott (1982), 4 - Cowling *et al.* (1987), 5 - van Leeuwen and Lamont (1986), 6 - Lamont and Barrett (1988), 7 - Ramsey (1988), 8 - Enright and Lamont (1989a), 9 - Enright and Lamont (1989b), 10 - Lamont *et al.* 1994, 11 - Chapters 5 and 6 of this thesis.

◆ $$\frac{\text{No. of follicles infructescence}^{-1}}{\text{No. of florets infructescence}^{-1}} \times 100$$

* After Cowling and Lamont (1985).

The low $\hat{t}$ rate recorded in P7 coupled with a significant *D*, which indicates matings between relatives, can be attributed to habitat modification induced by fire which temporarily reduced inflorescence production and had a detrimental effect on pollinator activity (van Leeuwen, unpublished data). Bamford (1986) reported similar fire-induced reductions in yearly inflorescence production in *B. attenuata* as did Zammit and Westoby (1987) for a number of other banksias. The consequences of changes in the flowering phenology of *Banksia* species have been observed to have a pronounced impact on pollinator behaviour (Collins 1980, Ramsey 1989, Vaughton 1990, Vaughton and Ramsey 1995).

Ayre and Whelan (1989) identified zygotic selection favouring embryos from outcrossed pollen sources (probably heterozygotes) as one of the proximate explanations for the low fruit:flower ratios observed in the Australian Proteaceae. Maternal control over seed production, attributed to early acting pre-zygotic incompatibility, as demonstrated by the arrest of pollen germination on the stigmatic surface, was reported in *B. prionotes* (Collins and Spice 1986). However, this was not observed by Lewis and Bell (1981) for *B. attenuata, B. menziesii, B. littoralis* and *B. telmatiaea*. Goldingay *et al.* (1991) inferred the existence of late acting pre-zygotic incompatibility in *B. ericifolia,* as inflorescences from self and autogamous pollination treatments did not initiate follicles and produced barren cones despite pollen tube penetration of the style. Maternal control in the form of late acting pre-zygotic or post-zygotic incompatibility must also occur in *B. attenuata, B. menziesii* and *B. spinulosa* as all exhibit high levels of outcrossing (Scott 1980, Carthew *et al.* 1988). Production of barren follicles and high seed abortion levels support post-zygotic selection in *B. tricuspis.*

The level of seed abortion recorded in P7, which was slightly higher than the pooled average across all populations (Table 2.11), was less that expected under the assumptions of a modified pollination system promoting consanguineous matings and selection favouring heterozygotes. However, significantly positive F_m estimates indicate survival of progeny originating from selfing and biparental inbreeding. This implies the operation of such a selection mechanism may not be driven by genetic considerations alone but is intrinsically linked to other selection mechanisms, such as the availability of nutrient resources (James and Kennington 1993). Vaughton (1991) demonstrated that the addition of cross-pollen and nutrients to inflorescences of *B. spinulosa* had an additive effect on fruit set over that observed following addition of only cross-pollen or nutrients. Numerous other studies have confirmed the importance of nutrients and pollen source in seed production in other *Banksia* species (Stock *et al.* 1989, Wallace and O'Dowd 1989, Goldingay & Whelan 1990).

Bet hedging may ensure the maximum recruitment of progeny into the seed bank of this weakly serotinous species thereby ensuring the maintenance of a sizeable seed bank and increasing the probability of individual seeds attaining maturity. It has been proposed as an explanation for the detection of progeny that have arisen from selfing and biparental inbreeding events. In *B. tricuspis,* the non-random abortion of seeds may be driven by resource limitations such as those induced by drought, availability of mineral nutrients within the inflorescence (Chapter 5) or spatial limitations in the developing cone. Evidence supporting the proposition of seed maximisation through bet hedging in *B. tricuspis* is evident in the form of the high fecundity of this species, given its high seed abortion level, compared with the predominantly outcrossing species *B. attenuata* and *B. menziesii* (Table 2.13).

2.4.4 *Conclusions*

In conclusion, *B. tricuspis* exhibits a predominantly outcrossing mating system in which significant inter-population differences exist. It is postulated that these differences are attributable to variations in habitat characteristics and disturbance history. Habitat modification may have a pronounced effect on the mating system of this species. Habitat modification induced by fire, the principal management tool used in the northern kwongan, impinges on the mating system of this species both intrinsically, in terms of affecting inflorescence production, and extrinsically, in terms of its impacts on pollinator assemblages and abundance. I contend that an increase in the 'natural' fire frequency could significantly impinge on the fitness of populations of *B. tricuspis* as the proportion of inbred to outbred seeds available for germination would increase. Land management authorities need to carefully consider the use of fire as a management tool in *B. tricuspis* communities as an increase in fire frequency could reduce the ability of this species to regenerate after disturbance and maintain population levels, as an increase in the level of inbreeding will reduced progeny fitness. Post-zygotic selection which favours heterozygotes but tolerates a low level of inbreeding appears to maintain outcrossing at levels below panmixia. I argue that *B. tricuspis* indulges in a type of bet hedging that promotes reproductive assurance. Zygotes with low fitness expectations are tolerated and their selection is regulated by a compromise between the long-term need to produce high quality seeds and the short-term need to maximise recruitment into the population.

Clearly, further research into the mating system and population biology of other *Banksia* species is required for an adequate understanding of the relationship between life history characteristics and genetic systems. Such research should be focussed on examining a number of hypotheses proposed in this chapter, in particular those regarding the influence of habitat modification on mating systems. Clarification of these hypotheses has implications for the management and perpetuation of many endangered plants. Perhaps the most appropriate manner to examine these hypotheses would be to investigate the mating system of a ubiquitous species occurring in numerous populations of varying size, endangerment and disturbance history. Concurrently, it may also be pertinent to undertake such investigations on critically endangered species before they are lost. Conducting research of this nature will also provide much needed information on the proposed fecundity advantage bestowed on resprouters and the genetic quality of their progeny.

Apart from examining the effects of habitat modification on the mating system it would also be desirable to investigate the validity of other propositions related to disassortative mating and selection favouring heterozygotes. This could best be achieved through an experimental design in which homozygous plants are hand pollinated with pollen from donors of identical and different genotypes. The experimental design should include provisions for xenogamous, consanguineous and geitonogamous matings and should be structured to ensure that the extent of competition between pollen types varies according to predetermined ratios. If time permitted it would also be rewarding to investigate the notion of 'heterozygote advantage' by documenting, over the generation time of this species, the patterns of recruitment and survivorship of seedlings of known genotypic quality.

2.5 References

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CHAPTER 3

FLORAL DAMAGE BY ANIMALS AND ITS IMPACT ON REPRODUCTIVE SUCCESS

3.1 Introduction

The reproductive success of a flowering plant is measured by the number of its offspring present in subsequent generations relative to other members of the population. In most plant species this number is considerably fewer than the potential number (Uma Shaanker *et al.* 1988). Low reproductive success is especially common in hermaphroditic plants (Stephenson 1981), usually due to the low conversion ratio of ovules to seeds. This ratio can be ascribed to a number of limiting factors which rarely act in isolation (Ehrlén 1992). Causes invoked to explain this include intrinsic maternal considerations (resources, breeding system) and extrinsic ecological factors (pollinator, disease, climate) (McCall and Primack 1987, Lee 1988, Hendrix 1988, Crawley 1989, Gutterman 1992).

The genus *Banksia* sets unusually few seeds compared with the number of ovules. Potential factors limiting seed production include resource availability (Paton and Turner 1985, Stock *et al.* 1989, Wallace and O'Dowd 1989), pollen limitation (Copland and Whelan 1989, Goldingay and Whelan 1990, Goldingay *et al.* 1991) and ovule/seed predation (Scott 1982, Abbott 1985, McFarland 1985, Zammit and Hood 1986, Lamont and van Leeuwen 1988, Vaughton 1990). Interactions have also been detected between pollen and resource availability (Lamont and Barrett 1988, Vaughton 1991) and predation and resource availability (Wallace and O'Dowd 1989).

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Predation of reproductive structures in banksias and its deleterious impacts on fecundity has been documented in many species (Scott and Black 1981, Cowling *et al.* 1987, Lamont and Barker 1988, Lamont and van Leeuwen 1988, Zammit and Westoby 1988, Wallace and O'Dowd 1989). For example, Vaughton (1990) noted that flower and seed predation reduced the reproductive success of *B. spinulosa* var. *neoanglica* by 50%, and Zammit and Hood (1986) showed an increase in *B. ericifolia* seed production when predators were excluded with the aid of insecticides. Scott (1982) reported that insect predation resulted in an average of 30% reduction in fecundity of six *Banksia* species, and Witkowski *et al.* (1991) noted that seed predation accounted for between 18% and 42% of the seed crop of three other species from southwestern Australia. In these studies, predation involved the destruction of ovules by insect larvae, seeds by insects and cockatoos and infructescences by parrots and cockatoos. The relationship between predators on banksias has not been examined in detail. There have been two studies on the relationship between phytophagous insects and cockatoo insectivory (Scott and Black 1981, Lamont and van Leeuwen 1988). These studies showed that bird activity is dependent on the level of insect activity and has the potential to benefit long-term fitness of the host species (Lamont 1994).

In this chapter, the relationship between *Banksia tricuspis* and its floral-damaging animals is examined. Particular attention is given to the relationship between insects and visiting birds and the implication of their interactions on the population dynamics and fitness of this species.

3.2 Materials and Methods

Banksia tricuspis is an endangered shrub or small tree endemic to the Gairdner Range, 220 km N of Perth, Western Australia. It occupies a number of habitats where it is emergent from the floristically rich sclerophyllous kwongan dominated by Myrtaceae, Restionaceae, Proteaceae and Epacridaceae (Lamont *et al.* 1982). Four flower-destroying agents - the Port Lincoln Ringneck Parrot *Barnardius zonarius*, larvae of a wood-boring moth *Arthrophora* sp., Tortricidae, the Little Corella

Cacatua pastinator and Carnaby's Black Cockatoo *Calyptorhynchus funereus latirostris* - have been recorded feeding on the inflorescences of *B. tricuspis* (van Leeuwen and Lamont 1986, Lamont and van Leeuwen 1988).

The impact of floral damage on the reproductive output of *B. tricuspis* was investigated by sampling three populations in the Lesueur National Park during March-December 1987. Counts were made of the number of (i) inflorescences plant^{-1}, (ii) damaged inflorescences plant^{-1} and the agent of damage, and (iii) infructescences plant^{-1}. An inflorescence was considered to have successfully converted into an infructescence if at least one follicle was present. All inflorescences on 300 plants were examined for floral damage. Damage by parrots was indicated by the presence of florets which had parts above the floral bracts removed. Predation by insects was indicated by frass over the rachis or tunnels and chambers in the rachis (noted by splitting the inflorescence). Damage by corellas and cockatoos was indicated by inflorescences which had been torn apart or had portions of the rachis missing. In instances where a distinction between parrot and cockatoo damage was not clear the event was ascribed to the latter.

The relationships between floral predation and the inflorescence attributes of length and presentation height were assessed on a sample of 50 random plants in population 1. The length and presentation height of all inflorescences on these plants was recorded in addition to the floral damage data outlined above. The number of rachis-boring insect larvae in a random sample of 300 inflorescences on 50 plants in a nearby adjacent population was also recorded.

Values for percentage predation were calculated for all data collected. Normality of data was examined through the Kolmogorov-Smirnov goodness of fit test statistic (Zar 1984) and arcsine or square root transformed where necessary. Back-transformed means and standard deviations are presented for most variables. Student *t* test statistics, chi-squared analysis, factorial and repeated measures ANOVAs incorporating Tukey-Kramer HSD pairwise comparison test, were used to compare data. Investigation of relationships between data sets was undertaken using the least squares linear regression model, where the significance of the relationship was determined using the Student's *t* test statistic (Zar 1984). Statistical analyses were undertaken with the aid of the SYSTAT computer program (Wilkinson 1992).

3.3 Results

Production for the year totalled 5 697 inflorescences for the 300 plants sampled. The mean number of inflorescences plant^{-1} of 19.0 ± 14.4 was positively skewed about the mean. About 2.5% of the sample produced no inflorescences and the greatest number was 84. All non-damaged inflorescences developed follicles thereby becoming infructescences. With the exception of eight parrot-damaged inflorescences, all damaged inflorescences failed to develop into infructescences.

Mean percentage floral damage plant^{-1} was lowest for parrot-induced damage which averaged 8.9% of the standing crop (Table 3.1). Damage by insects and cockatoos was considerably higher, averaging 62.6% and 57.4% respectively, although usually they damaged the same inflorescences. No predation was ascribed to corellas as they were not observed visiting inflorescences during this study. The number of inflorescences which developed into infructescences was 1 401 or 24.6% of the total number of inflorescences plant^{-1}.

Table 3.1 Mean (± sd) number of inflorescences plant^{-1} damaged by each type of animal for 300 plants of *Banksia tricuspis* in the Lesueur National Park during 1987.

	Mean	Minimum	Maximum
Inflorescences	19.0 ± 14.4	0	84
Parrot-damaged inflorescences	1.7 ± 2.1	0	25
Insect damaged inflorescences	1.8 ± 2.6	0	100
Cockatoo-damaged inflorescences	0.8 ± 1.3	0	60
Insect- plus cockatoo-damaged inflorescences	10.1 ± 10.0	0	100
Total number of damaged inflorescences	14.3 ± 13.2	0	100

The percentage of inflorescences damaged plant^{-1} showed a significant linear relationship with the number of inflorescences plant^{-1} ($t_{298} = 11.7$, $P < 0.001$), as was percentage floral damage by parrots ($t_{298} = 7.7$, $P < 0.001$) and insect plus cockatoos combined ($t_{298} = 11.3$, $P < 0.001$) (Figure 3.1).

Inflorescence production varied between the three populations sampled, population 2 having a mean significantly lower than the remaining other two populations (Table

3.2). The average level of floral damage of 7.1% by parrots did not vary significantly. Damage by insects and cockatoos only was also constant across populations at 9.3% and 3.9% respectively, but, when insect plus cockatoo were combined the level of damage was significantly higher in population 1. With a mean number of inflorescences damaged at 62.4%. damage in population 2 was less than in population 1.

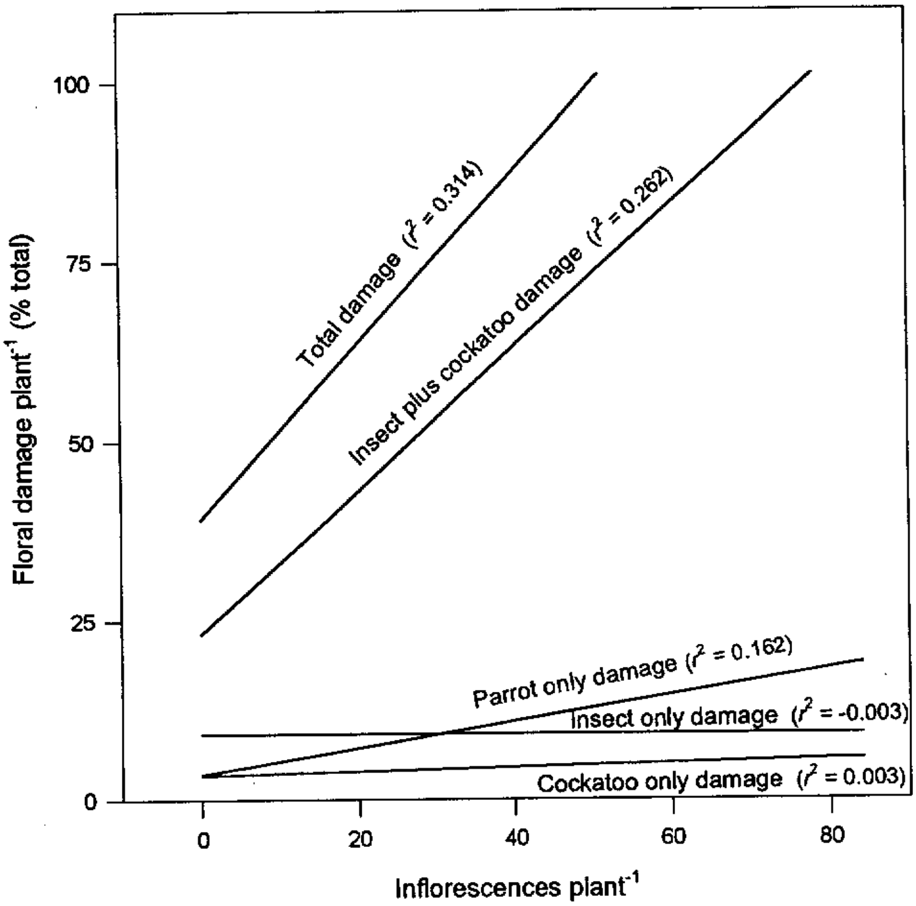

Figure 3.1 Linear relationship between percentage floral damage plant^{-1} by the various animals and the number of inflorescences plant^{-1} in 300 *Banksia tricuspis* plants during 1987.

Plants which were attacked had significantly more inflorescences than those which were not, independent of the predator (Table 3.3). Overall, the number of inflorescences plant^{-1} on those individuals in which inflorescences were attacked was similar to the population average. Plants attacked by parrots had a significantly larger number of inflorescences than the average ($t_{502} = 4.4$, $P < 0.001$) but those damaged by insects and cockatoos did not.

Table 3.2 Mean reproductive output and floral damage (mean ± sd) for three populations of *Banksia tricuspis* during 1987. Values in parentheses represent percentage of population sampled. Populations with different superscripts were significantly different, flora damage sources with different subscripts were significantly different (Tukey-Kramer HSD test, $P < 0.05$).

	Population 1	Population 2	Population 3
Sample size	70 (62)	80 (86)	150 (4)
Inflorescences plant^{-1}	21.5 ± 15.6^{a}	14.4 ± 11.0^{b}	20.2 ± 14.9^{a}
Parrot damage (%)	$7.8 \pm 7.2_{b}$	$6.6 \pm 6.3_{c}$	$6.9 \pm 6.2_{c}$
Insect damage only (%)	$7.1 \pm 6.7_{b}$	$10.6 \pm 18.2_{b}$	$9.6 \pm 15.5_{b}$
Cockatoo damage only (%)	$2.7 \pm 5.1_{c}$	$3.8 \pm 8.2_{d}$	$4.6 \pm 6.8_{d}$
Insect plus cockatoo damage (%)	$53.0 \pm 26.5^{a}_{a}$	$34.9 \pm 25.8^{b}_{a}$	$40.8 \pm 28.2^{b}_{a}$
Total damage	70.5 ± 27.1^{a}	55.9 ± 31.9^{b}	61.9 ± 31.3^{ab}

Table 3.3 Mean (± sd) number of inflorescences plant^{-1} visited by each animal type for 300 *Banksia tricuspis* plants during 1987.

	Damaged plants			Undamaged plants			
Animal type	mean	sd	*n*	mean	sd	*n*	*P*
Parrot	24.6	13.7	204	7.6	5.7	88	< 0.001
Insect	21.2	14.0	262	4.3	3.1	30	< 0.001
Cockatoo	21.5	14.0	258	4.6	3.0	34	< 0.001
Average	20.8	14.0	271	3.2	2.0	21	< 0.001

Only 21.8% of parrot-damaged inflorescences were also insect-damaged, and 54.6% of those inflorescences not damaged by parrots were predated by insects ($\chi^2 = 202.1$, $P < 0.001$). Floral damage by cockatoos was associated with inflorescence damage by insects ($\chi^2 = 2\,498.3$, $P < 0.001$), with 90.9% of cockatoo-damaged inflorescences also showing evidence of insect damage and conversely, 78.4% of inflorescences not damaged by cockatoos lacking signs of insect damage. The mean number of inflorescences plant^{-1} was 33.6 times greater for plants visited by cockatoos than those not visited (Table 3.4). The number of inflorescences plant^{-1} damaged by insects plus cockatoos was significantly larger than for any other category.

Table 3.4 Fate of inflorescences (mean ± sd) produced by 300 *Banksia tricuspis* plants during 1987 in relation to damage by insects and cockatoos.

Values in parentheses represent sample size.

	Inflorescences plant^{-1}		
	Plants visited by cockatoos	Plants not visited by cockatoos	Total
Inflorescences with larvae:			11.8 ± 11.0 (3 540)
damaged by cockatoos	10.0 ± 10.0 (3 013)	—	
not damaged by cockatoos	1.7 ± 2.5 (502)	0.1 ± 0.6 (25)	
Inflorescences without larvae:			5.5 ± 4.4 (1 642)
damaged by cockatoos	0.8 ± 1.3 (242)	—	
not damaged by cockatoos	4.3 ± 4.2 (1 276)	0.4 ± 1.5 (124)	
Total	16.8 ± 13.3 (5 033)	0.5 ± 1.7 (149)	

Length for the 1 199 inflorescences sampled on the 50 plants in sample population 1 ranged from 53 to 190 mm with a population mean of 127 ± 28 mm (Table 3.5). The damaged inflorescences were significantly larger than those that remained intact ($t_{1\,197} = 6.9, P < 0.001$). The size of parrot-damaged inflorescences were significantly larger than all other inflorescences. Within the sample population, inflorescence presentation height ranged from 21 to 278 cm with a mean height of 136 ± 48 cm (Table 3.5). No significant difference was recorded between damaged and intact inflorescence height, irrespective of predator. No relationship was detected between inflorescence length and presentation height.

A total of 912 insect larvae were recorded in rachises of the 300 *B. tricuspis* inflorescences sampled. Inflorescences contained from 1 to 24 larvae with a mean number per inflorescence of 3.0 ±1.2, but the most frequently recorded number per inflorescence was two. Insect number per inflorescence showed a significant linear relationship to inflorescence length ($r^2 = 0.2$, $t_{298} = 6.5$, $P < 0.001$), but no such relationship was evident for presentation height.

Table 3.5 Length and presentation height of *Banksia tricuspis* inflorescences relative to the agents of floral damage. Classes with different superscripts were significantly different (Tukey-Kramer HSD test, $P < 0.05$).

Floral damage class	*n*	mean	sd	min.	max.
Inflorescence length (mm)					
Parrot	120	137.4 ^{a}	2.4	67	190
Cockatoo	754	128.4 ^{b}	0.9	52	179
Insect	875	127.2 ^{b}	0.9	52	190
Insect plus cockatoo	718	125.5 ^{b}	1.0	52	189
Intact	235	115.3 ^{c}	1.7	67	179
Overall	1 199	127.3 ^{b}	0.8	52	190
Presentation height (cm)					
Parrot	120	132.3 ^{a}	4.6	45	241
Cockatoo	754	134.5 ^{a}	1.8	21	278
Insect	875	135.0 ^{a}	1.7	21	278
Insect plus cockatoo	718	134.6 ^{a}	1.8	21	266
Intact	235	138.8 ^{a}	3.3	23	262
Overall	1 199	135.7 ^{a}	1.4	21	278

3.4 Discussion

Floral damage by animals has a major impact on fruit production in *Banksia tricuspis*. Damage by parrots, phytophagous insects and cockatoos reduced infructescence number to fewer than 40% of the inflorescences produced (Figure 3.2). Reproductive success in *B. tricuspis* was controlled by the extent of floral damage in this study, as only intact inflorescences set any follicles. This contrasts with all banksia species previously studied, which have an abundance of sterile infructescences whether damaged or not (Whelan and Burbidge 1980, Abbott 1985, Lamont *et al.* 1991). Internal and other external constraints are therefore not critical at this stage in the life cycle of *B. tricuspis*, except as they may control the number of fruits and seeds produced per infructescence.

The overall level of inflorescence damage (62%) was significantly less than the 89% reported by Lamont and van Leeuwen (1988) for this species at population 1 two years earlier ($t_{299} = 4.1$, $P < 0.001$). This difference contrasts with inflorescence

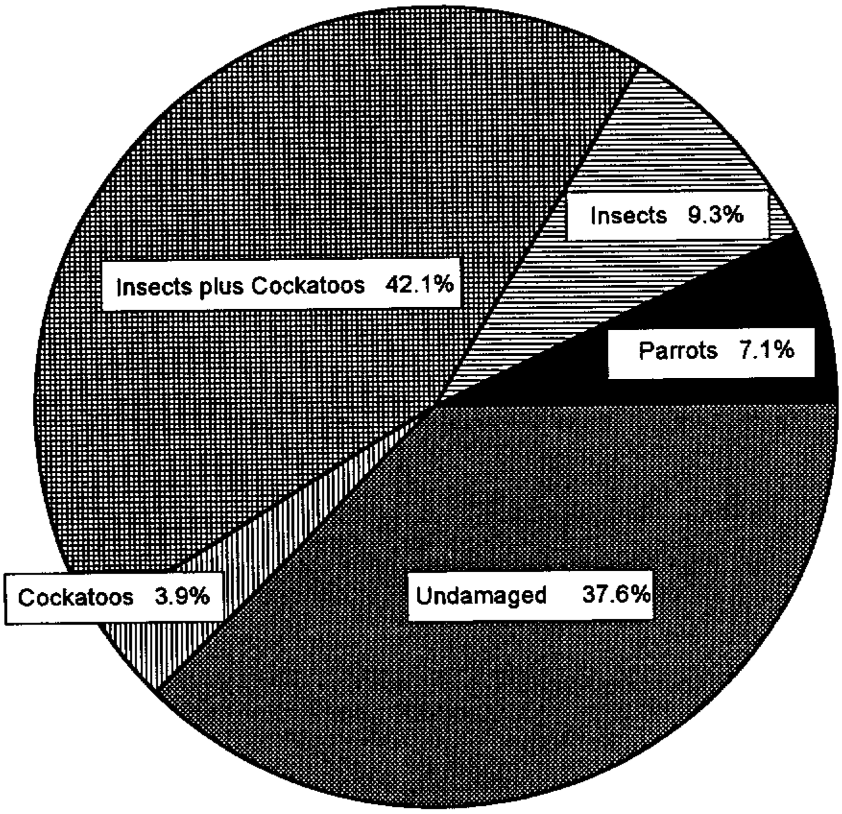

Figure 3.2 Proportion of inflorescences damaged by various animal groups for 300 *Banksia tricuspis* plants during 1987 in the Lesueur National Park. Only undamaged inflorescences produced fruit.

production which was three times greater per plant in the current study (t_{299} = 14.7, $P < 0.001$). The failure to detect numerical synchrony between reproductive output and rachis-boring larvae in the two studies suggests that the larger inflorescence crop during 1987 swamped the larvae population. Predation losses were therefore a lower fraction of the larger inflorescence crop. This difference between studies is akin to fruit "masting", where temporal cycling between high and low reproductive years ensures an increase in fitness through the overall reduction in the effect of predators (Janzen 1971, Hendrix 1988). It is argued that, during troughs in reproductive output, predator populations fall and remain low when host reproductive output increases suddenly, due to a lag in the ability of the predators to "catch up". Plant fitness increases as predator numbers are insufficient to exploit the additional resources. The acceptance of this interpretation requires that the ratio of reproductive output to rachis-boring larvae is higher than in the previous study. Since the number of larvae was 30% higher per invaded inflorescence, however, the ratio of inflorescences to larvae was actually lower than in the previous study. Why larvae should build up in the inflorescence rather than be distributed over more inflorescences is not clear. An understanding of such a functional response is critical to determining if predator saturation is an ultimate cause of masting.

Spatial variation in reproductive output and floral damage was also evident, one sample population being below that of the other two populations, but relative damage level for each of the three animal groups remained constant. Uniform levels of floral damage between populations may be attributed to the mobility and foraging behaviour of each agent. Variation in reproductive output between populations of this species has previously been reported (van Leeuwen and Lamont 1986) for inflorescence production which varied from 6.8 to 11.2 between two sample populations.

Undamaged inflorescences were significantly smaller than damaged ones, conforming with Scott's (1982) proposition that small inflorescences increase the likelihood of avoiding predation. The association was particularly strong for parrot visits, and insect numbers increased with inflorescence length. This predation avoidance strategy was also evident at the whole plant level, undamaged plants having significantly fewer inflorescences than those damaged. The competitive advantage of producing small or fewer inflorescences is questionable, however, as large inflorescences are purported to attract more pollinators and increase reproductive success (Pyke 1981, 1982). Indeed, visitation rates for honeyeaters (Meliphagidae) in *B. tricuspis* increased as inflorescences plant^{-1} increased (Chapter 7) and seeds infructescence^{-1} increased as inflorescence length increased (Chapter 6).

Floral damage by parrots impinges on the reproductive fitness of *B. tricuspis*, although the impact is much less than the damage caused by cockatoos. Parrots have been implicated in damage to *Banksia* inflorescences previously, although no attempt has been made to quantify the effect (McFarland 1985, Vaughton 1988). Damage by parrots results from the birds' attempts to gain unrestricted access to the floral nectaries on the rachis. Access is obtained by removing the styles and perianth thereby rendering the florets sterile. Avoidance of insect-damaged inflorescences may be related to nectar availability, as insect damage of the vascular tissue within the rachis would restrict nectar flow. It is also possible that floret damage results in the removal of instar larvae before they invade the rachis. Hood (1985) and Zammit and Hood (1986) report that insect larva are deposited when inflorescences are still in bud.

The damage from insect floral herbivory was of a similar magnitude to that reported for some other *Banksia* species, which also approached 50% (Scott 1982, Zammit and Hood 1986, Wallace and O'Dowd 1989, Vaughton 1990). The actual impact of insect floral herbivory, however, may be underestimated, as no consideration was given to predation of floral parts by instar larvae before they enter the rachis. Direct floral damage resulted in a reduction in reproductive output by 40% in *B. ericifolia* (Wallace and O'Dowd 1989, Vaughton 1990). Insect floral herbivory could have been greater but for the actions of numerous floral visitors. Ants and nectariferous birds have been implicated in the regulation of phytophagous insects in banksias (Scott 1982, Ford and Paton 1976, Lamont and van Leeuwen 1988). Indeed, only 6% of the inflorescences treated (caged) in 1986 to exclude insects and birds remained free of insect damage, and 16% of bird-excluded inflorescences remained intact. For the control and bird exposed treatments 57% and 62% remained intact respectively (van Leeuwen, unpublished data).

Floral damage by cockatoos accounts for 46% of reproductive output and can be attributed to their seeking insect larvae as part of their diet (Robinson 1965, Saunders 1980). Damage by cockatoos was somewhat less than by insects (51%), although cockatoos preferentially visited inflorescences infected by insects. In destroying 82.1% of insect-occupied rachises, however, they also inadvertently destroyed 9.4% of non-insect occupied inflorescences. Cockatoos enhance their ability to detect insect floral herbivores by preferentially visiting the larger inflorescences which are likely to contain more insects. They also favour plants with the largest number of inflorescences, which also contain the most insect-ravaged inflorescences. Cockatoo interactions with *B. tricuspis* depend on the presence of insect herbivores which have a significant negative effect on the reproductive success of this species. Any deleterious impact cockatoos confer on the *Banksia*, through the occasional destruction of insect-free rachises, is far outweighed by their overall beneficial regulation of future insect populations.

Carnaby's Cockatoo is endangered as a result of habitat fragmentation induced by agricultural clearing (Saunders *et al.* 1985, Saunders and Ingram 1987). The absence of this species from the Gairdner Range as a result of further habitat destruction could have a pronounced effect on the reproductive success of *B. tricuspis*. Immediate implications would be an increase in the survivorship of insects which, in subsequent seasons, may well severely limit reproductive success. Other impacts would be on nectar availability for pollinators and the size of the seed bank. The effect on the fitness of this resilient, long-lived *Banksia* may not be immediately apparent, but the loss of recruitment potential would eventually lead to a population decline and even extinction.

From this study, the fitness of *B. tricuspis*, as measured by its reproductive success, is governed by the extent of floral herbivory. Selection to enhance fitness and avoid floral herbivory favoured the least endowed inflorescences and least fecund individuals. The impact of interactions with floral herbivores on reproductive success ranged from minimal for parrots and cockatoos to severe for insects. Insect floral herbivory determines the pattern of floral damage by parrots and cockatoos, with the relationship between insect and cockatoo damage based on cockatoos eating larvae for food. A disintegration in the insect-cockatoo relationship could have a marked impact on the long term fitness of *B. tricuspis*.

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CHAPTER 4

BETWEEN YEAR VARIATION IN FECUNDITY AND FLORAL DAMAGE

4.1 Introduction

The level of reproductive success in hermaphroditic plants, in terms of the number of fruits or seeds set flower^{-1}, is low (Stephenson 1981, 1984, Sutherland 1987). This phenomenon, manifested in the production of apparently 'surplus' flowers, significantly impinges on the success of maternal reproduction by reducing realised seeds flower^{-1} to levels well below their potential. Sutherland and Delph (1984) reported average fruit sets of 22.1% in 187 self-incompatible hermaphroditic species, while Collins and Rebelo (1987) noted averages in the range 0.1% to 16.3% for 26 species of southern Australian Proteaceae. In most species, the intrinsic ceiling to reproductive success is determined by maternal resources which are allocated in a strategic manner to maximise plant fitness (Bazzaz *et al.* 1987). Hence, reproductive success below unity may be directly attributable to the high maternal resource cost of sexual reproduction (Horvitz and Schemske 1988).

4.1.1 *Resource* vs *pollen limitation*

Numerous factors, which may vary both temporally and spatially, have been proposed to explain the proximate cause of low numbers of seeds flower^{-1}. These factors entail both extrinsic and intrinsic constraints which are not necessarily mutually exclusive and can operate in synergy or interact with each other (Bierzychudek 1981, Stephenson 1992, Lawrence 1993). Factors most commonly cited as promoting few seeds flower^{-1} are maternal resource limitation and pollen limitation. Resource limitations are due to finite maternal assimilates and photosynthates precluding the conversion of fertilised ovules to mature seeds. This type of limitation is principally determined by extrinsic considerations, such as weather, soil condition, herbivory, disease or competition for nutrient and soil moisture resources within the community (McCall and Primack 1987, Ågren 1988). The mechanism of such resource constraints may be attributed to inter-ovary and ovary-vegetative structure competition or alternatively, the production of growth regulators which control source-sink interactions through the diversion and repartitioning of resources and the inhibition of some sinks (Lee 1988, Stephenson *et al.* 1988, Bangerth 1989, Guitián 1994).

Pollen limitation results from the inability to achieve maximum fertilisation due to insufficient acquisition of compatible pollen, which can primarily be attributed to temporal and/or spatial changes in the abundance of flowers (inflorescences) and/or pollinators (Campbell 1987, Ehrlén 1992, Karoly 1992). Other proximate factors attributed to few seeds flower^{-1} are genetic limitations and predation/pathogen limitations. Genetic limitations act via pre- and post-zygotic selection mechanisms which affect pollen tube, ovule and zygote mortality (Lloyd 1980, Charlesworth 1989), whereas predation/pathogen limitations impinge on the numbers of ovules and flowers which are available for fertilisation and subsequently, the numbers of fruits and seeds which reach maturity (Janzen 1971a, Herrera 1991, Siemens 1994).

Numerous studies have identified resource and pollen limitation as independent constraints limiting fecundity in natural populations (Bierzychudek 1981, Stephenson 1981, Lee and Bazzaz 1982, Campbell 1987, Johnston 1991). However, these two factors rarely act in isolation, interacting in complex mutualistic ways which preclude the detection of a strict dichotomy between the two (McCall and Primack 1987, Zimmerman and Pyke 1988a, Campbell and Halama 1993, Lawrence 1993). On the premise that resources are optimally allocated when there is equal limitation by all resources (Bloom *et al.* 1985), Haig and Westoby (1988a) propose that seed production will be restrained by both nutrient and pollen limitation. Campbell and Halama (1993) propose four scenarios that implicate both resources and pollen as constraints limiting maternal reproductive success. These scenarios include;

- Spatial and/or temporal heterogeneity in the availability of physical resources such that resource limitation is observed only when pollination is adequate and pollen limitation is observed only when resources are abundant. Within a population both limitations may operate in response to environmental, ecotypic and habit heterogeneity (Lawrence 1993).

- Differences in the mode and time of operation of the two factors relative to the reproductive development cycle and realised reproductive success. For example, pollen availability may limit fertilisation and zygote initiation while resource availability may influence later developmental stages (Horvitz and Schemske 1988). Similarly, seed set may be limited by resources while seeds fruit^{-1} may be limited by pollination levels.
- Resource availability indirectly influencing pollen availability through floral attractiveness. For example, resources may influence flower number, inflorescence size/architecture and nectar rewards which prejudice pollinator behaviour and pollination success (Wyatt, 1982, Klinkhamer *et al.* 1989, Real and Rathcke 1991, Hodges 1995).
- A relaxation of selective abortion mechanisms, which are pollen source driven, in conjunction with a corresponding increase in the availability of maternal resources (Lee 1988, Zimmerman and Pyke 1988b).

Haig and Westoby (1988a) submit that as pollen attraction effort (x) increases the number of effectively pollinated ovules (y_1) increases (pollination-limitation function), but the number of potentially provisioned seeds (y_2) decreases (provisioning-limitation function). The intersection of the two confronting functions delineates the maximum maternal reproductive success achievable with realised fecundity falling below this intersection and between the x axis intercepts of each function. If maternal reproductive success is limited by resources alone, fecundity could be increased by reducing the allocation of resources to a competing function such as pollinator attraction. Alternatively, if reproductive success is pollen limited, increasing pollinator attraction through the allocation of additional resources may increase fecundity (Lloyd 1980, de Jong and Klinkhamer 1989, Lawrence 1993).

The relative importance of resources *vs* pollen limitation in determining maternal reproductive success may be ascertained by correlating pollen attraction effort (x) against major components of plant fitness (seed number and mass) (y). If the relationship between pollen attraction effort and fitness components embodies a positive linear to exponential growth function then fecundity is assumed to be constrained by pollen limitation as described by the pollination-limitation model. If, on the other hand, the relationship embodies a negative linear to exponential decay function then resources limitation may be considered the principal constraining factor.

Plants respond to proximate constraints on reproductive success by adjusting the commitment of maternal resources to each successive reproductive stage, thus synchronising maternal investment with available resources and ensuring maximum plant fitness (Lloyd 1980, Stephenson 1984). This selective serial adjustment of maternal resources is achieved hierarchically from inflorescence to seeds by altering a) the number of flowers that are differentiated, b) the number of ovules flower^{-1}, c) the number of fruits and seeds that abort, and d) the mass of the mature seeds (Stephenson 1992). Such adjustments, and the ability to control resource allocation, enables a plant to maintain maximum levels of fecundity between successive years and ensures a high degree of maternal control over progeny.

4.1.2 *Temporal control and adjustment of fecundity*

Temporal divergence in the operation of proximate constraints impinging on the number of seeds flower^{-1} has been credited to yearly variation in reproductive success in a number of species (Ågren 1988, Mazer *et al.* 1989, Burrows and Allen 1991, Houle and Filion 1993, Guitián 1993). In many instances, maternal investment in reproductive success in one year influences fecundity in ensuing years, as well as subsequent vegetative growth and parental survival (Lovett Doust *et al.* 1986, Zimmerman and Aide 1989, Ackerman and Montalvo 1990). Climatic considerations which influence the initiation of reproductive structures, pollinator assemblages and their abundance, ovule fertilisation and maturation and fruit/seed abscission, together with resource availability (eg. soil moisture), have often been ascribed as the proximate causes of between year fluctuations (Campbell 1989, Owens *et al.* 1991). Similarly, variations in environmental considerations which affect pollinators, pathogens and herbivores (vegetative, floral and seed) and competition with heterospecifics may also induce between year fluctuations in reproductive success (Janzen 1971b, Weiner 1988). Intrinsic physiological control of maternal resources can also account for between year variation in seed production (Montalvo and Ackerman 1987).

Large between year fluctuations in fruits/seeds flower^{-1} are consistent with Lloyd's (1980) proposition that the serial adjustment of maternal investment in reproductive success is likely to occur at the fruiting stage when the fate of subsequent flowering is unpredictable. Such between year variation in flowering may confer a fitness advantage, whereby the over supply of flowers serves to match uncertainty in ovule and flower mortality as a result of unpredictable fluctuations in the availability of maternal resources, pollen, pathogens and herbivore pressures (Lee and Bazzaz 1982, Elmqvist *et al.* 1988, Kozlowski and Stearns 1989). This bet hedging scenario implies that there are unpredictable fluctuations between years in extrinsic proximate constraints commensurate with the overproduction of flowers and uncertainty in plant reproductive success. Ehrlén (1991) contends that bet hedging embraces the 'resource availability' (Guitián 1993) and 'reserve-ovules' hypotheses. The 'resource availability' hypothesis is based on a plant's ability to take advantage of favourable conditions, in terms of maternal resources, thus decreasing overall ovule mortality. This hypothesis confers homogeneity between seasons or populations in flower production (Ågren 1988). In contrast, reproductive success under the 'reserve-ovule' hypothesis remains constant, despite unpredictable mortality, as 'surplus' ovules (flowers) act as a reserve supply (Ehrlén 1991, 1993).

Conversely, sexual selection theory argues that the production of 'surplus' flowers is adaptive as an excess of flowers increases male fitness through increasing pollen donation (Stephenson and Bertin 1983). 'Surplus' flowers may also permit plants to selectively abort genetically inferior zygotes (Bawa and Webb 1984, Lloyd 1987), increase plant (inflorescence) attractiveness to pollinators (Stanton *et al.* 1986) and reduce predispersal seed predation (Siemens *et al.* 1992, Travis 1992).

The relative importance of 'surplus' flower production and commensurate few seeds flower^{-1} and the merits of contrary justifications for the phenomenon, can be judged by monitoring the pattern of fecundity across a number of years (Ågren 1988). If 'surplus' flowers serve mainly as a means of adjusting maternal investment to unpredictable variation in extrinsic constraints then variation between years in seeds flower^{-1} would be expected under the 'resource available' hypothesis. If, on the other hand, 'surplus' flowers are produced mainly to enhance male function (pollen donation), utilisation of reserve ovules, plant attractiveness or permit selective non-random abortion of inferior zygotes, then consistency between years in fecundity would be anticipated.

4.1.3 *Floral damage and compensatory responses*

Uncertainty in ovule and flower mortality may occur through the actions of flower feeding herbivores which have been demonstrated to have a pronounced effect on maternal and paternal reproduction success and plant fitness (Louda 1982, Paige 1992, Louda *et al.* 1990, Hendrix 1979). The interactions between plants and their flower herbivores are complex with plants reacting in a variety of ways which may affect resource allocation, photosynthesis rates and senescence/abscission (Hendrix 1984, McNaughton 1984). Such responses and a plant's inherent ability to regrow have important practical applications as they can offset reductions in biomass or reproductive potential normally associated with herbivory. The magnitude of herbivory effects may thus be mitigated through a bet hedging mechanism (Vail 1992) which increases partitioning of resources to undamaged flowers (Ehrlén 1992). Mitigating responses which ultimately result in greater fecundity and plant fitness include partial recovery (Bergelson and Crawley 1992), full compensation (Lowenberg 1994) and possibly, overcompensation (Paige and Whitham 1987, Paige 1992). Such responses are counterintuitive, however, as they imply that the loss of flowers and/or the response to such losses have no maternal cost. Such responses are plausible although, as the chief maternal reproductive costs are post-zygotic and flowers are generally considered to represent a lesser, albeit sometimes substantial, cost than seeds (Cruden and Lyon 1985, Delph 1990, Witkowski and Lamont 1996).

Compensatory responses to floral damage are most likely to occur when the probability of damage is high and restricted to short periods relative to a plant's development and life cycle (Vail 1992). Such responses will be induced by floral damage which occurs prior to or during early flowering and usually involves an increase in the quantity of reproductive tissues (Belsky 1986), but may also include increased fertilisation success of the remaining flowers and an increase in the average size of the ensuing seeds (Fenner 1985). Both maternal and paternal costs of herbivory are mitigated by such responses.

A compensatory response to floral herbivory would be expected if the production of 'surplus' flowers evolved primarily in response to the 'reserve ovule' bet hedging hypothesis (Louda 1982, Ehrlén 1991, 1993, Vail 1992). Under such a mechanism plants withhold maternal resources in anticipation of floral damage, compensating for such losses through increased pollination success and increased seed size. However, if 'surplus' flowers evolved primarily in response to selection driven by pollinators then no compensation should be expected. 'Surplus' flowers would simply attract more pollinators and no change in the number of seeds which mature would be expected with a change in the proportion of flower mortality.

The absence of a compensatory response to floral damage and consistency in seed flower^{-1} suggests reproductive synchrony between years which is a strategy individuals adopt to maximise fitness (Ims 1990a). In plants, synchronous reproduction is attributed primarily to resource matching where reproductive effort mirrors the availability of variable resources (Koenig *et al.* 1994). The pattern of reproductive effort under the assumptions of resource matching should be unpredictable unless the critical resources display a cyclic tendency or are not limiting (Sork *et al.* 1993). The operation of resource matching and its impact on synchronous reproduction is correlated with weather conditions (Houle and Filion 1993), which Norton and Kelley (1988) postulate is the deterministic variable for resource matching. Indeed, greater assimilation of maternal resources may be promoted by weather conditions in certain years thereby enabling a plant to increase its reproductive effort and output. This association between resources and climatic conditions has been referred to as the 'weather tracking' hypothesis (Sork *et al.* 1993), especially when fluctuations in reproductive effort are in direct response to variations in weather conditions.

4.1.4 *Reproductive synchrony and consistency*

Reproductive synchrony in plants is exemplified by the phenomenon of masting where populations engage in the periodic synchronous increased production of flowers, fruit or seeds (Janzen 1971a, Silvertown 1980, Crawley 1992). Masting is primarily found in long-lived plants, particularly trees, which are less affected by the cost of eschewed reproduction during indiscriminate years (Kelly 1994). Adaptive explanations for plant reproductive synchrony, particularly masting, focus on economy of scale hypotheses which propose that large reproductive efforts are more efficient than small ones (Norton and Kelly 1988). The principal economy of scale hypothesis cited is associated with predator saturation, where losses to predation are reduced by varying reproductive output such that predators are starved in low reproductive years and swamped in high reproductive years (Janzen 1971a). While predator saturation has been demonstrated in a number of tree species (Silvertown 1980, Ims 1990b), its importance as a selective force depends on the relationship and functional response of the predator (Nilsson and Wästljung 1987, Ims 1990a). Specialist predators may be successfully swamped in mast years. However, generalists, which are catholic in their foraging preferences, may cause high losses when fecundity is increased (Augspurger 1981, Nilsson and Wästljung 1987). Similarly, functional relationships with animals are not necessarily promoted through saturation (Sork 1993). In animal pollinated and animal-dispersed species it is desirable not to swamp pollinators and dispersers to ensure maximum output from the substantial input, although in a few instances, this logic of pollinator and disperser swamping has not been evident (Ims 1990a, 1990b).

Other hypotheses advanced in support of the economies of scale for reproductive synchrony include wind pollination, environmental prediction, animal attraction (pollination and fruit dispersal), accessory costs of reproduction and large seed size (Norton and Kelley 1988, Smith *et al.* 1990, Sork *et al.* 1993, Koenig *et al.* 1994). Most hypotheses, except the first two, are not well supported in the literature. The 'wind pollination' hypothesis implies that because of the undirected nature of pollen dispersal, pollen production concentrated in mast years should increase the efficiency of successful pollination. This is especially true when investment in flowers and fruits is considerable regardless of successful fertilisation and is supported by the incidence of this pollination syndrome in many of the masting species (Smith *et al.* 1990, Webb and Kelley 1993). The 'environmental prediction' hypothesis purports that plants can predict which years will be best for seedlings and is well supported in the Australian flora by cases of mast flowering triggered by fire as in the genus *Xanthorrhoea* and

Drosera, and in many species of kangaroo paw (Haemodoraceae) and terrestrial orchids (Orchidaceae) (Gill and Ingwersen 1976, van der Moezel *et al.* 1987, Lamont and Runciman 1993).

4.1.5 **Banksia tricuspis** - ***fluctuations in fecundity and floral damage***

The objective of this investigation was to document the temporal pattern of fecundity in a natural population of *Banksia tricuspis* over three consecutive years and assess the affect of inflorescence predatory and damaging animals on reproductive success. Plant fitness attributes, such as seed output and seed mass, are principal constituents of the management program for any species regardless of their endangerment (Pavlik 1987). A clear understanding of the constraints impinging on these attributes and their association with various plant fitness components (inflorescence output, infructescence output, viable seed output, aborted seed output, seed set and seed mass) are therefore paramount to the formulation of monitoring and recovery programs that promote the maintenance of existing populations and decrease the probability of extinction (Piggot 1992, Pavlik 1993).

Specifically, this investigation aimed to document the variation in components of fecundity and examine the: (1) interaction between these components and their influence on fitness attributes; and (2) impact on, and response of, *B. tricuspis* plants to flower mortality induced by interactions with inflorescence damaging animals. Answers were sought to the following questions. Firstly, what fecundity component(s) have the greatest impact on maternal reproductive success? This was tested by stepwise multiple regression where the components of plant fecundity were identified as independent variables and fitness attributes were dependent variables. Principal Component Analysis was also used to highlight important fecundity components impinging on plant fitness. Secondly, under natural conditions, does resource or pollen limitation play the most dominant role in limiting maternal reproductive success? This was tested by comparing plant fitness attributes for infructescences and whole plants (seed number and mass) against pollen attraction effort (total number of flowers). Thirdly, does the over production of flowers enable the species to adjust maternal investment? This was examined by comparing seed set between years and through changes in the within-year estimated coefficient of variation between different reproductive development stages. Fourthly, does floral damage have a detrimental effect on maternal reproductive success and do plants compensate for this impact? This question was addressed by comparing the various components of fecundity and attributes of plant fitness, with levels of flower mortality. Finally, does *B. tricuspis* indulge in synchronous reproduction? This was tested by examining for consistency among years and the pattern of reproductive success for each of the plant fitness components and attributes assessed. Interpretation of Spearman's rank correlations also addressed this synchronous reproduction question.

4.2 Materials and Methods

Between year variation in fecundity and floral damage was investigated over a three year period between 1986 and 1988 on plants of *Banksia tricuspis* in a population located in the Lesueur National Park (30° 09' 07" S, 115° 13' 05" E), 220 km N of Perth, Western Australia. This investigation documented the relationship between fecundity components and the fecundity attributes of seed number and seed mass, while examining the influence of floral damage on these relationships. Plant fecundity components were partitioned into those indicative of reproductive effort and those representative of maternal reproductive success.

4.2.1 *Plant reproductive effort*

Plant fecundity components characteristic of reproductive effort were documented by recording output and subsequent variance between years in the number of inflorescences plant^{-1}, infructescences plant^{-1} and fruiting rate (infructescences inflorescence^{-1}) in a sample of 30 plants sequentially selected along three transects within the sample population. Inflorescence production was recorded monthly throughout the March to October flowering period for each of the 30 plants, while conversion of inflorescences to infructescences was determined three months after the completion of flowering when follicle development had ceased. Cessation of follicle development was indicated by the discarding of desiccated florets and change in infructescence and follicle colour from green to grey-brown. The fruiting rate was determined as the percentage of inflorescences plant^{-1} that developed into non-barren infructescences.

4.2.2 *Maternal reproductive success*

Fecundity components indicative of maternal reproductive success were categorised into a dichotomy based on seed number and seed mass.

4.2.2.1 *Seed number*

Seed number fecundity components evaluated were viable seeds infructescence^{-1}, aborted seeds infructescence^{-1} and seed (viable) set infructescence^{-1}. Seeds infructescence^{-1} was determined each year for a selection of infructescences collected from a ten plant subsample randomly chosen from the 30 plant sample population. Infructescences were harvested in March following each flowering season to ensure that seeds had developed and were mature. A total of 178 infructescences were harvested over three years from the ten subsampled plants which represented the entire 1988 infructescence crop (87 infructescences), in conjunction with 59 and 37 randomly selected infructescences from the two preceding years, respectively. The entire infructescence crops from 1986 and 1987 were not sampled to ensure the persistence of a canopy stored seed bank in this endangered, weakly serotinous (Lamont 1991) species. Infructescence selection during these two years was based on the allocation of random numbers and accounted for approximately half of each year's crop. Determination of seeds infructescence^{-1} was achieved after the treatment of infructescences through burning and exposure to a wet-dry regime (Cowling and Lamont 1985). Seeds were ascribed to one of two categories, viable or aborted, where viable seeds were firm with an intact embryo and aborted (non-viable) seeds were malleable and lacked an embryo.

To facilitate the estimation of seed set infructescence^{-1}, the numbers of flowers occurring on 20 randomly selected inflorescences harvested during each season from plants not included in this study were counted. For each year's inflorescence crop a least squares linear regression was determined for the relationship between flower number (y) and infructescence length (x) in mm. Given homogeneity between yearly determined correlation coefficients for this relationship ($\chi^2_2 = 1.69$, $P > 0.05$) the estimated common correlation for the regression between x and y was $r_w = 0.96$. The regression was defined as:

$$y = 3.15x - 533.33.$$

This regression was highly significant ($F_{1,59} = 205.32$, $P < 0.001$) with 91.5% of the variance in flower number being predicted by infructescence length. Seed set infructescence^{-1} was therefore determined by the equation:

$$\left(\frac{\text{viable seeds per infructescence}}{\text{flowers per inflorescence x 2}}\right) \times 100$$

where flowers inflorescence^{-1} were calculated with reference to the previously determined regression incorporating infructescence length. The devisor in this equation, flowers inflorescence^{-1}, was multiplied by two to reflect the number of ovules flower^{-1}.

Seed number components were also determined for the ten plants in the subsample population during each year of this investigation. In 1988, the values for viable and aborted seeds plant^{-1} represented cumulative counts of the number of seeds present, as the entire infructescence crop was harvested. In the two preceding years, estimates were obtained by multiplying mean viable and aborted seeds infructescence^{-1} by the number of infructescences plant^{-1} for each plant. Seed set plant^{-1} was calculated by multiplying average seed set infructescence^{-1} by the fruiting rate for each individual plant.

4.2.2.2 *Seed mass*

The plant fecundity attributes of mass seed^{-1} (mg), seed mass infructescence^{-1} (g) and seed mass plant^{-1} (g) were also used to quantify maternal reproductive success. Mass seed^{-1} was quantified during each year of this investigation from the viable seeds produced by 15 infructescences collected indiscriminately with reference to infructescences remaining on each individual plant in the ten plants subsample population. Seeds were removed from their infructescences using the procedure described above and weighed. Seed mass infructescence^{-1} was determined by calculating cumulative seed mass for each of the 15 infructescences sampled during each year of this investigation. Seed mass plant^{-1} was estimated by multiplying average seed mass infructescence^{-1} by infructescences plant^{-1} for each of the ten subsample plants during each year.

4.2.3 *Floral damage*

Levels of floral damage were assessed monthly through the inspection of inflorescences on the 30 plant sample population for signs of parrot, insect or cockatoo damage. Records were maintained on the cause of damage and the fate of each inflorescence on each of the 30 plants in the sample population. Parrot damage was identified through the presence of florets which had some or all perianth parts above the floral bracts removed. Insect damage, which involved damage to the rachis, was detected through the presence of wilting florets, particularly prior to anthesis, by the presence of frass and silk at the base of florets and by the presence of tunnels and chambers in apparently barren infructescences (observed by splitting barren infructescences). Inflorescences which were torn apart or had portions missing were attributed to damage by cockatoos.

Yearly estimates of floral damage were calculated following amalgamation of monthly damage records to provide an estimate of the number of damaged inflorescences plant^{-1}, which was subsequently expressed as a percentage of the total inflorescence crop plant^{-1}.

4.2.4 *Data analysis*

Normality and homoscedasticity of the data were checked by examining symmetry (g_1), by the Kolmogorov-Smirnov goodness of fit statistic for a normal distribution (D), and Bartlett's test for homogeneity of variances between groups (Zar 1984). Significant departure from normality or heterogeneity of variances between groups was treated by square root or arcsine (percentage) transformations. Descriptive statistics presented are for back-transformed values.

Single factor, repeated measures ANOVA was employed to assess between year differences in the fecundity components used to assess reproductive effort. Post-hoc, pairwise, between subject coefficients, were generated to assess significance of differences between means (Wilkinson 1992). The significance of temporal variance in floral damage was also investigated using repeated measures ANOVA procedures, both for the pooled data set and for each agent of floral damage. Heterogeneity between years in seed number and seed mass fecundity components was investigated using a least squares ANOVA procedure as the majority of these tests represented unbalanced factorial designs. The significance of multiple range comparisons among means was determined by the Tukey-Kramer HSD test. This test has a smaller per comparison error than Fisher's Least Significance Difference test (Ott 1984), is recommended for unplanned multiple comparisons (Keppel 1982, Zar 1984) and is conservative when sample sizes are unequal (Neter *et al.* 1985). Detection of significant heterogeneity between years in seed numbers plant^{-1} and in seed mass infructescence^{-1} and plant^{-1} were investigated using repeated measures ANOVA procedures. When differences between years were not significant for any of the fecundity components and attributes investigated, pooled sample statistics were calculated.

The relationship and significance of interactions between and within plant fecundity components and attributes were appraised through least squares linear regression procedures. The significance of correlations was determined through ANOVA procedures, while pairwise and multiple comparisons among correlations were based on modified Z and χ^2 tests incorporating Fisher's z transformation (Zar 1984). In instances where no significant differences were detected between correlations the common correlation coefficient (r_w) was determined (Zar 1984).

The identification of best fit models describing the relationship between fecundity components and attributes was determined through stepwise multiple regression procedures and Principal Component Analysis. Fecundity components were assigned as independent variables while fecundity attributes were appointed as dependent variables. Seed set infructescence^{-1} and plant^{-1} was not included in the independent variable attribute set as this fecundity component was not considered an independent variable inasmuch as it was determined using viable seed number. Principal Component Analysis was used to verify identification of significant independent variables as determined through stepwise multiple regression procedures. The cosine of the angle between two variables on Principal Component vector plots was used to confirm stepwise multiple regression procedures, as this measure is analogous to the linear correlation between the two variables on an xy plot (Jolliffe 1986, Birks 1987).

Congruity between years in stepwise multiple regressions was determined by the procedure described above, incorporating Fisher's z transformation (Zar 1984). When homogeneity was detected between years a common stepwise multiple regression was determined for the contrasted independent and dependent variables.

The relationship between fecundity attributes and pollen attraction effort was determined through data interrogation and line/curve-fitting using a series of parametric models incorporating both linear and non-linear functions. The function which best described the data and related the dependent variable (fecundity attribute) to the independent variable (pollen attraction effort) was determined through the F statistic. The TableCurve 2D software (Jandel Scientific) was employed in line/curve fitting and determination of corresponding F statistic values.

Consistency and synchrony between seasons by individual plants was determined through the Spearman's rank correlation statistic. Coefficient of variation estimates were also determined to assess within and among year consistency in reproductive success (Silvertown 1980). Within year's coefficient of variation estimates, used to delineate the extent of intrinsic and extrinsic control over maternal reproductive success, were determined for inflorescences plant^{-1}, fruiting rate, and mass seed^{-1}. Common coefficients were estimated by averaging the sums of the estimated coefficients by the duration of the study (3 years). Testing for differences between coefficients was performed using the F statistic variance ratio tests (Zar 1984). Estimations of among year coefficients were determined by calculation of the coefficient of variation for each individual plant across the three study years and subsequently averaging, the summed variances by the number of plants sampled (Silvertown 1980, Sork *et al.* 1993).

4.3 Results

4.3.1 *Plant reproductive effort*

A total of 2 281 inflorescences were produced by the 30 sampled plants during the three years of this study. The total number of inflorescences produced fluctuated significantly between years ($\chi^2_2 = 50.75$, $P < 0.001$) varying from 865 in 1986 to 602 in 1987. Floral damage accounted for 61.5% of all inflorescences with the highest level of damage recorded in 1986, when 617 inflorescences were destroyed. This number was significantly different from the number of inflorescences destroyed in the two subsequent years ($\chi^2_2 = 63.60$, $P < 0.001$), although the overall proportion of the total inflorescence crop damaged remained constant. All barren infructescences exhibited damage by parrots, insect larvae or cockatoos. The number of non-barren infructescences produced, which totalled 742, did not vary significantly between years ($\chi^2_2 = 1.57$, $P > 0.05$) with a maximum of 260 infructescences present in 1988. The overall fruiting rate was 33.4% which varied from 28.9% in 1986 to 39.6% in 1987. As all non-damaged inflorescences developed into infructescences this fruiting rate was the reciprocal of floral damage. The difference between seasons in fruiting rate was not significant ($\chi^2_2 = 1.51$, $P > 0.05$) with an overall average of 247 infructescences produced per year. Fecundity components of reproductive effort for the 30 sampled plants were all asymmetrically distributed about the means ($D_{90} = 0.16$, 0.11, 0.19 and 0.11 for inflorescences, floral damage, infructescences and fruiting rate respectively, $P < 0.01$) with all distributions positively skewed ($g_{1\,90} = 1.69$, 1.34 and 1.11 for inflorescences, infructescences and fruiting rate respectively, $P < 0.001$) except for floral damage ($g_{1\,90} = -1.01$, $P < 0.001$) which was negatively skewed.

Between year variation in reproductive success plant^{-1}, as determined by inflorescence production, floral damage, infructescence development and fruiting rate in the 30 sampled plants, is presented in Figure 4.1. Homogeneity between years was evident for most attributes, with the exception of inflorescences plant^{-1} which in 1987 was significantly less than the two contiguous years (Table 4.1). This between year distinction in maternal reproductive success was also evident for the other plant attributes assessed. However, the differences were not significant.

Coefficient of variation estimates for inflorescences plant^{-1} and fruiting rate varied considerably within years, with significant heterogeneity detected between 1986 and 1987 for inflorescences plant^{-1} and between 1986 and the two succeeding years for fruiting rate (Figure 4.2). All within year estimated coefficients decreased between the two reproductive development stages, as exemplified by the significant difference between stages in the common estimated coefficients (62.4 ± 9.3% *vs* 33.4% ± 11.1 for inflorescences plant^{-1} and fruiting rate, respectively) ($F_{89,\,89} = 1.97$, $P < 0.01$). Among year coefficient of variation results for each of the fecundity components assessed were low indicating minimal variation between individuals across years in reproductive effort (Table 4.2).

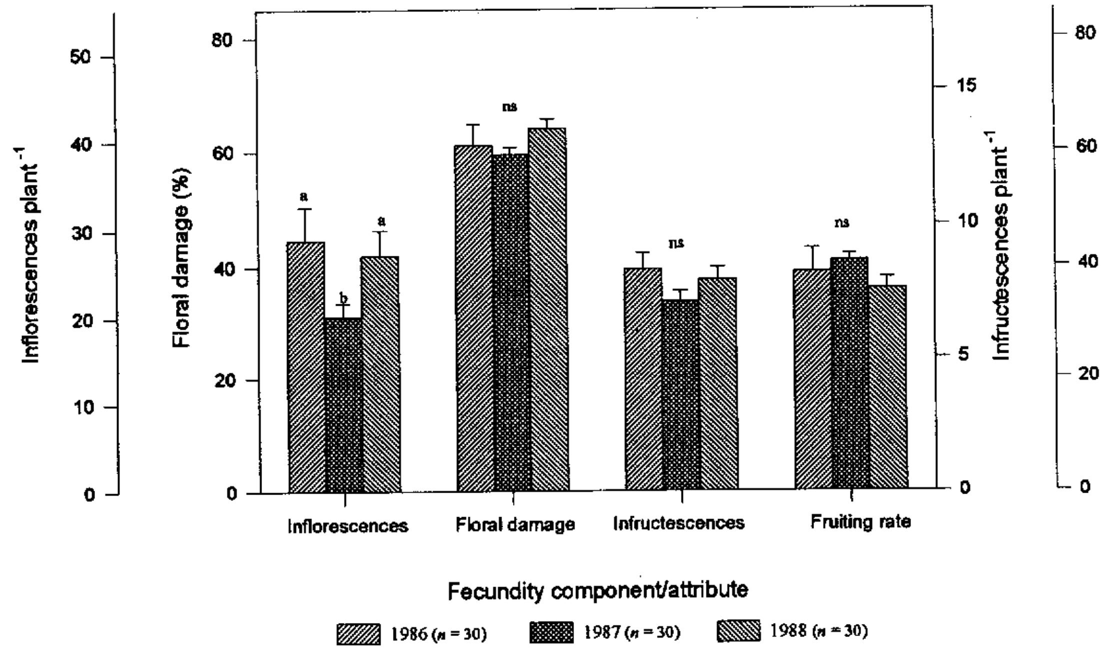

Figure 4.1 Variation in reproductive success plant^{-1} (mean ± SE) between 1986 and 1988 for 30 *Banksia tricuspis* plants. (Columns with different letters above their error bars were significantly different, Tukey-Kramer HSD, $P < 0.05$.)

Table 4.1 Statistics and significance of differences between years for attributes of reproductive effort used to assess fecundity in 30 *Banksia tricuspis* plants between 1986 and 1988.

Reproductive attribute	Year			$F_{2,58}$
	1986	1987	1988	
n	30	30	30	
Inflorescences plant^{-1}				
Mean ± SE	28.8 ± 3.8	20.1 ±1.6	27.1 ± 2.9	11.65***
Range	5 - 82	9 - 43	10 - 76	
Floral damage (%)				
Mean ± SE	61.1 ± 3.7	59.4 ± 1.3	64.0 ± 1.6	1.78
Range	20 - 81	45 - 70	49 - 79	
Infructescences plant^{-1}				
Mean ± SE	8.3 ± 0.6	7.8 ± 0.4	8.7 ± 0.5	3.72
Range	4 - 18	4 - 13	4 - 16	
Fruiting rate (%)				
Mean ± SE	39.3 ± 3.9	41.1 ± 1.4	36.0 ± 1.6	1.85
Range	19.0 - 83.3	30.0 - 55.6	21.0 - 51.0	

Significance: *** $P < 0.001$.

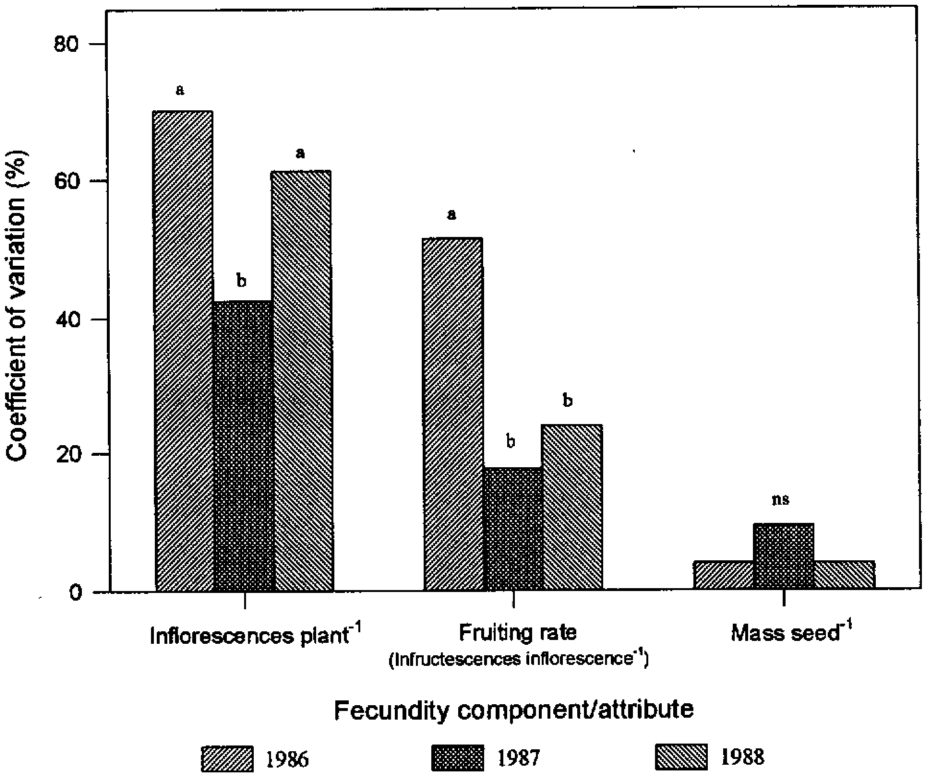

Figure 4.2 Coefficient of variation estimates demonstrating increasing maternal control over fecundity at various developmental phases in *Banksia tricuspis* between 1986 and 1988. (Columns with different letters above their error bars were significantly different, *F* statistic variance ratio test (Zar 1984), $P < 0.05$.)

Table 4.2 Among years' coefficients of variation for components and attributes of fecundity in *Banksia tricuspis* estimated between 1986 and 1988.

Fitness component/attribute	CV (%)
Inflorescences plant^{-1}	24.0 ± 3.4
Floral damage (%)	16.7 ± 3.9
Infructescences plant^{-1}	13.5 ± 1.3
Fruiting rate (%)	23.2 ± 2.6
Viable seeds infructescence^{-1}	17.3 ± 4.0
Viable seeds plant^{-1}	22.1 ± 4.3
Aborted seeds infructescence^{-1}	15.6 ± 3.2
Aborted seeds plant^{-1}	26.6 ± 4.9
Seed set infructescence^{-1}	24.9 ± 3.9
Seed set plant^{-1}	33.0 ± 3.9
Mass seed^{-1}	4.8 ± 1.2
Seed mass infructescence^{-1}	10.2 ± 2.6
Seed mass plant^{-1}	32.2 ± 4.6

Correlation coefficients between inflorescences plant^{-1} and the number of damaged inflorescences plant^{-1} were significantly positive for each year and homogeneous between years, with the common correlation coefficient estimated to be 0.98 (Table 4.3). The relationship between inflorescences plant^{-1} and infructescences plant^{-1} was also significantly positively correlated and homogeneous between years. Similarly the years and common correlation coefficient between damaged inflorescences plant^{-1} and infructescences plant^{-1} were all positive and significant. No correlations were determined for associations involving fruiting rate as it was the reciprocal of floral damage. Significant rank correlations, indicating uniformity and consistency between years by individuals in relation to reproductive success, were detected for all fecundity components (Table 4.4). These correlations also demonstrated significant accord between inflorescences plant^{-1} and floral damage, implying that the more fecund individuals experienced the higher levels of floral damage. Similarly, infructescences plant^{-1} was significantly associated with inflorescences plant^{-1}, suggesting that plants which produce few inflorescences also tend to produce few infructescences and *vice versa*. Rank correlations for fruiting rates were generally significantly negatively associated with inflorescences and infructescences plant^{-1}.

Table 4.3 Least squares correlation coefficients for linear regressions between attributes of reproductive effort and floral damage in *Banksia tricuspis* between 1986 and 1988.

Reproductive attributes	Year			r_w
	1986	1987	1988	
Inflorescences plant^{-1} *vs* damaged inflorescences plant^{-1}	0.98***	0.97***	0.99***	0.98***
Inflorescences plant^{-1} *vs* infructescences plant^{-1}	0.94***	0.93***	0.93***	0.94***
Damaged inflorescences plant^{-1} *vs* infructescences plant^{-1}	0.92***	0.87***	0.91**	0.90***

Significance: ** $P < 0.01$, *** $P < 0.001$.

The reproductive output of the ten subsample plants parallelled that of the original sample population of 30 plants. Consequently, between-year variation and rank correlations of maternal reproductive success among the 30 sampled plants and the

Table 4.4 **Matrix of Spearman's rank correlation for between year relationships among individual plants in reproductive effort between 30 *Banksia tricuspis* during 1986 to 1988.**

Reproductive	Inflorescence plant^{-1}			Floral damage			Infructescences plant^{-1}			Fruiting rate		
attribute	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988
Inflorescences plant^{-1}												
1986	-											
1987	0.84***											
1988	0.85***	0.76***										
Floral damage												
1986	0.93***	0.74***	0.84***									
1987	0.83***	0.78***	0.65***	0.81***								
1988	0.74***	0.59***	0.87***	0.80***	0.55**							
Infructescences plant^{-1}												
1986	0.86***	0.81***	0.70***	0.64***	0.69***	0.48**						
1987	0.69***	0.83***	0.79***	0.56***	0.49**	0.55**	0.81***					
1988	0.76***	0.78***	0.86***	0.63***	0.64***	0.57**	0.79***	0.87***				
Fruiting rate (%)												
1986	-0.92***	-0.73***	-0.84***	-	-	-	-0.65***	-0.57**	-0.64***			
1987	-0.75***	-0.82***	-0.53**	-	-	-	-0.59***	-0.44**	-0.52**	0.71***		
1988	-0.74***	-0.59***	-0.87***	-	-	-	-0.48**	-0.56**	-0.56**	0.79***	0.45**	-

Significance: ** $P < 0.01$, *** $P < 0.001$.

subsample population of ten plants were similar (Table 4.5). These results indicated that the ten plant subsample population was representative of the 30 plant sample for the fecundity components and attributes assessed.

Table 4.5 Comparison of differences between population means (n = 30) and sample means (n = 10) for components used to assess fecundity in *Banksia tricuspis* as determined by t tests, together with significance of between year variation (repeated measures ANOVA) and Spearman's rank correlations (r_s) for reproductive success in the ten sampled plants.

Reproductive attribute	Statistics				
	t_{38}	$F_{2,18}$	$r_{s\ 10}$		
				1986	1987
Inflorescences plant^{-1}					
1986	0.03				
1987	-0.05	3.94*	1987	0.93***	
1988	-0.08		1988	0.86**	0.85**
Floral damage (%)					
1986	0.01				
1987	-0.01	0.59	1987	0.80**	
1988	-0.09		1988	0.85**	0.66*
Infructescences plant^{-1}					
1986	-0.03				
1987	-0.02	1.45	1987	0.77**	
1988	-0.03		1988	0.89**	0.86**
Fruiting rate (%)					
1986	0.05				
1987	0.19	0.58	1987	0.80**	
1988	0.12		1988	0.85**	0.66*

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.3.2 *Floral damage*

Damage to inflorescences by the various animals remained constant between years (Figure 4.3). In all instances, except for cockatoos, 1987 was the year of least inflorescence damage. Insects were responsible for damaging the greatest numbers of inflorescences plant^{-1}, accounting for 49.2 ± 1.3% of the inflorescence crop over three years, while cockatoos accounted for 45.3 ± 1.4%. Parrots damaged the least number of inflorescences (7.0 ± 0.5%) while insect and cockatoo damage combined accounted for 40.0 ± 1.4% of inflorescences. All plants experienced some type of floral damage with at least one inflorescence plant^{-1} damaged.

The extent of floral damage plant^{-1} was significantly positively correlated with the number of inflorescences plant^{-1} for each agent of damage during each year (Table 4.6), indicating that floral damage increases with flowering display. Correlations between floral damage and inflorescences plant^{-1} were heterogeneous between years for all animal types, except parrots, which had a common correlation coefficient among years of 0.79.

4.3.3 *Maternal reproductive success*

4.3.3.1 *Seed number*

The numbers of viable and aborted seeds infructescence^{-1} over the three years of this study are presented in Table 4.7. The impact of insect damage to seeds was inconsequential, as seed predation accounted for only 23 (0.39%) of all firm viable seeds observed. Seed predation was ignored in subsequent data analyses. Overall, the numbers of viable and aborted seeds infructescence^{-1} were homogeneous between seasons (Figure 4.4). Similarly, repeated measures ANOVA results indicated homogeneity between seasons for both attributes when values for the 10 subsampled plants were interrogated (Table 4.7). On an infructescence^{-1} and plant^{-1} basis, 1986 was the year of poorest viable seed production and the year of greatest aborted seed production.

Yearly seed set infructescence^{-1} and plant^{-1} were low ranging from an average of 1.3% to 1.7% and 0.55% to 0.58% for seed set infructescence^{-1} and plant^{-1}, respectively. Both estimates exhibited an asymmetric distribution which was positively skewed (Table 4.8) during each year of the study and thus raw values were arcsine transformed. Seed set infructescence^{-1} displayed significant heterogeneity between years with 1987 being intermediate to both 1986 and 1988, the latter of which produced the greatest result (Figure 4.4). Conversely, seed set plant^{-1} was homogeneous between years with 1987 producing the greatest result. Among year estimates of the coefficient of variation for seed number fecundity components were low with the greatest variation being observed for seed set plant^{-1} (Table 4.2).

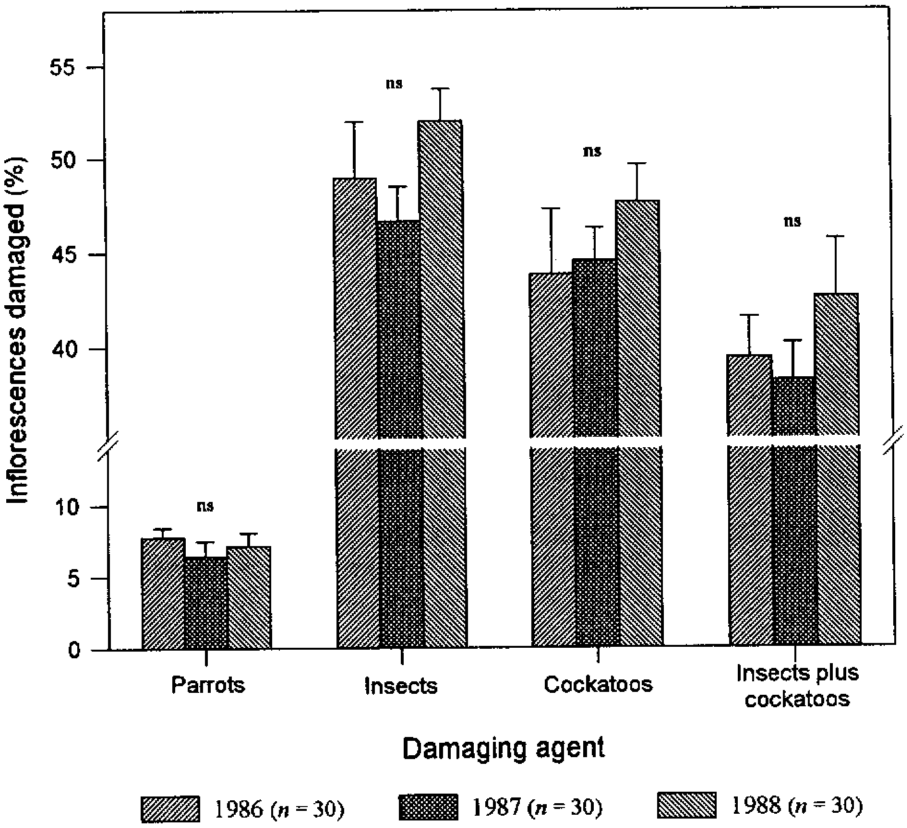

Figure 4.3 Variation in percentage inflorescence damage plant^{-1} (mean ± SE) by the various animal agents between 1986 and 1988 for 30 *Banksia tricuspis* plants. (No significant differences were detected between years for each damaging agent, Tukey-Kramer HSD, $P < 0.05$.)

Table 4.6 Chi-squared test for heterogeneity between correlation coefficients for the linear regressions between inflorescences plant^{-1} and floral damage for each animal agent responsible for inflorescence damage in 30 *Banksia tricuspis* plants between 1986 and 1988. (r_w = common correlation coefficient.)

Animal agent	Year			χ^2	r_w
	1986	1987	1988		
Parrots	0.83***	0.75***	0.77***	0.69	0.79***
Insects	0.69***	0.96***	0.88***	16.28***	-
Cockatoos	0.99***	0.95***	0.98***	9.02*	-
Insects plus cockatoos	0.98***	0.93***	0.98***	7.35*	-

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4.7 Statistics for seed number fecundity components and attributes determined for *Banksia tricuspis* between 1986 and 1988.

Reproductive attribute	Statistics		Between-years
	Mean ± SE	Range	
Viable seeds infructescence^{-1}	33.3 ± 1.2	2 - 97	$F_{2,175} = 2.30$
Aborted seeds infructescence^{-1}	18.3 ± 0.7	2 - 55	$F_{2,175} = 0.46$
Seed set infructescence^{-1} (%)	-	0.2 - 8.7	$F_{2,175} = 5.47$**
Mass seed^{-1} (mg)	-	18.2 - 98.3	$F_{2,175} = 16.53$***
Seed mass infructescence^{-1} (g)	1.9 ± 0.1	0.5 - 3.3	$F_{2,18} = 0.48$
Viable seeds plant^{-1}	278.1 ± 31.9	72 - 786	$F_{2,18} = 2.68$
Aborted seeds plant^{-1}	156.9 ± 12.9	36 - 340	$F_{2,18} = 0.73$
Seed set plant^{-1} (%)	0.5 ± 0.1	0.1 - 1.5	$F_{2,18} = 0.63$
Seed mass plant^{-1} (g)	15.5 ± 1.8	3.3 - 43.2	$F_{2,18} = 0.68$

Significance: ** $P < 0.01$, *** $P < 0.001$.

Table 4.8 Statistics on the significance of departure from zero and a normal distribution for seed set results obtained for *Banksia tricuspis* between 1986 and 1988 as determined by *t* test, Kolmogorov-Smirnov goodness of fit (*D*) and skewness (g_1).

Statistic	Year		
	1986	1987	1988
Seed set infructescence^{-1}			
n	32	59	87
t	13.35**	13.39***	22.47***
D	0.11	0.12	0.07
g_1	0.74	1.23**	0.95**
Seed set plant^{-1}			
n	10	10	10
t	5.06***	5.04***	9.86***
D	0.20	0.24	0.26
g_1	0.93	1.69*	0.98

Significance: * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$.

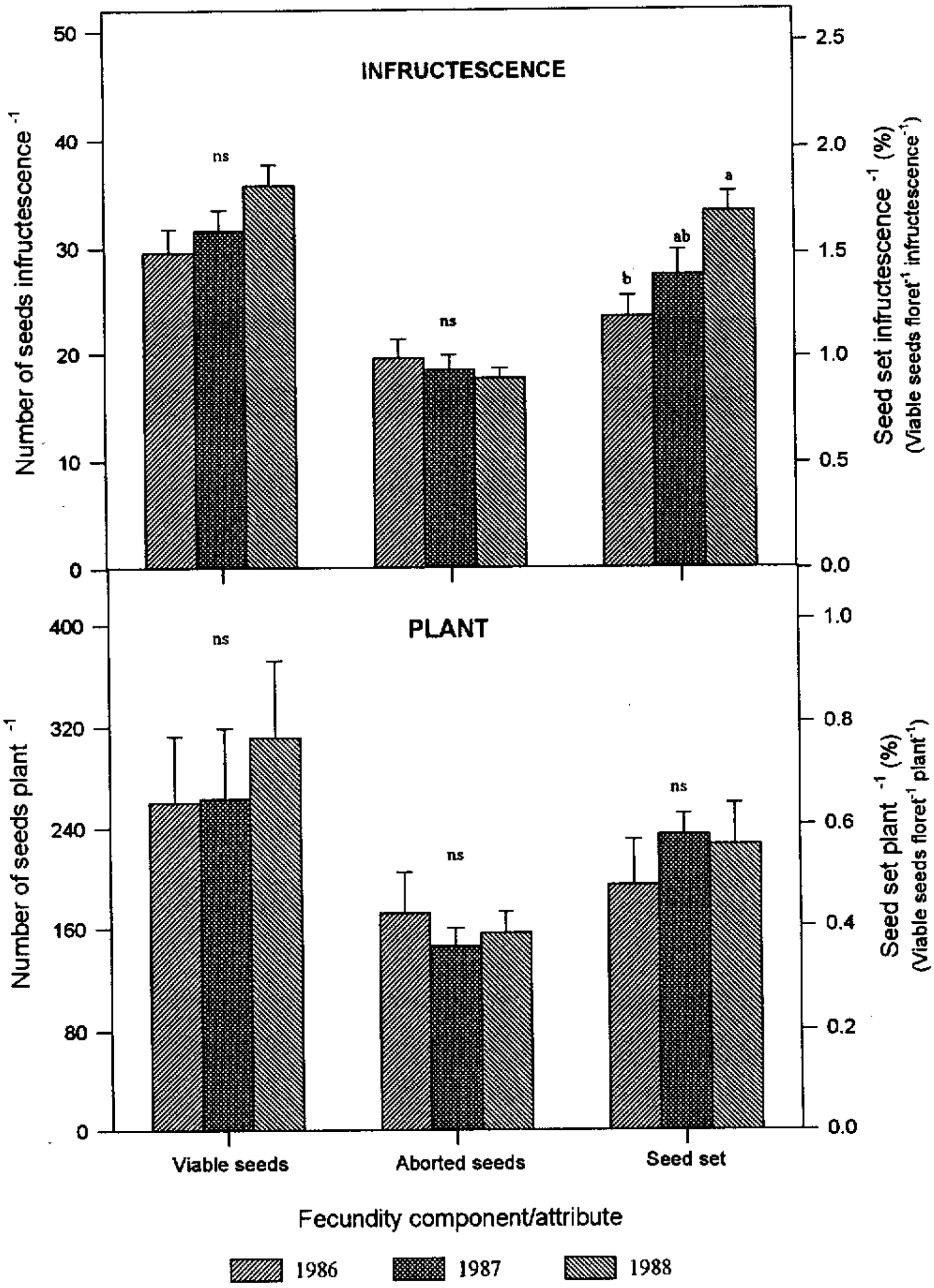

Figure 4.4 Infructescence and plant seed number fecundity attributes for 178 infructescences harvest from 10 *Banksia tricuspis* plant between 1986 and 1988. (Columns with different letters above their error bars were significantly different, Tukey-Kramer HSD, $P < 0.05$.)

Correlations between viable seeds infructescence^{-1} and plant fecundity components indicated that all interactions were positive during each year and homogeneous between years (Table 4.9). All interactions observed in 1988 were significant, as were all estimated common correlation coefficients. All correlations between fecundity components and aborted seeds infructescence^{-1} were similar between years, non-significant and suggested a negative trend for the association. Similarly, correlations between seed set infructescence^{-1} and the three fecundity components were homogeneous between years and suggestive of a positive association. Significant correlations were not identified for interactions occurring in 1988. However, all estimated common correlation coefficients for the pooled data were significant. Viable and aborted seeds plant^{-1} were all positively associated with the three plant fecundity components and were homogeneous between years (Table 4.9). Significant interactions were observed for inflorescences and infructescences plant^{-1} only. All correlations with seed set plant^{-1} were negatively associated with the fecundity components and correlations were similar between years. The estimated common correlation coefficient for the association between floral damage and seed set plant^{-1} was significant.

Stepwise multiple regression procedures were ambiguous in the identification of fecundity components which influence viable seeds infructescence^{-1} (Table 4.10). The common stepwise multiple regression was estimated as coefficients were homogeneous between years ($\chi^2_2 = 2.2$, $P > 0.05$). The most significant component which influences viable seeds infructescence^{-1} was infructescences plant^{-1}. However, this was only selected as a variable in the regression equation during 1987 and for the common estimated stepwise multiple regression. For contrast, results for viable seeds plant^{-1} were more elucidating, with infructescences plant^{-1} being identified as an important independent variable (Table 4.10). All estimated multiple stepwise regression coefficients were significant as were all the independent fecundity components selected. All regressions were also homogeneous between years ($\chi^2_2 = 0.7$, $P > 0.05$). Principal Component correlations comply with stepwise multiple regression estimates that propose infructescences plant^{-1} as the principal independent fecundity component influencing viable seeds infructescence^{-1} (Figure 4.5a, Table 4.11). Similarly, infructescences plant^{-1} were also identified as the principal independent fecundity component influencing viable seeds plant^{-1}, which complies with stepwise multiple regression predictions (Figure 4.5b, Table 4.11).

Table 4.9 **Least squares correlation coefficients and common correlation (r_w) for linear regressions between seed number fecundity components/attributes and plant reproductive effort in *Banksia tricuspis* between 1986 and 1988.**

Dependent variable	Independent variable					
	Inflorescences plant^{-1}		Infructescences plant^{-1}		Floral damage (%)	
	r	r_w	r	r_w	r	r_w
Viable seeds infructescence^{-1}						
1986	0.24	0.37***	0.21	0.34***	0.19	0.24**
1987	0.46***		0.46***		0.19	
1988	0.35***		0.34**		0.33***	
Aborted seeds infructescence^{-1}						
1986	-0.06	-0.07	-0.03	-0.04	0.03	-0.01
1987	-0.05		-0.03		0.11	
1988	-0.10		-0.10		-0.11	
Seed set infructescence^{-1}						
1986	0.22	0.23**	0.17	0.22**	0.12	0.16*
1987	0.29**		0.30*		0.02	
1988	0.22*		0.19		0.26*	
Viable seeds plant^{-1}						
1986	0.86***	0.75***	0.92***	0.79***	0.32	0.37*
1987	0.74***		0.81***		0.22	
1988	0.87***		0.92***		0.56*	
Aborted seeds plant^{-1}						
1986	0.64*	0.56***	0.76**	0.65***	0.33	0.32*
1987	0.65*		0.59*		0.39	
1988	0.55		0.66**		0.25	
Seed set plant^{-1}						
1986	-0.53	-0.29	-0.30	-0.17	-0.86***	-0.66*
1987	-0.12		0.04.		-0.55*	
1988	-0.34		-0.18		-0.46	

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4.10 Correlation coefficients and loadings on selected variables from stepwise multiple regressions of interactions between fecundity components and fecundity attributes in *Banksia tricuspis* between 1986 and 1988.

Fitness attributes	r	Constant	Inflorescences plant^{-1}	Floral damage	Infructescences plant^{-1}	Fruiting rate	Viable seeds infructecence^{-1} /plant^{-1}	Aborted seeds infructescence^{-1} /plant^{-1}
Viable seeds infructescence^{-1}								
1986	0.49	23.99	0.22					
1987	0.65**	4.61			4.03*			
1988	0.00	33.07						
Overall	0.38***	18.21			1.82**			
Seed mass infructescence^{-1}								
1986	0.96***	358.43					-64.62***	-13.82*
1987	0.92**	2 942.88	29.47	-47.65*			33.12***	
1988	0.99***	384.59				-6.03*	62.76***	
Overall	0.92***	473.91		-5.09*			53.43***	
Viable seeds plant^{-1}								
1986	0.92**	-73.99			40.22**			
1987	0.81**	-234.94			63.81**			
1988	0.87***	58.08	9.19**					
Overall	0.79***	-100.32			45.77***			
Seed mass plant^{-1}								
1986	0.98***	-3 582.44			1 035.59		41.24**	
1987	0.96***	1 911.55					51.35***	
1988	0.95***	-1 738.64					39.98***	
Overall	0.94***	2 213.89					50.65***	

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

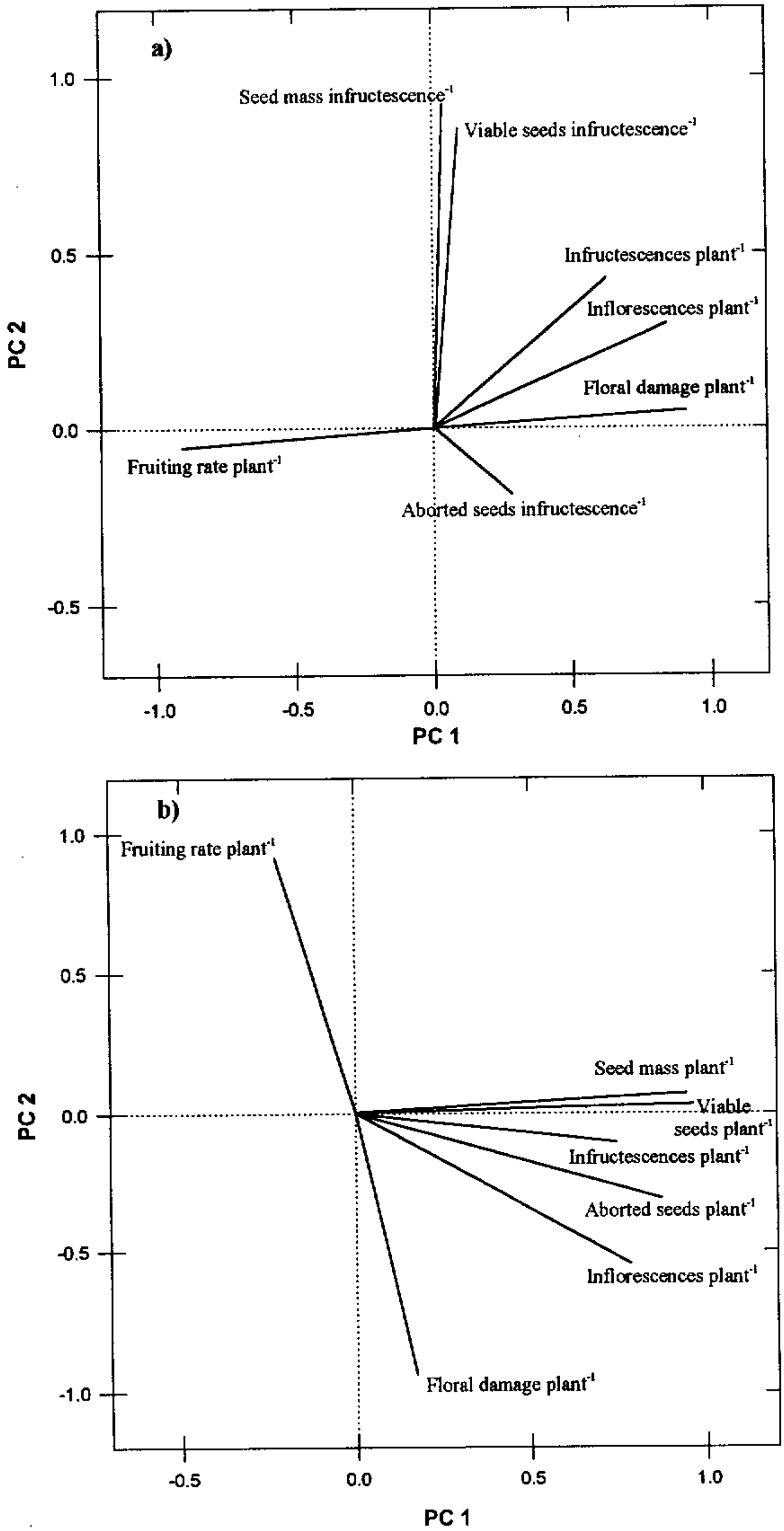

Figure 4.5 Principal Component plot of association between fecundity components and attributes in *Banksia tricuspis* for total reproductive effort between 1986 and 1988: a). infructescence^{-1}; b). plant^{-1}.

Table 4.11 **Cosine of angle between vectors plotted by Principal Component Analysis for determining the association between fecundity components and attributes in infructescences and plants of *Banksia tricuspis* between 1986 and 1988**. The cosine is equal to the correlation between the two variables.

Vectors	Fitness attributes							
	infructescence^{-1}				plant^{-1}			
	1986	1987	1988	Common	1986	1987	1988	Common
Viable seeds *vs*								
inflorescences	0.71	0.53	0.62	0.64	0.71	0.53	0.62	0.64
floral damage	0.21	0.32	0.57	0.41	0.21	0.32	0.57	0.41
infructescences	0.83	0.75	0.73	0.78	0.83	0.75	0.73	0.78
fruiting rate	0.04	-0.51	-0.26	-0.48	0.04	-0.51	-0.26	-0.48
aborted seeds	-0.20	-0.01	0.13	-0.03	-0.20	-0.01	0.13	-0.03
Seed mass *vs*								
inflorescences	0.67	0.71	0.53	0.57	0.67	0.71	0.53	0.57
floral damage	0.29	0.36	0.28	0.34	0.29	0.36	0.28	0.34
infructescences	0.81	0.69	0.71	0.73	0.81	0.69	0.71	0.73
fruiting rate	-0.36	-0.15	-0.61	-0.42	-0.36	-0.15	-0.61	-0.42
viable seeds	0.98	0.87	-0.99	1.00	0.98	0.87	-0.99	1.00
aborted seeds	0.00	0.03	-0.10	-0.10	0.00	0.03	-0.10	-0.10

The fecundity attribute of viable seed infructescence^{-1} was significantly associated with the number of florets infructescence^{-1}. However, only 18% of the variance in the equation was explained by the best fit model (Table 4.12). Sample correlation coefficients determined for each year were homogeneous despite a significant regression, which represented 60% of the variance in the best fit model, being detected in 1988. The overall best fit model for the association between viable seed infructescence^{-1} and florets infructescence^{-1} typified an exponential growth function (Figure 4.6a). Correlations determined for the best fit model estimated for the association between viable seeds plant^{-1} and florets plant^{-1} were all significant and homogeneous (Table 4.12). The common best fit model explained 61% of the variance in the association between the two variables and reflected a curvilinear upwards function (Figure 4.6b).

Table 4.12 Description and significance of the best fit function describing the relationship between fecundity attributes (seed output and mass) and pollinator attraction effort (florets plant^{-1}) in *Banksia tricuspis* between 1986 and 1988.

Fitness attribute	Best fit function	*F*	*r*	χ^2_2
Viable seeds infructescence^{-1}				
1986	$y = a + bx$	3.64	0.31	4.64
1987	$y = a + bx^{-2}$	0.19	0.14	
1988	$y = a + bx^{-1}$	19.84***	0.77***	
Common	$y = a + bx^{-1}$	9.28**	0.42**	
Viable seeds plant^{-1}				
1986	$y = a + bx$	43.31***	0.92***	0.34
1987	$y = a + bx^{3}$	21.98***	0.85***	
1988	$y = a + bx^{3}$	21.55***	0.86***	
Common	$y = a + bx^{2}$	44.73***	0.78***	
Seed mass infructescence^{-1}				
1986	$y = a + bx^{-1}$	4.36	0.50	1.43
1987	$y = a + be^{-x}$	0.64	0.28	
1988	$y = a + bx^{-1}$	9.66*	0.65*	
Common	$y = a + bx^{-1}$	7.32*	0.38*	
Seed mass plant^{-1}				
1986	$y = a + bx$	53.01***	0.92***	3.07
1987	$y = a + bx^{3}$	15.29***	0.80***	
1988	$y = a + bx^{3}$	6.95*	0.61*	
Common	$y = a + bx^{2}$	29.68***	0.72**	

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

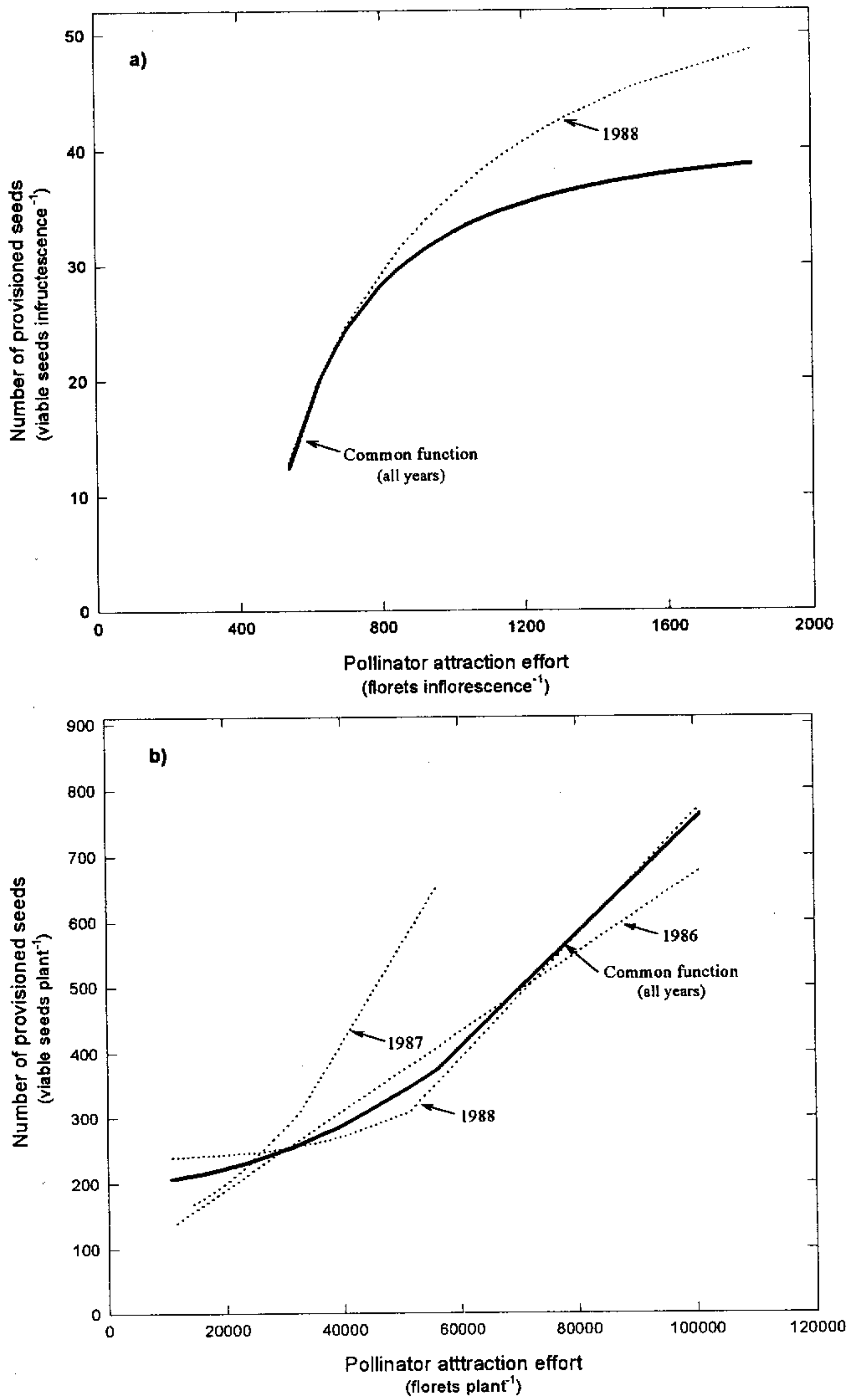

Figure 4.6 Significant best fit models for the association between provisioned seeds and pollinator attraction effort in *Banksia tricuspis* between 1986 and 1988: a) viable seeds infructescence^{-1} *vs* florets inflorescence^{-1}; b) viable seeds plant^{-1} *vs* florets plant^{-1}.

Rank correlations of viable seed infructescence^{-1} and seed set infructescence^{-1} for the ten subsample plants over the three years of this study indicated a propensity for plants to maintain reproductive output at a given rank or level with regards to other individuals within the population (Table 4.13). Aborted seed infructescence^{-1}, however, did not display a similar trend with the only significant parity being detected between 1987 and 1988. Notable negative correlations were detected for the interaction between viable seed infructescence^{-1} and aborted seed infructescence^{-1} and between aborted seed infructescence^{-1} and seed set infructescence^{-1}, for all interactions between each year. Rank correlations for the relationship between years of viable seed plant^{-1}, aborted seed plant^{-1} and seed set plant^{-1} were all positive and mostly significant, indicating predictability in maternal reproductive success among individuals (Table 4.13). Correlations between seed attributes were mostly weak and suggestive of a negative association for the interaction between aborted seed plant^{-1} and seed set plant^{-1}. The between-years correlations for viable and aborted seeds plant^{-1} were positively associated and, in some instances, significant.

Detection of significant rank correlations between viable seeds infructescence^{-1}, aborted seeds infructescence^{-1} and seed set infructescence^{-1} for the ten plants and the reproductive attributes of inflorescences plant^{-1}, infructescences plant^{-1} and floral damage was not achieved, despite the three fecundity components being significantly correlated, as demonstrated for this subsample of 10 plants (Table 4.5) and the population sample of 30 plants (Table 4.4). In the case of viable seeds infructescence^{-1}, the majority of correlations were positive while for aborted seeds infructescence^{-1} the majority of correlations were negative, especially those interactions with floral damage (Table 4.13). Seed set infructescence^{-1} was mostly positively associated with the three plant reproductive effort fecundity components between years, although not significantly so.

Between year rank correlations involving both viable seed plant^{-1} and aborted seed plant^{-1} were mostly positive and notable, indicating continuity between years in maternal reproductive success by individuals within the population (Table 4.13). Significant correlations were evident primarily for the interaction between infructescences plant^{-1} and aborted seed plant^{-1} over each year of the investigation.

Table 4.13 Matrix of Spearman's rank correlation for between year relationships among individual plants in seed number fecundity components used to measure reproductive success in *Banksia tricuspis*.

Reproductive attribute	Inflorescences plant^{1}			Infructescences plant^{1}			Floral damage (%)			Viable seeds infructescence^{-1} plant^{1}			Aborted seeds infructescence^{-1} plant^{1}			Seed set infructescence^{-1} plant^{1}		
	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988
Viable seeds infructescence^{-1}																		
1986	0.42	0.07	0.10	-0.03	0.33	0.25	0.37	-0.01	0.35									
1987	0.08	0.08	0.38	-0.01	0.31	0.09	0.24	0.04	0.46	0.84**								
1988	0.03	0.10	0.43	-0.08	0.34	0.14	0.24	0.04	0.46	0.84**	0.91***							
Aborted seeds infructescence^{-1}																		
1986	0.01	0.08	-0.15	0.21	0.24	0.02	-0.26	-0.13	-0.37	-0.49	-0.51	-0.84**						
1987	0.06	0.10	-0.22	-0.14	0.08	-0.18	-0.01	-0.09	-0.30	-0.26	-0.82**	-0.94***	0.29					
1988	-0.18	-0.15	-0.48	-0.09	0.23	-0.14	-0.25	-0.38	-0.53	-0.50	-0.72*	-0.96***	0.38	0.73*				
Seed set infructescence^{-1}																		
1986	0.08	0.02	0.28	-0.04	0.23	0.18	0.33	-0.01	0.27	-	-	-	-0.89**	-0.90**	-0.78*			
1987	0.19	0.19	0.34	-0.10	0.11	-0.08	0.43	0.24	0.58	-	-	-	-0.58	-0.86**	-0.61	0.73*		
1988	0.09	0.14	0.52	-0.07	0.42	0.19	0.33	-0.02	0.49	-	-	-	-0.48	-0.23	-0.39	0.72*	0.67*	-
Viable seeds plant^{-1}																		
1986	0.56	0.58	0.66*	0.71*	0.67*	0.79**	0.47	0.47	0.26									
1987	0.38	0.42	0.67*	0.53	0.57	0.29	0.51	0.24	0.65*	0.53								
1988	0.14	0.25	0.55	0.16	0.58	0.41	0.25	0.09	0.42	0.55	0.83**							
Aborted seeds plant^{-1}																		
1986	0.48	0.73*	0.50	0.84**	0.88**	0.85**	0.18	0.49	0.23	0.74*	0.35	0.27						
1987	0.61	0.81**	0.64*	0.71*	0.82**	0.92***	0.34	0.53	0.34	0.69*	0.34	0.34	0.79**					
1988	0.47	0.72*	0.66*	0.74*	0.93***	0.93***	0.36	0.33	0.12	0.67*	0.27	0.42	0.64*	0.82**				
Seed set plant^{-1}																		
1986	-0.72*	-0.66	-0.52	-0.22	-0.04	-0.09	-0.80	-0.74	-0.66*	-	-	-	0.12	-0.26	-0.07			
1987	-0.12	-0.14	0.25	-0.19	0.26	-0.12	0.09	-0.24	0.32	-	-	-	-0.03	-0.18	-0.02	0.27		
1988	-0.65	-0.49	-0.41	-0.41	-0.19	-0.10	-0.49	-0.51	-0.54	-	-	-	-0.04	-0.22	-0.18	0.66*	0.10	-

Significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Rank correlations between seed set plant^{-1} and the three fecundity components were predominantly negative but not significant.

4.3.3.2 *Seed mass*

Mass seed^{-1} statistics obtained from 1 380 seeds harvested from the 45 infructescences sampled over the three years of this study, were symmetrically distributed about the mean for each year (Table 4.14). Values for seed mass infructescence^{-1} and seed mass plant^{-1} were also symmetrically distributed about the mean for each year, with the exception of seed mass plant^{-1} during 1986. Significant temporal variation in mass seed^{-1} was detected with seeds produced in 1986 being significantly heavier that those produced in either 1987 or 1988 (Figure 4.7). Seed mass infructescence^{-1} and seed mass plant^{-1} however, were homogeneous between years (Table 4.7, Figure 4.7). The most successful year based on seed mass infructescence^{-1} and plant^{-1} appeared to be 1988. Coefficient of variation estimates for mass seed^{-1} were homogeneous between years (Figure 4.2). The estimated overall coefficient of 6.4 ± 1.9% was significantly less that the coefficient estimated for fruiting rate of 33.4% ($F_{89,\,29} = 2.69$, $P < 0.01$). Coefficients of variation estimated among years for seed mass indices were all generally low, with the exception of seed mass plant^{-1} which approached 33.2% (Table 4.2).

Table 4.14 The significance of departure from the normal distribution for mass seed^{-1} and seed mass results obtained for *Banksia tricuspis* between 1986 and 1988. (D = Kolmogorov-Smirnov goodness of fit, g_1 = skewness.)

Statistic	Year		
	1986	1987	1988
Mass seed^{-1}			
n	412	465	501
D	0.02	0.03	0.03
g_1	-0.10	-0.19	-0.21
Seed mass infructescence^{-1}			
n	15	15	15
D	0.15	0.12	0.21
g_1	-0.34	0.57	-0.23
Seed mass plant^{-1}			
n	10	10	10
D	0.32**	0.19	0.09
g_1	1.68	0.96	0.12

Significance: ** $P < 0.01$.

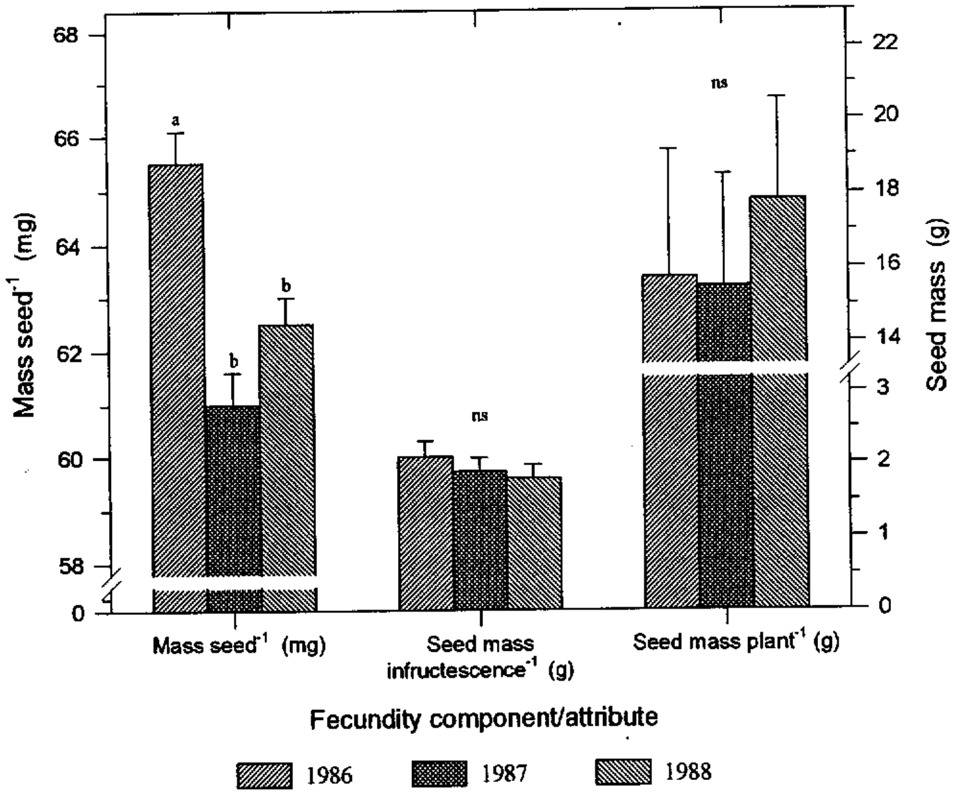

Figure 4.7 Variation in seed mass statistics for 1 380 viable seeds harvested from 45 infructescences on ten plants of *Banksia tricuspis* between 1986 and 1988. (Columns with different letters above their error bars were significantly different, Tukey-Kramer HSD, $P < 0.05$.)

The correlation between mass seed^{-1} and the number of inflorescences and infructescences plant^{-1} displayed heterogeneity between years (Table 4.15). This disparity between seasons was attributed to a significant correlation between mass seed^{-1} and inflorescences plant^{-1} in 1987 and between mass seed^{-1} and infructescences plant^{-1} in 1987 and 1988. All significant correlations between these two fecundity components and mass seed^{-1} were positive, unlike the significant correlations for floral damage which were negative. Interactions between mass seed^{-1} and floral damage were homogeneous between years and significant during 1986 and 1988. Seed mass infructescence^{-1} was not significantly correlated with inflorescence plant^{-1} or floral damage and the correlation with infructescences plant^{-1} was only significant during 1987 (Table 4.15). Correlation coefficients were mostly positive and homogeneous between years with the common correlation coefficients all notable except for the interaction with floral damage. Seed mass plant^{-1} was positively correlated with all

Table 4.15 Least squares correlation coefficients and common correlation (r_w) for linear regressions between seed mass fecundity attributes and plant fecundity components in *Banksia tricuspis* between 1986 and 1988.

Dependent variable	Independent variable											
	Inflorescences plant^{-1}		Infructescences plant^{-1}		Floral damage (%)		Viable seeds infructescence^{-1} plant^{-1}		Aborted seeds infructescence^{-1} plant^{-1}		Seed set infructescence^{-1} plant^{-1}	
	r	r_w	r	r_w	r	r_w	r	r_w	r	r_w	r	r_w
Mass seed^{-1}												
1986	-0.02		0.04		-0.11*	-0.09	0.06	-0.03	-0.07	-0.02	-0.02	
1987	0.16***		0.22***		-0.05		-0.07		0.05		-0.21***	
1988	-0.07		0.13**		-0.12**		-0.06		-0.02		-0.04	
Seed mass infructescence^{-1}												
1986	0.38	0.36*	0.40	0.40*	0.12	0.07	0.92***	0.91***	-0.18	-0.09	0.75***	0.68***
1987	0.47		0.60*		-0.12		0.86***		-0.24		0.60*	
1988	0.23		0.17		0.22		0.93***		0.15		0.66**	
Seed mass plant^{-1}												
1986	0.89***	0.79***	0.95***	0.87***	0.32	0.24	0.97***	0.95***	0.72**	0.68***	-0.13	0.25
1987	0.75**		0.86***		-0.8		0.96***		0.47		0.48	
1988	0.69*		0.73***		0.46		0.92***		0.78**		0.35	

Significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

three fecundity components with each correlation being significant for the interactions with inflorescences plant^{-1} and infructescences plant^{-1} (Table 4.15). All correlations with these fecundity components were homogeneous between years with the estimated common correlation coefficients being significant. The interaction with floral damage was homogeneous between years and positive despite a negative trend detected for the correlation in 1987.

Significant correlations between mass seed^{-1} and its association with the three seed fecundity variables were detected for the interaction between seed set and seed mass in 1987 only (Table 4.15). All interactions with viable and aborted seed infructescence^{-1} were homogeneous between years and the common correlation coefficients were negative. Significant heterogeneity between years was detected for seed set. Similar significant correlations were also detected for the association between seed mass infructescence^{-1} and seed set infructescence^{-1}, although homogeneity between years was evident (Table 4.15). Seed mass infructescence^{-1} was also significantly correlated with viable seeds infructescence^{-1} and each interaction was similar between years. Interactions between aborted seed infructescence^{-1} and seed mass infructescence^{-1} were generally negative, as indicated by the estimated common correlation coefficient. Correlations between the reproductive attributes of viable seeds plant^{-1}, aborted seeds plant^{-1} and seed set plant^{-1} were all homogeneous between years and mostly positive trending (Table 4.15). Interactions with viable and aborted seeds were mostly significant.

The principal independent fecundity component impinging on seed mass for both infructescences and plants was viable seed number (Table 4.10). This variable contributed significantly to the stepwise multiple regression equation determined for each yearly interaction and for the combined yearly interactions of both infructescences and plants. Each correlation estimated by the stepwise multiple regression procedure was significant. Submission of viable seed number as the principal fecundity component influencing seed mass is sustained through correlations estimated from the cosine of vectors displayed in Principal Component plots (Figure 4.5, Table 4.11).

Correlation coefficients determined from the best fit models for the association between seed mass infructescence^{-1} and florets infructescence^{-1} were homogeneous between years (Table 4.12). The common estimated best fit model was significant, as was the model estimated for 1988. Similarly, homogeneity was detected in correlation coefficients determined for the association between seed mass plant^{-1} and florets plant^{-1}. All yearly estimated best fit models were significant for the association with florets plant^{-1}, as was the common best fit model (Table 4.12). The relationship between seed mass and floret number embodied an exponential function for infructescences and a marked curvilinear upwards function for the plants (Figure 4.8).

Rank correlation results for seed mass infructescence^{-1} were non-significant between years indicating that seed mass infructescence^{-1} fluctuated between successive years among individual plants (Table 4.16). Conversely, seed mass plant^{-1} was homogeneous between successive years. Most rank correlations estimated between seed mass infructescence^{-1}, and the six other fecundity components assessed, were positive between interactions, although not significant. Significant interactions primarily verified correlation coefficient results. No significant correlations between successive years for the interaction between seed mass infructescence^{-1} and the other fecundity components were detected. Rank correlations between the reproductive attributes and seed mass plant^{-1} were predominantly positive with most interactions again verifying the linear correlation coefficient findings.

4.4 Discussion

Banksia tricuspis displays considerable investment in reproductive effort (number of inflorescences, infructescences and seeds), although percentage seed set is low and further reduced by floral damage. The most reproductively successful year for *B. tricuspis* was 1988 when viable seeds infructescence^{-1} and seed set infructescence^{-1} were at their greatest. However, the greatest reproductive effort was observed during 1986 when the number of inflorescences plant^{-1} was at a maximum. Seed set values for *B. tricuspis*, which averaged 2.9% over the three years of this investigation, are similar to those reported for other banksias (Lamont *et al.* 1985, Collins and Rebelo 1987). Typical of the genus, these low values were attributed to over 60% of inflorescences failing to develop into infructescences and over 98% of ovules on fruiting inflorescences failing to produce seeds (Hopper 1980, Whelan and Burbidge 1980, Whelan and Goldingay 1986, Lamont and Barrett 1988, Lamont *et al.*

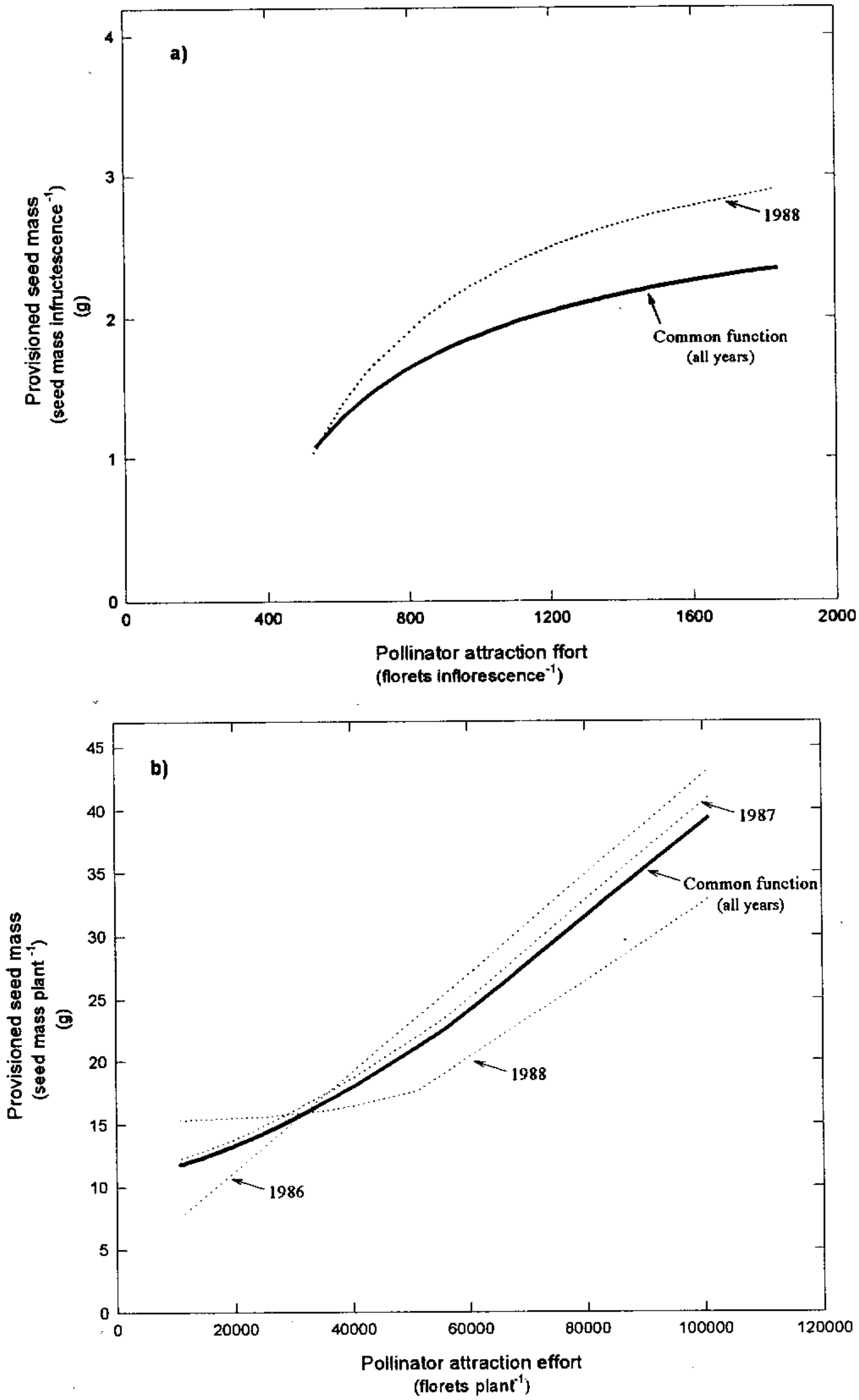

Figure 4.8 Significant best fit models for the association between provisioned seeds and pollinator attraction effort in *Banksia tricuspis* between 1986 and 1988: a) seeds mass infructescence^{-1} *vs* florets inflorescence^{-1}; b) seed mass plant^{-1} *vs* florets plant^{-1}.

Table 4.16 Matrix of Spearman's rank correlations for association between seed mass and plant fecundity components/attributes in *Banksia tricuspis* between 1986 and 1988.

Reproductive attribute	Inflorescences plant^{-1}			Infructescences plant^{-1}			Floral damage (%)			Viable seeds infructescences^{-1} plant^{-1}			Aborted seeds infructescences^{-1} plant^{-1}			Seed set infructescences^{-1} plant^{-1}			Seed mass infructescences^{-1} plant^{-1}		
	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988	1986	1987	1988
Seed mass infructescence^{-1}																					
1986	0.28	0.59^{*}	0.56^{*}	0.46	0.28	0.07	0.32	0.59^{*}	0.40	0.91^{***}	0.56^{*}	0.18	-0.02	0.27	0.14	0.79^{***}	0.25	-0.03			
1987	0.17	0.14	0.45	0.33	0.53^{*}	-0.16	0.18	-0.01	0.43	0.26	0.89^{***}	0.40	-0.10	-0.27	-0.12	0.23	0.70^{**}	0.14	0.35		
1988	0.01	-0.15	0.17	0.04	0.22	-0.19	0.03	-0.29	0.17	0.09	0.54^{*}	0.99^{***}	-0.49	-0.25	-0.01	-0.19	0.30	0.64^{**}	0.16	0.44	-
Seed mass plant^{-1}																					
1986	0.63	0.69	0.79^{**}	0.88^{**}	-0.95^{**}	0.87^{**}	0.38	0.38	0.31	0.82^{**}	0.54	0.49	0.88^{**}	0.84^{**}	0.81^{**}	-0.04	0.21	-0.23			
1987	0.26	0.36	0.65^{*}	0.27	0.72^{*}	0.37	0.27	0.06	0.54	0.36	0.87^{**}	0.86^{**}	0.47	0.39	0.29	0.03	0.71^{*}	-0.06	0.69^{*}		
1988	0.08	0.21	0.50	0.17	0.56	0.41	0.18	0.09	0.31	0.56	0.76^{*}	0.96^{***}	0.47	0.25	0.22	0.21	0.69^{*}	0.31	0.49	0.78^{*}	-

Significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

1993) . Fecundity appeared to be largely controlled by extrinsic factors, particularly floral damage, although synergistic interactions with intrinsic mechanisms is apparent.

4.4.1 *Fecundity components and reproductive success*

Reproductive success in *B. tricuspis*, as determined by seed mass, is principally influenced by seed output which was highly correlated with infructescence number (Table 4.9). This fecundity component was itself correlated with inflorescence number which impinged significantly on the majority of fecundity components assessed. The existence of strong correlations for associations interacting with inflorescence number indicated that plant fecundity is largely controlled by initial reproductive effort (inflorescence plant^{-1}). This implies that the most fecund individuals are those which indulge in the greatest flower production. Rank correlation results for the same interactions support this proposition (Table 4.13), as do the results of pollinator attraction effort against seed output and mass (Figure 4.6 and 4.8).

The detection of significant associations between inflorescence number and other fecundity components may be attributed to temporal and spatial disparity in a plant's allocation of reproductive resources. The pattern of resource allocation may be determined by plant size (Waller 1988, Klinkhamer *et al.* 1990, de Jong and Klinkhamer 1994), genetic makeup and thus superiority of individuals (Silander 1984, Geber 1990), plant age (Morrison and Myerscough 1989, Gutterman 1992), microhabitat quality (nutrients and moisture status) (Marshall and Watson 1992), climatic considerations (Sork *et al.* 1993) or the synergistic interaction of these variables (Davis 1981, Bazzaz and Ackerly 1992). Plant size may provide a plausible explanation for many of these associations as the success of all fecundity components and attributes was positively correlated with size (van Leeuwen, unpublished data). Plant size itself may also be a function of these deterministic variables. Heterogeneity in plant size has previously been invoked to explain variation in fecundity between road verge and non-edge *B. hookeriana* plants (Lamont *et al.* 1994).

Alternatively, the fecundity association with inflorescence number may be attributed to a pollen/pollinator mechanism in which a larger reproductive effort confers increased pollination success, as enumerated in the 'pollinator attraction' hypothesis for the production of 'surplus' flowers and the commensurate low seed flower^{-1} ratios (Stanton *et al.* 1986). This explanation, however, does not account for the disparity in reproductive effort (inflorescences plant^{-1}) in the first instance, which can be ascribed to the operation of mechanisms controlling reproductive allocation, particularly plant size, climatic conditions and genetic makeup (Bazzaz and Ackerly 1992). Carthew (1993) submitted that the absence of congruity among plants of *B. spinulosa* between years was attributable to genetic or micro-environmental considerations.

The detection of a positive correlation between the number of infructescences plant^{-1} and seed output and seed set also suggests that the reproductive allocation of resources may influence fecundity. An increase in seed output and seed set may simply reflect an increase in plant resource availability, as an individual's endowment with infructescences is inherently correlated with plant size (van Leeuwen, unpublished data). Alternatively, the increase in seed output could be promoted through increased pollination success bestowed by increased plant attractiveness. Plant attractiveness would be enhanced through increased inflorescence numbers, which, in this instance, was significantly correlated with infructescence number. Plant attractiveness, in terms of inflorescence number, is known to bias pollinator behaviour in this species (van Leeuwen, unpublished data, Chapter 7).

The detection of a positive association between mass seed^{-1} and infructescences plant^{-1} indicates intrinsic factors also control fecundity. This association implies that the better flowering plants have the greatest resource reserves, as mass seed^{-1} is the most sensitive indicator of resource status (Harper *et al.*1970). Correlations involving seed mass and infructescences plant^{-1} were usually significant, which can be attributed to a numerical association, where seed output infructescence^{-1} and plant^{-1} increase as cone and plant numbers increased.

Similar variation in fecundity has been reported in a number of other banksias and, although the peculiarity of such variation differs among species, most fluctuations have been attributed speculatively to maternal resource limitations associated with climatic variation (Copland and Whelan 1989, Vaughton 1991). Indeed, resource limitation of inflorescence production is evident in a number of species, as demonstrated in *B. spinulosa* where the addition of nutrients induces a corresponding increase in inflorescence production (Vaughton 1991), and the difference in fecundity of *B. hookeriana* between nutrient enriched (roadside) and customary (non-edge) habitats (Lamont *et al.* 1994).

Mass seed^{-1} in 1986 was significantly greater than in the two subsequent seasons. By contrast, seed output and seed set infructescence^{-1} in this season were at their lowest. This result could be interpreted as a limitation in maternal resources. However, marked correlations between mass seed^{-1} and both seed output and seed set were not detected, thus repudiating this interpretation. The strong correlation between seed output/seed set infructescence^{-1} and the number of inflorescences/infructescences plant^{-1} suggests that maternal resources were not limiting. Increased pollination success through greater pollinator attraction is also a credible explanation for these correlations. The failure to detect disparity in the number of aborted seeds infructescence^{-1} despite fluctuations in inflorescence numbers suggests that pollen/pollinator limitation provides a plausible explanation for the levels of fecundity observed. Resource limitations would be apparent if the proportion of aborted seeds increased with inflorescence number and the corresponding reduction in resource reserves.

It appears that inflorescence number sets the upper limit to reproductive success in *B. tricuspis* and governs the level of fecundity during subsequent development phases, especially for the fecundity attributes of seed output and mass. The nature of the control exerted by inflorescence number may be determined by intrinsic maternal resources and interactions with extrinsic factors, particularly pollen/pollinator limitations.

4.4.2 *Resource* vs *pollen limitation*

Undoubtedly the greatest constraint impinging on the fecundity of *B. tricuspis* is floral damage. This constraint reduced the potential to achieve maximum maternal reproductive success to levels which were generally less than 40% of what was possible in the absence of damage. Realised levels of fecundity, as indicated by seed set (Figure 4.4), were also considerably less than the levels possible, indicating that other constraints were operating to restrict reproductive success. Pollen limitation is advanced as the most likely factor to explain this shortfall in fecundity, although resource availability sets the upper limits to fecundity through flower production (Bazzaz *et al.* 1987).

The operation of pollen/pollinator limitation as a constraint impinging on fecundity is evident by the increase in fecundity (seed output and mass) with increased allocation of maternal resources (florets plant^{-1}) (Figure 4.6 and 4.8). The significant correlation between inflorescence number, which is resource controlled, and the fecundity attributes of seed output and mass also may implicate pollen limitation as a fecundity constraint. The extent of pollen limitation as a constraint varied between reproductive development phases. Pollen limitation is not evident in the inflorescence to infructescence development phase as no undamaged barren infructescences were recorded. Conversely, overall plant fecundity, and to a lesser extent infructescence fecundity, appear to be restricted by pollen limitation during later developmental phases, as indicated by the best fit model for the functions describing pollinator attraction effort *vs* provisioned seeds (Figure 4.6 and 4.8). These models indicate that whole plant fecundity is significantly constrained by pollen limitation and that resource limitation is apparently lacking. This is especially true for models between seed mass and pollinator attraction effort as the dependent variable, seed mass, is a sensitive indicator of maternal resource status (Haig and Westoby 1988b). This result was not only evident for the pollinator attraction effort of the entire inflorescence crop but was also detected for the best fit models of pollinator attraction effort versus fecundity, when pollinator attraction effort was determined for undamaged inflorescences only (van Leeuwen, personal observation).

Pollen limitation of whole plant fecundity may be attributed to a suite of factors which are extrinsic and intrinsic in operation (Bertin 1988, Stephenson 1992). Explanations invoked for *B. tricuspis* focus on reductions in paternal fitness and plant attractiveness (maternal fitness) as a consequence of floral damage. Paternal fitness is reduced under this scenario as a consequence of a contraction in the pollen pool size following a reduction in the number of intact inflorescences present within the population. Plant attractiveness is detrimentally altered as a consequence of the numerical loss of inflorescences, as pollinator behaviour in *B. tricuspis* is influenced by inflorescence endowment (van Leeuwen, unpublished data, Chapter 7). The non-random nature of floral damage, (Chapter 3) where large inflorescences are preferentially attacked, may also detrimentally affect plant attractiveness through changes in pollinator rewards (nectar and pollen) and alterations in the visual cues which attract pollinators. Competition among pollinators for reduced nectar and pollen resources may also promote pollen limitation, especially if pollinators are specialists and/or territorial. Most pollinators of *B. tricuspis* exhibit territorial behaviour and thus competition for nectar and pollen resources increases with a reduction in inflorescence number (van Leeuwen, unpublished data). The extent to which such changes in plant attractiveness impinge on pollinators will be governed by the functional relationship between the two.

Pollen limitation of infructescence fecundity was also evident, although the effect was of a smaller magnitude to that observed for whole plant fecundity. Infructescence fecundity appears to be approaching the point where resource limitation could be invoked as a constraint limiting further fecundity gains. Differences in the best fit models describing the relationships between pollinator attraction effort and both infructescence and plant fecundity imply serial adjustment in maternal resources between development phases (Lloyd 1980). During the inflorescence development phase (Figure 4.6 and 4.8) resources appear to be abundant. However, at the subsequent fruit development phase, resource limitations become imminent. This disparity in best fit models could be attributed to the low cost of flowers relative to substantial post-zygotic maternal costs (Delph 1990, Witkowski and Lamont 1996).

The more pronounced impact of the pollen-limitation function on whole plant fecundity could be attributed to the significantly larger pollinator attraction effort of this physiological unit. However, the nature of the best fit model did not alter as a consequence of floral damage, as evident by the detection of similar best fit models, when pollinator attraction effort of undamaged infructescences was employed as the independent variable (van Leeuwen, personal observation). The disparity between infructescence and whole plant best fit models in registering the importance of resource and pollen limitation and, in particular, the imminent approach of resource limitation in infructescence fecundity, reinforces the notion of 'surplus' flower production. Impending, although not yet apparent, resource limitation of infructescence fecundity also demonstrates that *B. tricuspis* synchronises maternal investment with available maternal resources.

Overall, maternal resources appear not to constrain reproductive success in *B. tricuspis.* This is especially true for whole plant fecundity attributes. However, the distinction between resource and pollen limitation for infructescence fecundity is not as unequivocal. The proposition that maternal resources are not limiting is supported by the successful conversion of all non-damaged inflorescences to infructescences and the failure to detect significant departure between years in the fecundity of individuals relative to other plants in the population. Consistency between years or non-randomness in reproductive success and low variance among years in fecundity components and attributes also supports this proposition (Ågren 1988).

Conversely, marked variance in seed output, as extrapolated through follicle production, has been attributed to nutrient and pollen limitation in other banksias. Vaughton (1991) demonstrated that seasons of low inflorescence visitation by nectarivorous birds (pollen limitation) in *B. spinulosa* corresponded with periods of low fecundity and that nutrient supplementation through the addition of fertiliser increased fecundity in this species.

4.4.3 *Temporal consistency and adjustment of maternal resources*

Between year plasticity in fecundity among the reproductive components and attributes assessed was usually not significant. However, exceptions were observed in the case of inflorescences plant^{-1}, seed set infructescence^{-1} and mass seed^{-1}. Low among years' estimates of the coefficient of variation for most of the fecundity components and attributes assessed also confers continuity in reproductive success between years in this species (Table 4.2).

The failure to detect significant disparity between years in the fecundity component of infructescences plant^{-1}, despite marked yearly differences in inflorescences plant^{-1} with which it was significantly correlated, supports the proposition that *B. tricuspis* efficiently matches reproductive effort with available maternal resources. The effective matching of reproductive effort (inflorescences plant^{-1}) to maternal resources is also supported by the failure to detect any notable departure between years in the fecundity of individual plants relative to other plants in the population, as indicated by significant rank correlations (Table 4.13).

Morrison & Myerscough (1989) contend that minimal between year disparity in fecundity indicates consistency and predictability in resource availability and environmental conditions impinging on reproductive success. This proposition is acceptable in *B. tricuspis*, where heterogeneity between years was absent for most fecundity components and attributes and between years consistency in reproductive effort and fecundity of individuals was evident, as indicated by significant accord in rank correlation and minimal variance as estimated by the coefficients of variation. The lignotuberous habit of the species (George 1981) may promote between year consistency in resource availability, providing a buffer against deleterious environmental conditions. Similarly, consistency between seasons in floral damage (Figure 4.1) suggests predictability in the major extrinsic constraint limiting fecundity. Consistency in fecundity between years also appears to occur in other banksias (Copland and Whelan 1989, Whelan and Goldingay 1986). However, in one instance, significant variance in seed output has been demonstrated, where the most successful season was almost double that of the other seasons (Carthew 1993).

Results for the coefficient of variation among individual plants within years support the notion of predictability in fecundity, especially the significant reduction in variability with advancement through the different reproductive stages. The considerable variation in inflorescences plant^{-1} was significantly greater than that detected for infructescences inflorescence^{-1}, which in turn was significantly greater than the variance in mass seed^{-1} (62% *vs* 33% *vs* 6.4% respectively). This result implies that intrinsic control over fecundity increases through the various reproductive phases. The significant reduction in variation between the inflorescence (flower) and infructescence (fruit) developmental phases demonstrates that the greatest variance in fecundity is detected when the intrinsic control over fecundity, and thus the predictability of reproductive success, is at its lowest. Coincidentally, the principal extrinsic control influencing fecundity, floral damage, operated between these two reproductive phases. Between year homogeneity in infructescence production, despite heterogeneity in inflorescence production and the significant reduction in variance between these two reproductive stages, also implies that plants are able to repartition maternal resources. This finding supports Lloyd's (1980) proposition of serial adjustment in maternal investment occurring preferentially at the fruiting phase rather than the flowering phase.

A cyclic or bimodal trend in maternal reproductive success, as tentatively suggested for three of the fecundity indices assessed (inflorescences plant^{-1}, infructescences plant^{-1} and mass seed^{-1}), may be indicative of a resource reimbursement strategy where reproductive success in one season detrimentally influences fecundity in subsequent seasons (Zimmerman and Aide 1989). This strategy has been observed in a number of other plants and is driven by constraints associated with a tradeoff between reproductive output and resource limitation (Ackerman and Montalvo 1990). This reimbursement strategy is rejected as a factor influencing fecundity in this species, as between year differences in most of these cyclic variables were not significant and rank correlations results indicated significant accord between years in the fecundity of individuals. The lignotuberous habit of *B. tricuspis,* acting as a resources reserve enabling this species to maintain reproductive effort at a level where fecundity in one year does not impinge on the potential of subsequent years, supports the repeal of this strategy.

B. tricuspis is able to maximise fecundity through maternal control over resources in response to limiting constraints, as clearly demonstrated by consistency and minimal variance between years in most fecundity components and attributes. This maternal control of fecundity is highlighted by the reduced variance in fitness components between developmental phases and by uniformity in fruit production despite heterogeneity in flower production. The reserve ovule bet hedging hypothesis accounting for 'surplus' flower production (Ehrlén 1991, Stephenson 1992), which pronounces consistency in fecundity between years, is promoted by reproductive success in *B. tricuspis*. Conversely, disparity in fecundity is embraced by the enhanced male function and selective, non-random abortion hypotheses (Ågren 1988, Morgan 1993).

4.4.4 *Responses to floral damage*

The extent of inflorescence loss caused by the agents of floral damage was a similar magnitude to that reported previously (Chapter 3), with the greatest loss being ascribed to the activities of the rachis boring *Arthrophora* moth larvae. Damage by the moth larvae was closely mirrored by cockatoo damage with 40% of inflorescences being attacked by both agents. The extent of floral damage by each of the agents was positively correlated with the number of inflorescence plant^{-1} suggesting that increases in reproductive effort may not necessarily increase fecundity or plant fitness. This association supports the proposition that floral display acts as a visual cue for floral damaging agents (Chapter 3, Lamont and van Leeuwen 1988).

A compensatory response to floral damage was detected in *B. tricuspis*, indicated by the positive interaction between seed output (viable seeds infructescence^{-1}) and floral damage (Table 4.9). This response was also evident for the interaction between seed set infructescence^{-1} and floral damage and indicates that seed output and the number of seeds flower^{-1} on infructescences increased as the level of flower mortality increases. Results from Principal Component Analysis determined correlations also indicated that floral damage had a moderately positive influence on seed output. This response is best described as partial recovery (Bergelson and Crawley 1992), as seed set plant^{-1} was very low and significantly negatively associated with floral damage due to the reciprocal increase in flower mortality with floral damage. If a full compensatory response was achieved, no correlation between the fecundity components would be expected while an overcompensatory response would be represented by a significantly positive correlation.

This partial recovery response is attributed to an increase in pollination success and thus amended paternal reproductive success. An increase in pollination success would be predicted with a rise in floral damage, as the reduction in nectariferous sources (inflorescence) associated with floral damage would propel pollinators to increase the duration of foraging bouts at inflorescences, thereby increasing the probability of pollination. Pollination success may also increase due to a rise in the incidence of xenogamous matings following an increase in the proportion of inter-plant foraging bouts. The incidences of deleterious geitonogamous and consanguineous mating may also be reduced by such an increase in inter-plant foraging and may also enhance pollination success.

The failure to detect an association between aborted seeds and floral damage could be interpreted as support for the notional increase in xenogamous matings while the absence of a negative correlation tenuously implies that deleterious matings remain constant regardless of floral damage. Such propositions must, however, be considered with caution as pre-zygotic abortion has not been considered. The repartitioning of maternal resources among the remaining undamaged flowers, thereby facilitating an increase in post-zygotic survivorship, is rejected as the mechanism propelling this compensatory response as no complementary negative interaction between floral damage and aborted seeds was detected. Absence of such an interaction also implies that genetic considerations rather than maternal resources principally determine the pattern of post-zygotic selection.

No compensatory response was observed in results for mass seed^{-1} or seed mass, which remained constant despite increases in floral damage and the increased postulated partitioning of material resources among undamaged inflorescences. This result complies with the predictions of the optimal seed size model (Haig and Westoby 1988b) which proposes that plants will respond to changes in resource availability by a change in seed number rather than size.

The presence of this compensatory response, albeit only partial recovery, complies with the notion that 'surplus' flowers function primarily in response to a bet hedging mechanism. The reserve ovule hypothesis (Ehrlén 1991, 1993), which states that reproductive success remains constant and surplus ovules/flowers act as a reserve supply to replace unpredictable mortality, conforms with the results discussed for *B. tricuspis*. In this instance, 'surplus' flowers provide an opportunity to increase fecundity, as indicated by the increase in seed output with increasing floral damage.

The disproportionate magnitude of the compensatory response in relation to the level of floral mortality is puzzling, although it may be attributed to pollen and possibly resource limitation impinging on infructescence fecundity. Failure to detect a more symmetrical response and the realisation that infructescence fecundity is approaching the limits of maternal resources suggest that 'surplus' flowers may also function to enhance pollinator attraction in this species. The identification of a compensatory response in *B. tricuspis* complies with expectations for this phenomenon (Vail 1992), as this species experiences high levels of floral mortality which are predictable and are restricted to short periods in an enduring life cycle.

4.4.5 *Synchronous non-random reproductive success*

Fecundity in *B. tricuspis* is synchronous, constant and predictable between years which is attributed to the intrinsic control exerted over maternal resources and the predictability of the principal extrinsic constraint, floral damage. This proposition is supported by the low among years coefficient of variation estimates for all fitness components and attributes (Table 4.2), which were below the level indicative of constant reproductive output (40%) (Kelly 1994). These coefficient of variation estimates must, however, be interpreted with caution as an interval approaching 10 years is recommended to substantiate patterns of reproductive success in plants (Kelly 1994). Synchrony between years is also indicated by significant associations in rank correlations, indicating that the reproductive effort and fecundity of individual plants remains constant between seasons with regards to other individuals. It is postulated that the intrinsic control of maternal resources ensuring continuity in reproductive success is facilitated by the lignotuberous habit of this species.

Predictability in floral damage does not support the notion of an inflorescence herbivory avoidance mechanism akin to masting in this species, as suggested in Chapter 3. Synchrony between inflorescence production and floral damage, as demonstrated by significant correlations between the two variables and proportional synchrony between years in the pattern of inflorescence production and floral damage, also rebuts masting. This synchrony between inflorescence number and agents of floral damage implies a functional relationship in which the animal agents are not dependent on the *Banksia*. This interpretation is verified by the realisation that the parrots and cockatoos are facultative granivorous species, while the insect larvae also invade the rachis of more abundant *Banksia* spp. in the Lesueur area (van Leeuwen, personal observation). The facultative nature of the relationship between the plant and damaging agent may also account for the observed consistency in floral damage, despite the amplification of inflorescence number during particular years.

The attraction of pollinators (Kelly 1994) is suggested as the most likely explanation for synchronous reproduction in *B. tricuspis*. Coefficient of variation results for inflorescence production comply with the premise of this economy of scale hypothesis, in that within year variation in flower number was high (62.4%) and among years variation was moderate to low (24.0%) (Koenig *et al.* 1994). This hypothesis focuses on competition among trees for pollinators and is driven by the premise that large floral displays attract more pollinators (Augspurger 1981). Ims (1990a) submits that pollinator attraction is the proximate explanation for synchrony and confers constant reproductive success, which is supported here.

4.4.6 *Conclusions*

Fecundity and plant fitness in *B. tricuspis* is governed by the maternal allocation of resources which do not appear to be limiting. Reproductive synchrony, continuity and predictability in fecundity and compensatory responses to flower mortality all imply that resources are unconstrained. Floral damage is the primary extrinsic constraint limiting fecundity, while pollen/pollinator limitation is suggested as the proximate constraint inhibiting subsequent increases in plant fitness. Resource limitation of infructescence fecundity appears to be imminent despite no complementary limitation of plant fecundity. Repartitioning of resources and compensation for flower mortality imply a high level of intrinsic control over reproductive success in this species.

The production of 'surplus' flowers in *B. tricuspis* appears to have two possible adaptive advantages. Firstly, enhancement of plant attractiveness to pollinators as suggested through: a) economies of scale hypotheses for synchronous reproduction; b) increased seed output with proportional increases in flower mortality; and c) increased fecundity with increased inflorescence number. Secondly, the over production of ovules enables this species to utilise reserve supplies when pollination success is enhanced in response to floral mortality, as evident in the compensatory response to flower mortality. This bet hedging advantage is also promoted by consistency and low variability in fecundity between years. 'Surplus' flowers may also serve an ultimate function in terms of reducing the effect that flower mortality has on fecundity. The over production of relatively inexpensive flowers enables the plant to allocate resources during later, more resource expensive, developmental phases in a manner that limits the effects of flower mortality, maximises resource utilisation and enhances fecundity.

Verification of the propositions discussed in this chapter could be achieved through rigorous experimentation. The primary hypothesis requiring attention is the role of resource versus pollen limitation in constraining fecundity. The importance of each factor could be investigated through carefully orchestrated fertiliser supplementation and hand pollination trials which highlight the influence of these constraints on both infructescence and plant fecundity. The addition of fertilisers may augment maternal resources while hand pollination may mitigate pollen limitation. If fecundity or the surrogates of progeny fitness increased following fertiliser supplementation then resource considerations could be cited as limiting, whereas pollen considerations could be cited if no increase in fecundity followed hand pollination treatments. Such experiments would need to be carefully planned to avoid the many avenues of bias which may be introduced through, for example, a time lag between fertiliser uptake and utilisation and genetic incompatibility problems between pollen donors and the maternal parent.

Experimentation could also be used to investigate the extent to which floral damage constrains reproductive success. This could be achieved by preventing the agents of floral damage from attacking inflorescences and by subsequently documenting changes in reproductive success which may eventuate. If reproductive success remains constant following this treatment then resource limitation considerations could be advanced as a constraint on fecundity; however, if reproductive success increased it may be refuted. Pollen supplementation through hand pollination would need to be undertaken in such an experiment to eliminate the ambiguity conferred by pollen limitation considerations. A similar experimental approach, excluding the agent of floral damage, would be required to test the hypothesis that this *Banksia* responds to changes in resource availability by increases in seed number rather than in seed size. For such an experiment, increases in seed number relative to the control would confirm this proposition while increases in seed size would disprove it.

Continued monitoring of the reproductive success of *B. tricuspis* over many years would also be beneficial to confirm the proposition of reproductive consistency and thus the reserve ovule bet hedging hypothesis for the production of 'surplus' flowers.

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CHAPTER 5

FECUNDITY AND PROGENY FITNESS PATTERNS WITHIN INFRUCTESCENCES

5.1 Introduction

Plants which produce 'surplus' flowers are physiologically incapable of providing the necessary nutritional resources to form mature zygotes (fruits/seeds) from all of their fertilised ovules (Stephenson 1981). Under such circumstances, zygotes in excess of maternal resource provisions are aborted (Bangerth and Ho 1984). Thus, the upper limits to a plant's reproductive success, which may be strongly affected by the relationship between flowering phenology, pollination success, paternal parentage and the nutritional relationship among its flowers, is determined by the availability of maternal resource provisions. Abortion of zygotes, synchronising maternal investment with resource provisions, concurs with the proposition of serial adjustment at successive reproductive stages (Lloyd 1980). This serial adjustment enables a plant to maintain maximum levels of fecundity, in spite of the randomness of pollination (Mulcahy 1979), between reproductive bouts, and ensures a high degree of maternal control over the resultant progeny (Lee 1988). Differential or non-random patterns of zygote survival, as is typical in many plants exhibiting 'surplus' flowers (Stephenson and Bertin 1983, Sutherland 1986, Uma Shaanker *et al.* 1988, Diggle 1995), exemplifies the ability of the maternal parent to adjust paternity of its progeny (Marshall and Ellstrand 1986, Marshall and Folsom 1991), regulating offspring number (Rocha and Stephenson 1990, Venables 1992) and genetic quality (Bawa and Webb 1984, Rocha and Stephenson 1991). Non-random zygote survival has been proposed as one of the hypotheses for the apparent wasteful and inefficient production of 'surplus' flowers (Stephenson 1981, 1992, Lee 1984, 1988).

Mechanisms of abortion which promote non-random patterns of zygote survival between plants are usually dependent on the number of developing zygotes, paternal parentage, time of zygote initiation and flower position, or some combination of these factors (Stephenson 1981, 1992, Stephenson and Bertin 1983, Lee 1988). Abortions based on the number of developing zygotes generally conform with the assumption that abortion increases as the number of developing fruits increase, and that those fruits which are abandoned usually have the least number of developing zygotes (Bertin 1982, Lee and Bazzaz 1982). The relationship between zygote number and abortion is usually positively correlated with pollination intensity (Snow 1982, Wilson *et al.* 1987) and may be influenced by the physiological independence of the reproductive unit (Watson and Casper 1984, Vaughton 1993, Guitián 1994).

Selective abortion, mediated in response to paternal parentage, has been attributed primarily to the deleterious effects of self pollination, increasing the opportunity for the expression of lethal recessives and pre- and post-zygotic incompatibility systems (Seavey and Bawa 1986, Barrett 1988). However, abortion driven by paternal parentage may also be attributed to mate choice and the mechanisms responsible for non-random patterns of fertilisation. These include disparity in maternal sporophytic vigour as a consequence of insufficient pollen tube competition, divergence in the competitive ability of microgametophytes from contrasting paternal sources, and genetic incongruity between contrasting paternal and maternal sporophytes (Bertin 1988, Schlichting *et al.* 1987, Stephenson 1992, Niesenbaum and Casper 1994). Selective abortion based on paternal parentage, incompatibility systems and mate choice represents a genetic sieve which selects those pollen and ovule genotypes most likely to make the greatest contribution to the fitness of the maternal sporophyte while permitting regulation of reproductive success with finite maternal resource provisions (Stephenson and Bertin 1983).

Time of zygote initiation and position of the zygote within the reproductive unit have a pronounced effect on maternal reproductive success, which is noticeable both between and within whole plants (eg. early *vs* late flowering, terminal *vs* axillary inflorescence presentation) and inflorescences (eg. early *vs* late flowering florets, apical (distal) *vs* peduncular (proximal) florets) (Stephenson 1980, 1992, Wyatt 1980, Stephenson and Bertin 1983, Lee 1988, Gutterman 1992). The ensuing pattern of zygote survival is generally described as one of first-fruit dominance where florets initiated and fertilised first have a greater chance of survival than those initiated and fertilised at latter stages in the phenological sequence (Ho 1992, Guitián 1994). First-fruit dominance confers a temporal advantage to those inflorescences opening early in the flowering season and to florets located at the proximal end of acropetalous inflorescences. First-fruit dominance also confers a spatial advantage in that zygotes on axillary inflorescences and in peduncular positions on acropetalous inflorescences are located more favourably to resource provisions, thereby pre-empting the nutrient supply to more apical or distal reproductive organs (Stephenson *et al.* 1988).

The non-random pattern of zygote survival and its impact on maternal reproductive success and progeny fitness in response to variations in the temporal and spatial advantages conferred through position effects varies among species (Lee 1988, Stephenson 1992). In some instances, especially the Leguminosae, the highest probability of zygote maturation and progeny fitness is associated with the apical end of the inflorescence (Lee and Bazzaz 1986, Nakamura 1988, Rocha and Stephenson 1990, 1991, Herring 1994). Conversely, some taxa display the highest probability and greatest fitness in peduncular positions (Wyatt 1981, Stephenson 1992 Ehrlén 1993, Guitián 1994). In other species (Stanton 1984, Horowitz *et al.* 1976), zygotes originating from middle positions in the inflorescences display a higher probability of zygote maturation and progeny fitness than in more apical or peduncular positions.

Hypotheses invoked to explain the non-random patterns of zygote survival in response to time of initiation and position effects mainly focus on late formed zygotes being starved of the necessary nutritional resources (Watson and Casper 1984, Lee 1988). The two primary explanations, which are not necessarily mutually exclusive, focus on 'competition' and 'inhibition' hypotheses (Guitián 1994, Ganeshaiah and Uma Shaanker 1994). The explanations range from simple competition between competing sinks (maturing zygotes) for the limited maternal resource provisions, through to the production of growth hormones that regulate source-sink interactions and effectively divert resources to the more vigorous, earlier initiated sinks and away from later initiated, weaker sinks (Lee 1988, Stephenson *et al.* 1988). The production of growth regulators by early developing zygotes that actively inhibit and induce abortion of later zygotes has also been proposed as an explanation (Tamas *et al.* 1979, Bangerth 1989).

Recently, the process of self organisation has also been presented as a possible explanation for the differential abortion of zygotes (Ganeshaiah and Uma Shaanker 1992, 1994). This model proposes that the probability of any zygote receiving sufficient resources to develop successfully is a function of: a) its ability to attract maternal resources relative to other zygotes; and b) the volume of maternal resources already received. This self organisation of maternal resources is initially propelled by asymmetry between developing zygotes and reinforced by the development of an autocatalytic feedback process which promotes a dominance hierarchy among developing zygotes, culminating in the abortion of those zygotes which fail to receive sufficient maternal resources (Ganeshaiah and Uma Shaanker 1994). This model assumes that initial asymmetry between zygotes is a consequence of random drift (Ganeshaiah and Uma Shaanker 1992). However, the development of a dominance hierarchy between zygotes, which supplements the process of self organisation, may be encouraged by first-fruit dominance or through genetic incongruity between zygotes (Ganeshaiah and Uma Shaanker 1994, Uma Shaanker *et al.* 1995).

Inadequate development of the vascular network associated with terminal reproductive structures and apical portions of inflorescences, has been invoked to explain the non-random pattern of zygote maturation (Van Steveninck 1957, Lee and Bazzaz 1982, Vaughton 1993). Inadequacies in the vascular network of some inflorescences has also been credited with a reduction in the number of ovules flower^{-1} (Solomon 1988, Thomson 1989, Diggle 1995). Pollinator foraging behaviour at an inflorescence, which typically mimics the acropetalous anthesis pattern of many inflorescences (Waddington 1983, Waser 1983, Collins and Spice 1986), has also been promoted as an explanation for the non-random pattern of zygote maturation observed in many plants (Lee 1988). Pollinator behaviour would promote a greater incidence of xenogamous pollination at the base of the inflorescence with a differential increase in the incidence of geitonogamous pollination with movement towards the most recently opened florets (Lamont 1982, Wyatt 1983). Zygotes originating from xenogamous fertilisation are inherently genetically superior and perhaps more vigorous that those originating from geitonogamous fertilisation and are thus stronger genetic sinks for maternal resources (Horovitz et al. 1976, Nakamura 1986, Stephenson 1992). The higher rates of seed abortion in outbreeding compared with inbreeding species supports the notion that stronger sinks develop as a consequence of xenogamous fertilisation (Weins *et al.* 1987, Bawa and Buckley 1989).

Non-random zygote maturation determined by an inability to provision all fertilised ovules may not only be manifested in the level and pattern of zygote survival but also in attributes of progeny fitness such as mass seed^{-1}, germination vigour and reproductive output of subsequent offspring (Stephenson and Winsor 1986, Nakamura 1988, Rocha and Stephenson 1990, 1991 Gutterman 1992). For example, in some species with acropetalous inflorescences, both seed number and mass seed^{-1} vary systematically with floral position, with apical florets producing significantly fewer and smaller seeds than the more peduncular florets (Delvin 1989). Similarly, the vigour of progeny from peduncular positions often outperforms the progeny from more apical positions (Rocha and Stephenson 1991). Non-random survival of zygotes that selects for progeny of the highest fitness (heaviest seeds, greatest germination vigour, enhanced reproductive output) is adaptive especially when competition for maternal resources is not severe (Bawa and Webb 1984, Mazer 1987). In many instances where selection favours progeny with the greatest fitness, the non-random pattern of zygote survival may extend to the abortion of potentially viable zygotes (Lee and Bazzaz 1982, Casper 1984, 1988, Nakamura 1986, Rigney 1995).

It has been proposed that low levels of maternal reproductive success within the genus *Banksia* (Collins and Rebelo 1987) enable maternal plants to regulate progeny fitness through the differential maturation of zygotes (Ayre and Walker 1989, Walker and Whelan 1991). Strong differential selection is suggested in this genus through the juxtaposition between high outcrossing rates, which approach panmixia (Scott 1980, Carthew *et al.* 1988, Vaughton and Carthew 1993), and pollinator foraging behaviour that promotes mostly geitonogamous and consanguineous (mating with sibs) pollination events (Collins and Spice 1986, Ramsey 1989, Vaughton 1990, Vaughton and Ramsey 1991). Indeed, Vaughton and Carthew (1993) experimentally demonstrated selective abortion of selfed embryos in the partially self-compatible but highly outcrossing *B. spinulosa* var. *neoanglica* which they attributed to competition among embryos for limited maternal resources with selfed zygotes representing uncompetitive sinks. Non-random survival of zygotes, as evident by low fruit set in the apical portion of the infructescence, has also been demonstrated for this species (Vaughton 1988). Vaughton (1993) attributed this pattern to smaller ovules in the apical portion of the inflorescence which conferred differentiation as a consequence of inter-ovary competition for limited maternal resources. Non-random patterns of seed set and zygote survival have also been observed in other species of *Banksia* (McFarland 1985, Zammit and Hood 1986, Stock *et al.* 1989, Wallace and O'Dowd 1989) and in other genera of Australian Proteaceae (Goldingay and Whelan 1993, Harris and Whelan 1993).

Differences in the distribution of follicles over the infructescences of the sympatric *B. integrifolia* and *B. spinulosa* var. *collina* were attributed to inconsistencies in the pattern of nectar production within inflorescences of these two species and to the subsequent discord in honeyeater behaviour while probing inflorescences for nectar (McFarland 1985). However, Wallace and O'Dowd (1989) noted that differential pollinator visitation was unlikely to explain the non-random pattern of follicle production observed in *B. spinulosa* var. *cunninghamii*. Alternatively, they suggested that florets located towards the top of the inflorescence may be functionally andromonoecious (Johnson and Briggs 1975) or that fertilised peduncular ovules may pre-empt maternal resource provisions to more distally located ovules. Conversely, Goldingay and Whelan (1993) argued that the non-random preponderance of fruits in the apical third of *Telopea speciosissima* infructescences was probably attributable to enhanced pollination success as a consequence of greater honeyeater visitation at this stage of inflorescence opening. Scott (1982) proposed that a random distribution of follicles within the infructescence reduced insect damage by increasing the burrowing time of destructive insect larvae. However, no dependent relationship between insect damage and non-random fruit production has been observed (Wallace and O'Dowd 1989). Similarly, damage to seeds by insect predators was independent of the non-random pattern of fruit production in both *B. ericifolia* and *B. oblongifolia* (Zammit and Hood 1986).

This chapter examines the pattern of reproductive output within inflorescences and infructescences of *Banksia tricuspis* in a natural population. The aim was to determine if non-random intra-infructescence patterns exist in various components of maternal fecundity and progeny fitness. Specifically, the objectives of the study were to determine if maternal fecundity, as inferred through fruit and seed set and the probability of zygote survival, is non-random within inflorescences, and if progeny fitness, as deduced through the surrogates of mass seed^{-1}, seed nutritional content and outcrossing estimates, is heterogeneously partitioned according to position. Fulfilment of these objectives were achieved through examining intra-inflorescence patterns of floret, follicle and viable seed production which enabled the determination of fruit and seed set and calculation of zygote survival probabilities. Additionally, mass seed^{-1}, seed total nitrogen and phosphorus content, observed heterozygosity and minimum variance mean estimates of outcrossing were investigated for intra-infructescence patterns. Relationships between components of fecundity and fitness in this *Banksia* were also examined, especially with regard to any non-random patterns observed. The project outcomes are discussed with reference to (i) the relative importance of initiation time and position effects on reproductive output; (ii) the role of resource and pollen limitation in delineating the intra-infructescence patterns observed; and (iii) the possible adaptive significance of 'surplus' flowers in this species.

5.2 Materials and Methods

Non-random patterns in maternal reproductive success in *Banksia tricuspis* infructescences were investigated by examining maternal fecundity and progeny fitness within infructescences produced on ten plants from a natural population in the Lesueur National Park. The population was located about 3.9 km E of Mt Lesueur and 10.5 km SE of Mt Peron (30° 10' 11" S, 115° 14' 14" E) near the coastal town of Jurien, about 220 km north of Perth. This species is a long-lived, woody shrub to small tree which produces conspicuous bright golden yellow inflorescences that are usually terminal or on short lateral branchlets. Inflorescences consist of up to 2 500 florets arranged orthogonally around a woody central rachis with anthesis occurring basipetally over a two week period (George 1981, van Leeuwen and Lamont 1986). Infructescences (fruiting inflorescences) support prominent elliptic follicles (dehiscent fruits) which contain up to two viable seeds that mature two to four months after the completion of flowering (van Leeuwen and Lamont 1986).

This investigation was conducted on inflorescences produced during the 1987 flowering season which were subsequently harvested as infructescences in March 1988 after follicles and seeds had matured. To minimise the effects of contrasting plant sizes, which significantly affect maternal reproductive success in this species (van Leeuwen, unpublished data), plants of a similar size were selected in the sampling strategy for this investigations. Plant size was estimated as canopy volume ($0.5236 \times H \times W_1 \times W_2$, where H is canopy height, W_1 is maximum canopy width and W_2 is canopy width perpendicular to W_1) with all ten sampled plants having a canopy volume greater than 16.90 m^3. Canopy volumes of this size represent estimates in the upper quartile of canopy volumes for this species (van Leeuwen, personal observations). The average canopy volume for the ten plants sampled was 18.04 ± 0.19 m^3.

Non-random patterns of maternal reproductive success within the reproductive structures of *B. tricuspis* were assessed by partitioning inflorescences and infructescences into ten intervals (referred to here as 'positions') based on the overall length of the reproductive structure and distance along the rachis from its apex (distal end). Previous studies highlighting intra-infructescence variation in banksias (McFarland 1985, Zammit and Hood 1986, Vaughton 1988, 1993, Stock *et al.* 1989, Wallace and O'Dowd 1989) have divided the inflorescence/infructescence into apical, middle and basal thirds only. This classification was not accepted in this investigation, however, as morphological and anatomical considerations associated with apical and basal tapering of the reproductive structures and the associated reduction in floret numbers in these regions (George 1981) might unduly prejudice results for adjacent positions. An exception was made for seed nutrient content where the infructescence was divided into apical, middle and basal thirds because of the restrictions on obtaining enough seed material for chemical analyses.

5.2.1 *Maternal fecundity*

5.2.1.1 *Floret production*

Floret number inflorescence^{-1} was determined from a sample of three inflorescences collected from each of the ten sample plants during June of the 1988 flowering season. Inflorescences were selected indiscriminately based on the allocation of random numbers with the stipulation that they not be damaged by predatory insect larvae or foraging cockatoos. Florets position^{-1} was determined by removing and counting all florets in each of the ten inflorescence positions. Normality of direct count data was checked prior to statistical interrogation by examining symmetry (g_1), fit of the data to a normal distribution (Kolmagorov-Smirnov statistic) and homogeneity of variances between positions (Bartlett's statistic). Repeated measures ANOVA procedures (Winer 1971, Kirby 1993) were utilised to interrogate variation in the number of florets position^{-1}, where the variable associated with each of the ten plants was distinguished as a trial factor facilitating the detection of any between plant effects. Multiple comparisons between the mean number of florets position^{-1} were assessed by the conservative, although robust, Tukey-Kramer multiple range *posteriori* test (Zar 1984, Fry 1993). Because positions were not independent, Spearman's rank correlation statistic (r_s) was employed to illustrate any relationship between floret number and position on the inflorescence. Similarly, this statistic was also used to investigate relationships between floret number and the other fecundity and fitness attributes assessed (as described in the next sections).

5.2.1.2 *Follicle and seed production*

Estimates of follicle and seed production were obtained from a sample of 90 infructescences collected from the ten sample plants. Nine infructescences were collected indiscriminately from each plant through the allocation of random numbers when more than nine infructescences were present. Follicle number infructescence^{-1} and position^{-1} was determined by direct counts. Determination of seed attributes were achieved after treatment of the infructescence through burning and exposure to a wet-dry regime (Cowling and Lamont 1985). The number of seeds infructescence^{-1} and position^{-1} were determined by direct counts and seeds were ascribed to one of two categories, viable or aborted, where viable seeds were firm with an intact embryo and aborted seeds were malleable and lacked an embryo. Subsequently, follicles were assigned to one of three fecundity categories based on the number of viable seeds they contained. These fecundity categories were follicles with 2 viable seeds, follicles with 1 viable and 1 aborted seed, and follicles with 2 aborted seeds. Insect damaged seeds, as indicated by the presence of borers and frass within the follicles, were ignored as they accounted for only 0.24% of the viable seeds collected.

To enable calculation of fruit and seed set estimates for each position, for infructescences harvested in March 1988, the number of florets position^{-1} was estimated with reference to its least squares linear regression equation (Table 5.1). These linear regressions were determined for each position using floret number (y) and position width (mm) (x) data obtained from the 30 inflorescences harvested in June 1988. The overall regression defining the relationship between floret number and inflorescence length was highly significant ($y = 17.07x - 391.74$, $F_{1,29} = 245.09$, $P < 0.001$) with 89.8% of the variance in floret number predicted by inflorescence length. This regression was similar to that reported in other concurrent investigations (Chapter 4) ($Z = 0.41$, $P > 0.05$). Fruit set was determined using the equation:

$$\left(\frac{\text{follicles per infructescence position}}{\text{florets per inflorescence position}}\right) \times 100.$$

Seed set was determined using the equation:

$$\left(\frac{\text{viable seeds per infructescence position}}{\text{florets per inflorescence position} \times 2}\right) \times 100.$$

The devisor in the seed set equation was multiplied by two to reflect the assumed presence of two functional ovules floret^{-1} (Walker and Whelan 1991, Vaughton 1993). Zygote survival, where a zygote was defined as an initiated seed, was calculated by dividing the number of viable zygotes (viable seeds) into the total number of zygotes initiated (viable and aborted seeds combined) in each infructescence position. In all instances, results were expressed as percentages.

Normality of direct count data on follicle, viable and aborted seed numbers was checked prior to statistical analysis and square root transformed when significant departure was detected. Descriptive statistics presented are for back-transformed values. Repeated measures and two-way ANOVA procedures (Wilkinson 1992) were utilised to interrogate intra-infructescence position variations in follicle and seed attributes together with the probabilities of zygote survival. In all ANOVA inquiries individual plants were included as a trial factor to emphasise any between subject effects. In interrogations where follicle and zygote fecundity categories were included as independent variables these categories were also included as trial factors. Multiple comparisons between means were undertaken with Tukey-Kramer multiple range test. Paired t test statistical procedures were utilised to test for significant disparity between viable seeded follicle and zygote survival categories at each of the ten positions. Estimates of fruit and seed set were normalised by the arcsine transformation procedure (Fry 1993) and interrogated for significant disparity between positions using the multiple comparison procedures described above.

Table 5.1 Least squares linear regression values for each of the ten infructescence positions determined from floret number (y) and inflorescence position width in mm (x) data obtained from 30 *B. tricuspis* inflorescences harvested in June 1988.

Infructescence position		a	b	r_{30}	$F_{1,29}$
1	Apical	-49.82	12.41	0.82***	29.88***
2		-51.67	12.87	0.88***	47.91***
3		-51.94	12.94	0.92***	76.03***
4		-52.47	13.06	0.94***	142.50***
5		-54.59	13.59	0.96***	253.90***
6		-56.71	14.12	0.97***	392.10***
7		-55.65	13.86	0.94***	186.43***
8		-54.06	13.46	0.96***	201.74***
9		-52.73	13.13	0.92***	67.86***
10	Peduncular	-50.35	12.54	0.90***	68.62***

Significance: *** $P < 0.001$.

Correlations for follicle and seed attributes with position on the infructescence and with each other were examined by the Spearman's rank correlation procedure. Stepwise linear regression procedures (Zar 1984) were employed to define the best fit function for the relationship of fruit and seed set probabilities to position. The significance of these regressions were investigated using ANOVA testing procedures (Zar 1984) with TableCurve 2D software (Jandel Scientific). Similar stepwise procedures were also used to define the relationship between position and the associated probability of the various follicle fecundity attributes. In most instances, polynomial functions were selected as the best models describing the relationship between position and maternal fecundity attributes. Zygote survival and its relationship with position was also examined through this stepwise operation. The effect of ovule position along the infructescence on the independent fecundity attributes of seed set and zygote survival was assessed using Principal Component Analysis procedures (Wright 1994). This analysis permits the determination of correlations between independent attributes and ovule position, as inferred by the cosine of the angle between vectors on a Principal Component plot in the presence of other independent attributes (Jolliffe 1986, Duigan and Kovach 1991).

5.2.2 *Progeny fitness*

Progeny fitness was assessed by determining mass seed^{-1}, seed nutrient content and outcrossing estimates for the ten plants and the seed samples collected from their 90 harvested infructescences.

5.2.2.1 *Mass seed^{-1}*

Mass seed^{-1} was determined for all viable seeds removed from follicles on the 90 infructescences sampled. A total of 2 134 seeds was weighed and their position on the infructescence, together with the fecundity category (see 5.2.1.2) of the follicle from which they originated, was recorded. Seed mass infructescence^{-1} was determined by summing mass seed^{-1} results for the entire seed crop produced by each of the 90 infructescences sampled. A two-way ANOVA, with plants and positions as trial factors, was used to detect significant incongruity along the infructescence after the data were checked for normality and homogeneity of group variances. The significance of differences between position means and the relationship of mass seed^{-1} to other fecundity and fitness attributes was investigated using statistical and multivariate procedures already detailed (see 5.2.1.2).

5.2.2.2 *Nutrient content*

The levels of total nitrogen and phosphorus (mg g^{-1}) present in de-winged seeds were determined from bulked samples of viable seeds collected from the largest and smallest of the nine infructescences harvested for each of the ten sampled plants. Seeds were assigned to apical, middle or basal positions on the infructescence and, where sufficient material was present, two replicates from each seed lot were analysed. Total nitrogen was determined on 1 g samples by the Kjeldahl technique followed by colorimetric analysis of the diluted digest on a Tecator auto-analyser. Concentrations of total phosphorus were determined on 0.2-0.5 g perchloric acid digested samples using the molydbenum blue colorimetric method (Grimshaw *et al.* 1989).

Variation in nutrient content (mg g^{-1}) between positions was determined with a repeated measures ANOVA procedure which included two trial factors (replicates and plants) in the design (Wilkinson 1992, Kirby, 1993). Missing values arising as a consequence of insufficient seed material for the analysis of two replicates in some seed lots was treated by substitution using the mean value for that position (Kirby 1993). Arithmetic means were determined for each of the positions across all plants and contrasted using the Tukey-Kramer multiple range test. A similar multiple contrast was also performed on the nutrient content (mg) of individual seeds position^{-1}.

5.2.2.3 *Outcrossing estimates*

Intra-infructescence differentiation in outcrossing and levels of heterozygosity were assessed on viable seeds collected from three of the ten sampled plants which previous investigations had shown were homogeneous for the polymorphic enzyme system phosphoglucomutase (PGM-1, E.C. 5.4.2.2) (Chapter 2, population 5). Viable seeds were bulked for each position for each of the three homogeneous plants with the exception of position 1 which was excluded from the analyses because of insufficient seed material. The number of seeds (progeny) position^{-1} assessed for each plant (family) ranged from a minimum of five to a maximum of 22.

Each of the sample seeds were germinated on moistened filter paper at 15°C for 14 days after which time the emerging radical and associated embryonic material was separated from the testa and remaining cotyledonous material and homogenised in a Tris HCl buffer system (Chapter 2). Electrophoresis was conducted using a Helena Laboratory's cellulose acetate plate arrangement. The homogenate was electrophoresed on a Tris EDTA Maleate buffer system (Chapter 2, Hebert and Beaton 1989). Isozyme activity was detected using the methodology described by Coates (1988) with the PGM staining recipe similar to that presented by Wendel and Weeden (1989) with slight modifications for 1.75% agar overlays (Chapter 2).

Progeny genotypes were determined by direct interpretation of segregation pattern on zymograms. Subsequently, arithmetic means (± standard errors) for observed average heterozygosity, and the levels of heterozygosity expected under Hardy-Weinberg equilibrium for the progeny arrays from each position were determined.

Significant divergence between observed and expected average levels of heterozygosity was examined with chi-squared goodness-of-fit procedures (Swofford and Selander 1981). Single ($\hat{t}_s$) locus mean estimates of outcrossing for each position on the three homogeneous plants were determined using the procedure outlined by Ritland (1983). This procedure requires the maternal genotype to be known and is based on the number of novel alleles (outcrossed progeny) present in the progeny array of the homogeneous parent. This procedure prejudices estimates of outcrossing downwards as progeny of heterozygous parents are ignored because matings are ambiguous (Brown *et al.* 1989). Minimum variance mean estimates of outcrossing for each position and each plant were calculated (El-Kassaby *et al.* 1987). The significance of differences between these minimum variance mean estimates of outcrossing were determined by the non-parametric Kruskal-Wallis test (Fry 1993) while multiple comparisons among the nine positions were investigated using Dunn's multiple comparison test (Zar 1984).

Rank correlation and least squares linear regression procedures were employed to compare the relationship of outcrossing estimates to position on the infructescence and other fecundity and fitness attributes assessed. The significance of linear regressions were determined by ANOVA testing. The effect of ovule position along the infructescence on outcrossing estimates was investigated through Principal Component procedures.

5.3 Results

5.3.1 *Maternal fecundity*

5.3.1.1 *Floret production*

The number of florets inflorescence^{-1} produced during the 1988 season was symmetrically distributed about the mean of 1 240 ± 53 (Table 5.2). Between subject results from the repeated measures ANOVA indicated homogeneity between the ten plants in florets inflorescence^{-1} (Table 5.3). However, significant heterogeneity was detected in floret number with reference to position. Middle inflorescence positions were significantly more floriferous with floret numbers decreasing symmetrically towards the apical and peduncular positions (Figure 5.1). The correlation between position and florets position^{-1} was negative, although not significant (Table 5.4).

Table 5.2 **Descriptive statistics for selected attributes of reproductive success from ten *Banksia tricuspis* plants harvested during 1988.**

Reproductive attributes	Mean ± SE	Range	*n*	g_1
Florets inflorescence^{-1}	1 240 ± 53	498 - 1 883	30	-0.231
Follicles infructescence^{-1}	23.8 ± 1.0	8 - 60	90	1.099***
Viable seeds infructescence^{-1}	30.5 ± 1.4	9 - 83	90	1.143***
Aborted seeds infructescence^{-1}	17.2 ± 0.9	4 - 39	90	0.655*
Seed mass infructescence^{-1} (g)	1.44 ± 0.07	0.42 - 3.57	90	1.169***

Significance: * $P < 0.05$, *** $P < 0.001$.

Table 5.3 **Descriptive statistics and repeated measures ANOVA results for selected reproductive success attributes over ten position effects on 30 inflorescences and 90 infructescences harvested from ten *Banksia tricuspis* plants.**

Reproductive attributes	Mean ± SE	Range	Source of variance	df	*F*
Florets position^{-1}	124.0 ± 4.1	38 - 240	Plant	9	1.004
			Position	9	48.489***
			Position *x* plant	81	0.460
Follicles position^{-1}	2.4 ± 0.5	0 - 17	Plant	9	2.733**
			Position	9	43.895***
			Position *x* plant	81	1.042
Viable seed position^{-1}	3.4 ± 0.6	0 -22	Plant	9	2.488**
			Position	9	46.041***
			Position *x* plant	81	0.977
Aborted seeds position^{-1}	1.7 ± 0.3	0 - 16	Plant	9	1.936
			Position	9	19.971***
			Position *x* plant	81	0.951
Mass seed^{-1} position^{-1} (mg)	60.8 ± 2.9	18.2 - 117.8	Plant	9	1.449
			Position	9	32.795***
			Position *x* plant	81	0.922

Significance: ** $P < 0.01$, *** $P < 0.001$.

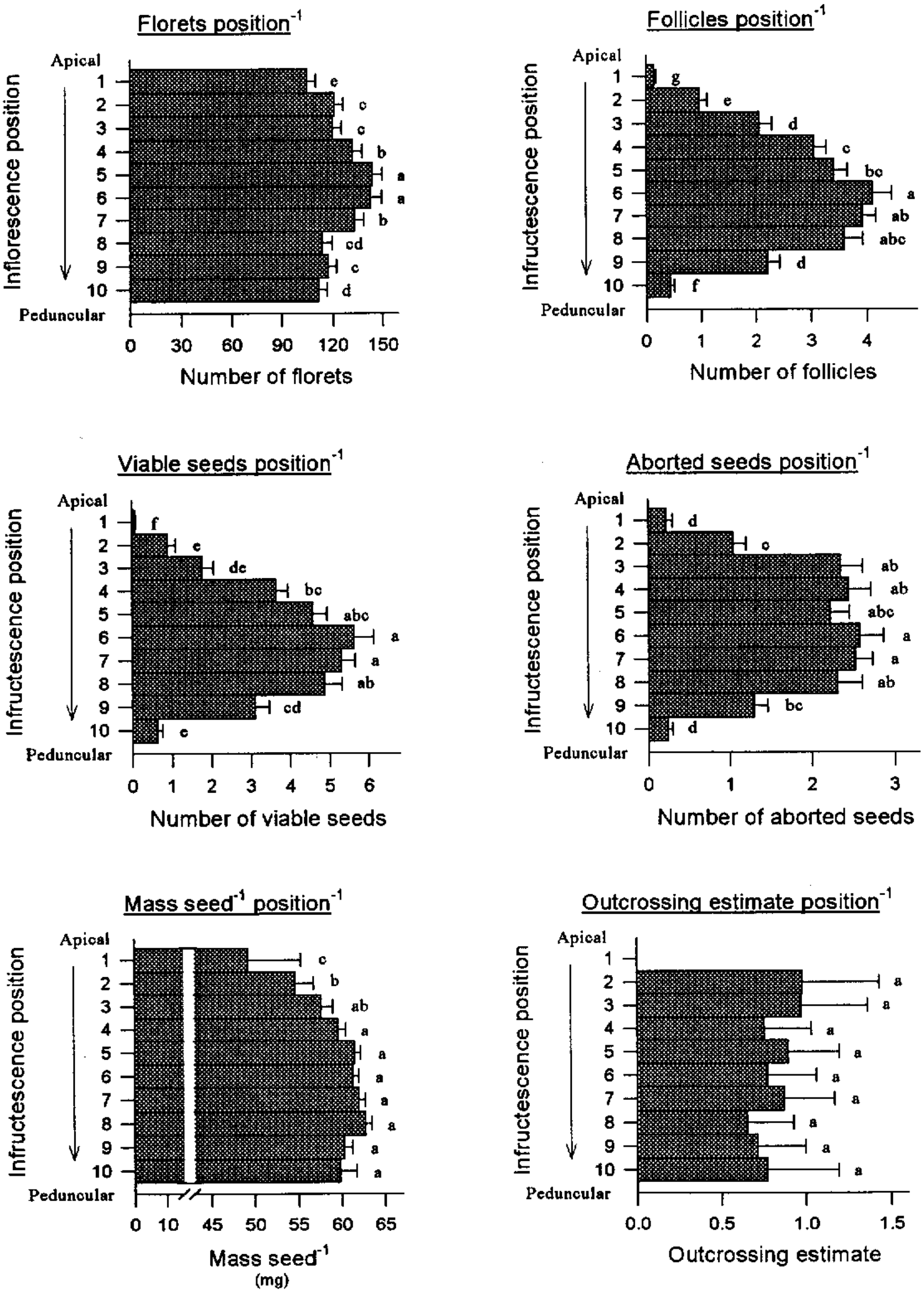


Figure 5.1 Influence of position on the inflorescence/infructescence on mean (± SE) reproductive success in *Banksia tricuspis*. (Horizontal columns with different letter(s) adjacent to their error bars were significantly different (Dunn's multiple comparison for outcrossing estimates (Zar 1984), and Tukey-Kramer multiple range test for remaining attributes, $P < 0.05$).)

Table 5.4 Results of Spearman's rank correlation analysis of the relationship between position on the inflorescence/infructescence and each of the fecundity and fitness attributes assessed.

Reproductive attribute	Position	Florets position^{-1}	Follicles position^{-1}	Viable seeds position^{-1}	Aborted seeds position^{-1}	Mass seed^{-1} position^{-1}
Florets position^{-1}	-0.079					
Follicles position^{-1}	0.333	0.709*				
Viable seeds position^{-1}	0.333	0.709*	1.000*			
Aborted seeds position^{-1}	0.115	0.697*	0.867*	0.867*		
Mass seed^{-1} position^{-1}	0.685*	-0.079	0.333	0.333	0.115	
Outcrossing estimate position^{-1}	-0.717*	0.450	-0.233	-0.233	-0.033	-0.483

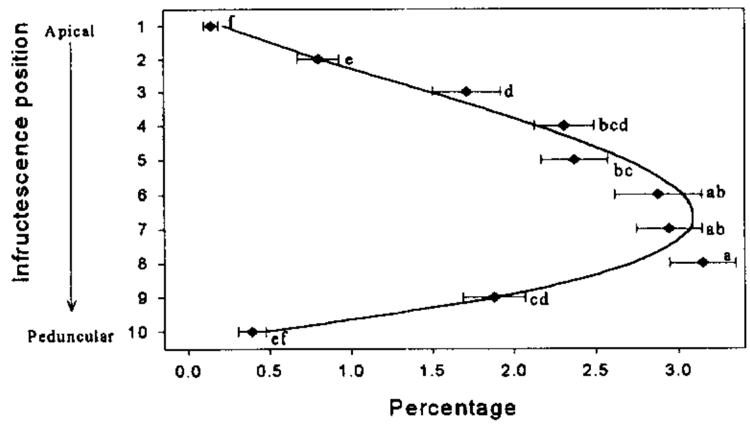
Significance: * $P < 0.05$.

5.3.1.2 *Follicle and seed production*

The distribution of follicles, and viable and aborted seeds infructescence^{-1} was significantly asymmetrical and positively skewed (Table 5.2). Significant heterogeneity was detected in follicle, viable seed and aborted seed numbers relative to position on the infructescence (Table 5.3) with apical and peduncular positions producing significantly fewer follicles and seeds than middle positions (Figure 5.1). Between subject results also indicated significant heterogeneity in follicle and viable seed numbers between the ten sampled plants. However, no significant differences were evident for aborted seeds. Similarly, no significant differentiation was detected for the interaction between the ten sampled plants and position for each of the fecundity attributes assessed (Table 5.3).

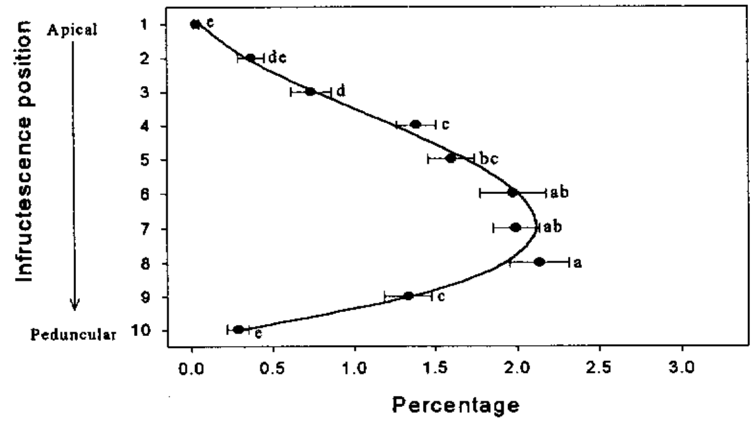
The probability of a floret developing into a follicle (fruit set) increased progressively along the infructescence from the apex (0.1%) to position 8 (3.2%), after which there was a marked decline to the most peduncular position (0.4%) (Figure 5.2). A similar pattern was detected in the probability of an ovule becoming a viable seed (seed set) where the probabilities ranged from a low of 0.03% at position 1 to a high of 2.1% at position 8 (Figure 5.2). The probability of an ovule aborting

Fruit set



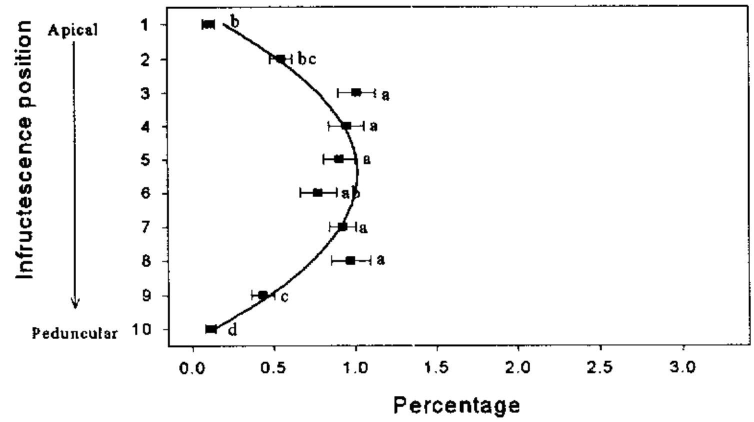
Best fit function: $y = -2.0e{-}03 - 3.0e{-}03x + 1.3e{-}03x^2 - 1.5e{-}04x^3$, $r^2 = 0.96$, $F_{3,6} = 49.488$, $P < 0.001$

Viable seed set



Best fit function: $y = -4.6e{-}06 - 8.0e{-}04x - 1.8e{-}03x^2 - 1.4e{-}04x^3$, $r^2 = 0.98$, $F_{3,6} = 119.713$, $P < 0.001$

Aborted seed set



Best fit function: $y = -2.1e{-}03 + 4.5e{-}3x - 4.2e{-}04x^2$, $r^2 = 0.96$, $F_{2,7} = 17.189$, $P < 0.001$

Figure 5.2 Mean (± SE) probability and line of best fit for fruit, viable and aborted seed set in ten positions along 90 *Banksia tricuspis* infructescences. (Error bars with different letter(s) indicate a significant difference between positions (Tukey-Kramer multiple range test, $P < 0.05$).)

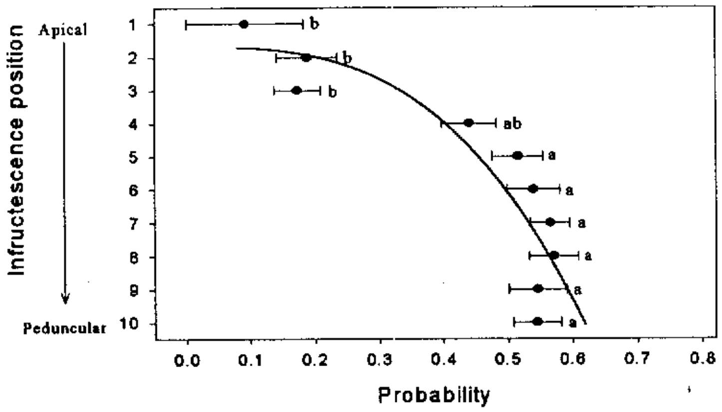
ranged from a low of 0.1% at position 1 to a high of 1.0% at position 3, although similarly high probabilities were also obtained for positions 4 to 8 (Figure 5.2). In all instances fruit and seed set were similar in the most apical and peduncular positions. The best linear functions describing the distribution of these probabilities were standard polynomial equations. A quadratic function provided the best fit for the probability of an ovule aborting while a cubic function provided the best fit for the probability of fruit and seed set (Figure 5.2). All three functions were significant with high coefficients of determination.

The probability of a follicle containing two aborted seeds decreased from the apex to the peduncular position on the infructescence (Figure 5.3). Conversely, the probability of a follicle containing two viable seeds increased in a reciprocal manner, as demonstrated by the significant negative rank correlation between values at each position ($r_{s\,10} = -0.85$, $P < 0.001$). The probability of a follicle containing one viable and one aborted seed remained constant over all positions. ANOVA results indicated that differences between plants in the probability of a follicle containing either two viable, two aborted, or one viable and one aborted seed was constant between the ten plants sampled (Table 5.5). However, position had a significant effect on the probability of a follicle containing either two viable or two aborted seeds. For both these follicle categories, the three apical positions differed significantly from the remaining distal positions (Figure 5.3).

The distribution of follicle categories over the infructescence indicated heterogeneity between positions. This intra-infructescence disparity promoted significant discord in the abundance of each follicle category over the entire infructescence and at each position (Table 5.6a). Differences in the number of each follicle at each position were significant for all positions excluding positions 2 and 3. In the first three apical positions, follicles containing two aborted seeds were the most numerous, while in the remaining positions follicles with two viable seeds dominated (Table 5.6b).

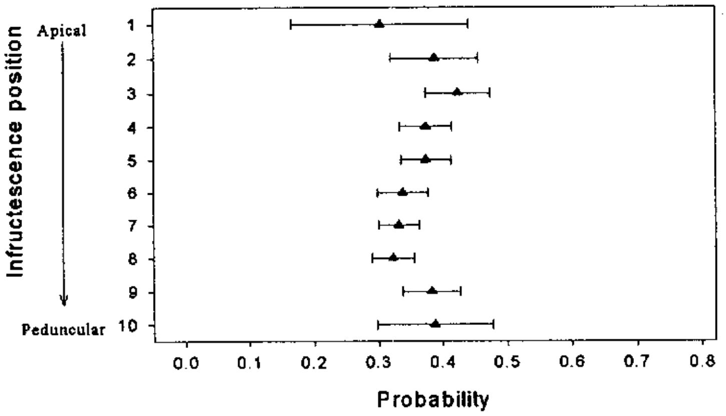
The probability of a follicle containing only one viable seed for those follicles which contained viable seeds decreased with progression towards the peduncular

Two viable seeds position^{-1}

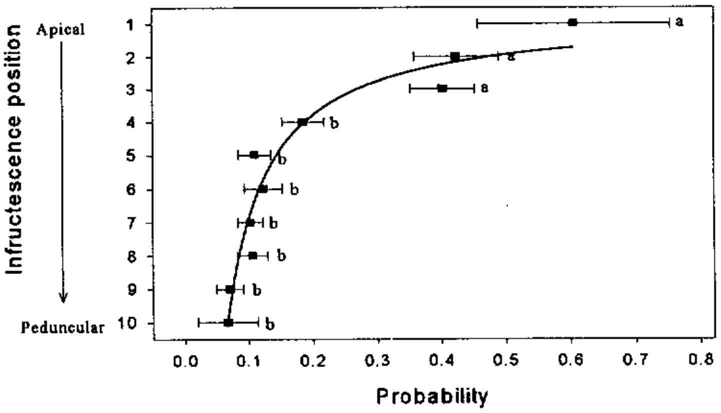


Best fit function: $y = 9.31 - 35.21x^3$, $r^2 = 0.80$, $F_{1,8} = 32.215$, $P < 0.001$

One viable seed and one aborted seed position^{-1}



Two aborted seeds position^{-1}



Best fit function: $y = 10.30 - 0.62/x$, $r^2 = 0.94$, $F_{1,8} = 115.21$, $P < 0.001$

Figure 5.3 Position effect on the probability (mean ± se) of a follicle containing either two viable seeds, one viable and one aborted seeds or two aborted seeds along the infructescence of *Banksia tricuspis*. (Error bars with different letter(s) indicate a significant difference between positions (Tukey-Kramer multiple range test, $P < 0.01$.))

Table 5.5 Two-way ANOVA results on the probability of the three follicle categories occurring in apical to peduncular positions along the rachis on 90 *Banksia tricuspis* infructescences.

Source of variance	df	Follicle category Two viable seeds	 One viable and one aborted seed	 Two aborted seeds
Plant	9	1.802	1.496	0.979
Position	9	12.322***	0.576	14.819***
Position *x* plant	81	0.843	0.872	0.996

Significance: *** $P < 0.001$.

Table 5.6 Abundance of follicle fecundity categories from apical to peduncular positions on 90 *Banksia tricuspis* infructescences: a) results of repeated measures ANOVA with two trial factors; b) mean (± SE) number of follicles with two viable seeds, one viable and one aborted seed, and two aborted seeds position^{-1}. (values with different letter(s) are significantly different, Tukey-Kramer multiple range test, $P < 0.05$.)

a).

Source of variance	df	*F*
Plant	9	1.732
Follicle category	2	74.033***
Position	9	41.231***
Plant *x* follicle category	9	1.232
Position *x* plant	81	0.740
Position *x* follicle category	18	14.634***

Significance: *** $P < 0.001$.

b).

Infructescence position	Follicle category two viable seeds	 one viable & one aborted seed	 two aborted seeds	Significance $F_{2,267}$
1 Apical	0.02 ± 0.02^{b}	0.04 ± 0.02^{ab}	0.18 ± 0.07^{a}	3.785*
2	0.51 ± 0.17	0.38 ± 0.08	0.67 ± 0.13	1.209
3	0.78 ± 0.20	0.99 ± 0.14	1.33 ± 0.21	2.196
4	2.16 ± 0.24^{a}	1.49 ± 0.19^{ab}	0.98 ± 0.19^{b}	7.905***
5	2.93 ± 0.28^{a}	1.60 ± 0.19^{b}	0.62 ± 0.13^{c}	30.046***
6	3.82 ± 0.43^{a}	1.80 ± 0.23^{b}	0.78 ± 0.18^{c}	26.841***
7	3.67 ± 0.29^{a}	1.69 ± 0.16^{b}	0.84 ± 0.17^{c}	45.456***
8	3.22 ± 0.33^{a}	1.63 ± 0.22^{b}	0.67 ± 0.16^{c}	27.470***
9 ↓	2.19 ± 0.29^{a}	0.94 ± 0.12^{b}	0.36 ± 0.12^{b}	22.673***
10 Peduncular	0.44 ± 0.11^{a}	0.19 ± 0.05^{b}	0.04 ± 0.03^{b}	7.479***

Significance: * $P < 0.05$, *** $P < 0.001$.

end of the infructescence. Conversely, the probability of a follicle containing two viable seeds for those follicles which contained viable seeds increased (Figure 5.4). Incongruity between positions for the probability of a viable seeded follicle containing one or two viable seeds was detected (Table 5.7). However, no significant differences were detected between individual plants or for the interaction between plants and positions. Similar unity in abundance, both between plants and in the two viable seeded follicles categories was also detected, while incongruity with respect to position was evident (Table 5.8a). As reflected in follicle numbers, viable seeded follicles with only one viable seed represented the most abundant follicle category until position 5, after which follicles with two viable seeds predominated (Table 5.8b). In all instances the differences, however, were not significant with the exception of position 3.

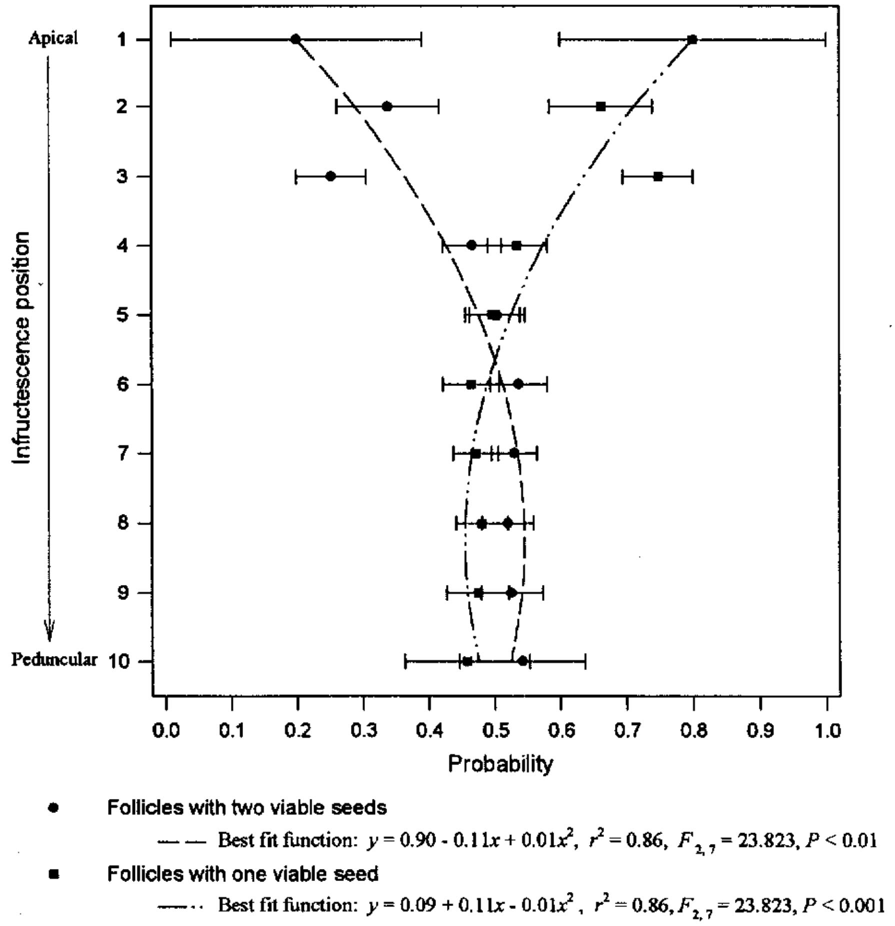


Figure 5.4 Distribution from apical to peduncular infructescence positions in the mean (± SE) probability of a *Banksia tricuspis* follicle with viable seeds containing either one or two viable seeds.

Table 5.7 Results of repeated measures ANOVA with one trial factor (plants) for the probability of a viable seeded follicle containing either one or two viable seeds for 90 *Banksia tricuspis* infructescences.

Source of variance	df	*F*
Plant	9	1.065
Position	9	3.635***
Position *x* plant	81	0.776

Significance: *** $P < 0.001$.

Table 5.8 Abundance of viable seeded follicles containing either one or two viable seeds from apical to peduncular positions on 90 *Banksia tricuspis* infructescences: a) results from repeated measures ANOVA with two trial factors; b) mean (± SE) distribution and paired *t* test statistics on the number of follicles with viable and aborted seeds infructescence position^{-1}.

a).

Source of variance	df	*F*
Plant	9	1.628
Follicle category	1	1.253
Position	9	34.064***
Plant *x* follicle category	9	0.734
Position *x* plant	81	0.757
Position *x* follicle category	9	8.443***

Significance: *** $P < 0.001$.

b).

Infructescence position	Viable seeded follicle category		Significance	
	One seed	Two seeds	df	*t*
1 Apical	0.4 ± 0.2	0.1 ± 0.1	10	2.609*
2	0.8 ± 0.1	0.5 ± 0.2	44	0.842
3	1.5 ± 0.2	0.6 ± 0.1	60	2.237*
4	1.6 ± 0.2	1.2 ± 0.1	83	3.625***
5	1.8 ± 0.2	1.7 ± 0.2	79	7.733***
6	2.0 ± 0.2	2.2 ± 0.2	79	6.656***
7	1.7 ± 0.2	1.8 ± 0.1	87	9.227***
8	1.9 ± 0.2	1.9 ± 0.2	71	7.478***
9 ↓	1.2 ± 0.1	1.4 ± 0.2	68	6.308***
10 Peduncular	0.7 ± 0.2	0.8 ± 0.2	24	4.042***

Significance: * $P < 0.05$, *** $P < 0.001$.

The probability of a zygote maturing once fertilised increased with progression towards the peduncular end of the infructescence (Figure 5.5). The best fit function describing the change in probability was a cubic polynomial equation which was significant with a marked correlation between variables. Multiple comparisons among the mean probabilities of zygote survival at each position indicated a significant dichotomy (Table 5.9), with survival significantly reduced in apical regions of the infructescence, positions 1 to 3 (Figure 5.5). However, the probability of survival in the remainder of the infructescence remained uniformly high between positions. This result was supported by repeated measures ANOVA outcomes where both positions and the fate of a zygote were grouped as trial factors (Table 5.10). The ANOVA indicated significant heterogeneity in the number of viable and aborted zygotes with reference to position. The dichotomy between viable and aborted zygote position^{-1} was also highlighted as significant. These findings were not impacted by any interaction between the ten plants. Paired *t* test outcomes of the number of viable and aborted zygotes at each position verify heterogeneity in the abundance of each zygote type along the infructescence, with two of the three apical positions dominated by aborted zygotes (Table 5.11). In the remaining peduncular positions, where viable zygotes dominated, the differences were also significant.

Table 5.9 Two-way ANOVA results for the differential pattern of zygote survival from apical to peduncular positions for 90 *Banksia tricuspis* infructescences.

Source of variance	df	*F*
Plant	9	1.312
Infructescence position	9	18.239***
Infructescence position *x* plant	81	1.094

Significance: *** $P < 0.001$.

The rank distribution of viable seeds along the inflorescences was significantly correlated with both follicle and floret distribution (Table 5.4). Aborted seed distribution exhibited a similar pattern although the relationship with follicles position^{-1} was not as marked as the relationship detected for viable seeds. The relationship between viable and aborted seeds was significant. However, both attributes were poorly correlated with position on the infructescence. Principal

Component plot results (Figure 5.6, Table 5.12) indicated that seed set was poorly correlated with position on the infructescences and moderately correlated with zygote survival. Zygote survival on the other hand was significantly correlated with position.

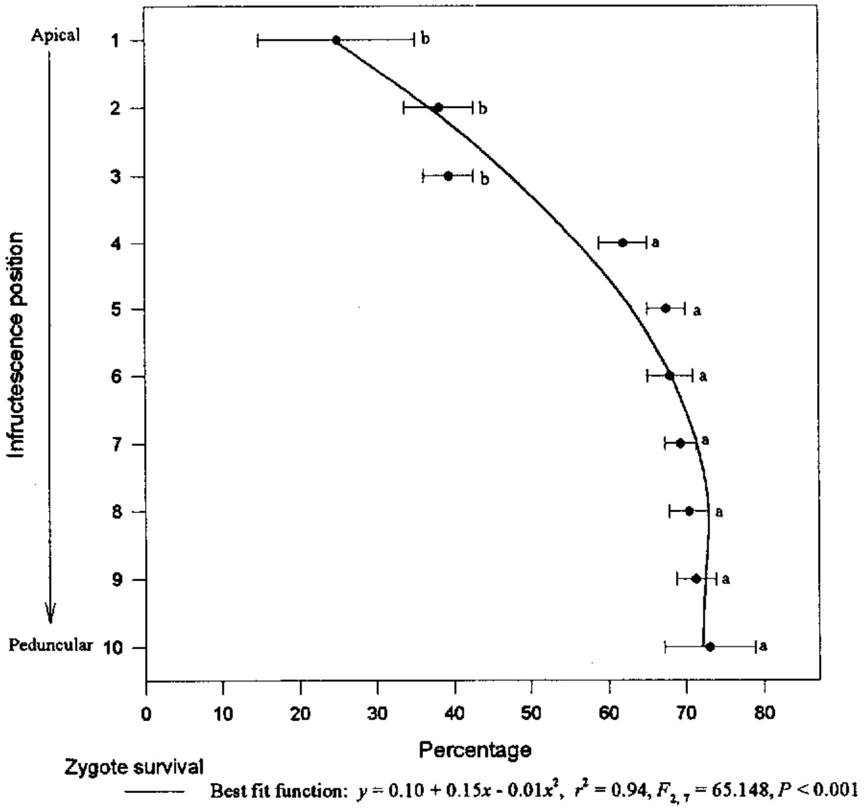


Figure 5.5 Pattern of zygote survival (mean ± SE) from apical to peduncular positions for 90 *Banksia tricuspis* infructescences. (Error bars with different letters indicate significant differences between positions (Tukey-Kramer multiple range test, $P < 0.01$)).

Table 5.10 Results from repeated measures ANOVA with two trial factors for the pattern of zygote survival from apical to peduncular positions on 90 *Banksia tricuspis* infructescences.

Source of variance	df	*F*
Plant	9	1.536
Zygote type	1	121.548***
Position	9	43.895***
Plant *x* zygote type	9	1.297
Position *x* plant	81	1.042
Position *x* zygote type	9	22.782***

Significance: *** $P < 0.001$.

Table 5.11 Significance of the difference between total mean (± SE) numbers of viable and aborted zygotes position^{-1} for 90 *Banksia tricuspis* infructescences.

Infructescence position		Number of zygotes		*t*
		viable	aborted	
1	Apical	0.1 ± 0.0	0.3 ± 0.1	2.609*
2		0.9 ± 0.2	1.0 ± 0.2	0.842
3		1.7 ± 0.3	2.3 ± 0.3	2.237*
4		3.6 ± 0.3	2.4 ± 0.3	3.625***
5		4.6 ± 0.4	2.3 ± 0.2	7.733***
6		5.6 ± 0.5	2.6 ± 0.3	6.656***
7		5.3 ± 0.4	2.5 ± 0.2	9.227***
8		4.9 ± 0.4	2.3 ± 0.3	7.478***
9	↓	3.1 ± 0.4	1.3 ± 0.2	6.308***
10	Peduncular	0.6 ± 0.1	0.2 ± 0.1	4.042***

Significance: * $P < 0.05$, *** $P < 0.001$.

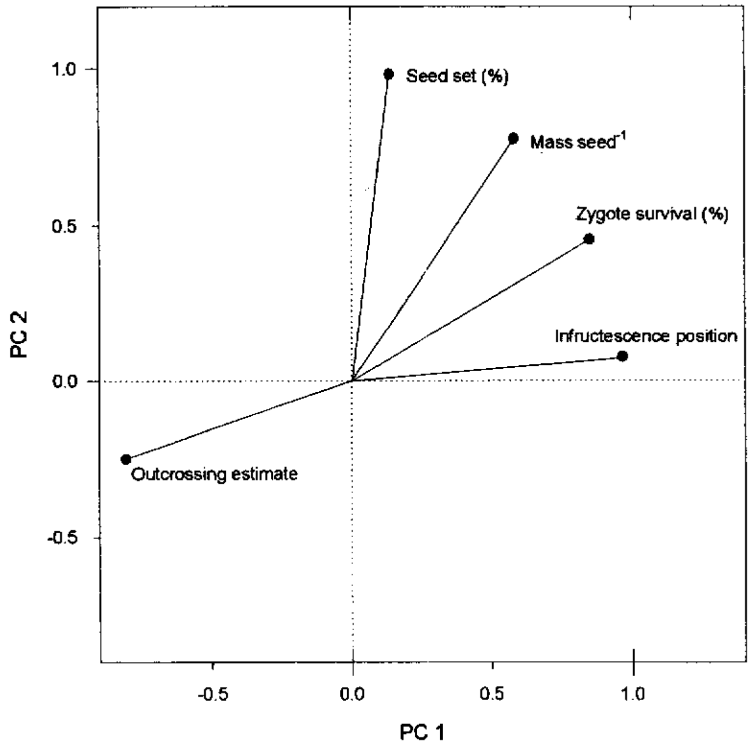


Figure 5.6 Principal Component plot of infructescence position and associated reproductive attributes for *Banksia tricuspis*. (PC 1 accounts for infructescence position, zygote survival (%) and outcrossing estimates, PC 2 accounts for mass seed^{-1} and seed set (%).)

Table 5. 12 Matrix of correlations between vectors determined by Principal Component Analysis plot as presented in Figure 5.6 for ten infructescence positions and associated reproductive attributes in *Banksia tricuspis*.

	Infructescence position	Seed set (%)	Zygote survival (%)	Mass seed^{-1}
Seed set (%)	0.21			
Zygote survival (%)	0.91	0.60		
Mass seed^{-1}	0.64	0.88	0.89	
Outcrossing estimate	-0.98	-0.41	-0.94	-0.80

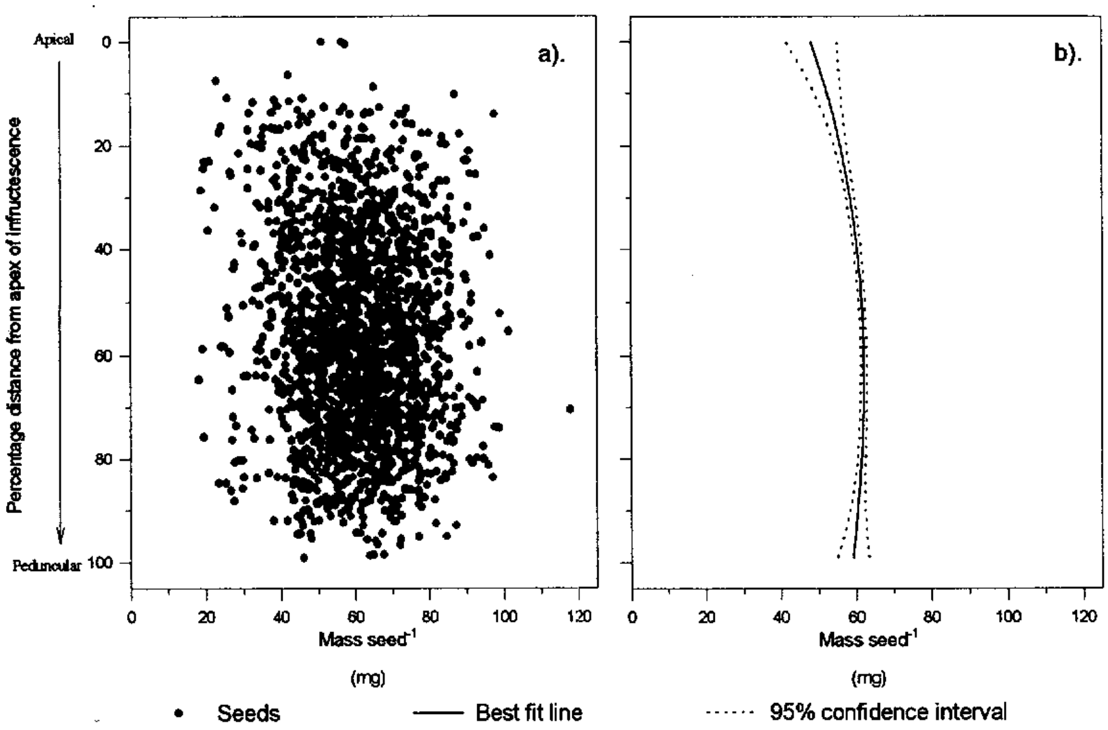
5.3.2 *Progeny fitness*

5.3.2.1 *Mass seed^{1}*

Mass seed^{-1} ranged from 18.2 to 117.8 mg and was symmetrically distributed about the mean for the 2 134 seeds sampled (g_1 = -0.08, $P > 0.05$). Mass seed^{-1} was uniform between the ten plants sampled, however, varied significantly with regard to position (Table 5.3). The heaviest seeds were located in the middle and peduncular portions of the infructescence while those in positions 1 and 2 were significantly lighter than the remainder and also differing significantly from one another (Figure 5.1).

Mass seed^{-1} over the ten positions was positively correlated with position along the infructescence indicating that mass seed^{-1} increased towards the peduncular end of the reproductive organ (Table 5.4). Mass seed^{-1} was not significantly correlated with the fecundity components examined previously. The identification of a significant quadratic polynomial equation (Figure 5.7) for the relationship between mass seed^{-1} and distance from the infructescences apex support this observation. Seed mass infructescence^{-1} varied significantly between the 90 infructescences sampled and was distributed asymmetrically about the mean (Table 5.2). No significant inter-plant differences in seed mass infructescence^{-1} were identified ($F_{9,79} = 1.94$, $P > 0.05$).

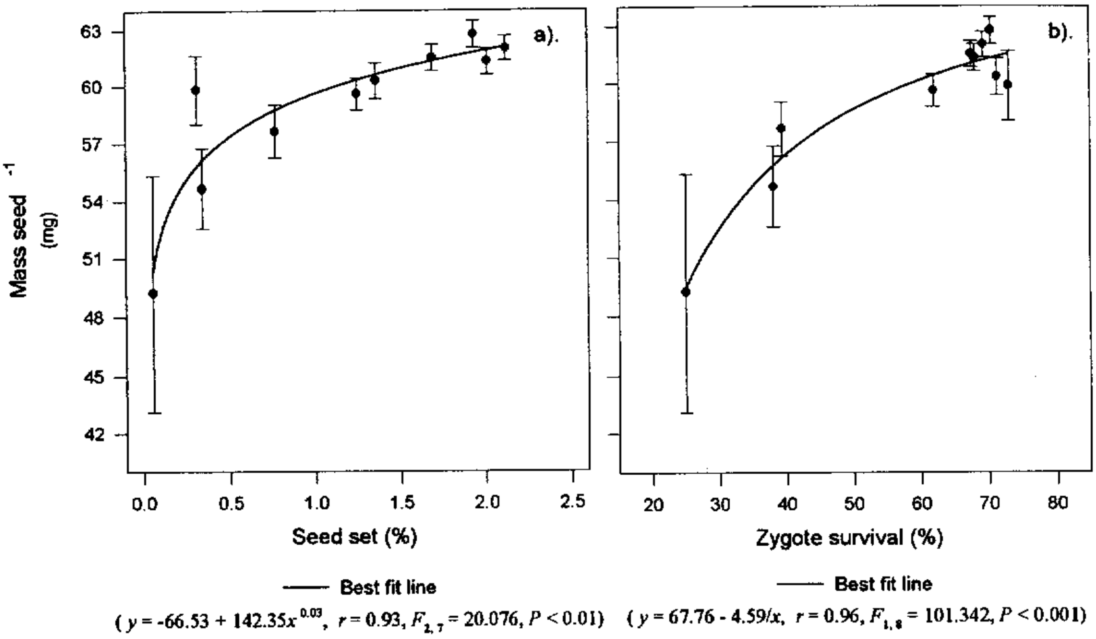
Mass seed^{-1} position^{-1} was poorly correlated with the number of viable and aborted seeds and the correlation for both regressions did not differ significantly ($Z_{2,\infty}$ = 0.817, $P < 0.05$). The common correlation coefficient determined for these regressions (r_w = 0.05) was also not significant. Similar outcomes, indicating poor association between variables, were also evident in the outcome of rank correlations presented in Table 5.4.



Best fit function: $y = 49.38 + 0.39x - 0.003x^2$, $r^2 = 0.02$, $F_{2,2131} = 18.61$, $P < 0.001$

Figure 5.7 Distribution of mass seed^{-1} along the infructescence of *Banksia tricuspis*: a) data for the 2 134 seeds; b) best fit line and 95% confidence interval.

Correlations of mass seed^{-1} position^{-1} with seed set and zygote survival were markedly positive (Table 5.12) and approximated an increasing but diminishing best fit function (Figure 5.8) which indicated that progeny fitness increases with maternal fecundity. Comparisons with the pattern of seed set (Figure 5.2) and zygote survival (Figure 5.5) illustrate that the progressive increase in mass seed^{-1} from apical to middle positions was mirrored by a corresponding increase in both seed set and zygote survival. However, in peduncular positions the decline in mass seed^{-1} was only complemented by a reduction in seed set as zygote survival remained constant.



($y = -66.53 + 142.35x^{0.03}$, $r = 0.93$, $F_{2,7} = 20.076$, $P < 0.01$) ($y = 67.76 - 4.59/x$, $r = 0.96$, $F_{1,8} = 101.342$, $P < 0.001$)

Figure 5.8 Relationship between mean (± SE) mass seed^{-1} position^{-1} and the reproductive attributes of: a) seed set (%); and b) zygote survival (%) on 90 *Banksia tricuspis* infructescences.

5.3.2.2 *Nutrient content*

Significant differentiation in the partitioning of seed nitrogen and phosphorus along the infructescence was detected. This pattern was constant between the ten plants sampled (Table 5.13a). The concentration of nitrogen and phosphorus tended to be lower in seeds from apical positions on the 10 infructescences sampled (Table 5.13b). The partitioning of these nutrients over the remainder of the infructescence was similar in both middle and peduncular positions. The nutrient content of individual seeds in each of the three position also varied significantly (Table 5.13b). Peduncular seeds contained larger amounts of both nutrients than those from middle and apical positions which also differed markedly from one another.

5.3.2.3 *Outcrossing estimates*

Observed average heterozygosity of the three plants was greater than expected under Hardy-Weinberg equilibrium (Table 5.14). This excess of heterozygotes was also evident for each of the nine positions examined, although the difference between observed and expected heterozygosity was only significant in positions 4 to 7.

Table 5. 13 Descriptive statistics on the concentration of nitrogen and phosphorus in the viable seeds of *Banksia tricuspis* harvested from the apical, middle and peduncular infructescence positions: a) results of repeated measures ANOVA procedures with two trial factors; b) multiple comparisons of nutrient content (mean ± SE) between positions.

a).

Nutrient	Mean ± SE (mg g^{-1})	Range	Source of variance	df	*F*
Nitrogen	111.39 ± 0.46	102.70 - 121.40	Plant	9	2.345
			Replicate	1	0.259
			Position	2	18.035***
			Position x plant	18	2.951
			Position *x* replicate	2	0.037
Phosphorus	12.42 ± 0.05	11.38 - 13.22	Plant	9	0.659
			Replicate	1	0.486
			Position	2	11.965***
			Position *x* plant	18	0.544
			Position *x* replicate	2	0.013

Significance: *** $P < 0.001$.

b).

Value	Significance	Nutrient	Position [a]		
			Apical	Middle	Peduncular
mg g^{-1}	$F_{2,38}$ = 9.626**	N	108.58 ± 0. 59 [b]	112.01 ± 0.30 [a]	113.85 ± 0.91 [a]
	$F_{2,38}$ = 16.348***	P	12.16 ± 0.10 [b]	12.63 ± 0.05 [a]	12.31 ± 0.07 [ab]
mg $seed^{-1}$ $position^{-1}$	$F_{2,19}$ = 163.04***	N	5.75 ± 0.03 [c]	6.72 ± 0.01 [b]	7.11 ± 0.09 [a]
	$F_{2,19}$ = 219.86***	P	0.64 ± 0.02 [c]	0.76 ± 0.01 [b]	0.78 ± 0.02 [a]

Significance: ** $P < 0.01$, *** $P < 0.001$.

[a] Values with different letter(s) are significantly different (Tukey-Kramer multiple range test, $P < 0.05$).

A single ($\hat{t}_s$) locus outcrossing estimate of 0.803 ± 0.069 was obtained from the three homogeneous plants. This estimate did not differ significantly from panmixia ($\hat{t}_s$= 1) ($t_2 = 2.86$, $P > 0.05$) and was uniform between the three plants ($H_2 = 5.274$, $P > 0.05$). Incongruity between positions in the minimum variance mean estimates of outcrossing were evident, with the trend suggesting an overall decline in outcrossing towards the peduncular end of the infructescences (Figure 5.1). This differentiation was, however, not significant, with multiple comparisons between positions failing to detect any significant discord ($H_8 = 9.136$, $P > 0.05$).

Table 5.14 Observed and expected average (± SE) heterozygosity frequencies for the progeny from nine positions along the infructescences for three Pgm-1 homogeneous *Banksia tricuspis* plants.

Infructescence position	Heterozygosity		χ^2
	Observed	Expected	
2 Apical	0.53 ± 0.17	0.40 ± 0.11	1.697
3	0.50 ± 0.11	0.39 ± 0.08	3.033
4	0.41 ± 0.07	0.34 ± 0.05	4.179*
5	0.50 ± 0.06	0.42 ± 0.04	8.306**
6	0.41 ± 0.07	0.33 ± 0.04	4.276*
7	0.47 ± 0.08	0.36 ± 0.05	5.986*
8	0.35 ± 0.06	0.29 ± 0.04	2.794
9 ↓	0.34 ± 0.06	0.31 ± 0.05	3.105
10 Peduncular	0.60 ± 0.07	0.49 ± 0.04	2.400
Overall	0.44 ± 0.07	0.34 ± 0.05	35.341***

Significance: * $P < 0.05$, ** $P < 0.01$.

Outcrossing estimates position^{-1} were negatively correlated with all maternal fecundity and progeny fitness attributes, with the exception of florets inflorescence^{-1} where the correlation was negative (Table 5.4). The only significant rank correlation was with position itself. Outcrossing estimates were negatively correlated with seed set, zygote survival and mass seed^{-1}, although the correlations were only significant for zygote survival (Figure 5.6, Table 5.12). Principal Component results indicated that position on the infructescence was negatively correlated with outcrossing estimates and that this relationship was the strongest for any of the reproductive attributes assessed (Figure 5.6, Table 5.12).

The distribution of observed heterozygosity position^{-1} was similar to that reported for outcrossing estimates ($r_9 = 0.72$, $P < 0.05$). Correlations with other maternal fecundity and progeny fitness components were thus similar to those identified for outcrossing.

5.4 Discussion

5.4.1 *Position effects on maternal fecundity and progeny fitness*

Non-random patterns in maternal reproductive success along infructescences of *B. tricuspis* was apparent for all fecundity and fitness attributes assessed. In most instances, the patterns were characterised by asymmetrical distributions centred on the middle to middle-peduncular regions of the infructescence tapering gradually towards the apex and sharply towards the peduncle. Exceptions to this pattern were observed for zygote survival, mass seed^{-1} and the nutrient content of seed which increased towards the peduncular end of the infructescence, and outcrossing estimates which exhibited a slight, non-significant, diminishing trend towards the peduncle.

Position on the infructescence may affect maternal fecundity and progeny fitness as a consequence of either temporal or spatial variance. This may confer a competitive advantage on zygotes in one position over another (Lee 1988, Stephenson *et al.* 1988). A temporal advantage is usually demonstrated through the phenomenon of first-fruit dominance where early initiated zygotes have greater access to the limited maternal resources and are less confined by anatomical constraints imposed with the development of fruiting structures (Ho 1992, Guitián 1994). In this basipetalous flowering *Banksia*, a competitive advantage would be conferred on zygotes originating near the apex if first-fruit dominance was prevalent. Clearly, the reduced viable seed numbers, depressed levels of fruit and seed set, high probabilities of zygote abortion, low mass seed^{-1} and the reduced nutrient content of seeds from apical positions in this species dispel any temporal advantage associated with first-fruit dominance. Furthermore, uniformity in zygote survivorship, mass seed^{-1} and nutrients content position^{-1}, imply consistency in the availability of maternal resource provisions between zygotes originating in peduncular (late initiated) and middle (intermediate initiation) positions and supports the rejection of first-fruit dominance. First-fruit dominance may, however, confer a genetic quality advantage on *B. tricuspis* as illustrated by the slightly greater outcrossing tendency of progeny from apical positions and the negative association between outcrossing estimates and position (Table 5.12).

The rejection of first-fruit dominance as an explanation for variation in reproductive success along infructescences of *B. tricuspis* also implies an absence of physiological constraints associated with plant hormonal and growth regulatory controls. These controls divert nutritional resources to earlier initiated zygotes ('competitive' hypothesis) or inhibit and abort younger, later initiated, zygotes ('inhibition' hypothesis) (Lee 1988, Guitián 1994). Such controls have been invoked to account for the observed pattern of reproductive success in other species (Wyatt 1982, Stephenson 1992, Obeso 1993, Guitián 1994). The failure of mass seed^{-1} to decline with the reduction in viable seeds infructescence^{-1} and seed set in peduncular positions also implies an absence of physiological controls and suggests that source-sink interactions may be poorly developed. Maternal resource status apparently has a major impact on source-sink relationships with the physiological control over such interactions being relaxed when selective pressures (resource limitations) are suppressed (Peel 1974, Lee 1988).

The spatial advantage conferred by position within the infructescence is related to the proximity of the sink (ovule, zygote) to the nutrient source. It predicts that sinks closer to the nutrient source are better provisioned with resources and are able to pre-empt nutrients destined for more distal sinks, especially when resource provisions become limited (Wyatt 1981, Stephenson 1992). A spatially determined position advantage is evident in *B. tricuspis* where zygotes from near-peduncular positions have the highest probability of survival and the progeny produced in these positions have large nutritional reserves as inferred through larger mass seed^{-1} (Figure 5.1 and 5.7) and seed nutrient content (Table 5.13b). Similar intra-infructescence spatial effects have been observed in other *Banksia* species where follicles infructescence^{-1} and mass seed^{-1} were greatest in middle and peduncular positions (Zammit and Hood 1986. Vaughton 1988, 1993, Wallace and O'Dowd 1989). Wallace and O'Dowd (1989) speculated the consistent development of follicles in basal portions of *B. spinulosa* var. *cunninghamii* infructescences may be attributed to developing zygotes pre-empting the nutrient supply to more distal zygotes thereby inhibiting development.

The non-random pattern of reproductive success observed in this species does not appear to conform with the notion of the self-organised movement of resources among developing zygotes (Ganeshaiah and Uma Shaanker 1992, 1994). The marked positive correlation between mass seed^{-1}, which is indicative of a zygote's ability to attract resources, and zygote survival conflicts with predictions from this hypothesis. Temporal differences in the schedule of ovule fertilisation over an inflorescence, which may vary by as much as 18 days (van Leeuwen, unpublished data), and the consistency in resource provisioning of aborted zygotes as indicated by their nutritional content and size (van Leeuwen, unpublished data, Lamont and van Leeuwen 1988) suggests that zygote survival is not based on a dominance hierarchy between ovules promoted by random drift.

This variation in reproductive success between infructescence positions in *B. tricuspis* may in part be attributed to ontogenetic considerations restricting number of florets at the apical and peduncular positions. George (1981) noted the existence of areas at the apex and base of *Banksia* inflorescences were bracts and bracteoles were present but florets absent, while the terminal tapering nature of the inflorescence may also contribute to a reduction in floret number. Ontogenetic considerations have also been implicated in the pattern of fruit and seed development along the flower axis in other species (Diggle 1995). Analogous to ontogenetic considerations are physiological considerations where fewer flowered portions of the inflorescence represent weaker physiological sinks incapable of competitively competing with more floriferous positions for nutrient resource provisions. Clearly in *B. tricuspis*, developmental considerations promoting a reduction in floret number and the corresponding physiological implications of such a reduction may help explain the pattern of reduced maternal fecundity and progeny fitness in apical positions. However, the failure to observe a commensurate reduction in maternal fecundity and progeny fitness in the peduncular positions, which experienced a similar reduction in floret number challenges this explanation.

I suggest that inadequacies in the vascular network of *B. tricuspis* infructescences, in concert with ontogenetic and physiological constraints which limit resource provisions to developing florets, ovules and zygotes, particularly in the apical regions, might be the principal intrinsic mechanism promoting the observed intra-infructescence pattern of fecundity and fitness observed in this species. Inadequacies in the vascular network towards the infructescence apex were implied through the positive correlation between position and mass seed^{-1} where heavier seeds were located near the peduncle (Figure 5.7) despite their initiation later than the lighter seeds nearer the apex. The pattern of seed nutrient content also support this proposition. Inadequacies in the vascular network of inflorescences impinging on resource provisions to apical positions has been reported in other species (Van Steveninck 1957, Lee and Bazzaz 1982, Solomon 1988, Diggle 1992) and in *B. spinulosa* var. *neoanglica* was assumed responsible for a reduction in the size of apical ovules and consequently smaller seeds (Vaughton 1993).

5.4.2 *Resource and pollen limitation of zygote survival and fitness*

I hypothesise that the non-random pattern of reproductive success in infructescences of *B. tricuspis* may be attributed to intra-inflorescence variance in the operation of both resource and pollen limitation mechanisms. These mechanisms act mutualistically to promote a differential pattern in the trade-off between maternal fecundity (quantity) and progeny fitness (quality) along the infructescence. This differentiation between fecundity and fitness is exemplified in the contrast between increasing zygote survival and decreasing outcrossing estimates with distance from the apex (Figures 5.5 and 5.1 respectively).

In apical positions on the *B. tricuspis* infructescence, it appears that resource limitation reduces reproductive success to levels below realised pollination success, as demonstrated through low zygote survival. Such resource limitation, as previously suggested, may be promoted by inadequacies in the vascular network of apical positions or by physiological deficiencies in the source-sink relationships of apical positions as a consequence of diminished floret abundance. Whatever the cause of resource limitation, the consequences appear to be an increase in the selective forces which prevent all zygotes from maturing. Elevated outcrossing estimates and heterozygosity in excess of expectation in apical positions implies the operation of genetic based 'decisions' which influence the pattern of zygote survival. These decisions, which are probably influenced by zygote vigour and the expression of genetic incompatibility systems (Rocha and Stephenson 1990, 1991, Rigney 1995), represent mate (female) choice (Stephenson and Bertin 1983). Indeed, uniformity in the nutrient content and size of aborted zygotes (van Leeuwen, unpublished data, Lamont and van Leeuwen 1988) is indicative of genetic control influencing zygote survival (Weins *et al.* 1987). These incompatibility systems, which are not necessarily rigid mechanisms governing selfing, can regulate progeny quality and enable the maternal parent to influence paternal parentage (Bertin 1982, Westoby and Rice 1982, Seavey and Bawa 1986) Non-rigid incompatibility systems are apparent in this species as demonstrated by outcrossing estimates below panmixia which indicate a tolerance of geitonogamous and consanguineous matings (Chapter 2).

If the non-random pattern of reproductive success in *B. tricuspis* infructescences is the consequence of mate choice, a relaxation in constraints on nutritional resource provisions should be accompanied by a reduction in the selective pressures controlling zygote survival. Consequently, progression towards the peduncular end of the infructescence and the commensurate increase in resource provisions, as demonstrated by enhanced mass seed^{-1} and seed nutritional content, was reciprocated by an increase in zygote survival. The decreasing probability of a follicle containing only one viable seed as opposed to two viable seeds with progression towards the peduncle (Figure 5.4) also indicates a mitigation in selective forces. In plants homogeneous for the enzyme Pgm-1 this acquiescence in selective forces controlling zygote survival was highlighted by a slight decline in outcrossing estimates (Figure 5.1) and a reduction in the abundance of heterozygous progeny (Table 5.14).

The proposal that zygote survivorship is a function of genetic quality and maternal resource provision such that a reduction in the latter is complemented by a reduction in zygote survivorship conforms with propositions for non-random reproductive success in other species (Casper 1988, Westoby and Rice 1982, Rigney 1995). The detection of a negative correlation between outcrossing estimates and zygote survival (Table 5.12) indicating an increase in the genetic quality of progeny with a decrease in zygotic survival, supports this proposition. Similar associations between outcrossing rate and both seed set and mass seed^{-1} also substantiate this proposition.

Attributing the non-random pattern of reproductive success in this species to maternal resource provisioning decisions which are influenced by zygote genetic quality implies that pollen limitation differentially affects reproductive success, as zygote genetic quality is determined by paternal considerations. Pollen limitation, expressed in terms of pollen load, appears to be an important paternal consideration in this species as demonstrated by the excess in heterozygous progeny from homogeneous maternal parents. Such excesses imply disassortative mating and non-random selection favouring heterozygous progeny (Brown 1979).

I attribute differential patterns in the operation of pollen limitation in *B. tricuspis*, especially pollen load, to the consequences of variation in inflorescence attractiveness (conspicuousness) and resultant pollinator foraging behaviour (Chapter 7). The frequency and duration of pollinator visits to inflorescences of this species are directly proportional to the number of open florets on an inflorescence (Chapter 7). This relationship indicates that pollinators preferentially visit and forage for the longest duration on the most floriferous inflorescences, those on which the 'advancing front' (Collins and Spice 1986) has progressed to middle and middle-peduncular positions. Pollinator visits and duration of foraging bouts were shorter on inflorescences with only a small portion of the most apical florets open. Similar foraging behaviour was observed on inflorescences with the most peduncular florets open and apical florets senescing. The consequences of such assortative pollinator foraging are likely to approximate a normal curve distribution with pollination success favouring middle positions, which experience inflated levels of xenogamous pollination, in comparison with apical and peduncular positions which experience minimal pollination with high levels of geitonogamy and consanguineous matings. The symmetrical shape of fruit and seed set against position (Figure 5.2) in this species is similar to a normal distribution and supports the proposition that pollen limitation as a consequence of pollinator foraging behaviour influences the reproductive success of this species. Lower outcrossing estimates in the middle positions, although not significantly different from apical or peduncular positions, may also support this proposition.

The proposition advanced to explain the observed pattern of maternal reproductive success in infructescences of *B. tricuspis* requires that maternal resource provisions are limited. However, this requirement has not been substantiated. It has been assumed that genetic considerations impinge on the probability of zygote survival and the vigour with which these considerations are invoked is dependent on position inconsistencies in the vascular network or source-sink relationships along the infructescence. I have previously argued (Chapter 4) that resource provision constraints appear to be minimal in this species. Consistency between plants in the level of reproductive success for most inflorescence/infructescence attributes assessed in this study also suggest that resource provisions are not limiting. Similarly, the failure to detect negative relationships between fecundity attributes and mass seed^{-1} also suggests that resource provisions were not limiting, especially in inflorescences. Negative relationships between fecundity components and mass seed^{-1}, which can be expected when resources are limited, usually indicates a trade-off between maternal reproductive success (quantity) and progeny provisioning (quality) (Smith and Fretwell 1974, Haig and Westoby 1988, Venable 1992). The trade-off is usually expressed as a reduction in the number of viable seeds, as mass seed^{-1} is the least plastic of all plant morphological traits (Harper *et al.* 1970, Winn 1991, Sakai and Sakai 1995) and is the concluding phase in the hierarchical sequence of serial adjustments which plants undertake to synchronise maternal investment with resource provision (Lloyd 1980). In this study, viable seeds position^{-1}, seed set and zygote survival were all positively correlated with mass seed^{-1} (Figure 5.8, Tables 5.4 and 5.12).

The proposed accord between resource and pollen limitation of reproductive success in infructescences of *B. tricuspis* concurs with propositions proposed for other species (Lee 1988, Zimmerman and Pyke 1988, Marshall and Folsom 1991, Rigney 1995). Campbell and Halama (1993) suggested the concordant relaxation of zygote selective pressures (which are pollen load driven) with a corresponding increase in maternal resources, as one of four possible scenarios implicating both resource and pollen limitation in constraints impinging on reproductive success in *Ipomopsis aggregata*.

The abortion of zygotes as a consequence of the post-zygotic expression of lethal genes could be an alternative proposition to explain the differential pattern of reproductive success in *B. tricuspis.* This proposition implies certainty in the abortion of incompatible zygotes and may operate when resource provisions are not limited, as is suggestive of quality-quantity correlations. The observed differential pattern of reproductive success is still attributable to the constraints imposed by pollen limitation through the differential pattern of pollinator foraging and ensuing inadequacies in pollen load. This proposition has one major shortcoming, however, as it does not accommodate the sustained survival of zygotes in peduncular positions when such positions are expected to experience high levels of geitonogamous and consanguineous pollen transfer by pollinators (Chapter 7).

5.4.3 *Adaptive considerations*

Differential reproductive success implies the selective abortion of zygotes which shields maternal plants from the randomness of pollination, permitting the regulation of offspring parentage and maximisation of progeny fitness under conditions of resource limitation (Lee 1988, Rocha and Stephenson 1991, Stephenson 1992). The present results suggest that differential abortion regulates reproductive success in infructescences of *B. tricuspis* when resources are limited (apical positions). Conversely, when resource limitations are relaxed (middle and peduncular positions) mechanisms controlling the decision to abandon zygotes are moderated and accordingly maternal fecundity increases. I suggest that genetic considerations influenced by pollen limitation (load) are the mechanisms controlling selective abortion decisions (Westoby and Rice 1982, Seavey and Bawa 1986, Campbell and Halama 1993).

The differential maturation of zygotes driven by resource provision considerations which are affected by genetic influences conforms with the 'selective abortion' hypothesis for the production of 'surplus' flowers (Stephenson and Bertin 1983). This concurrence is enhanced by the proposal that zygotes in this *Banksia* vary in their fitness as a consequence of pollen limitation factors, primarily pollen load. In addition, those individuals of the lowest fitness (selfed zygotes) are rejected in order to increase investment in those with higher fitness expectations (Kozlowski and Stearns 1989). Problematically, an inadequacy of selective abortion in *B. tricuspis,* which does not conform with the hypothetical expectation of the maternal parent identifying the best zygote without error as early as possible, is the proposed tolerance of progeny of diminished fitness as inferred by the reduced levels of outcrossing. The high probability of zygote survival in peduncular positions where geitonogamous and consanguineous pollination events dominate (Chapter 7), also implies that certainty in maternal choice is not stringent. Early identification and abandonment of low fitness zygotes, however, does occur as demonstrated by the low nutrient content and absence of endosperm in abandoned individuals (van Leeuwen, personal observation, Lamont and van Leeuwen 1988).

The response along the *B. tricuspis* infructescence to an abatement in resource limitations partially conforms with the 'bet-hedging' hypothesis advocated for the production of 'surplus' flowers (Udovic 1981, Ayre and Whelan 1989). The notion of 'resource availability', which asserts that maternal parents adjust fecundity upwards to take advantage of favourable conditions (Guitián 1993), is particularly relevant. The delaying of maternal decisions to promote abortion to as late a stage as possible, a primary requirement of the 'bet-hedging' hypothesis (Kozlowski and Stearns 1989), however, is not forthcoming. Uniformity in the morphological characteristics of aborted zygotes and minimal variance in their low nutrient content (van Leeuwen, personal observation, Lamont and van Leeuwen 1988) suggest that abortion occurs uniformly early in the maturation process. As noted by Kozlowski and Stearns (1989), 'bet-hedging' and 'selective abortion' hypotheses for 'surplus' flowers are not strict alternatives and may operate in concert. The proposition that variation in the mechanisms influencing the maternal decision to abort zygotes vacillates with changes in the availability of resource provisions along the infructescence, provides the opportunity for both selective abortion and bet-hedging to increase maternal fecundity and progeny fitness in *B. tricuspis.*

Zygote overproduction is fundamental to the operation of selective abortion and/or bet-hedging mechanisms which permit plants to adjust maternal investment thereby increasing plant fitness (Lloyd 1980). Contingencies which increase the opportunities for zygote overproduction, such as 'surplus' flowers, therefore should be advantageous and favourably selected. 'Surplus' flowers increase the opportunity to overproduce zygotes in *B. tricuspis* through increasing inflorescence attractiveness to pollinators as demonstrated by the preferential foraging of pollinators on the most floriferous and attractive structures (Chapter 7). Increases in inflorescence attractiveness culminating in enhanced pollinator foraging raise the opportunities for selective abortion in this species as the probability of unsuitable geitonogamous and consanguineous matings, inferred through the duration of foraging bouts and outcrossing estimates, increases with inflorescence attractiveness (Chapter 6 and 7). Similarly, the opportunities for bet hedging increase with inflorescence attractiveness as pollination success, inferred through the number of foraging bouts and zygote abortion, (Chapter 6 and 7) is positively correlated with inflorescence attractiveness.

5.4.4 *Conclusions*

Reproductive success in infructescences of *B. tricuspis* is differentially influenced by progeny position along the rachis. This effect was not due to the initiation time of developing zygotes. I suggest that this effect is due to the differential provisioning of maternal resource along the infructescence. The pattern of zygote survival and levels of outcrossing were sensitive to position effects although they exhibited opposing correlations.

The pattern of fruit and seed set (relative to floret production) favours middle positions on the infructescence. This pattern probably mimics the pattern of pollen receipt (Chapter 7) and possibly the degree of pollen limitation. Conversely, the pattern of zygote survival probably mimics the pattern of resource availability which is postulated to increase towards the peduncular end of the infructescence, as evident by greater mass seed^{-1} and higher nutrient content of seeds in this region. The difference between these two functions may be attributed to an acquiescence in the selective forces determining zygote survival, which may occurs in conjunction with a commensurate increase in resource provisions. The negative correlation between outcrossing estimates and mass seed^{-1} supported the suggestion of an acquiescence in selective forces with an increase in resource provisions.

This scenario infers the abortion of the least vigorous progeny when maternal resources are limited which conforms with the notion of mate choice and the proposition that the genetic threshold determining selection can alternate with resource provisions. Inherently, this scenario also implies a high level of intrinsic control over reproductive success thereby minimising the randomness of pollination experienced by this species. Allowing and promoting zygotes of lower quality to abort, thus releasing resources for other zygotes with higher fitness expectation, bestows a greater fitness advantage to the maternal plant. 'Surplus' flower production promoting enhanced inflorescence attractiveness is advantageous under this scenario as it increases the opportunity for greater genetic selection, via selective abortion and bet hedging, as a consequence of reduced pollen limitation.

Future work into the influence of position on the inflorescence rachis should focus primarily on two avenues of investigation. Firstly, the pattern and uniformity in maternal resource distribution along the entire inflorescence should be investigated. Documenting the uniform distribution of maternal resources will requiring rigorous ontogenetic and cytological examination of the inflorescence's vascular network. This examination should also investigate variability in the viability and vigour of ovules along the rachis. Carefully designed experimental trials which facilitate comparisons in resource provisioning between zygotes of identical genetic quality, from contrasting positions on the inflorescence, may also provide beneficial information on the patterns of resource provisioning along the inflorescence.

The influence on reproductive success of variations in maternal resource and genetic quality considerations which promote non-random patterns of zygote provisioning and abandonment should also be experimentally investigated. Methodical hand pollination experiments are promoted as a suitable method to document the pattern of zygote survival and progeny provisioning under contrasting conditions of competition between zygotes of assorted genetic quality. The experimental design should permit comparisons between maternal fecundity and progeny provisioning attributes when no competition, based on genetic considerations, is apparent (single pollen genotype), through to conditions of strong competition (several pollen genotypes). This competition cline would be promoted by varying the extent of geitonogamous, consanguineous and xenogamous matings achieved through hand pollination and should alternate along the rachis to document inflorescence position effects.

This hand pollination experimental design would also facilitate a comparison between genotypes and their ability to influence source-sink relationships and zygote provisioning. The proposition that progeny with the greatest fitness expectations are selectively favoured would be appraised by such experiments, especially if zygotes develop in isolation of different genotypes and thus under non-competitive genetic conditions.

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CHAPTER 6

CORRELATION OF INFLORESCENCE SIZE AND PRESENTATION HEIGHT WITH REPRODUCTIVE OUTPUT

6.1 Introduction

A major feature of the floral display of many hermaphroditic plants is the aggregation of florets into inflorescences. Variation in the display traits of inflorescences, such as floret number, arrangement, pattern of anthesis, colour, odour, and nectar rewards influence male and female sex functions which ultimately affect plant fitness (Zimmerman 1983, Galen and Stanton 1989, Diggle 1992, Mitchell 1994, Harder and Barrett 1995, 1996). This is especially true of non-autogamous zoophilous plants where animal-mediated pollen flow is essential for reproductive success (Galen and Stanton 1989). Thus, 'attractive' structures (Morgan 1992a) are a prerequisite for pollen dissemination and receipt and provide an important source of variation in successful pollination (Murcia 1990, Podolsky 1993).

Natural selection should favour inflorescences which enhance reproductive success through the attraction of the most efficient and abundant pollinators (Waser 1983). This is pertinent to plants with low overall reproductive success, where strong selection should promote displays which increase the probability of pollination (Schemske 1980). Indeed, increasing a plant's attractiveness has been proposed as one of the ultimate functional explanations for the phenomenon of 'surplus' flowers (Sutherland and Delph 1984, Stephenson 1992). Increases in reproductive success through pollen supplementation, suggesting insufficient pollination, support this hypothesis (Sutherland 1987, Zimmerman and Pyke 1988, Ehrlén 1992, Burd 1994). However, the failure to observe augmented fecundity in some species (Guitián 1993, Muenchow and Delesalle 1994) and the void between the realised (eg. fruit set, seed set) and intrinsic ceilings to reproductive success (eg. ovules floret^{-1}, florets inflorescence^{-1}, inflorescences plant^{-1}) indicate that this hypothesis may not be the only explanation for 'surplus' flowers (Sutherland 1986a).

Sexual selection theory, in particular the "pollen donation hypothesis" (Charnov 1982), predicts that fecundity in resource-limited plants drives the evolution of display traits which enhance the probability of progeny siring on other individuals in a population. Thus, it has been proposed that floral displays, which achieve high visitation rates and enhance pollen transfer among individuals, are favoured by selection through male function (Willson and Ratchke 1974, Stephenson and Bertin 1983, Queller 1983, Sutherland and Delph 1984). Studies supporting this proposition cite a reduction in fruit set with a commensurate increase in pollen transfer and the apparent failure of many pollinated flowers to develop into fruits, as indicative of selection driven by male function (Willson and Price 1977, Sutherland 1986b, Horvitz and Schemske 1988). Selection associated with female function also appears important in determining floral display traits as the production of large floral displays permits the regulation of progeny quality through male competition and female choice, via the selective abortion of undesirable progeny (Marshall and Ellstrand 1986, Stephenson and Wilson 1986, Sutherland 1987, Rocha and Stephenson 1990). As noted by Wyatt and Broyles (1994) and Wilson *et al.* (1994), the character of a plant's floral display probably represents a compromise between the processes that enhance both male and female reproductive success.

Any fitness advantage conferred through differentiation in the display characteristics of an inflorescence which increases its attractiveness will be influenced intrinsically by the ability of the plant to provision the inflorescence and the commensurate increased number of progeny (Davis 1981). As noted by Stanton and Preston (1988), in instances where reproductive success is limited by maternal resource provisions, fecundity may be enhanced by conserving provisions rather than investing in structures to increase attractiveness. In such instances plants are unlikely to experience enhanced fecundity with increased allocation to attraction. Conversely, plants which are pollen limited are unlikely to gain any fecundity advantage from the production of more ovules and commensurate increase in inflorescence size. The potential clearly exists for conflict between the desire to attract pollinators and the need to conserve resources for progeny provisioning. As has been noted, the mode of operation of these conflicting requirements on the resource provisions of a plant are not necessarily mutually exclusive but may operate and interact in concert (Stephenson 1992, Lawrence 1993).

Verification of such conflicting requirements on resource provisions is often demonstrated by quality-quantity trade-offs among progeny (Smith and Fretwell 1974) and is usually manifested in resource limited plants through variations in seed number while seed size (mass) remains constant. Numerous studies, however, indicate that considerable plasticity exists within and among individuals in the size of their seeds (Nakamura 1988, Winn 1991, Mehlman 1993), which has been attributed to several adaptive factors (Sakai and Sakai 1995).

Progeny quality is also an important measure of plant fitness that is affected by inflorescence display traits (Wyatt 1982, Marshall and Folsom 1991). This association is attributed to the dependence of progeny quality on pollen flow and hence the pattern of pollen receipt and dissemination promoted through pollinator foraging behaviour (Campbell 1991, Stanton *et al.* 1991, Campbell and Halama 1993). It may therefore be argued that display traits promote selective pollination and/or enhance the opportunity for mate choice (Stephenson and Bertin 1983, Waser 1983, Marshall and Follson 1991). For example, the increased attractiveness of large inflorescences may entice pollinators over a larger foraging area reducing the probability of consanguineous (mating with sibs) pollen receipt and increasing the potential for the expression of high quality pollen (Mulcahy 1983). A large inflorescence may also experience a greater diversity of pollen, enhancing the opportunity for mate choice (Palmer *et al.* 1988). Similarly, small inflorescence displays may reduce the opportunity for stigma clogging and deposition of geitonogamous (fertilisation by pollen from a flower on the same plant) pollen (Wyatt 1982, Klinkhamer and de Jong 1993). In such cases, the potential increase in progeny quality enhances the female functional role even if no association with progeny quantity is observed (Schmid-Hempel and Speiser 1988).

Display characteristics of an inflorescence may not necessarily be driven by selection based on sex function or progeny quality-quantity trade-offs, but may represent a response to other functional considerations (Wyatt 1982). Reductions in the opportunity for fruit and seed predation (Hendrix 1988, Crawley, 1992), enhancement of the probability of fruit and seed dispersal (Janzen 1971, Jardano 1992) and physiological considerations associated with the translocation of nutrient assimilates (Lee 1988) may also selectively influence inflorescence display traits. For example, large inflorescence displays may increase the probability of subsequent dispersal in plants with a frugivorous dispersal syndrome (Van der Pijl 1982). However, in other species such a display may increase the opportunity for nectar robbing or floret, fruit and seed damage by herbivorous animals (Scott 1982). Similarly, elevated inflorescence displays may increase the flight potential of wind dispersed progeny while the same display on another species may be disadvantaged through assimilate deprivation promoted by the physical distance from nutrient sources (Lee 1988).

The number of florets on an inflorescence and its presentation are important floral traits influencing reproductive success in plants (Schemske 1980, Pyke and Paton 1983, Harder and Cruzan 1990, Harder and Barrett 1985). Generally, augmentation of floret number which increases display size enhances the capacity of an inflorescence to attract more pollinators, thereby increasing pollen receipt and dissemination and enhancing reproductive success (Willson and Rathcke 1974, Willson and Price 1977, Queller 1983, Schmid-Hempel and Speiser 1988, Campbell 1989a, Andersson 1991, Ohara and Higashi 1994). Similarly, inflorescences presented in a conspicuous manner may be more attractive and receive more pollinator visitors that those which are obscure and cryptic (Pyke 1981, Pyke and Paton 1983, Burd 1994). Such positive associations, however, may not always exist as pollination success may sometimes decline with total floret number (Willson *et al.* 1979, Wyatt 1980, Muechow and Delesalle 1994). Such positive associations may also be undermined by inadequacies in the fidelity of the pollinating agents and factors which influence their foraging behaviour (Ford *et al.* 1979, Ramsey 1989, Burd 1994).

The display traits of inflorescences on *Banksia* species have a marked impact on the relationship between reproductive success and pollination. Initially, Rourke and Weins (1977), Carpenter (1978) and Holm (1978) proposed a dichotomy between display traits which promoted different pollinator assemblages and pollination syndromes. However, the catholic and opportunistic predisposition of most Australian pollinators has fostered the dismissal of any such dichotomy in subsequent work (Ford *et al.* 1979, Hopper 1980). Carpenter and Recher (1979) attributed this dichotomy in inflorescence display characteristics to natural selection driven by the fecundity advantage bestowed on nonresprouting species over resprouters. More recent research on *Banksia* inflorescence displays has indicated that abundance (McFarland 1986, Ramsey 1989, Copland and Whelan 1990), size (MacFarland 1986, Paton and Turner 1985, Goldingay and Whelan 1990), nectar and pollen rewards (Collins and Spice 1986, Vaughton 1990) and colour (Lamont and Collins 1988) all influence pollinator attraction and affect reproductive success.

Inflorescence size is usually positively associated with reproductive success in banksias. Paton and Turner (1985) reported that the conversion ratio of large inflorescences to infructescences was much greater than that of small inflorescences in *B. ericifolia,* which they attributed to the inability of small inflorescences to receive sufficient xenogamous pollen. Differential patterns in fruit set between infructescences of *B. integrifolia* and *B. spinulosa* growing sympatrically have also been attributed, in part, to variation in inflorescence size (McFarland 1985). Similarly, Goldingay and Whelan (1990) identified a positive association between inflorescence size and fruit set in *B. spinulosa* which they purported implied an absence of pollen limitation, although no such association was observed in the sympatric *B. paludosa.* The effect of inflorescence presentation height, which is a function of both height and visibility, on reproductive success in banksias has not been well reported, although considerable variation is evident both within and between species (Holm 1978, George 1981, Collins and Rebelo 1987, Carthew 1993). Ramsey (1989) noted that the presentation height of inflorescences in *B. menziesiii* influenced pollinator foraging with the most conspicuous inflorescences being visited the most frequently.

This chapter reports on the influence of inflorescence size (length) and presentation height (height above the ground) on the quantity (fecundity) and quality (fitness) of reproductive success in *Banksia tricuspis.* The aim of the study was to determine the relationship between the fecundity and fitness attributes of an infructescence while sympatrically investigating the effects of display traits on these variables. Concurrent studies have demonstrated that both display traits influence reproductive success through: a) the deleterious impacts of floral damage (Chapter 3); and b) pollinator attraction (Chapter 7). Specifically, this study investigated the variability and relationships between follicles and viable seeds per infructescence, fruit and seed set, zygote survival, mass seed^{-1} and outcrossing estimates, and attempted to correlate this variability to inflorescence length and presentation height. Outcomes of this investigation are discussed with reference to the possible role of resource and pollen limitation, the influence of floral displays on reproductive strategies and the adaptive nature of inflorescence displays in this species.

6.2 Materials and Methods

This investigation into the correlation of display traits on the reproductive success of *B. tricuspis* was undertaken in a natural population located approximately 4.3 km ENE of Mt Lesueur and 9.8 km SE of Mt Peron within the Lesueur National Park, approximately 220 km north of Perth. A typical *B. tricuspis* plant produces up to 80 conspicuous, golden yellow, cylindrical inflorescences which are born terminally or occur on short lateral branches throughout the canopy. Inflorescences, which contain up to 2 500 florets, attract honeyeaters, invertebrates and small mammals that forage for nectar and pollen in the vicinity of freshly opened florets and effect pollination (van Leeuwen and Lamont 1986). Zoophily is essential for the production of robust infructescences (cones) which contain numerous follicles (fruits), as *B. tricuspis* is not capable of spontaneous autogamy (van Leeuwen and Lamont 1986). The number of infructescences sired by a plant, which may range up to 20 in a season, is considerably less than the number of inflorescences produced by a plant (up to 85 in a season), as a consequence of invertebrate and avian floral damage (Chapter 3).

A sample of five adult plants was selected from the population of 268 during April 1988. In order to reduce the effects of differentiation in plant size on the plasticity of display traits and reproductive success attributes, selection of the sample plant was designed to achieve homogeneity in canopy volume between individuals and to the population average. Crown volume was employed as an index of plant size, which previous investigations have demonstrated significantly impacts on reproductive success in this species (van Leeuwen, unpublished data). Canopy volume was determined using the formula 0.5236 x H x W_1 x W_2, where H was maximum canopy height, W_1 was maximum canopy width and W_2 was canopy width perpendicular to W_1

(Witkowski *et al.* 1991). The average population estimate of crown volume (9.80 ± 1.08 SE m^3) was determined by recording canopy variables on 76 plants in five transects established through the population. One plant per transect for those individuals which had a crown volume that approached the population average ± 95% confidence interval (± 2.15 m^3) was selected through the allocation of random numbers to all qualifying candidates.

6.2.1 *Display traits*

Inflorescence length and presentation height were selected as independent display traits which may affect reproductive success in *B. tricuspis*. Inflorescence length, which is a direct measure of display size, is an intrinsic function of floret number while presentation height provides an indication of the conspicuousness of the inflorescence. Both traits provide a measure of inflorescence attractiveness to pollinators and have previously been identified as differentially influencing the level of reproductive success achieved in this species (Chapter 3 and Chapter 7). These traits are also known to affect plant reproductive output in other *Banksia* and proteaceous taxa (McFarland 1985, Paton and Turner 1985, Pyke 1987, Goldingay and Whelan 1990). Inflorescence length (mm) and presentation height (cm) were recorded for all infructescences produced by the five sample plants in the 1987 flowering season.

To determine fruit and seed set on the sampled infructescences an estimate of florets inflorescence^{-1} for 1987 was obtained from a sample of ten inflorescences harvested from the five sample plants in April 1988. Two inflorescences, the largest and smallest on each of the sampled plants, were collected and floret numbers recorded. An estimate of floret numbers for the harvested infructescences was then obtained using the linear regression model for florets inflorescence^{-1} in 1988, which was:

$$\text{floret number} = 14.42 \text{ inflorescence length} - 391.07$$

This model was significant ($r = 0.98$, $F_{1,9} = 232.46$, $P < 0.001$) accounting for 95% of the variance in floret number.

6.2.2 *Reproductive success attributes*

Five fecundity and two fitness attributes were recorded from the infructescence harvested from the five sampled plants.

6.2.2.1 *Fecundity attributes*

The number of follicle and viable seeds per infructescence were recorded for each of the harvested infructescences. Follicles infructescence^{-1} were determined by direct counts while viable seeds infructescence^{-1} were determined by recording the number of firm intact seeds extracted from infructescences after exposure to a burn treatment and wet-dry regime (Cowling and Lamont 1985). Subsequently, percentage fruit and seed set, together with zygote survival, where a zygote was defined as an initiated seed, were determined for each of the harvested infructescences using the follicles infructescence^{-1} and viable seeds infructescence^{-1} data. Fruit set was determined using the equation:

$$\left(\frac{\text{follicles infructescence}^{-1}}{\text{florets inflorescence}^{-1}}\right) \times 100$$

while seed set was determined using the equation:

$$\left(\frac{\text{viable seeds infructescence}^{-1}}{\text{florets inflorescence}^{-1} \times 2}\right) \times 100$$

In both equations, florets inflorescence^{-1} was determined using the previously defined regression model. The devisor in the equation for seed set was doubled to reflect the assumed presence of two functional ovules floret^{-1}.

Zygote survival was determined by dividing the number of viable zygotes (ie. viable seeds) infructescence^{-1} by the total number of zygotes initiated which was the sum of viable and aborted seeds infructescence^{-1}. Aborted seeds were readily identifiable, being malleable and lacking an embryo.

6.2.2.2 *Fitness attributes*

The fitness of progeny produced by infructescences with varying display traits was assessed on surrogate attributes by determining mass seed^{-1} and outcrossing estimates for each of the harvested infructescences. Mass seed^{-1} was determined for all viable seeds extracted from follicles on harvested infructescences while outcrossing estimates were obtained for each infructescence through an appraisal of progeny genotypes, as detected by isozyme electrophoresis.

Electrophoresis was performed on the emerging radical and associated embryonic material of germinating seeds which had been imbibed for 14 days on moistened filter paper at 15°C. The number of seeds (progeny) assessed for each infructescence (family) ranged from a minimum of six to a maximum of 22, although the mean was 21.5 progeny family^{-1}. Electrophoresis was conducted using a cellulose acetate plate arrangement (Helena Laboratories) following the procedures and buffer recipes detailed in Chapter 2. Infructescence samples were assayed for the polymorphic enzyme system phosphoglucomutase (PGM-1, E.C. 5.4.2.2) as this was the only polymorphic system of 17 isozyme systems examined (van Leeuwen, unpublished data) and had sufficient allelic frequency for mating system estimations. Activity of the Pgm-1 isozyme on the cellulose acetate plates was detected using the methodology described by Coates (1988) and Wendel and Weeden (1989) with a slight modification for 1.75% agar overlays. The maternal parentage of each of the five sampled plants was also determined by conducting electrophoresis on pollen samples collected from freshly opened florets in 1988.

Single locus ($\hat{t}_s$) estimates of outcrossing were calculated for each infructescence using the joint maximum likelihood estimation of the mixed-mating model, as determined by the procedures and computer programs developed by Ritland and Jain (1981). These programs permitted single family estimations of outcrossing when maternal parentage is known, especially when progeny numbers are low. Single locus estimates of outcrossing were also determined from the population of five plants from the 185 progeny family^{-1}.

6.2.3 Data analyses

Normality (symmetry, Kolmagorov-Smirnov test) and homogeneity of group variances (Bartlett's test) for raw data was examined prior to statistical interrogation and corrected where necessary. Normality of percentage values was achieved by arcsine transformation while minimisation of homogeneity of variance in mass seed^{-1} was accomplished through a logarithmic transformation according to the ladder of powers (Velleman and Hoaglin 1981). Descriptive statistics presented are for back-transformed values. A minimum variance mean, single locus, estimate of outcrossing infructescence^{-1} was calculated for each plant from outcrossing estimates obtained for each infructescence by the procedures defined by El-Kassaby *et al.* (1987).

Homogeneity between plants was investigated using a one way ANOVA for all reproductive success attributes, except outcrossing estimates where the nonparametric Kruskal-Wallis test was utilised (Fry 1993). Pairwise comparisons between plants were investigated using the Tukey-Kramer multiple range *posteriori* test (Zar 1984, Fry 1993) for all one way ANOVA interrogations, while Dunn's multiple comparison was used for the nonparametric outcrossing estimate interrogations (Zar 1984).

The relationship between display traits and reproductive success attributes was examined using least squares linear regression where the significance of the regression was tested by ANOVA procedures (Zar 1984, Fry 1993). Assumptions of this linear model were checked through residual plots and the Durbin-Watson autocorrelation test (Kirby 1993). Relationships between the two independent display traits and each of the reproductive success attributes were investigated using a mixed models regression procedure which decomposed the effect of each variable on the dependent attributes (Wilkinson 1992). Homogeneity between plants in correlation coefficients was examined using chi-squared procedures where acceptance of the null hypothesis permitted the calculation of a common correlation coefficient (Zar 1984).

Determination of the best fit linear regression model describing the relationship between pollinator attraction effort (florets inflorescence^{-1}) and the number of provisioned seeds (viable seeds infructescence^{-1}) was achieved by stepwise selection, among models generated by the TableCurve 2D software (Jandell Scientific), using the test statistic F as described by Zar (1984).

Pairwise associations between display traits and reproductive success attributes were assessed by Spearman's rank correlation, while multiple comparisons between variables were examined by Kendall's coefficient of concordance which was tested for significance by Friedman's chi-squared statistic. A judicious assessment of an inflorescence's attractiveness was obtained by averaging the sum of the combined rank scores for both display traits for each of the 44 infructescences. The relationship of this character set with each reproductive success attribute was then examined using Spearman's rank correlation procedures.

Principal Component Analysis (PCA) procedures were used to test for association between display traits and reproductive success attributes and to identify the independent variables impinging on each fecundity and fitness attribute. PCA permitted the determination of correlations between independent variables in the presence of all variables, as inferred through the cosine of the angle between vectors (variables) on a Principal Component plot (Jolliffe 1986, Wright 1994). Corroboration of PCA results was undertaken using multiple, reduced and stepwise regression procedures for each reproductive success attribute. Multiple regressions were performed on the entire collection of variables with the significance of the estimate model determined by ANOVA procedures (Zar 1984, Fry 1993). In reduced regression procedures the independent variable least correlated with the chosen dependent variable, of variable pairs shown to be highly correlated in the PCA plot, was eliminated from the subsequent regression model. Similarly, non-independent variables were also excluded from reduced regression models. Stepwise selection was initiated with all independent variables selected and proceeded using the interactive stepping routine presented in SYSTAT where tolerance and F statistic values were used as criteria to eliminate variables from the model (Wilkinson 1992).

6.3 Results

6.3.1 *Display traits*

Inflorescence length and presentation height were both normally distributed about the mean and exhibited homogeneity of variances between the five plants (Table 6.1). Departure from normality was not evident in either mean inflorescence length or presentation height. The association between inflorescence length and presentation height was similar for all plants ($\chi^2_5 = 2.82$, $P > 0.05$) with the common correlation between the two traits representing a weak association ($r = 0.18$, $F_{1,42} = 1.44$, $P > 0.05$) (Figure 6.1). Floret number inflorescence^{-1} in 1987, as estimated from the 1988 regression between inflorescence length and floret number, ranged from 799 to 2 385,

Table 6.1 **Descriptive statistics and results of between plant comparisons for heterogeneity in display traits and reproductive success attributes for 44 infructescences of *Banksia tricuspis* harvested in 1988** (g_1 = symmetry, D = Kolmogorov-Smirnov goodness of fit).

Display traits / reproductive attributes	Mean ± SE	Range	g_1	D_4	$F_{4,39}$
Inflorescence length (mm)	119 ± 4	81 - 189	0.572	2.590	0.923
Presentations height (cm)	164 ± 7	79 - 268	-0.171	1.282	1.741
Follicles infructescence^{-1}	24.6 ± 1.2	7 - 40	-0.095	2.220	7.239***
Viable seeds infructescence^{-1}	27.2 ± 1.5	8 - 51	0.578	6.840	10.206***
Fruit set (%)	1.1 ± 0.1	0.4 - 4.0	1.181**	6.614	3.052*
Seed set (%)	1.0 ± 0.1	0.2 - 1.9	0.233	3.330	4.751**
Zygote survival (%)	55.5 ± 1.3	39.7 - 82.7	1.021**	3.634	3.368*
Mass seed^{-1} (mg)	61.9 ± 0.4	57.7 - 66.8	0.209	21.142***	0.632
Outcrossing estimate [a]	0.831 ± 0.144	0.470 - 0.963	-	-	1.975

[a] Kruskal-Wallis test employed to investigate interplant differences. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

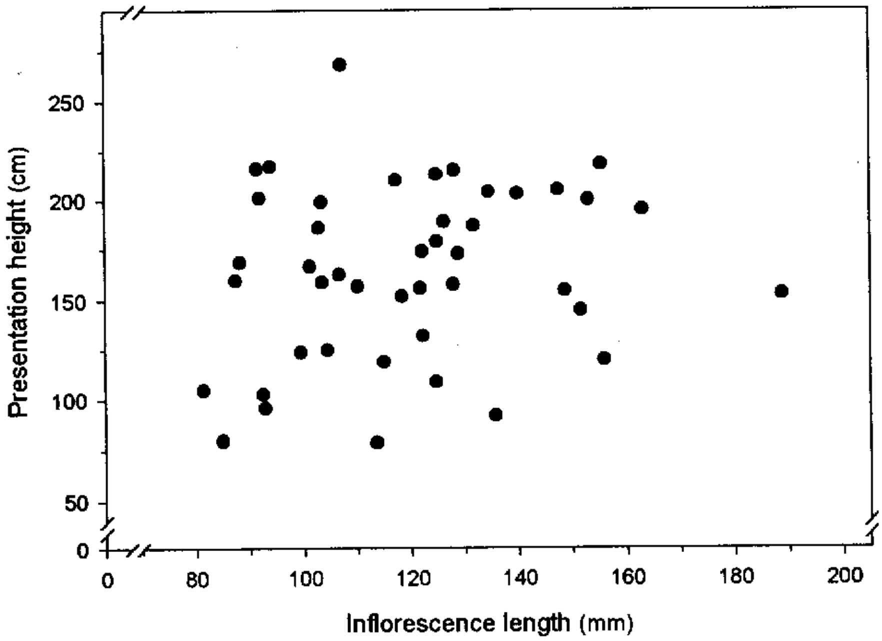

Figure 6.1 **Relationship between inflorescence length and presentation height in 44 infructescences of *Banksia tricuspis* harvested from five plants during 1988.**

with a mean of 1 335 ± 54. As this trait is dependent on inflorescence length it was assumed not to be correlated with presentation height.

6.3.2 *Reproductive success attributes*

6.3.2.1 *Fecundity attributes*

Totals of 1 081 follicles and 1 196 viable seeds were produced by the 44 infructescences sampled from the five *B. tricuspis* plants. All fecundity attributes exhibited homogeneity between group variances for the five plants examined. However, fruit set and zygote survival were asymmetrically distributed (Table 6.1). Significant heterogeneity between plants was detected in the mean estimates of each fecundity component. In all cases, except zygote survival, this heterogeneity was attributed to incongruity between the most fecund and barren plants (Tukey-Kramer multiple range test, $P < 0.05$). The between plant incongruity associated with zygote survival was attributed to one plant exhibiting significantly greater zygote survival than the remaining four individuals (Tukey-Kramer multiple range test, $P < 0.05$).

All fecundity attributes were either negatively or not correlated with inflorescence length (Figure 6.2). The least squares linear regression, which was homogeneous between the five plants for each attribute (common correlation coefficient equals 0.23, 0.19, -0.43, -0.45, and 0.12 for follicles infructescence^{-1}, viable seeds infructescence^{-1}, fruit set, seed set and zygote survival respectively, $P > 0.05$), was significant for both fruit and seed set only. No such associations was detected for follicles infructescence^{-1}, viable seeds infructescence^{-1} or zygote survival. Homogeneity between plants in correlations between each of the fecundity attributes and inflorescence presentation height was also detected (common correlation coefficient equals 0.63, 0.35, 0.46, 0.25 and -0.46 for follicles infructescence^{-1}, viable seeds infructescence^{-1}, fruit set, seed set and zygote survival respectively, $P > 0.05$). Follicles infructescence^{-1}, viable seeds infructescence^{-1} and fruit were all significantly positively correlated with presentation height while no association was detected for the relationship between presentation height and seed set (Figure 6.2). In contrast, zygote survival was significantly negatively correlated with presentation height (Figure 6.2). Significant associations were detected between all fecundity variables, except fruit and seed set, for interactions with both independent variables

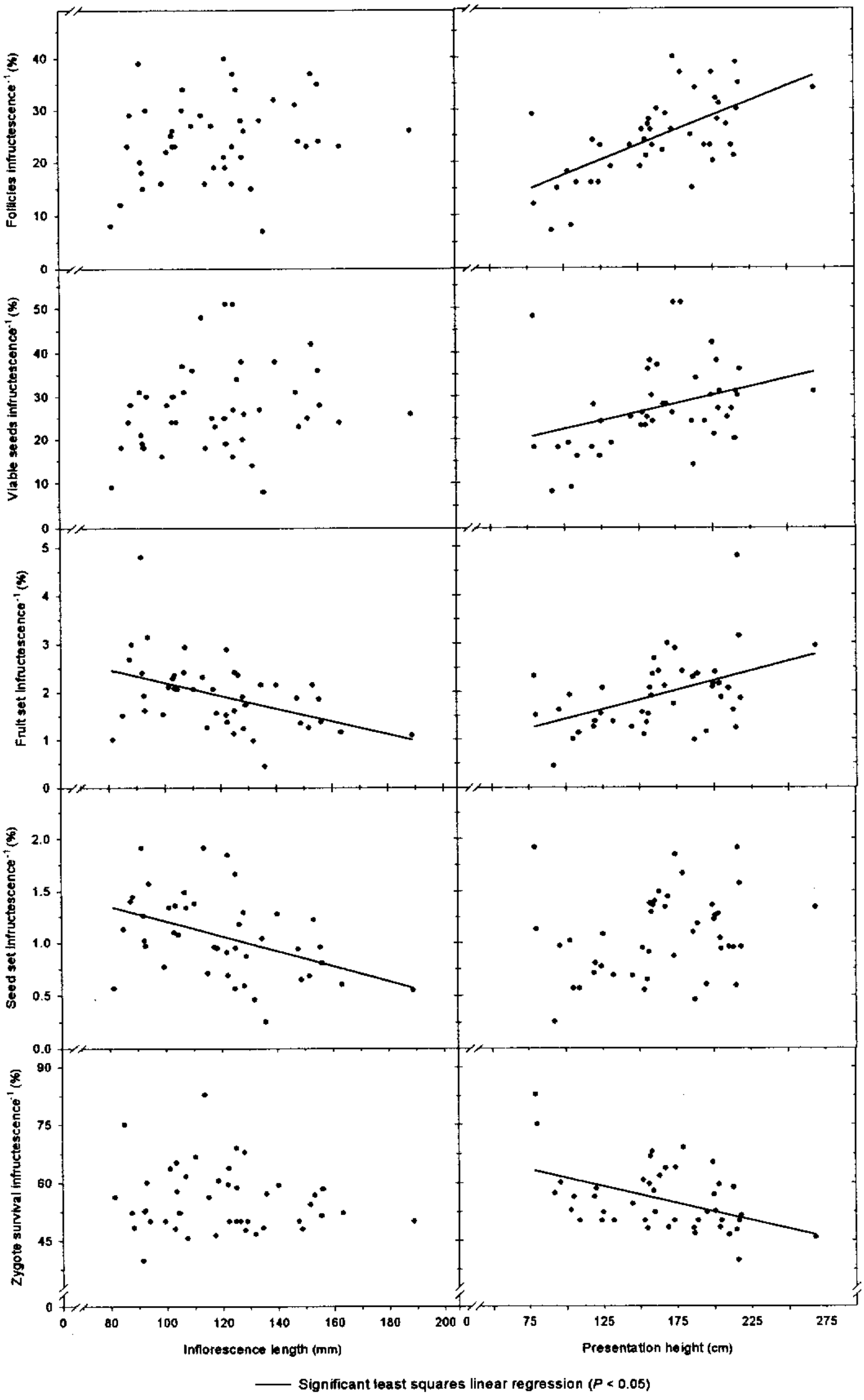

Figure 6.2 **Scatter plots and linear regression models for the association between display traits and fecundity attributes on 44 *Banksia tricuspis* infructescences.**

Table 6.2 Regression and ANOVA results for the interaction between both independent display traits and each of the dependent fecundity and fitness attributes recorded for 44 *Banksia tricuspis* infructescences.

Reproductive attributes	r	$F_{1,42}$	P
Follicles infructescence^{-1}	0.578	21.071	0.000
Viable seeds infructescence^{-1}	0.360	6.254	0.016
Fruit set	0.061	0.155	0.696
Seed set	0.079	0.266	0.609
Zygote survival	-0.376	6.900	0.012
Mass seed^{-1}	0.351	5.902	0.019
Outcrossing estimate	0.812	81.14	0.000

(inflorescence length and presentation height) indicating an increase in realised fecundity and zygote abortion with inflorescence attractiveness (Table 6.2).

6.3.2.2 *Fitness attributes*

Mass seed^{-1} for the 1 196 viable seeds collected from the 44 *B. tricuspis* infructescences was asymmetrically distributed (g_1 = 1.44, $P < 0.01$) about the mean of 61.8 ± 0.5 mg and ranged from 18.8 to 101.4 mg. Mass seed^{-1} infructescence^{-1} was, however, normally distributed (Table 6.1) although significant heterogeneity between variances for each of the individual infructescences was detected (D_{43} = 112,39, $P < 0.001$). Similar heterogeneity in group variance was also detected between the five sampled plants (Table 6.1). After log normalisation no significant differences were detected in the distribution of mass seed^{-1} infructescence^{-1} among the five plants.

Mass seed^{-1} infructescence^{-1} was positively correlated with both inflorescence length and presentation height, although in the case of the latter, the relationship was not significant (Figure 6.3). The correlation coefficient of these associations, determined for each plant, were homogeneous (χ^2_5 = 7.34 and 8.51 for inflorescence length and presentation height respectively, $P > 0.05$). The interaction between the independent variables of inflorescence length and presentation height also appeared to have a marked effect on mass seed^{-1} infructescence^{-1}, although only 12.3% of the variance in mass seed^{-1} infructescence^{-1} was accounted for by the interaction between these display traits (Table 6.2).

Single locus outcrossing estimates for the population of five plants were not significantly different from panmixia ($\hat{t}_s = 0.842 \pm 0.125$, $t_5 = 1.26$, $P > 0.05$) and were comparable to those determined for other undisturbed natural populations of *B. tricuspis* where a small number of plants were examined (Chapter 2). Similar levels of outcrossing, which were homogeneous between infructescences, were also determined for the minimum variance means estimate for the 44 infructescences (Table 6.1). Outcrossing estimates were significantly correlated with both inflorescence length and presentation height (Figure 6.3) and the interaction between these two independent variables (Table 6.2). The correlation coefficients for each interaction on each of the five plants were similar (χ^2_5 = 0.57 and 3.89 for inflorescence length and presentation height respectively, $P > 0.05$). The correlation coefficient and slope of the linear regression determined for the interaction of both display traits with estimates of outcrossing were homogeneous ($F_{1,41}$ = 0.273 and $F_{1,84}$ = 0.947 respectively, $P > 0.05$).

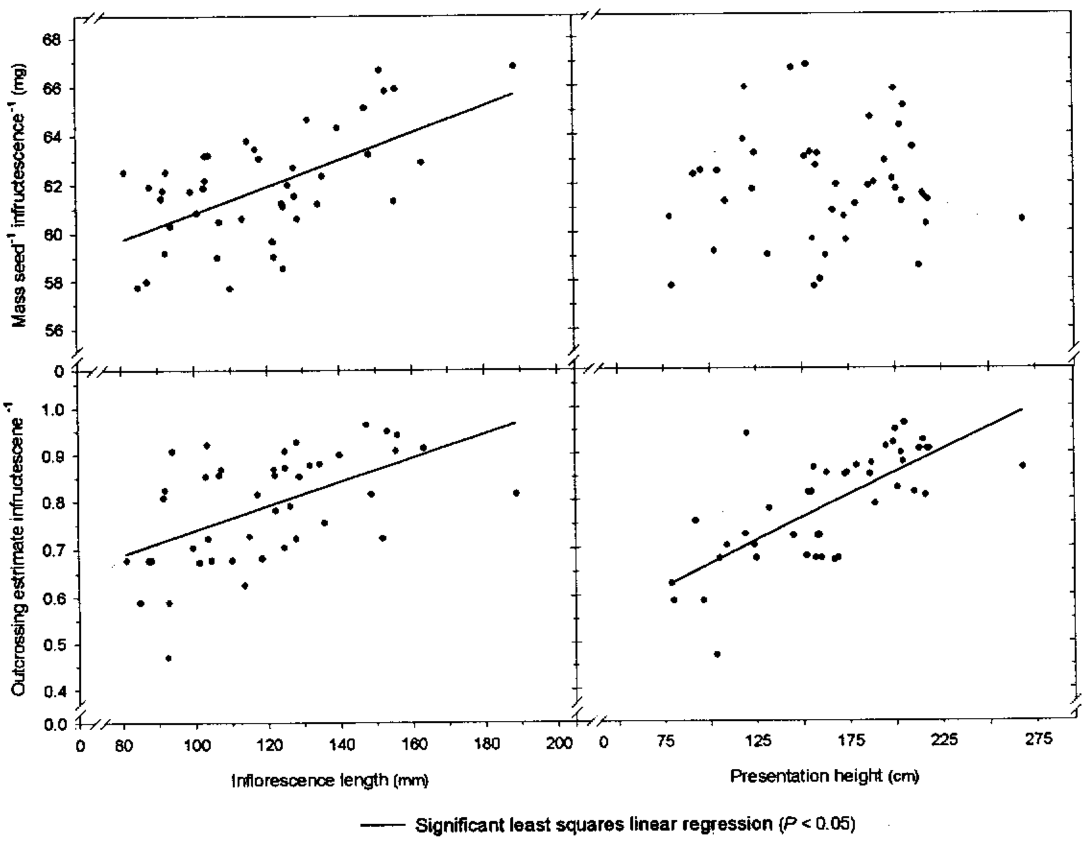

Figure 6.3 **Scatter plot and linear regression models for the association between display traits and fitness attributes of 44 *Banksia tricuspis* infructescences.**

6.3.3 *Interaction between pollinator attraction effort, fecundity and fitness*

The association between the number of viable seeds infructescence^{-1} and pollinator attraction effort was represented by an increasing but diminishing function, which was significant at the 5% level (Figure 6.4). This function, however, accounted for only 9% of the variance in viable seed numbers. New best fit linear functions were not determined for the association between each of the other reproductive attributes and pollinator attraction effort as all were identical to the functions determined for the association with inflorescence length.

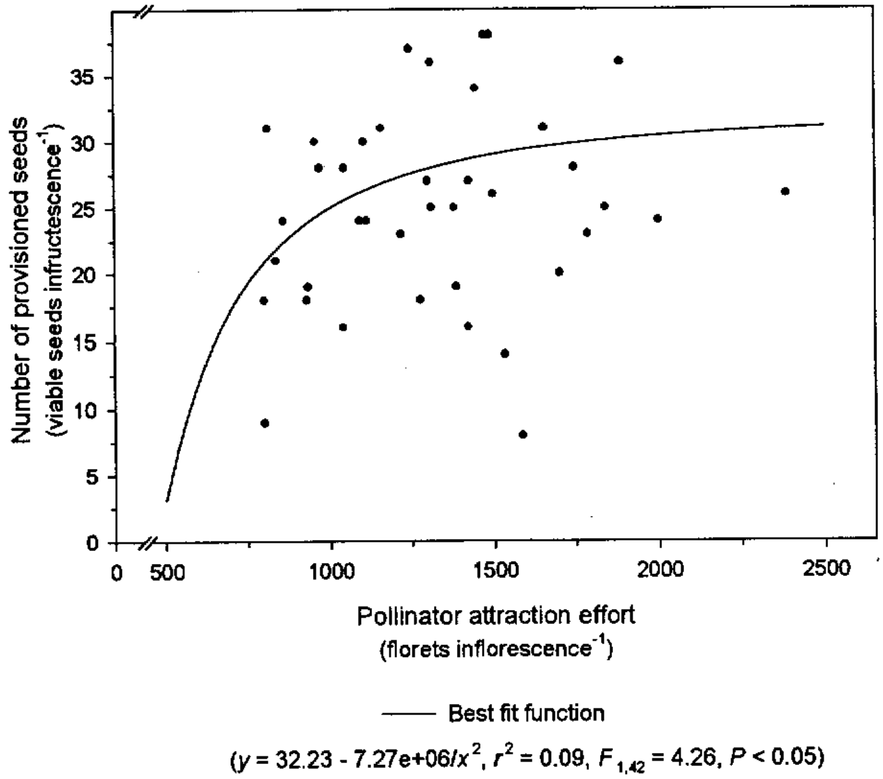

Figure 6.4 Relationship between pollinator attraction effort and number of provisioned seeds for 44 *Banksia tricuspis* infructescences.

6.3.4 *Interaction between display traits and reproductive success attributes*

Results from Spearman's rank (Table 6.3) and PCA correlations (Figure 6.5, Table 6.4) of the association between inflorescence length and presentation height concurred with least squares linear regression results and indicated a weak association between the two display variables (Figure 6.1).

Table 6.3 **Results of Spearman's rank correlation statistic for the association between display traits and reproductive success attributes for 44 infructescences of *Banksia tricuspis*.**

Reproductive attributes	Inflorescence length	Presentation height	Follicles infructescence^{-1}	Viable seeds infructescence^{-1}	Fruit set	Seed set	Zygote survival	Mass seed^{-1}
Presentation height	0.180							
Follicles infructescence^{-1}	0.244	0.609***						
Viable seeds infructescence^{-1}	0.209	0.468**	0.915***					
Fruit set	-0.426**	0.462**	0.700***	0.670***				
Seed set	-0.468***	0.282	0.628***	0.718***	0.925***			
Zygote survival	-0.101	-0.420**	-0.068	0.292	0.038	0.340*		
Mass seed^{-1}	0.471**	-0.026	-0.029	-0.100	-0.361*	-0.430**	-0.199	
Outcrossing estimate	0.587***	0.707***	0.421**	0.347**	0.031	-0.095	-0.232	0.212

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Spearman's rank correlation indicated that the majority of reproductive attributes were significantly associated with inflorescence length, although the nature of the association varied from positive for mass seed^{-1} and outcrossing estimates to negative for fruit and seed set (Table 6.3). The other fecundity attributes were not significantly associated with inflorescence length. Significant rank correlations were also detected for associations with presentation height, as illustrated by the declining trend in zygote survival and increases in most fecundity attributes and outcrossing estimates. Results obtained from PCA correlations (Figure 6.5, Table 6.4) were mostly similar to those determined for rank correlations. PCA results indicate that inflorescene length was highly positively associated with mass seed^{-1} and outcrossing estimates while conversely being negatively associated with zygote survival. Contrastingly, presentation height was associated strongly with follicle and viable seed number infructescence^{-1} and also outcrossing estimates. Interestingly zygote survival was strongly, negatively associated with mass seed^{-1}.

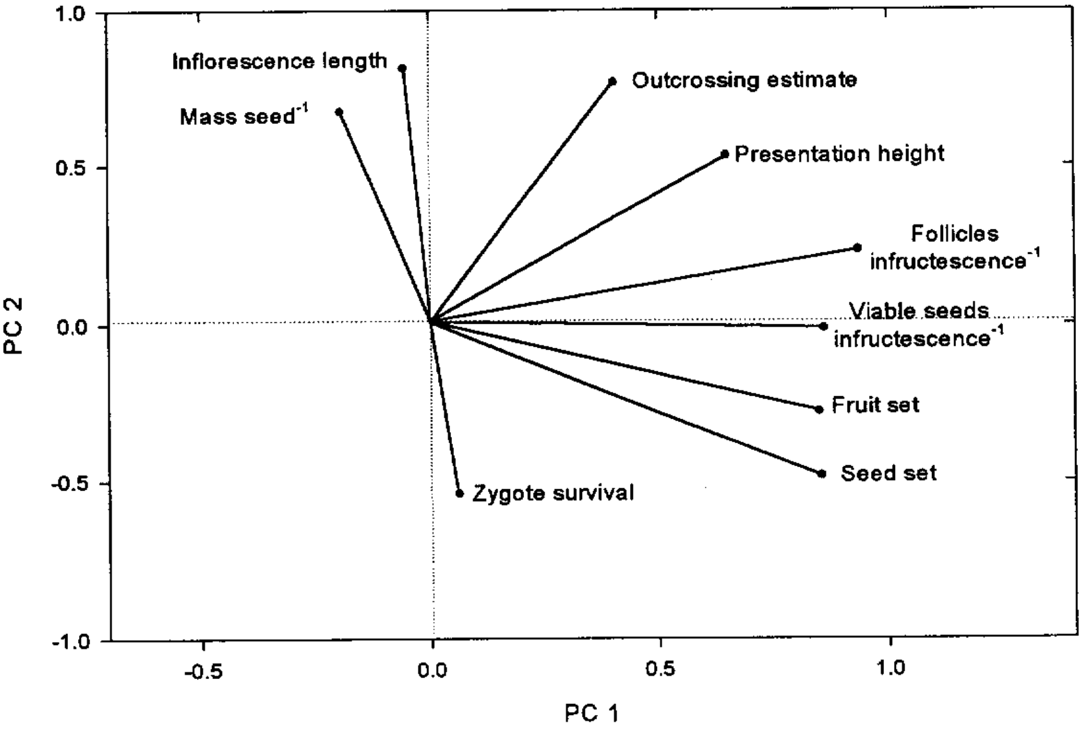

Figure 6.5 Principal Component plot of the interaction between display traits and fecundity/fitness attributes for 44 *Banksia tricuspis* infructescences. (PC 1 accounted for follicles infructescence^{-1}, viable seeds infructescence^{-1}, percentage fruit set, percentage seed set and presentation height. PC 2 accounted for inflorescence length, outcrossing estimates, mass seed^{-1}, and percentage zygote survival.)

Table 6.4 Matrix of correlations between vectors determined by Principal Component Analysis plots for relationship between display traits and reproductive success attributes for 44 ***Banksia tricuspis*** infructescences.

Reproductive attributes	Inflorescence length	Presentation height	Follicles infructescence^{-1}	Viable seeds infructescence^{-1}	Fruit set	Seed set	Zygote survival	Mass seed^{-1}
Presentation height	0.44							
Follicles infructescence^{-1}	0.22	0.94						
Viable seeds infructescence^{-1}	0.14	0.87	0.98					
Fruit set	-0.16	0.74	0.92	0.98				
Seed set	-0.33	0.62	0.85	0.93	0.99			
Zygote survival	-0.99	-0.44	-0.10	0.07	0.26	0.42		
Mass seed^{-1}	0.95	0.34	0.00	-0.17	-0.37	-0.53	-0.99	
Outcrossing estimate	0.82	0.93	0.74	0.63	0.45	0.31	-0.73	0.67

Significant association between both display traits and each individual reproductive attribute, indicating concordance between variables, was detected for associations with the dependent variables of mass seed^{-1} and outcrossing estimates only (Table 6.5). These results concur with least squares linear regressions of the combined effect of both independent variables on these dependent attributes. However, no significant association was detected for follicles infructescence^{-1} or zygote survival, despite least squares regression procedures identifying a significant association between these attributes and both display traits combined (Table 6.2). Overall concordance between display traits and reproductive attributes was minimal. However, the association that did exist was significantly different from no association at all (W = 0) (Table 6.5). Concordance among variables was also minimal for interactions involving either inflorescence length or presentation height separately, with the association involving the latter display trait being significantly different from no association at all (Table 6.5).

Table 6.5 Results of Kendall's coefficient of concordance (*W*) and Friedman's chi-squared test statistic (χ^2_r) for the association between both display traits and each of the reproductive attributes assessed for the 44 *Banksia tricuspis* infructescences.

Reproductive attributes	Test statistic	
	W	χ^2_{r43}
Follicles infructescence^{-1}	0.195	50.739
Viable seeds infructescence^{-1}	0.195	50.739
Fruit set (%)	0.381	49.194
Seed set (%)	0.332	42.818
Zygote survival (%)	0.257	33.187
Mass seed^{-1}	0.472	60.919*
Outcrossing estimate	0.660	85.171***
All reproductive success attributes	0.203	61.101*
All reproductive success attributes *vs* inflorescence length only	0.183	47.125
All reproductive success attributes *vs* presentation height only	0.235	60.642*

Significance: * $P < 0.05$, *** $P < 0.001$

Inflorescence attractiveness, as determined by the mean rank score of inflorescence length and presentation height combined, significantly influence all infructescence fecundity and progeny fitness attributes, except percentage fruit and seed set (Table 6.6). Most of the associations with inflorescence attractiveness were positive. However, opposing relationships were detected with fruit and seed set and zygote survival. These results indicate that as pollinator attraction effort or the investment in attractiveness increased, fecundity and fitness also appreciated.

Table 6.6 Results of Spearman's rank correlation for the association between inflorescence attractiveness and reproductive success attributes for 44 infructescences of *Banksia tricuspis*. Inflorescence attractiveness was determined as mean rank score of inflorescence length and presentation height combined.

Reproductive attributes	Inflorescence attractiveness
Follicles infructescence^{-1}	0.528***
Viable seeds infructescence^{-1}	0.406**
Fruit set	-0.005
Seed set	-0.153
Zygote survival	-0.349*
Mass seed^{-1}	0.299*
Outcrossing estimate	0.853***

Significance: * $P < 0.05$, *** $P < 0.001$

Follicles and viable seeds infructescence^{-1} were significantly correlated with presentation height only (Table 6.3 and 6.4). In both cases the correlation with inflorescence length was poor. Multiple regression procedures indicated that both fecundity attributes were significantly correlated and were also significantly associated with both fruit and seed set (Table 6.7). These correlations were spurious, however, as these variables were not independent - both percentage fruit and seed set were directly determined from the number of follicles and viable seeds infructescence^{-1}. Subsequently, in reduced and stepwise regression models where dependent variables were excluded, viable seeds infructescence^{-1} were identified as the reproductive variable accounting for most of the variance in follicles infructescence^{-1}. Conversely, follicles infructescence^{-1} was identified as the only reproductive variable influencing viable seeds infructescence^{-1}. Inflorescence presentation height was identified as the dominate display variable predicting both follicles and viable seeds infructescence^{-1} thus confirming the Spearman's rank and PCA correlations (Table 6.7).

Table 6.7 **Multiple, reduced and stepwise regression models for the interaction between display traits and reproductive attributes on 44 infructescences in *Banksia tricuspis*.**

Reproductive success attributes	Variables										Significance	
	Constant	Inflorescence length (mm)	Presentation height (cm)	Follicles infructescence^{-1}	Viable seeds infructescence^{-1}	Fruit set (%)	Seed set (%)	Zygote survival (%)	Mass seed^{-1} (mg)	Outcrossing estimate	*r*	*F*
Follicles infructescence^{-1}												
multiple	8.59	0.02	0.01*		0.82***	7.58***	-13.44***	-0.12*	-0.040	-2.05	0.99	663.19***
reduced	-0.93	-0.02	0.08***		0.59***					-8.68	0.94	81.50***
stepwise	-2.46		0.07***		0.60***						0.94	161.72***
Viable seeds infructescence^{-1}												
multiple	-9.91	0.01	-0.01	1.08***		-9.61***	19.90***	0.09	0.03	2.44	0.99	792.18***
reduced	31.90	0.01	-0.10***	1.36***					-0.52	11.50	0.92	43.43***
stepwise	6.03*		-0.08***	1.36***							0.91	104.29***
Fruit set (%)												
multiple	-0.03	0.01	-0.01	0.07***	-0.07***		2.10***	-0.01	0.00	0.17	0.99	628.34***
reduced	-0.10	0.02***	0.01***		0.04***				0.04	-1.05	0.88	23.22***
stepwise	1.97	-0.02***	0.01***		0.04***						0.89	36.97***
Seed set (%)												
multiple	-0.09	-0.00*	0.00	-0.03***	0.030**	0.43***		0.01	-0.00	-0.08	0.99	874.12***
reduced	1.90**	-0.00***	-0.03**	0.05***					-0.019	0.21	0.94	56.76***
stepwise	1.43***	-0.00***	-0.02***	0.05***							0.96	96.73***
Zygote survival (%)												
multiple	51.42***	0.08	-0.01	1.16*	0.66	-9.05*	28.10*		-0.10	0.34	0.97	75.52***
reduced	49.04*	0.36**	-0.06	-1.46***			37.32***		-0.62	9.16	0.86	17.44***
stepwise	51.01*	0.39***	-0.04	-1.49***			37.73***		-0.62		0.86	20.97***
Mass seed^{-1} (mg)												
multiple	58.89***	0.07*	-0.01	-0.31	0.19	2.78	-3.56	-0.08		0.35	0.64	3.02*
reduced	57.18***	0.05**	0.00			-0.92				-0.36	0.60	5.47***
stepwise	55.29***	0.06***									0.58	21.47***
Outcrossing estimate												
multiple	0.29	0.00	0.00***	-0.02	0.02	0.17	-0.37	0.00	0.00		0.85	11.18***
reduced	0.32***	0.00	0.00***	0.00		-0.02					0.84	22.83***
stepwise	0.27***	0.00***	0.00***								0.85	46.69***

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fruit set appeared to be associated with all display traits and reproductive attributes, with the exception of zygote survival and outcrossing estimates (Table 6.3). PCA correlations (Table 6.4) suggested that the relationship between fruit set and inflorescence length was weaker than indicated by the Spearman's rank correlation. However, this result was not verified by regression model procedures which identified inflorescence length as a significant predictive variable (Table 6.7). Multiple regression procedures for fruit set indicated a significant relationship with seed set, which was confirmed by Spearman's rank and PCA correlation results (Tables 6.3 and 6.4). Seed set, however, was excluded from the reduced and stepwise regression models for fruit set as it represented a non-independent variable. This non-independence was attributed to the unifying reliance of both variables on floret number. Likewise, fruit set was excluded as a variable in the reduced and stepwise regression models for seed set.

Rank correlations indicated that seed set was poorly correlated with both presentation height and estimates of outcrossing (Table 6.3) which was verified by the multiple and reduced regression models (Table 6.7). Reduced models also indicated that seed set was poorly correlated with mass seed^{-1} which contradicts the rank correlation results where a significant association was detected. PCA correlations indicated that seed set was moderately correlated with presentation height which was identified and selected as a significant predicting variable during reduced and stepwise regression interrogations.

Zygote survival was negatively associated with all traits and attributes, except viable seeds infructescence^{-1} and fruit and seed set. Rank correlations indicated that this negative association was significant for presentation height only, while the positive correlations were only significant for the association with seed set (Table 6.3). Correlations between vectors on the PCA plot (Figure 6.5) indicated that zygote survival was highly negatively correlated with inflorescence length and mass seed^{-1}. However, only inflorescence length was selected as a significant predictor in the regression procedures undertaken (Table 6.7). Attributes identified as significant predictors accounting for the variation in zygote survival among the 44 infructescences were inflorescence length, follicles infructescence^{-1} and seed set.

Presentation height, which was significantly associated with zygote survival in rank correlation results, was not a significant predictor in these regression models.

No significant associations was detected between mass seed^{-1} and presentation height for either rank or PCA correlations. However, mass seed^{-1} was significantly correlated with inflorescence length and the two fecundity attributes of fruit and seed set, although the association between these attributes was opposing (Tables 6.3 and 6.4). PCA correlations also indicated that mass seed^{-1} was significantly correlated with zygote survival. However, this was not supported by regression models where zygote survival was either excluded as an independent variable because of its marked association with inflorescence length or was not selected as a predicting variable in the stepwise analysis. Correlation results indicated that mass seed^{-1} remained constant with increasing infructescence fecundity and increased with estimates of outcrossing. Multiple, reduced and stepwise regression models identified inflorescence length only as a significant predicting variable which accounted for 33.6% of the variance in mass seed^{-1} for the most significant regression model (Table 6.7).

Single locus, minimum variance mean estimates of outcrossing were poorly associated with fruit and seed set, with the latter variables exhibiting negative associations. Significantly positive rank correlations were detected with follicles and viable seeds infructescence^{-1}. A marked negative association was evident with zygote survival while a moderate association was detected with the fitness attribute of mass seed^{-1} (Tables 6.3 and 6.4). Outcrossing estimates were significantly associated with both display traits, although PCA correlations suggested that the association with inflorescence length was not as marked as that with presentation height (Figure 6.5, Table 6.4). Presentation height was identified as the most significant predictor variable influencing outcrossing estimates among the 44 infructescences for all calculated regression models (Table 6.7). In the stepwise regression model, inflorescence length was also identified as a significant predictor value which, combined with presentation height, accounted for 70.1% of the variance in estimates of outcrossing.

6.4 Discussion

The size and presentation of inflorescences has a pronounced correlation with reproductive output in *B. tricuspis.* Correlation and association tests indicated that inflorescence presentation height accounts for much of the variation in maternal fecundity and progeny fitness, while inflorescence size explained most of the variation in resource provisioning of progeny.

The size of the inflorescence display in this *Banksia* was not correlated with maternal fecundity, although it had a marked association with progeny fitness. All maternal fecundity attributes were either significantly negatively correlated or not associated with inflorescence length (Figure 6.2). A fecundity advantage conferred through increasing inflorescence size has been observed in many plant species (Schmid-Hempel and Speiser 1988, Rodríguez-Robles *et al.* 1992, Ohara and Higashi 1994), including some banksias (Paton and Turner 1985, McFarland 1985, Goldingay and Whelan 1990). However, this advantage was not evident in this investigation. The failure to detect a corresponding increase in fecundity with display size may be attributed to the receipt of inappropriate (geitonogamous and consanguineous) pollen which increases the opportunity and intensity of selection based on female choice. While large inflorescence displays in this species invite and receive more pollinators, which were attracted over greater distances, such displays also entice longer foraging bouts at the inflorescence and increase the probability of intra-plant or near neighbour foraging movements (Chapter 7). Such movements increase the opportunity for geitonogamous and consanguineous pollination (Lamont 1982, Harder and Barrett 1995, 1996). The increasing probability of zygote abortion with inflorescence length supports the receipts of inappropriate pollen loads on large inflorescences in this species.

In contrast, inflorescence size enhanced progeny fitness, as indicated by significantly positive associations with both progeny provisioning and estimates of outcrossing (Figure 6.3). The association with mass seed^{-1} can be attributed to two possible scenarios, both influenced by maternal resource provisions. Firstly, the association may be attributed to chemically mediated processes whereby large inflorescences function as dominant sinks for nutrient provisions at the expense of smaller inflorescences and are thus more successful at provisioning their developing zygotes. Such inflorescences are inherently stronger sinks because of the provisioning requirements associated with increased floret numbers (Ho 1992). Increases in the vascular supply to fecund inflorescences, which occurs in response to sink strength, has been reported in other *Banksia* species (Lamont and Barrett 1988, Vaughton 1993). Secondly, this association may be explained through the notion of a quality-quantity trade-off (Smith and Fretwell 1974, Haig and Westoby 1988, Venables 1992) whereby increases in mass seed^{-1} are achieved through reductions in seed number. While Spearman's rank (Table 6.3) and PCA (Table 6.4) correlations between maternal fecundity attributes and mass seed^{-1} tentatively supported such an explanation, the rejection of all four fecundity attributes as predictor variables in the best fit regression model for mass seed^{-1} (Table 6.7) repudiate this trade-off explanation. Similar associations between progeny size and the level of resource provisions available to inflorescences and individuals have been reported in other species (Winn and Werner 1987, Venables 1992). The selection of a reproductive system based on progeny quality in preference to quantity, as appears to be evident for inflorescence size in *B. tricuspis,* may be considered a selective response in this resprouting species (Carpenter and Recher 1979).

The concurrent increases in outcrossing estimates with inflorescence size were primarily attributable to the effects of inflorescence length on pollinator movements, whereby large inflorescences attracted pollinators from greater distances (Chapter 7), potentially enhancing the xenogamous component of the pollen load received by a floret. However, the paradoxical increase in outcrossing estimates with larger inflorescence size, when this also enhances the opportunity for geitonogamous pollination suggests that nonrandom selection based on pollen type may be occurring in the species (de Jong *et al.* 1993, Klinkhamer and de Jong 1993). This proposition is corroborated by the decrease in zygote survival with increasing inflorescence length, indicating that inflorescence size enhances the opportunity for sorting among possible mates.

Inflorescence presentation, as expressed through height above the ground, had a marked effect on maternal fecundity attributes. All attributes, with the exception of seed set, exhibited significant association with presentation height, although in the case of zygote survival the association was negative. The positive association with most attributes indicates that fecundity increases with the height of the inflorescences. This can be attributed to the increased attractiveness and conspicuousness of such displays for pollinators. Inflorescences displayed towards the upper crown of *B. tricuspis* plants preferentially receive move pollinator visits and the flight distance travelled to reach such inflorescences is greater than for inflorescences displayed close to the ground (Chapter 7). The modal distance travelled by pollinators to inflorescences displayed towards the upper crown is smaller than those displayed towards the ground, which paradoxically increases the probability of geitonogamous and consanguineous pollinator movements (van Leeuwen, unpublished data). The contracting probability of zygote survival with increasing presentation height may be a consequence of such geitonogamous and consanguineous pollen transfer events.

Progeny provisioning was not affected by inflorescence presentation height, despite the probability that inflorescences presented higher on a plant are located further away from the source of resource provisions. Similarly, no quality-quantity trade-off was detected as a function of presentation height, with mass seed^{-1} increasing in concert with fecundity attributes (Table 6.4). The ability of distantly located inflorescences to provision progeny more efficiently than those located closer to resource provisions may be attributed to enhancements in sink strength following fertilisation, which promotes establishment of a dominance hierarchy among the developing infructescences for resource provisions. Such enhancements are advocated by chemically mediated processes and hormonal actions which permit inflorescences with large numbers of developing zygotes to outcompete those with fewer (Lee 1988).

Inflorescence presentation height had a marked influence on outcrossing estimates with higher inflorescences experiencing the greatest outcrossing. These high outcrossing estimates were recorded despite considerable opportunity for ovule fertilisation following geitonogamous and consanguineous pollination (Chapter 7). This result may be explained by non-random post-zygotic selection based on female choice (Lyons *et al.* 1989, Waser 1993) which reputedly influences reproductive success in other species (Stephenson and Winsor 1986, Marshall and Ellstrand 1988), including some banksias (Scott 1980, Vaughton and Carthew 1993). The positive correlation between presentation height and the probability of zygote abortion supports this notion of female choice.

I suggest that inflorescence attractiveness considerably enhances female fertility and function in *B. tricuspis*. Fertility is enhanced through higher realised maternal fecundity and progeny fitness while function is enhanced through female choice and the differential provisioning of progeny. The enhancement in female function is apparent in the association between zygote survival and both display traits, especially presentation height, and is manifested in amplified outcrossing estimates above those presumably achieved through pollination alone. The negative association between inflorescence attractiveness and fruit and seed set (Table 6.67), indicating an excess of florets inflorscence^{-1} to that required for maximum maternal reproductive success, also suggests an important male functional role for inflorescences in this species (Willson and Ratchke 1974, Bertin 1988). Indeed, differential rates of pollen removal, which are associated with inflorescence attractiveness, have been observed in this species (van Leeuwen, unpublished data) and are also suggested through the influence of inflorescence displays on pollinator foraging movements and behaviour (Chapter 7). The importance of the male functional role is also demonstrated by estimates of outcrossing which approach unity in this non-autogamous species.

I suggest that female inflorescence function may be more important in this species than male function, albeit at the cost of proportional floret fecundity, as mechanisms which select and promote progeny of a superior quality are considered to be adaptive in resprouting species (Carpenter and Recher 1979). Floral display characters which increase inflorescence attractiveness may, however, operate in concert to enhance both functional roles (Campbell *et al.* 1991, Wilson *et al.* 1994) and, as argued by Wyatt (1980), are only important if they increase their relative contribution to the next generation. Consequently, both inflorescence length and presentation height are likely to be important inflorescence attributes affecting plant fitness through both sex functions in *B. tricuspis*.

6.4.1 *Constraints on reproductive success*

Both pollen and resource limitation appear to constrain the reproductive success of *B. tricuspis* infructescences, as indicated by the best fit model of pollinator attraction effort versus provisioned seeds (Figure 6.4) (Haig and Westoby 1988). This model is similar to one identified previously (Chapter 4) which supposedly illustrates an acquiescence in pollen limitation with the concurrent implementation of resource limitation as inflorescence size increases and thus attraction effort is augmented. This best fit model therefore predicts that inflorescence attractiveness increases with attraction effort to a point where constraints associated with the availability of resource provisions preclude any further increase in fecundity. Small inconspicuous inflorescences are pollen limited while reproductive success in more attractive inflorescences progressively becomes constrained by resource provisioning considerations under this model. The detection of negative associations between both fruit and seed set and progeny provisioning, indicated by the decline in mass seed^{-1} with increasing fecundity floret^{-1}, tentatively support the assertion that resource limitation constraints were impinging on reproductive success.

Confounding this assertion, however, is the marked association between attraction effort (particularly inflorescence length) and mass seed^{-1}, which indicated that resource provisions were most abundant in inflorescences which invested more resources in attraction effort (Figure 6.3, Table 6.3). Similarly, consistency in mass seed^{-1} with increases in realised viable seed number infructescence^{-1} rebut the proposal that resources were limiting. Decreases in zygote survival with augmented attraction effort also confound the suggestion of an acquiescence in pollen limitation as inflorescence attraction increased. This paradoxical situation and the role of both pollen and resource limitation in the reproductive success of *B. tricuspis* infructescences could be clarified by a competition-dependent model where female choice and the asymmetrical allocation of resources provisions based on sink strength control reproductive success.

This competition-dependent model proposes that attractive inflorescence displays receive a more diverse pollen load than smaller/inconspicuous displays and subsequently, the attractive inflorescences initiate more zygotes which are subjected to intense post-zygotic selective pressures through female choice and sorting among compatible mates, as determined by their genotype and that of neighbouring sibs. The intensity of the selective pressures between compatible mates are moderated by the availability of maternal resource provisions which are furnished as a function of the inflorescence's sink strength. Consequently, estimates of progeny fitness (eg. outcrossing rate) for attractive inflorescences are amplified above levels achieved at pollination. This selective model ensures maintenance of maternal fecundity and selection of the most superior progeny under a) conditions of abundant pollination when competition and selection among compatible mates is intense; and b) when pollination is restrained and selfing prevails. Reproductive assurance is the ultimate consequence of this reproductive strategy (Barrett 1988, Waser 1993).

This mechanism is suggested in *B. tricuspis* infructescences by a) an increase in pollinator visitation rate (Chapter 7); b) a rising tendency in fruit and seed production (Figure 6.2); c) decreases in zygote survival (Figure 6.2); and d) increases in mass seed^{-1} and outcrossing estimates (Figure 6.1) with an increase in inflorescence attractiveness. The uniformity in size and nutrient status of aborted zygotes (Lamont and van Leeuwen 1988, van Leeuwen, personal observation), suggested maternal genetic control over zygote survival (Seavey and Bawa 1986, Weins *et al.* 1987). This supports the competition-dependent model and indicates post-zygotic selection via differential provisioning and abandonment of zygotes (Lyons *et al.* 1989). Synchronous post-zygotic selection or sorting among compatible mates at an early developmental stage conserves resource provision (Seavey and Bawa 1986). This proposed mechanism conforms with the proposition that maternal parents are selectively favoured to differentially provision progeny based on the progeny's genotype (Bertin 1982, Temme 1986), with resource provisions being withheld from less vigorous compatible mates (Westoby and Rice 1982). The proposal also adheres to suggestions that the differential provisioning of progeny based on pollen source is most likely in species exhibiting reduced progeny fitness following selfing, ie. inbreeding depression (Marshall and Ellstrands 1986, Weins *et al.* 1987). The positive correlation between mass seed^{-1} and estimates of outcrossing tentatively illustrate inbreeding depression in this species, as mass seed^{-1} decreases with increased estimates of selfing. This model also ensures that maternal resources are allocated in a strategic manner maximising plant fitness. Low quality zygotes and inferior compatible mates are abandoned in the presence of higher quality individuals allowing amplification of outcrossing levels while fecundity is assured when pollination limitation is constraining. The model is similar to that proposed for intra-inflorescence variations in reproductive success in this *Banksia* (Chapter 5).

This competition-dependent model predicts that attractive (large and/or conspicuous) inflorescences receive abundant pollinator visits which transfer a large, diverse array of pollen types that fertilise many ovules. The resultant developing zygotes experience intense post-zygotic selection through female choice based on their genotype and that of neighbouring sibs which embellishes estimates of outcrossing. Selective mechanisms which preferentially increase estimates of outcrossing between ovule fertilisation and progeny production (preferential outcrossing) have been observed in a number of other species (Griffin *et al.* 1987, Barrett 1988, Rigney *et al.* 1993). The intensity of post-zygotic selection in attractive inflorescences may not be attributed only to female choice eliminating weaker, presumably selfed, zygotes but may also represent differential selection among compatible mates on the basis of relatedness to the maternal parent (Waser 1993). Rigney *et al.* (1993) reported nonrandom seed set from outcrossed pollen on the basis of distance away from the maternal parent in *Erythronium grandiflorum.*

This post-zygotic selection model is mediated by competition for resource provisions among the developing zygotes, such that intense selection occurs in infructescences with large numbers of fertilised ovules. Provisioning of progeny may also be enhanced in such attractive inflorescences as a consequence of chemically mediated sink augmentation following enhanced fertilisation (Ho 1992). This provisioning proposition is supported by evidence of increased maternal resource allocation to inflorescences as a consequence of the number of zygotes initiated (Winn 1991). Large inflorescence displays may also inherently confer a progeny provisioning advantage in response to strong established sinks developed in accord with enlarged floret numbers. Small and inconspicuous inflorescences which are not as attractive to pollinators as larger and conspicuous inflorescences (Chapter 7) are therefore likely to exhibit higher levels of selfed progeny as a consequence of inadequate pollen transfer; reduced mass seed^{-1} as a consequence of inferior sink strength; and higher zygote survival in response to moderated post-zygotic selection pressures promoted by less intense sorting between compatible mates.

Outcrossing estimates which approach unity despite considerable opportunity for geitonogamous and consanguineous pollination, especially on attractive inflorescence displays where the duration of pollinator foraging bouts and frequency of near neighbour pollinator movements are elevated (Chapter 7), are also explained by this selective model. The detection of similar high estimates of outcrossing in other *Banksia* species (Scott 1980, Carthew *et al.* 1988, Vaughton and Carthew 1993), which similarly experience considerable opportunity for geitonogamous pollination (Ramsey 1989, Vaughton 1990), is consistent with this proposed model This is especially the case in *B. spinulosa* var. *neoanglica* where outcrossing approaches unity, pollinator behaviour promotes geitonogamy, autogamous fertilisation occurs in the absence of pollination and differential resource provisioning influences fecundity (Vaughton 1988, 1990, 1991, Vaughton and Carthew 1993). The variation in outcrossing estimates with attraction effort observed in this species demonstrates that pollinator behaviour influences mating system estimates (Wyatt 1983, Karoly 1992).

The competition-dependent model differentially invokes both pollen and resource limitation as constraints on reproductive success. Both factors may function in concert (Stephenson 1992, Lawrence 1993) to ensure synchronisation of maternal investment with available resources, which will enable a plant to maintain maximum levels of reproductive output under random extrinsic influences. Pollen limitation is advanced as the primary constraint impinging on reproductive success in *B. tricuspis* infructescences. Pollen limitation constrains the reproductive success of unattractive inflorescences through a lack of sufficient pollen transfer, as illustrated by low fruit and seed production infructescence^{-1} and minimal post-zygotic selection which permits ample selfing. Attractive inflorescences are also pollen limited as a consequence of inadequacies in the genetic composition of the pollen load received, as demonstrated by high levels of zygote abortion. Therefore, the increasing but diminishing function of the best fit model describing the relationship between pollinator attraction effort and number of provisioned seeds (Figure 6.4) does not represent the intensification of resource limitation with increased investment in attractiveness, but appears to be a function of insufficient pollen transfer and promiscuous pollen loads. The failure of zygote abortion to decrease with increased attraction effort (inflorescence length) also suggests pollen limitation was constraining (Figure 6.2).

Resource limitation arguably may be cited as constraining reproductive success as illustrated by the variance in progeny provisioning with attraction effort. However, chemically mediated processes in response to sink strength may account for these differences. The linear function of mass $seed^{-1}$ versus investment in attractiveness also verifies that resource provisions were not limiting (Figure 6.3).

6.4.2 *Adaptive considerations*

The display traits of *B. tricuspis* inflorescences are correlated with plant fitness surrogates, particularly through the effects of inflorescence size and presentation height on pollination success and female choice. These inflorescence traits are also instrumental in predicting male function (Chapter 7, van Leeuwen, unpublished data) and the extent of floral damage (Chapter 3) which also regulates fitness in this species. Display traits probably represent a compromise between processes that simultaneously affect all selective forces influencing reproductive success (Campbell 1989b, Morgan 1992b, Wyatt and Broyles 1994). Reproductive mechanisms in this *Banksia* which promote pollination success and provide the opportunity for nonrandom zygote survival, thus amplifying progeny fitness (progeny provisioning and outcrossing estimates), may be adaptive as they confer a fitness advantage on this resprouter. Resprouting plants are purported to indulge in the selection of superior quality reproduction as an individual's inherent resilience to selective forces permits survival and perpetuation in fluctuating, unpredictable environments (Carpenter and Recher 1979, Lloyd 1979, Jarne and Charlesworth 1993). The cost of this reproductive strategy is reduced fecundity and is clearly demonstrated in differences between resprouting and nonsprouting congeneric pairs (Bellairs and Bell 1990). Differences in estimates of fecundity and fitness have been reported between congeneric resprouting and nonsprouting *Banksia* species (Cowling *et al.* 1987, Zammit and Westoby 1987). Floral displays which therefore enhance the opportunity to increase the fitness advantage conferred to progeny in this species should be favoured selectively.

It is proposed that large conspicuous inflorescence displays, which are synonymous with the production of 'surplus' flowers (Stephenson 1992), promote a progeny fitness advantage in *B. tricuspis* through two functional avenues. Firstly, large conspicuous displays attract more pollinators (Chapter 7) which increases the incidence of pollen transfer (male function) and moderates pollen limiting constraints. The tendency for follicle and seed numbers infructescence^{-1} to increase with inflorescence length and presentation height, indicating the additive influence of floral attractiveness on maternal fecundity, supports this assertion. Secondly, such floral displays appreciate the utilisation of reserve ovules through increased pollination which enhances the opportunity for female choice. This nonrandom selection of zygotes occurs as a consequence of the receipt of promiscuous pollen loads. Declining zygote survival with increases in both inflorescence length and presentation height, indicating increased selection among developing zygotes, supports this assertion.

Therefore, I suggest that the production of 'surplus' flowers in this species serves to increase progeny fitness through a relaxation in pollen limiting constraints (pollinator attraction hypothesis) and an enhancement in the opportunity for female choice as a consequence of selective abortion (selective abortion hypothesis) and the oversupply of ovules (bet hedging hypothesis). The pollinator attraction hypothesis is favoured as a reduced investment in attraction effort was complemented by reduced fruit and seed production, although the interdependence of attraction effort and fruit set is reason for caution (Stephenson 1979). The survival of 'poor quality' progeny in infructescences with limited choice between compatible mates (unattractive inflorescences), the lower resource provisioning of such progeny and accord between fruit and seed set and the quality of pollen received are symbolic of the selective abortion hypothesis (Richardson and Stephenson 1991). High ovule mortality due to a lack of fertilisation and the independence of zygote survival and seed production provided support for the bet hedging hypothesis (Ehrlèn 1993). Advancing three of the five (Ayre and Whelan 1989, Ehrlèn 1993) possible adaptive functions for 'surplus' flowers in *B. tricuspis* conforms with the notion that the ultimate causes of 'surplus' flowers may not be mutually exclusive, but interact and function synergistically (Karoly 1992, Ehrlen 1992, Guitián 1993).

While large conspicuous inflorescences confer a fitness advantage, such displays also increase the opportunity for floral damage as demonstrated by the incidence of parrot, insect and cockatoo attack on large inflorescences, which is significantly greater than the extent of damage reported for small inflorescences (Chapter 3). This contradiction between the function of inflorescences to attract pollinators, thereby enhancing the opportunity for female choice, and the requirement to avoid floral damage may be attributed to the expectation that this species should selectively indulge in progeny of the highest quality. The cost of this fitness maintaining strategy is the production of large numbers of inflorescences which are subsequently damaged, although this cost may be minimal as such structures are inexpensive compared with seeds (Delph 1990, Witkowski and Lamont 1996). The detection of consistency between seasons in floral damage (Chapter 4) ensures that this reproductive cost is predictable and thus permits certainty with regards to pollination success. This contradictory situation highlights Wyatt's (1982) cautionary comments regarding attributing inflorescence display features to selection driven by sex function and progeny quality-quantity considerations alone and in isolation from other selective forces.

6.4.3 *Conclusions*

Inflorescence attractiveness is correlated with reproductive success in *B. tricuspis* and may effect maternal fecundity and progeny fitness. Investment variation in inflorescence attractiveness influences the quality and quantity of progeny available for future generations. Increased investment in attractiveness augments progeny quantity and amplifies quality, while reduced attractiveness is reciprocated by a decline in both plant fitness values. Ultimately, the randomness of pollinator limitation and thus pollen transfer and load receipt are presented as the principal constraints impinging on the fecundity and fitness of *B. tricuspis* infructescences. In responding to these extrinsic constraining forces it is proposed that this *Banksia* has selected a reproductive strategy which provides a fitness assurance while maximising fecundity. This reproductive strategy selects progeny of the highest quality under conditions of intense competition between mates for resource provisions and moderates these selective pressures when competition is minimal. The production of 'surplus' flowers may therefore be considered adaptive as it increases the opportunity to maximise progeny fitness at the expense of maximum fecundity, due to the uncertainty of pollinator limitation. 'Surplus' flowers enhance attraction effort, providing the opportunity to mitigate pollen limitation and serve as a contingency, preventing any reduction in progeny fitness.

Experimental manipulation of inflorescences through the use of pollen analogues would be a useful method for investigating the proposition that display attractiveness influences the type and volume of pollen received by an inflorescence. This experiment should be designed to document the receipt and dilution rates of a pollen analogue between inflorescences which vary in length and presentation height. The sophistication of the experiment could be augmented through the use of numerous pollen analogues which are strategically applied to various positions on an inflorescence and between inflorescences which vary in their attractiveness. Such a design would permit an estimation of the likely extent of geitonogamous compared with xenogamous pollen receipt. It would also elucidate how inflorescence attractiveness influences intra- and inter-inflorescence pollen dispersal.

Further descriptive interrogations are required to detect differences in ovule viability and vigour which may be indicative of variations in inflorescence attractiveness, particularly inflorescence size. Descriptive investigations, especially analyses of whole plant nutrient and assimilate distribution patterns, need to be undertaken to ascertain whether inflorescences are equally provisioned with maternal resources and represent comparable nutrient sinks, irrespective of their size or distance from nutrient sources.

Hand pollination experiments are also required to test the proposition that disassortative mating is promoted by pollen limitation, especially under varying conditions of resource competition. The design of such an experiment should ensure that inflorescences of differing attractiveness are hand pollinated with mixtures of pollen which vary across the spectrum of possible matings. Subsequent analyses should endeavour to determine if fecundity and the surrogates of plant fitness vary between inflorescences treated with only one pollen type compared to those treated with multiple pollen types. Concurrently, these analyses should also attempt to determine how resource considerations, which may vary with inflorescence attractiveness, influence mate choice and affect reproductive success. This could be accomplished by comparing estimates of fecundity and fitness from inflorescences of contrasting attractiveness which were treated with mixed pollen loads.

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CHAPTER 7

INFLUENCE OF INFLORESCENCE DISPLAY ON POLLINATOR FORAGING

7.1 Introduction

The transport of pollen has important consequences for reproductive success and fitness in outcrossing zoophilous species (Galen and Stanton 1989). Accordingly, the efficiency with which pollinators transfer and deposit pollen is markedly influenced by a plant's floral display (Barrett and Harder 1996). Displays which reduce the randomness of pollination by increasing the frequency of appropriate pollinating visits should be favoured (Waser 1983). A reduction in the randomness of pollination will increase both the male and female functional roles in reproductive success and ultimately enhance plant fitness (Bell 1985, Mitchell 1994).

Plants respond to selective forces propelled by the abundance and efficiency of pollinators and the requirement to maximise fitness through an array of floral designs and architectural displays (Barrett and Harder 1996). Flower abundance, flower size, inflorescence size, nectar quality and quantity, time and rate of anthesis, flower colour and volatile attractants are all floral traits which influence pollinator activity and behaviour at flowers (Waddington 1983, Waser 1983, Andersson 1988 1996, Zimmerman 1988, Real and Rathcke 1991, Podolsky 1992, Rodríguez-Robles *et al.* 1992, Muenchow and Delesalle 1994).

Generally, the most successful displays at enticing pollinators promote higher rates of pollen transfer and receipt to and from a larger proportion of the plant population which augments male fitness through outcrossed seed siring success (Harder and Barrett 1996). Nevertheless, attractive displays also enhance female function, as higher visitation rates facilitate male competition and enhance the opportunity for mate choice among fertilised embryos (Stephenson and Winsor 1986, Marshall and

Ellstrand 1988). The effects of attractive displays on male and female reproductive function is not necessarily mutually exclusive, and generally converges with increasing attractiveness to enhance overall plant fitness (Sutherland 1986, Campbell *et al.* 1991, Wilson *et al.* 1994).

Flower abundance and size, whether measured for individual reproductive units, amalgamations of units (inflorescences) or for an entire plant are prominent elements in the attractiveness of reproductive organs which influence pollination success (Willson and Rathcke 1974, Willson and Price 1977, Waser 1983). Generally, an increase in either or both elements equates to an increase in pollination and reproductive success, as pollinators preferentially visit the most attractive displays which provide a stronger signal (Campbell 1989, Andersson 1991, 1996) and may offer greater rewards in terms of pollen and nectar (Thomson 1988). Such attractive displays may also increase the duration of the foraging bout which can also augment the probability of reproductive success (Ohara and Higashi 1994). However, in some instances, the relationship between attractiveness and reproductive success may waiver as increases in attraction effort confer negative or no appreciable increase in maternal fecundity or progeny fitness (Willson and Price 1977, Wyatt 1980, Muenchow and Delesalle 1994).

The production of attractive floral displays confers two mating costs which paradoxically impinge on reproductive success through both male and female functional roles. These costs are most apparent in plants which display many flowers simultaneously (Klinkhamer and de Jong 1993). Firstly, the increased duration of foraging bouts enhances the opportunity for geitonogamous pollen receipt which can lead to self fertilisation and inbreeding depression (Hessing 1988, Charlesworth 1989, Klinkhamer and de Jong 1990). Secondly, increases in geitonogamous pollen deposition potentially reduce the amount of pollen exported to conspecifics, thereby reducing the success of the plant as a pollen parent (Klihkhamer and de Jong 1993, Harder and Barrett 1995). This mating cost predicament also extends to pollen transfer among conspecifics, especially in populations which exhibit genetic structuring as a consequence of restricted pollen and seed dispersal. In such cases, attractive displays may increase the opportunity for consanguineous matings and biparental inbreeding (Waller 1993).

The mating costs of attractive display are moderated, however, as such displays increase the opportunity for mate choice through the receipt of diverse pollen loads, as a consequence of longer foraging bouts and increases in the opportunity for the receipt of acceptable pollinator visits from genetically unrelated conspecifics (Schmid-Hempel and Speiser 1988). This compensatory interaction between attractiveness and mating costs is promoted by an increase in the receipt of acceptable pollen with increased visitation and a decrease in the opportunity for geitonogamy and consanguineous matings as a consequence of the dilution in volume of self pollen available for transfer to flowers on the same plant or adjacent sibs.

As attractive displays are traits which increase overall male and female reproductive success and are designed to maximise a plant's fitness they should be favoured through natural selection (Charnov 1982, Broyles and Wyatt 1990, Cohen and Dukas 1990). This is especially true for species where reproductive success is pollen-limited, as an enhancement in attractiveness should promote an increase in the opportunity for pollination thereby diminishing this limiting constraint. The production of 'surplus' flowers by many plants (Stephenson 1981, Sutherland 1987, Ehrèl 1991), which Cohen and Dukas (1990) suggest confers a considerable fitness advantage, has been attributed to a number of ultimate functional explanations of which enhancement in attractiveness of the reproductive organ to pollinators is one (Schemske 1980, Waser 1983, Stanton *et al.* 1986, Ayre and Whelan 1989). The 'attraction' hypothesis for 'surplus' flowers is supported by investigations which demonstrate that flowers above the optimum number required for realised reproductive success serve to enhance the attractiveness of the reproductive organ (Firmage and Cole 1988, Stanton and Preston 1988, Campbell 1989, Podolsky 1992, Rodríguez-Robles *et al.* 1992, , Ohara and Higashi 1994, Andersson 1996).

In the Australian flora, pollinators respond to differences in floral displays and the rewards they present in ways that have significant impacts on the pattern of pollen transfer and receipt within a population and thus the levels of maternal fecundity and progeny fitness which constituents achieve (Hopper and Burbidge 1978, Hopper and Moran 1981, Paton and Ford 1983, Collins and Rebelo 1987, Collins *et al.* 1990, Sampson *et al.* 1996). Within the genus *Banksia* the attractiveness of the floral display has been implicated in the stochasticity of pollinator foraging and the efficiency with which pollinators transfer and deposit pollen (see review by Collins and Rebelo 1987, Ramsey 1988a, 1989, Vaughton 1990, Goldingay *et al.* 1991, Ramsey and Vaughton 1991, Carthew 1993, 1994).

In many *Banksia* pollination studies the abundance of pollinators, particularly honeyeaters (Meliphagidae), and the frequency of inflorescence visits is dependent on flowering phenology/intensity, inflorescence abundance/density and the volume of nectar rewards presented (Newland and Wooller 1985, Paton and Turner 1985, McFarland 1986a, Collins and Briffa 1982, Vaughton 1990, Carthew 1993). Generally, the greater the attraction effort, the greater the utilisation by pollinators and the higher the level of reproductive success (Hopper 1980, McFarland 1986a, Copland and Whelan 1989,) although nutrient provisioning considerations (Lamont *et al.* 1994), selection to mitigate pollen limitations (Goldingay and Whelan 1990) and deleterious impacts of inflorescence consumers (Zammit and Hood 1986) may affect these relationships.

In *Banksia* populations, the abundance of pollinators and the frequency of their visits to inflorescences generally increases as the number of open florets and the volume of nectar available increases (McFarland 1986a, Ramsey 1989, Vaughton 1990). This association can promote considerable competition between sympatric plants, whether conspecific or heterospecific, for the services of pollinators (Ramsey 1995) and may saturate the pollinator population or increase the opportunity for interspecific pollen deposition (Copland and Whelan 1989). The association between nectar availability and pollinator visits may, however, not represent an increasing functional relationship, as the frequency of visits to inflorescences may increase proportionally with a decrease in nectar availability (Paton and Ford 1983, McFarland 1986b). This paradoxical relationship may arise as a consequence of the energetic requirements of pollinators and/or as a repercussion of aggressive interactions with other pollinators (Collins and Briffa 1982, Paton and Ford 1983).

The intensity of flowering and the abundance of inflorescences within a *Banksia* population are also purported to affect pollinator movements and thus the frequency of geitonogamy and the opportunity for siring outcrossed seed. Vaughton (1990) and Carthew (1994) reported a disproportionate increase in the frequency of inter-plant movements between consecutive inflorescence visits towards the beginning and conclusion of the flowering season in *B. spinulosa*, which was attributed to temporal and spatial heterogeneity in the availability of nectar. Ramsey (1989) also observed differences in inter-plant movements which were primarily promoted by aggressive interaction among competing honeyeaters, where the aggressive interactions were influenced by increases in flowering intensity. Conversely, Copland and Whelan (1989), in part, attributed low fruit set in late flowering *B. ericifolia* plants to inappropriate geitonogamous pollination, as a consequence of restricted pollinator movements. Similarly, incongruity between flowering seasons in the reproductive success of some *Banksia* species has been attributed to significant inter-seasonal heterogeneity in the abundance of pollinators which was promoted by significant inter-seasonal incongruity in the production of inflorescences and inflorescence flowering intensity (Vaughton 1991, Carthew 1993).

The size of an inflorescence, which is proportional to the number of florets, may also influence pollinator visitation. Numerous investigations have demonstrated that reproductive success increases with inflorescence size, which may be attributed to an increase in the frequency of pollinator visits and the appropriateness of the pollen deposited (Paton and Turner 1985, Goldingay and Whelan 1990). Non-random patterns in reproductive success were attributed to variations in pollinator foraging promoted by differences in inflorescence size in sympatric *B. integrifolia* and *B. spinulosa* plants (McFarland 1985).

The frequency and duration of honeyeater visits can also be influenced by the phenology of an individual inflorescence. Collins and Spice (1986) observed that honeyeaters preferentially visit inflorescences on which anthesis had commenced and that the frequency of inflorescence probing was greatest in areas immediately adjacent to the 'advancing front', an area where pollen and nectar were most abundant. Similarly, Ramsey (1988a, 1988b) observed a direct link between inflorescence phenology and pollinator visitation, which was highlighted by increases in the rate of anthesis with pollinator visitation as floret opening occurs during honeyeater probing in *B. menziesii*. The non-random distribution of fruits towards the middle and peduncular ends of some *Banksia* infructescences has also been attributed to assortative pollination visitation among inflorescences with the middle to middle-peduncular regions being preferentially visited (McFarland 1985).

This relationship between inflorescence phenology and pollinator visitation may be attributed to morphological and/or colour cues (Collins and Rebelo 1987) which enhance attractiveness, thereby enticing pollinators. The morphological cues may be associated with the distribution of dehisced florets or the provision of more stable perching platforms by undehisced florets. The colour cues may be provided by a sharp demarcation between dehisced and undehisced florets along the 'advancing front' (George 1981), or may be associated with a change in receptivity of the inflorescence. Colour cues have been invoked previously to explain the dichotomy in pollinator visitation between reproductively receptive-nectar producing inflorescences and reproductively senescing-nectar void inflorescences of *B. ilicifolia* (Lamont and Collins 1988). Spatial variation in the presentation and conspicuousness of *Banksia* inflorescences and their effects on pollinators has also been reported for *B. menziesii* (Ramsey 1989).

This chapter investigates the influence of the *Banksia tricuspis* inflorescence display on pollinator foraging behaviour. The principal intent of this investigation was to determine if inflorescence attractiveness differentially affects the opportunity for pollen transfer and deposition by avian pollinators, the most frequent floral visitors in this species (van Leeuwen and Lamont 1986). The objective was to determine if non-random patterns of fecundity and fitness reported within and between infructescences in previous investigations (Chapter 5 and 6) were attributable to assortative pollinator foraging. Fulfilment of this objective was achieved through observing and recording the foraging behaviour of pollinators and the display traits of the inflorescences which they visited. The investigation outcomes are discussed with reference to the attractiveness of the floral display in terms of: (i) the position of the 'advancing front'; (ii) size; (iii) presentation (conspicuousness) of the inflorescences; and (iv) the potential effect of the pollinators' foraging behaviour on plant fitness.

7.2 Materials and Methods

This investigation into the influence of inflorescence display on the foraging behaviour of diurnal avian pollinators was conducted on a natural population of *Banksia tricuspis* located in the Lesueur National Park, approximately 220 km north of Perth. The study population was located 3.6 km NE of Mt Lesueur and 7.9 km SE of Mt Peron (30° 09' 07" S, 115° 13' 05" E) and consisted of 87 mature plants, unevenly distributed over approximately 8 ha. Plants in this population were emergent and dominated the low kwongan heath in the study area. Other nectivorous species within or in close proximity to the study population included *B. attenuata*, *B. grossa*, *B. leptophylla*, *B. menziesii*, *Calothamnus quadrifidus*, *C. sanguineus*, *C. torulosus*, *Dryanda sessilis*, *D. shuttleworthiana*, *Eucalyptus calophylla*, *E. drummondii* and *Lambertia multiflora*.

B. tricuspis produces conspicuous bright golden-yellow inflorescences that are usually terminal or on short lateral branchlets within the crown. Inflorescences consist of up to 2 500 florets arranged orthogonally around a woody central rachis with anthesis occurring basipetally over a two week period (George 1981, van Leeuwen and Lamont 1986). Flowering commences in March, peaks in July-August and continues through to the beginning of October. Florets are protandrous with pollen deposited on the pollen-presenters prior to anthesis on a verrucose collar located below the apically positioned stigmatic groove. Spontaneous autogamy is therefore not possible. The stigmatic groove, which is not receptive at anthesis, appears to reach maximum receptivity 36 hours after anthesis (van Leeuwen and Lamont 1986).

Inflorescences are visited by numerous animals which act as pollinators of this zoophilous species (van Leeuwen and Lamont 1986). Honeyeaters (Meliphagidae) are the most frequent visitors to inflorescences with seven species previously observed foraging for nectar and carrying pollen of this species. The Honey Possum (*Tarsipes rostratus*), Honey Bee (*Apis mellifera*) and thynnid wasps are also frequent visitors to inflorescences.

In July 1987, a transect of 480 m was established throughout the thickest part of the *B. tricuspis* population. Within the bounds of this transect were 38 *Banksia* plants with numerous inflorescences at various stages of flowering. The location of each plant was plotted, with individual plants colour coded around the trunk for easy identification. A total of 111 flowering inflorescences, where flowering inflorescences were defined as those on which anthesis had begun but had not yet extended to the most peduncular florets, were recorded on the 38 plants during two, six day observation periods. Each flowering inflorescence was colour coded directly below the peduncle on the supporting inflorescence branch and its location within the population of 38 plants recorded.

Traits used to investigate the independence of foraging attributes on the floral display of *B. tricuspis* were: a) the position of anthesis along the inflorescence axis; b) inflorescence length; and c) presentation height. The position of anthesis along the axis was recorded for each flowering inflorescence subsequent to its division into 10 equal portions (Chapter 5). Anthesis position (the position of the 'advancing front') was recorded at the beginning of each observation period, which culminated in numerous inflorescences belonging to two contrasting position categories. Therefore position related analyses were increased to a sample size of 156 individuals. Inflorescence length (to the nearest mm) and presentation height (to the nearest cm) were measured for each flowering inflorescence.

During the two observation periods, records on the foraging activities of birds which visited flowering inflorescences on the 38 plants were recorded. Observations on the behaviour of individual birds during a foraging bout were made for as long as possible with the aid of binoculars. A foraging bout was defined as commencing with perching on a *B. tricuspis* inflorescence and concluded when a subsequent movement was to a non-*B. tricuspis* inflorescence roost. These observations were made over two four hour periods each day, the first commencing at dawn while the latter commenced at 2 pm. Data collected for each individual inflorescence visit included:

- the position of the 'advancing front' on the inflorescence visited;
- the length and presentation height of the inflorescence visited;
- the total time spent at an inflorescence;.
- the distance between consecutive visits to inflorescences; and
- the type of inter-inflorescence movement between consecutive inflorescence visits.

The total time spent at an inflorescence during a foraging visit was measured in seconds and included both floret probing and perching time. The type of inter-inflorescence movement was grouped into three categories comprising:

- intra-plant movements where the next inflorescence visited was on the same plant;
- near neighbour movements where the next inflorescence visited was on an adjacent tree that was less then 10 m distant; and
- long distant movements where the next inflorescence visited was on a plant at least 10 m removed.

The 10 m cut-off between near neighbour and long distance movements was inferred from field data, which indicated that the average distance between adults and seedlings in the first year after recruitment was 2.78 m and that less than 0.7% of 298 one year old seedlings were dispersed greater than 10 m away from the nearest adult plant (van Leeuwen and Lamont 1986). In this scheme, I propose that intra-plant movements promote geitonogamous matings, while long distance movements promote xenogamous matings. Consanguineous matings (mating between siblings) were assumed to dominate near neighbour inter-inflorescence movements as a consequence of restricted seed(ling) dispersal characteristics.

7.2.1 *Data analyses*

Records for all avian inflorescence visitors were combined prior to analysis. Normality and homoscedasticity of all data used for parametric statistical tests were checked prior to statistical interrogation and transformed where necessary to fulfil model assumptions. Presentation height and visitation number data were log transformed, while proportional data for the frequency of each inter-inflorescence movement category was arcsine transformed. Values presented in tables and figures were back transformed (Zar 1984).

Homogeneity between inflorescences and the position of the 'advancing front' in foraging attributes was checked using *G*-test procedures (Sokal and Rohlf 1995), while heterogeneity among inter-inflorescence movement categories was investigated using a Tukey-type multiple comparison procedure for proportional data following arcsine transformation (Zar 1984). Variation among values for observed foraging attributes were illustrated using box and whisker plots (Kirby 1993). Correlations between and agreement among rankings of foraging attributes and floral display traits were investigated using Spearman's rank correlation and Kendall's coefficient of concordance, where Friedman's chi-squared statistic was used to determine the significance of any agreement (Zar 1984).

Significant heteroscedasticity among inflorescence positions necessitated the use of the Kruskal-Wallis ranks protocol to investigate homogeneity between 'advancing front' positions in foraging attributes (Sokal and Rohlf 1995). Multiple comparisons among inflorescence positions was undertaken using Dunn's non-parametric multiple comparison procedure (Zar 1984), as equality in the number of data points for each inflorescence position was not attained. Coefficient of variation estimates were used to investigate the variation in inter-inflorescence movement distances at each 'advancing front' position. The significance of differences between coefficients of variation was investigated using pairwise variance ratio test procedures (Zar 1984).

Least squares linear regression procedures were employed to determine the dependence of foraging attributes on the display traits of inflorescence length and presentation height. The significance of the regression model was examined by ANOVA procedures (Kirby 1993). Normality, equal variance and independence assumptions of the linear regression model were checked through *posteriori* test procedures (Kirby 1993). Assumptions of linearity were tested using a corrected ANOVA protocol constructed from the standard regression ANOVA table and a one-way ANOVA table, in which the replicated dependent values (y) for each independent value (x) were assigned to different groups (Fry 1995). In regressions where the assumption of linearity was not fulfilled, the dependent values were not transformed as the assumptions of normality, homoscedasticity and independence were already fulfilled and any subsequent transformation would not abide by these assumptions (Zar 1984). Pairwise and multiple comparisons between correlation coefficients were investigated using Z test and Tukey-type multiple comparison procedures (Zar 1984).

7.3 Results

A total of 2 015 foraging visits were recorded for the 111 inflorescences of *Banksia tricuspis* observed during this investigation. Four species of honeyeater, the Western Spinebill (*Acanthorhynchus superciliosus*), New Holland (*Phylidonyris* *novaehollandiae*), Tawny-crowned (*P. melanops*), and Brown (*Lichmera indistincta*), together with the Silvereye (*Zosterops lateralis*), were observed foraging at inflorescences. No attempt was made to interrogate the statistical data to distinguish between the foraging activities of these pollinators.

The display traits of inflorescence length and presentation height were both positively skewed, although this asymmetry was only significant for the latter trait, which was corrected through log transformation (Zar 1984) (Table 7.1). A significant correlation between inflorescence length and presentation height was detected (r_{111} = 0.366, $P < 0.001$), which indicates collinearity (Kirby 1993) between traits. This significant correlation implied an increase in inflorescence attraction effort (number of florets) with an amplification in the height of inflorescence presentation.

Table 7.1 Descriptive statistics and significance of departure from normality for the independent display traits recorded from 111 *Banksia tricuspis* inflorescences. (g_1 = skewness, D = Kolmogorov-Smirnov goodness of fit.)

Display trait	Mean ± SE	Range	g_1	D
Inflorescence length (mm)	130.9 ± 2.3	86 - 184	0.193	0.070
Presentation height (cm)	161.2 ± 4.6	81 - 289	0.566*	0.122***

Significance: * $P < 0.05$, *** $P < 0.001$.

All pollinator foraging statistics were significantly positively skewed and not representative of normally distributed populations (Table 7.2, Figure 7.1). All statistics displayed significant heterogeneity between individuals suggesting a dependency by pollinator foraging on inflorescence display traits. Similarly, the categories of inter-inflorescence movement were also positively skewed and differed noticeably from normal distributions (Table 7.2, Figure 7.1). *G*-test results indicated that the frequency of each inter-inflorescence movement were not independent of the inflorescence display traits.

Near neighbour pollinator movements were the most frequent, accounting for 49.3% of all movements, followed by intra-plant and long distance movements which accounted for 28.2% and 22.5% respectively. Among the 2 015 foraging movements observed, significant incongruity was detected in the proportion of each category

($q_{\infty,3} = 360.40$, $P < 0.001$), with pairwise comparisons indicating all three categories differing significantly.

Table 7.2 Foraging statistics and significance of departure from normality for 2 015 visits by pollinators to 111 inflorescences of *Banksia tricuspis*. (g_1 = skewness, D = Kolmogorov-Smirnov goodness of fit, G-test = log-likelihood ratio test.)

Foraging statistic	Mean ± SE	Range	*n*	g_1	*D*	*G*-test
Duration of visit (s)	19.7 ± 0.2	4 - 62	2 015	0.392***	0.101***	7 905.61***
Distance between visits (m)	6.6 ± 0.1	0.3 - 35.4	2 015	0.969***	0.094***	716.50***
Intra-plant	4.1 ± 0.2	1 - 19	140	1.579***	0.157***	195.40**
Near neighbour	6.8 ± 0.4	1 - 29	147	1.393***	0.147***	1 492.29***
Long distance	4.3 ± 0.4	1 - 29	106	2.796***	0.235***	361.81***

Significance: ** $P < 0.01$, *** $P < 0.001$.

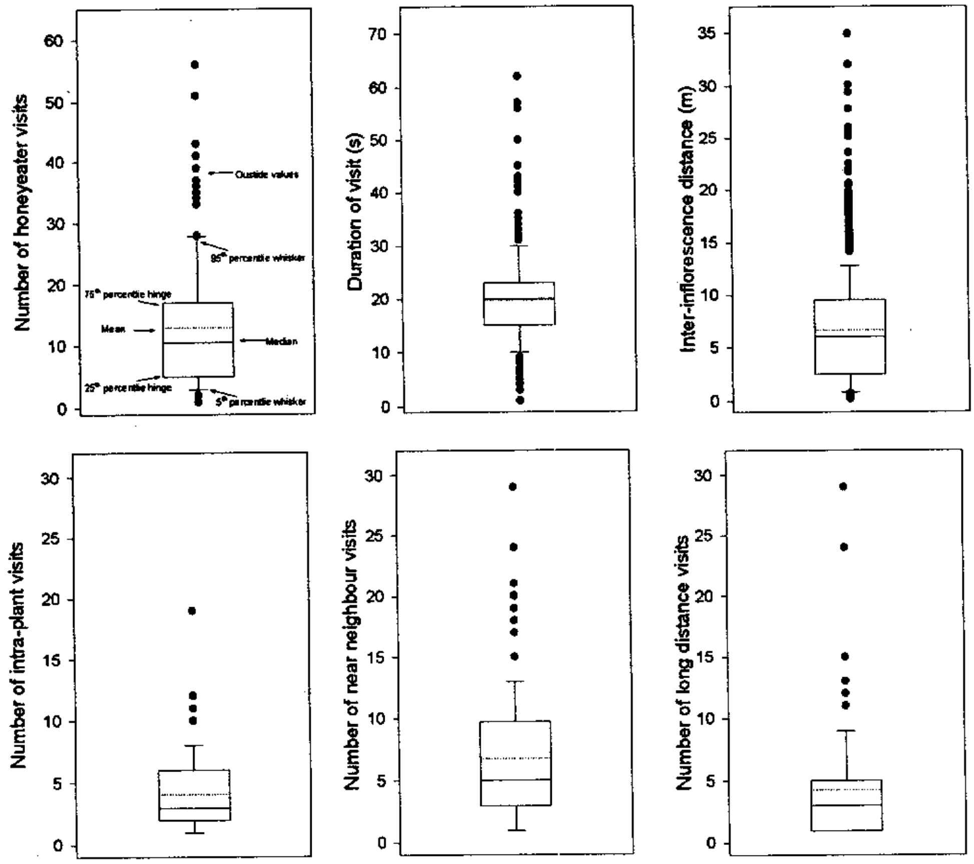

Figure 7.1 Box and whisker plots of foraging statistics for 2 015 pollinator visits to 111 *Banksia tricuspis* inflorescences. (• = outside values beyond 5th and 95th percentiles, whiskers represent 5th and 95th percentiles, box hinges represent 25th and 75th percentiles, solid box line represents median, dotted box line represents mean.)

Average estimates of visitation duration and inter-inflorescence movement distances for each inflorescence were significantly correlated ($r_{s\,111} = 0.43$, $P < 0.001$), as was the relationship of these two attributes with the number of visits received by an inflorescence ($r_{s\,111} = 0.62$ and 0.50 respectively, $P < 0.001$). Significant association was also detected between all three foraging attributes using Kendall's coefficient of concordance, implying that high visitation rates were likely to be observed on inflorescences which receive longer visits. Such inflorescences were also associated with greater average inter-inflorescence movements ($W = 0.68$, $\chi^2_{r_{110}} = 223.48$, $P < 0.001$). Likewise, significant association was also detected in the average frequency of each category of inter-inflorescence movement recorded for each of the 111 inflorescences ($W = 0.65$, $\chi^2_{r_{110}} = 214.89$, $P < 0.001$).

7.3.1 *Position of anthesis* vs *pollinator foraging*

The frequency of foraging visits to *B. tricuspis* inflorescences was dependent on the position of the 'advancing front' along the inflorescence ($G_{10} = 1\,464.9$, $P < 0.001$). The average number of visits was greatest for inflorescences on which anthesis had progressed to middle-peduncular positions (Figure 7.2). The average rate of visitation in these inflorescence positions differed markedly ($H_9 = 98.27$, $P < 0.01$) from the visitation rate for inflorescences on which either fewer or more florets had opened. The frequency of visits to preceding apical positions decreased progressively towards the apex, while in the two ensuing peduncular positions the frequency of visits also decreased markedly (Figure 7.2). The duration of an inflorescence visit by pollinators was also dependent on the position of the 'advancing front' along the inflorescence ($H_9 = 336.48$, $P < 0.001$), with the average time of a visit being greatest at position 7 (Figure 7.2). The shortest visitation duration was observed for the most apical position.

The distance of inter-inflorescence movements was similarly dependent on the position of the 'advancing front' along the inflorescence ($H_9 = 60.96$, $P < 0.001$), with the average distance of an inter-inflorescence movement being similar in positions 4 to 8 (Figure 7.2). The shortest inter-inflorescence movements were observed at the apex and peduncular ends of the inflorescence. In most instances, inter-inflorescence

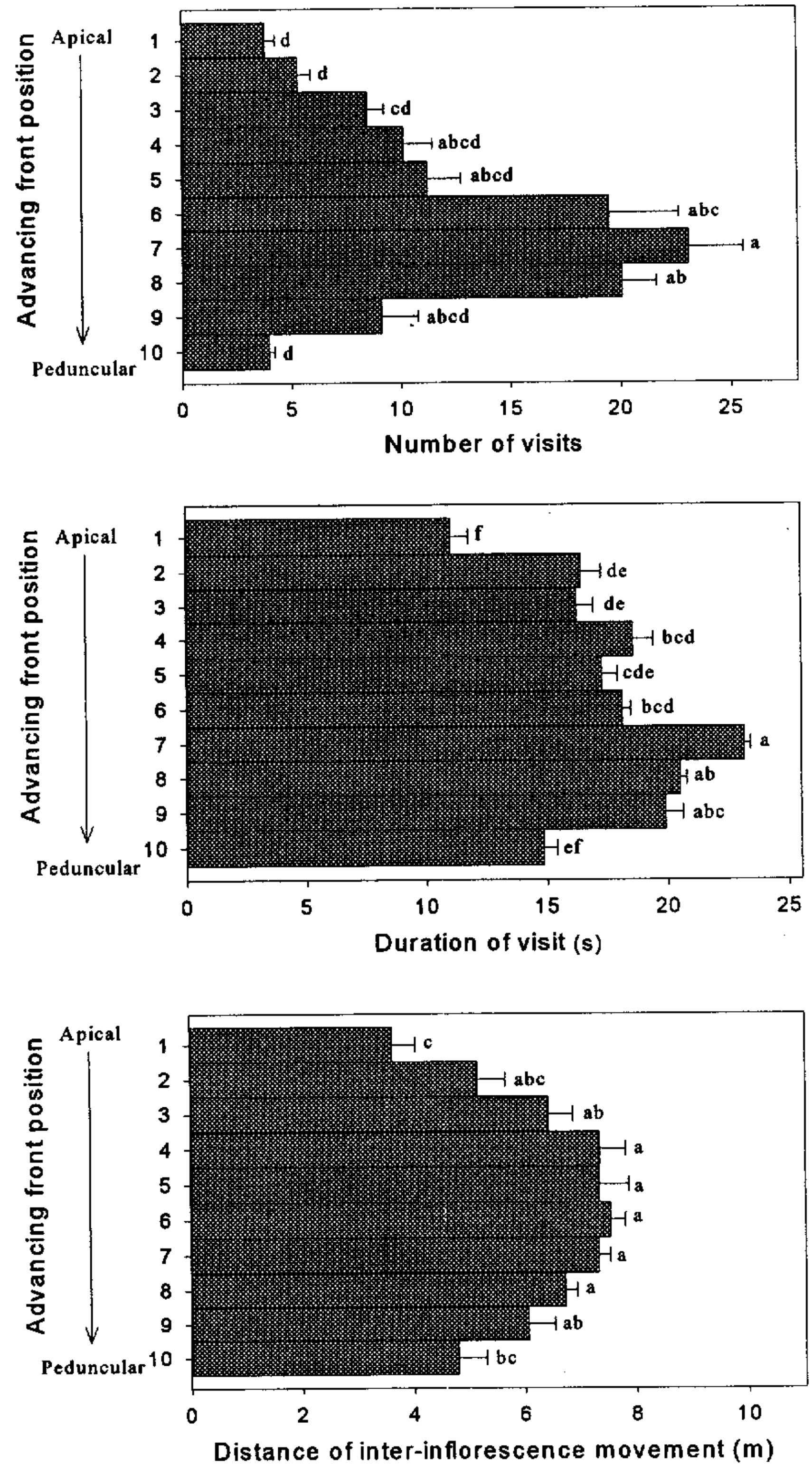

Figure 7.2 Influence of 'advancing front' position on mean (± SE) foraging attributes recorded from 2 015 pollinator visits to 111 *Banksia tricuspis* inflorescences. (Horizontal columns with different letter(s) adjacent to their error bars were significantly different (Dunn's multiple comparison procedure (Zar 1984)), $P < 0.05$.)

movements were significantly positively skewed indicating a profusion of movements below the reported mean value (Table 7.3). Coefficient of variation values ranged from 60.7 % to 89.8% and exhibited a significantly negative association with the average inter-inflorescence movement distance ($r_{s_{10}}$ = -0.86, P < 0.001). Pairwise variance ratio test results indicated that the coefficient of variation for inter-inflorescence movements was significantly higher in apical and peduncular positions than for estimates from positions towards the middle of the inflorescence (Table 7.3).

Table 7.3 Relationship between 'advancing front' position along the inflorescence and the distance (m) of inter-inflorescence movements for 2 015 foraging visits to 111 *Banksia tricuspis* inflorescences. (Coefficient of variation (V) values with different letter(s) were significantly different, Variance ratio test, P < 0.05.)

'Advancing front' position		N	Distance		g_1	V
			Median	Range		
1	Apical	57	3.40	0.2 - 16.7	1.48***	86.8^{a}
2		69	5.30	0.3 - 16.4	-0.54	80.8^{a}
3		110	5.75	0.0 - 21.0	0.99***	72.0^{a}
4		91	6.30	0.2 - 18.4	0.54*	60.7^{b}
5		112	6.10	0.2 - 19.0	0.61***	75.6^{a}
6		311	6.00	0.2 - 30.0	0.90***	67.2^{ab}
7		577	6.60	0.2 - 32.0	1.05***	69.0^{ab}
8		502	6.25	0.2 - 34.9	1.19***	70.0^{ab}
9		91	6.80	0.2 - 18.0	-0.48	72.9^{a}
10	Peduncular	75	3.90	0.2 - 16.1	0.61*	89.8^{a}

Significance: * P < 0.05, *** P < 0.001.

The position of anthesis along the inflorescence had a marked effect upon the category of inter-inflorescence movement (G_{18} = 89.75, P < 0.001). For all positions, excluding position 3, long distance movements were the least frequent and represented the smallest proportion of foraging visits received (Figure 7.3). Near neighbour movements significantly dominated in all 'advancing front' positions, except position 5. Within each of the movement categories the frequency of foraging visits was dependent upon position (G_{10} = 304.2, 791.2 and 459.3 for intra-plant, near neighbour and long distant movements respectively, P < 0.001). In all categories, the average number of pollinator visits were greatest in middle to middle-peduncular positions (Figure 7.4). Kruskal-Wallis results indicated significant heterogeneity between positions for each of the movement categories (H_9 = 62.69, 85.07 and 31.49 for intra-plant, near neighbour and long distant movements respectively, $P < 0.001$). Dunn's multiple comparison results indicated that the average number of foraging visits decreased towards the apex and peduncle from positions 6 to 8 for both inter-plant and near neighbour movements. No significant differences between positions along the inflorescence were detected for long distance movements, although the tendency was for a similar apical and peduncular decline from a middle and middle-peduncular peak (Figure 7.4).

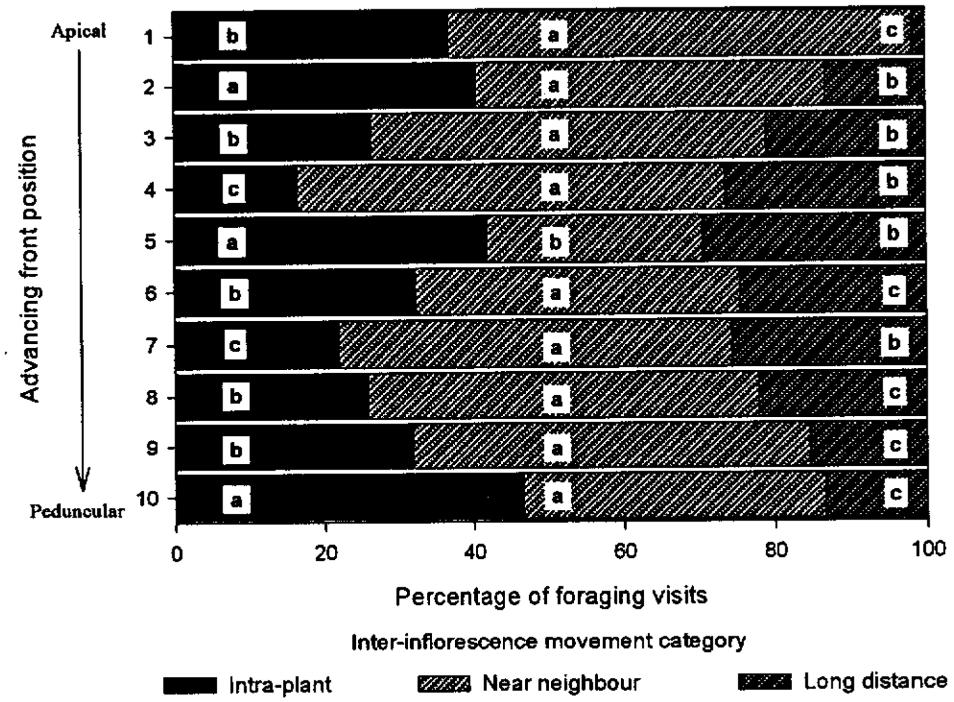

Figure 7.3 Proportion of inter-inflorescence movement categories received at each 'advancing front' position along the *Banksia tricuspis* inflorescence for 2 015 pollinator visits. (Inter-inflorescence movements at each anthesis position with different letters were significantly different, ($P < 0.05$)).

Kendall's coefficient of concordance results indicated that positions along the inflorescence which experience high visitation rates also received longer visits which followed greater inter-inflorescence movements (W = 0.65, $\chi^2_{r_9}$ = 23.41, $P < 0.01$). This concordance, however, was not marked for the association between the distance

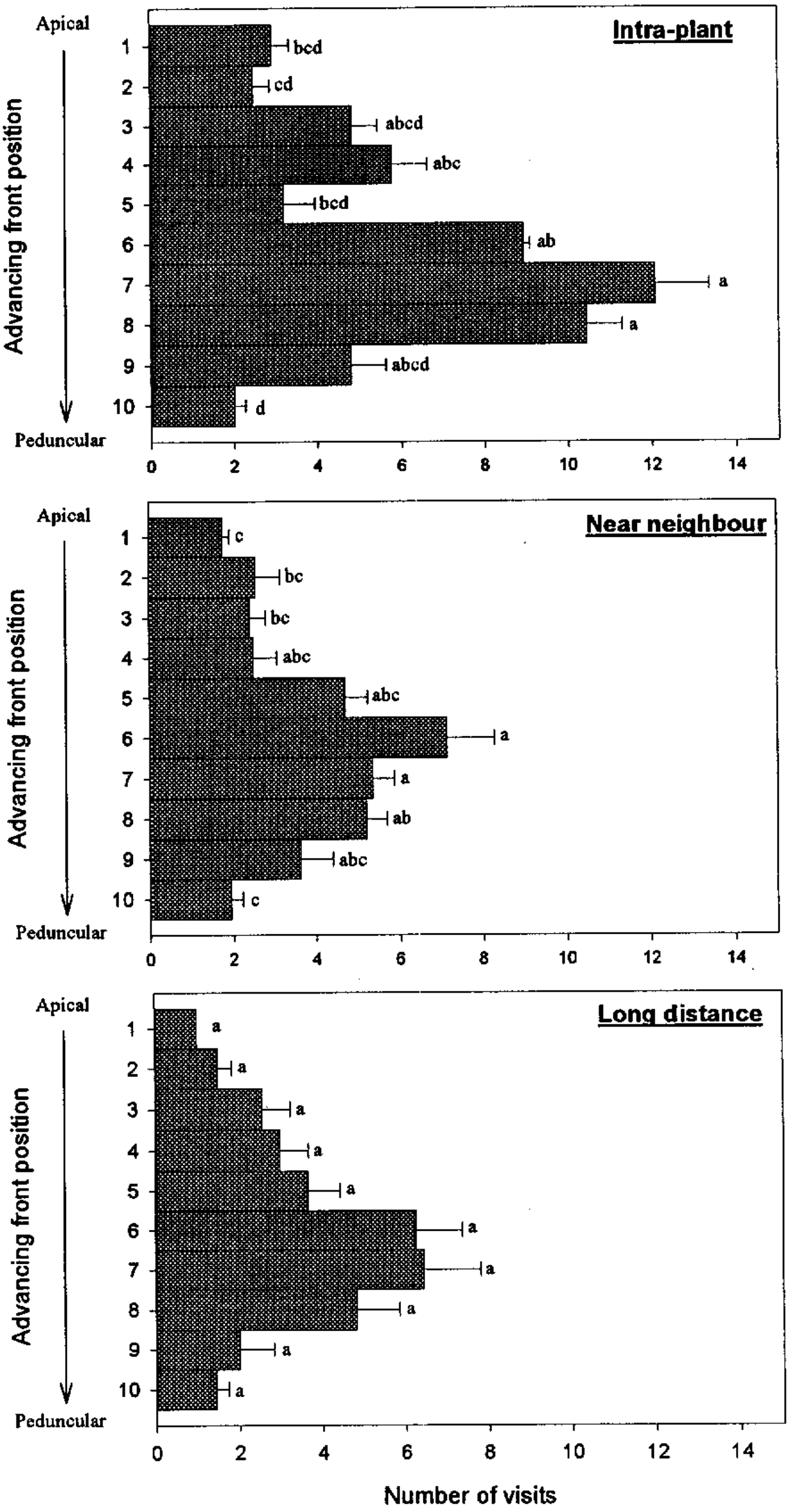

Figure 7.4 Influence of the 'advancing front' position on the mean (± SE) number of pollinator visits received in each inter-inflorescence movement category. (Horizontal columns with different letter(s) adjacent to their error bars were significantly different (Dunn's multiple comparison test (Zar 1984)), $P < 0.05$.)

of inter-inflorescence movements and visitation time, as indicated by non-significant Spearman's rank correlation results ($rs_{10} = 0.58$, $P > 0.05$). The frequency of each inter-inflorescence movement category was also significantly associated with 'advancing front' position ($W = 0.66$, $\chi^2_{r_9} = 24.84$, $P < 0.01$).

7.3.2 *Inflorescence length* vs *pollinator foraging*

The number of visits, their duration and the distance of inter-inflorescence movements were all significantly correlated with inflorescence length (Table 7.4, Figure 7.5). The least squares linear model describing the relationship of the independent variable (length) to both visitation duration and the distance of inter-inflorescence movements deviated significantly from linearity (Table 7.4). Lack of fit for both these significant models was attributable to significant variation between replicate observations of both dependent variables for each of the 111 inflorescences.

Table 7.4 Corrected ANOVA results of significance and deviation from linearity of regression models for inflorescence length and foraging attributes (*y*) in *Banksia tricuspis*.

Source of variance	Foraging attributes (*y*)						df
	Number of visits		Duration of visit		Distance between visits		
	F	*P*	*F*	*P*	*F*	*P*	
Between groups of *y*	0.717	0.891	10.745	0.000	4.322	0.000	68
Linear regression	10.119	0.003	133.478	0.000	52.045	0.000	1
Deviation from linearity	0.576	0.978	8.422	0.000	3.543	0.000	67

The number of near neighbour and long distance inter-inflorescence movements were also correlated with inflorescence length, with both foraging attributes increasing as length expanded (Table 7.5). The least squares linear model adequately described the relationship between long distance movements and inflorescence length. However, the model for near neighbour movements did not conform with linearity expectations (Table 7.5). The strength of the correlation for both inter-inflorescence

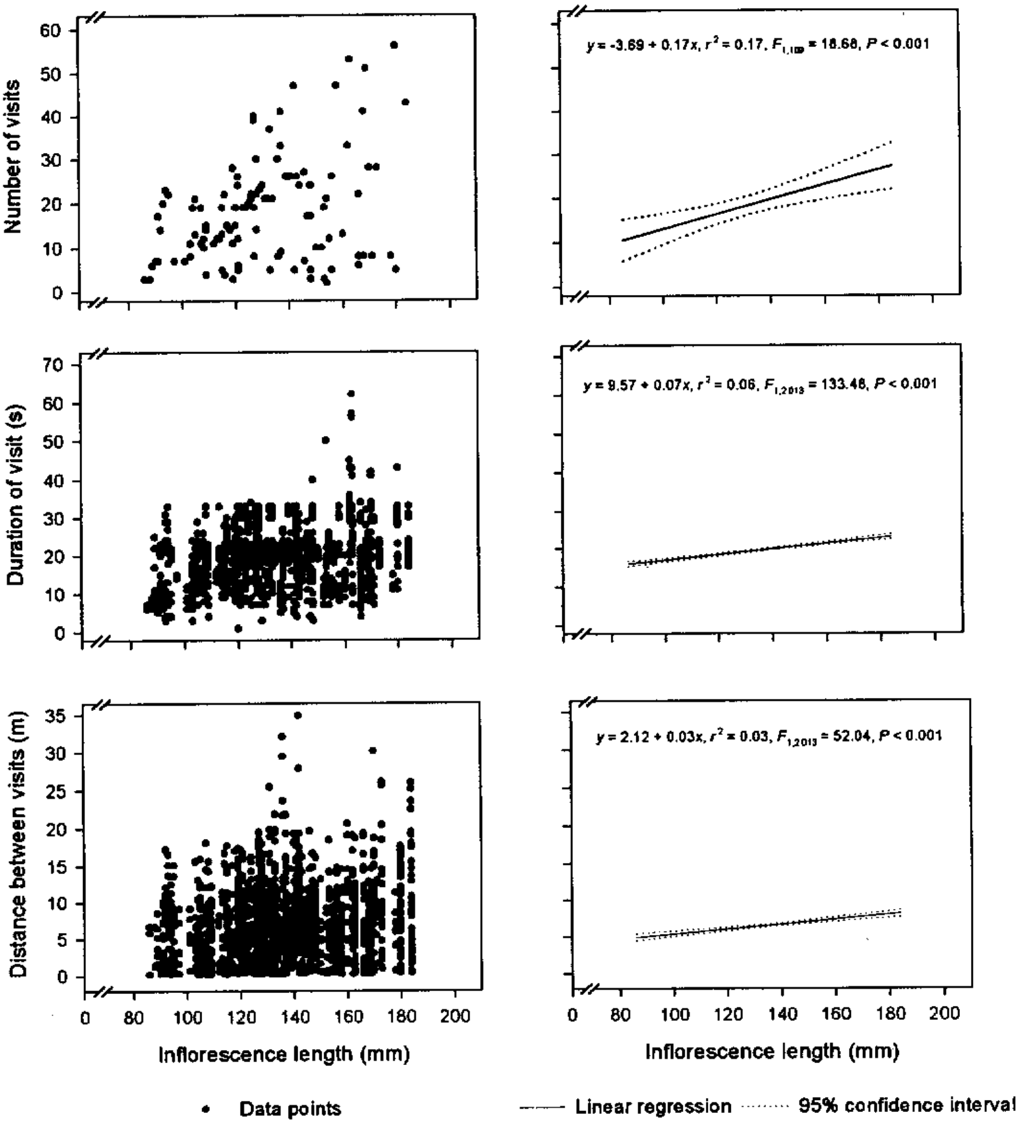

Figure 7.5 Distributions and linear regressions for the association between inflorescence length and foraging attributes in *Banksia tricuspis*.

movement categories and inflorescence length were analogous (Table 7.5). The number of intra-plant movements was independent of inflorescence length. The proportion of long distance foraging movements, as a percentage of all movements, increased significantly with inflorescence length, with the independent variable accounting for 11.2% of the variation in the dependent variable (Table 7.5, Figure 7.6). No significant relationships were detected between the proportion of other inter-inflorescence movement categories and inflorescence length.

Table 7.5 Corrected ANOVA results of significance and deviation from linearity of regression models for visits per inter-inflorescence movement category (*y*) and inflorescence length in *Banksia tricuspis*. (Identical letters after significant linear regressions indicate no significant difference between correlation coefficients, $P < 0.05$).

Source of variance	Inter-inflorescence movement category (*y*)						df
	Intra-plant		Near neighbour		Long distance		
	F	*P*	*F*	*P*	*F*	*P*	
Number of visits							
Between groups of *y*	0.839	0.744	1.061	0.424	0.616	0.963	68
Linear regression	0.813	0.372	10.119	0.003^{a}	10.402	0.002^{a}	1
Deviation from linearity	0.842	0.739	0.932	0.607	0.469	0.997	67
Proportion of all visits							
Between groups of *y*	1.055	0.433	0.744	0.863	0.767	0.837	68
Linear regression	3.642	0.063	0.821	0.369	10.561	0.002	1
Deviation from linearity	0.745	0.861	1.016	0.486	0.620	0.960	67

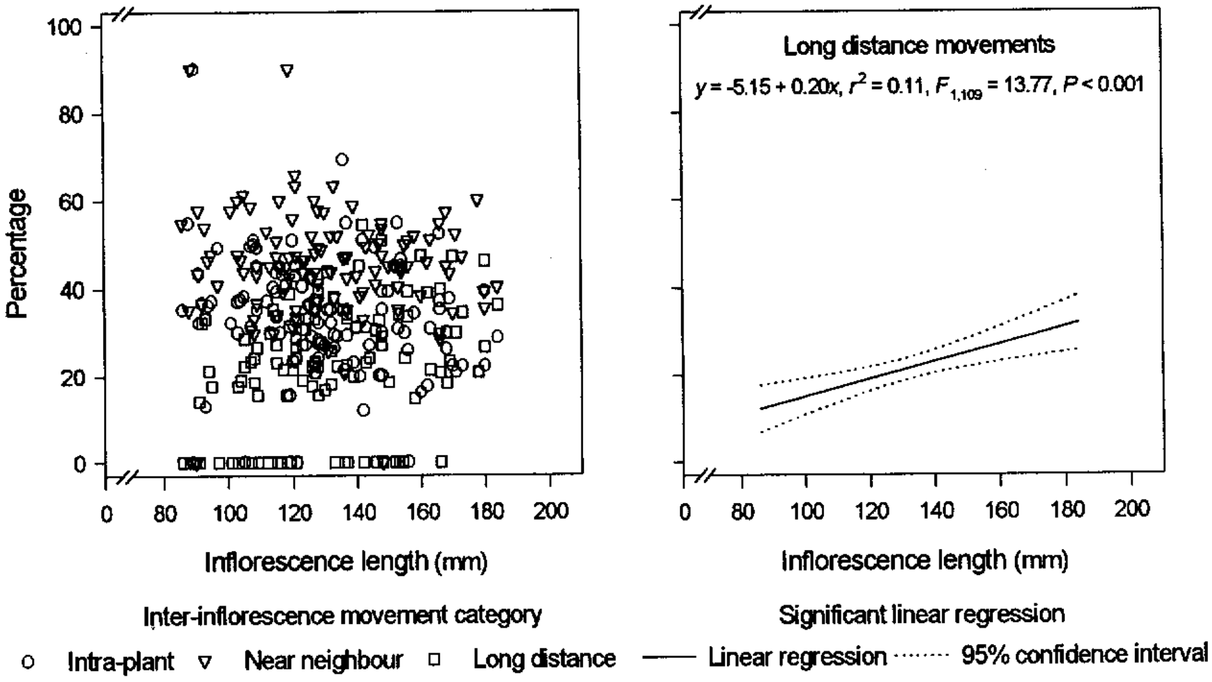

Figure 7.6 Relationship between inflorescence length and the proportion of inter-inflorescence movement categories received by inflorescences of *Banksia tricuspis*.

Within each of the inter-inflorescence movement categories, significant correlations between inter-inflorescence movement distance and inflorescence length were detected for intra-plant and long distance movements (Table 7.6). In both instances, the distance of movement increased with inflorescence size and the correlation coefficient of both regressions were similar. However, the least squares linear model for each association differed significantly from linearity. For long distance movements, this lack of fit was attributable to significant variation among the replicated observations of the dependent variable (Table 7.6).

Table 7.6 Corrected ANOVA results of significance and deviation from linearity of regression models for the distance of each inter-inflorescence movement category (*y*) and inflorescence length in *Banksia tricuspis*. (Identical letters after significant linear regressions indicate no significant difference between correlation coefficients, $P < 0.05$.)

	Inter-inflorescence movement category (y)								
Source of variance	Intra-plant			Near neighbour			Long distance		
	F	P	df	F	P	df	F	P	df
Between groups of y	1.155	0.199	67	1.607	0.002	67	1.926	0.000	58
Linear regression	6.474	0.011^a	1	0.755	0.385	1	10.085	0.002^a	1
Deviation from linearity	1.074	0.332	66	1.619	0.002	66	1.765	0.001	57

Average estimates of all foraging attributes for each inflorescence were significantly correlated with inflorescence length (Table 7.7). A significant association between the three foraging attributes and inflorescence length was therefore detected. Similarly, the frequency of all categories of inter-inflorescence movements were significantly correlated with inflorescence length and all four variables displayed significant associations (Table 7.7).

7.3.3 *Presentation height* vs *pollinator foraging*

All three foraging attributes were positively correlated with presentation height (Figure 7.7), although the least squares linear regression models differed significantly from linearity in all instances (Table 7.8). In the case of both visitation duration and inter-inflorescence movement distance, the lack of fit was attributed to significant variation among replicate observations of the dependent variables.

Table 7.7 Spearman's rank correlation coefficient (r_s) and Kendall's coefficient of concordance (W) for association between foraging attributes and display traits in *Banksia tricuspis*. (Different letters after significant correlations indicate significant differences between coefficients, $P < 0.05$.)

Foraging attributes	Display traits: Inflorescence length $r_{s_{III}}$	Inflorescence length W^1	Presentation height $r_{s_{III}}$	Presentation height W^1
Number of visits	0.27**^{b}	0.55***	0.42***^{a}	0.61***
Duration of visit (s)	0.21*^{b}		0.25***^{a}	
Inter-inflorescence distance (m)	0.35***^{a}		0.66***^{a}	
Inter-inflorescence movement				
Intra-plant visits	0.02	0.50***	-0.06	0.54***
Near neighbour visits	0.22*^{b}		0.44***^{a}	
Long distance visits	0.34***^{a}		0.56***^{a}	

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ^{1} Significance of $W = \chi^2_r$ (df = 110).

Table 7.8 Corrected ANOVA results of significance and deviation from linearity of regression models for presentation height and foraging attributes (y) in *Banksia tricuspis*.

Source of variance	Number of visits F	Number of visits P	Duration of visit F	Duration of visit P	Distance between visits F	Distance between visits P	df
Between groups of y	1.575	0.051	12.963	0.000	5.582	0.000	61
Linear regression	31.627	0.000	167.963	0.000	173.952	0.000	1
Deviation from linearity	1.057	0.423	9.656	0.000	1.901	0.000	60

Similar deviation from linearity was also detected for significant linear regression models between the number of near neighbour and long distance foraging visits and the independent variable of presentation height (Table 7.9). The number of intra-plant movements received by an inflorescence appeared to be independent of presentation height. The proportion of inflorescence visits which culminated from long distance movements increased significantly as presentation height increased (Figure 7.8), although significant incongruity between replicate dependent variables promoted marked deviation from linearity in the regression model (Table 7.9). A similar result was obtained for the proportion of intra-plant movements, although the linear regression model indicated a significantly negative dependence on presentation height (Table 7.9, Figure 7.8).

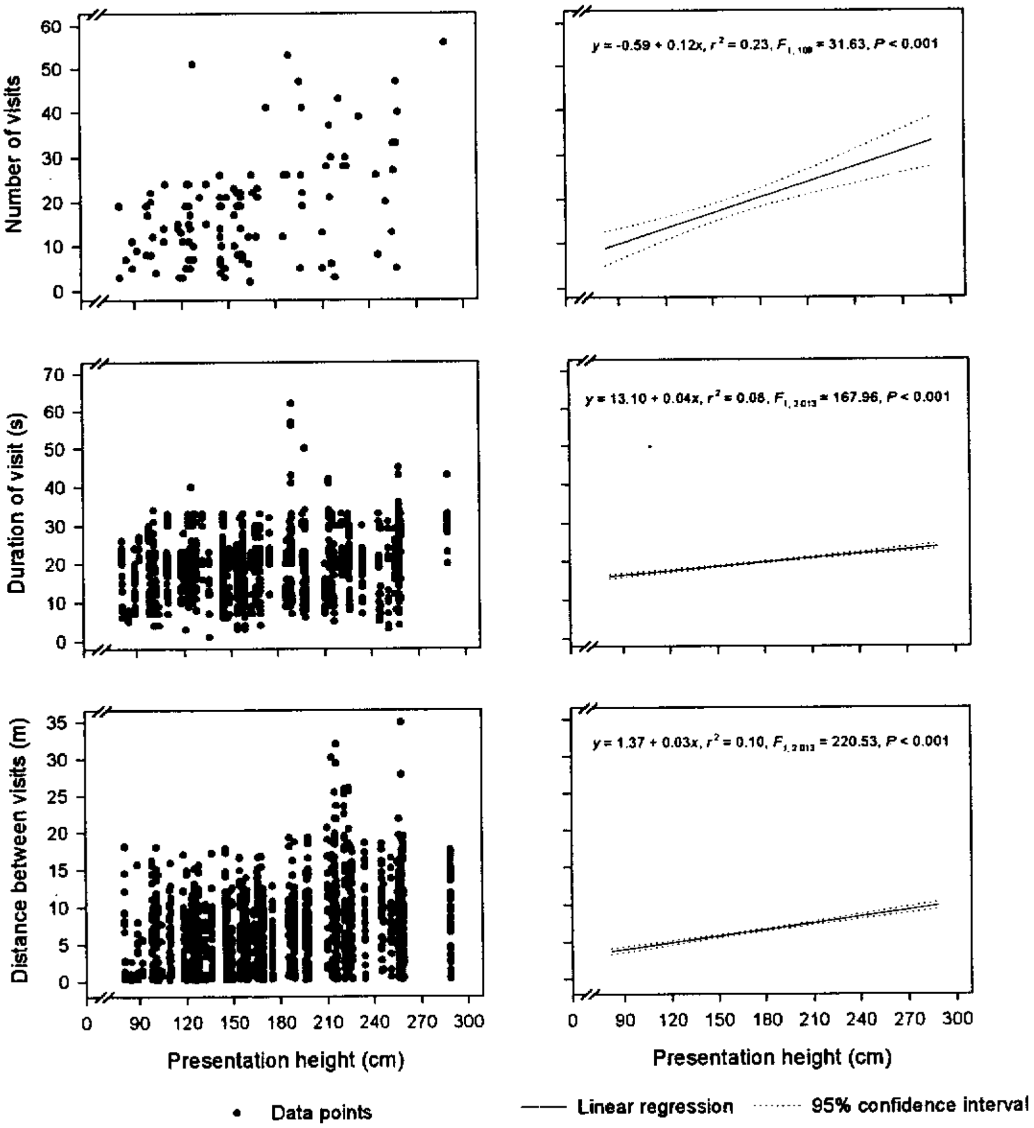

Figure 7.7 Distributions and linear regressions for the association between presentation height and foraging attributes in *Banksia tricuspis*.

Significant least squares models were determined for the relationship between presentation height and inter-inflorescence movement distances within each of the three movement categories (Table 7.10). In all instances, the regression model deviated significantly from linearity, which for all inter-inflorescence movement categories were related to significant incongruity in replicated dependent variable observations.

Table 7.9 Corrected ANOVA results of significance and deviation from linearity of regression models for visits per inter-inflorescence movement category (*y*) and presentation height in *Banksia tricuspis*. (Different letters after significant linear regressions indicate a significant difference between correlation coefficients, $P < 0.05$.)

Source of variance	Inter-inflorescence movement category (*y*)						df
	Intra-plant		Near neighbour		Long distance		
	F	*P*	*F*	*P*	*F*	*P*	
Number of visits							
Between groups of *y*	1.008	0.492	1.283	0.184	2.978	0.000	61
Linear regression	0.149	0.700	18.937	0.000^{b}	78.712	0.000^{a}	1
Deviation from linearity	1.022	0.471	0.990	0.517	1.416	0.105	60
Proportion of all visits							
Between groups of *y*	2.439	0.000	1.539	0.060	1.767	0.020	61
Linear regression	59.472	0.000^{b}	0.495	0.484	53.563	0.000^{a}	1
Deviation from linearity	1.317	0.610	1.554	0.056	0.935	0.600	60

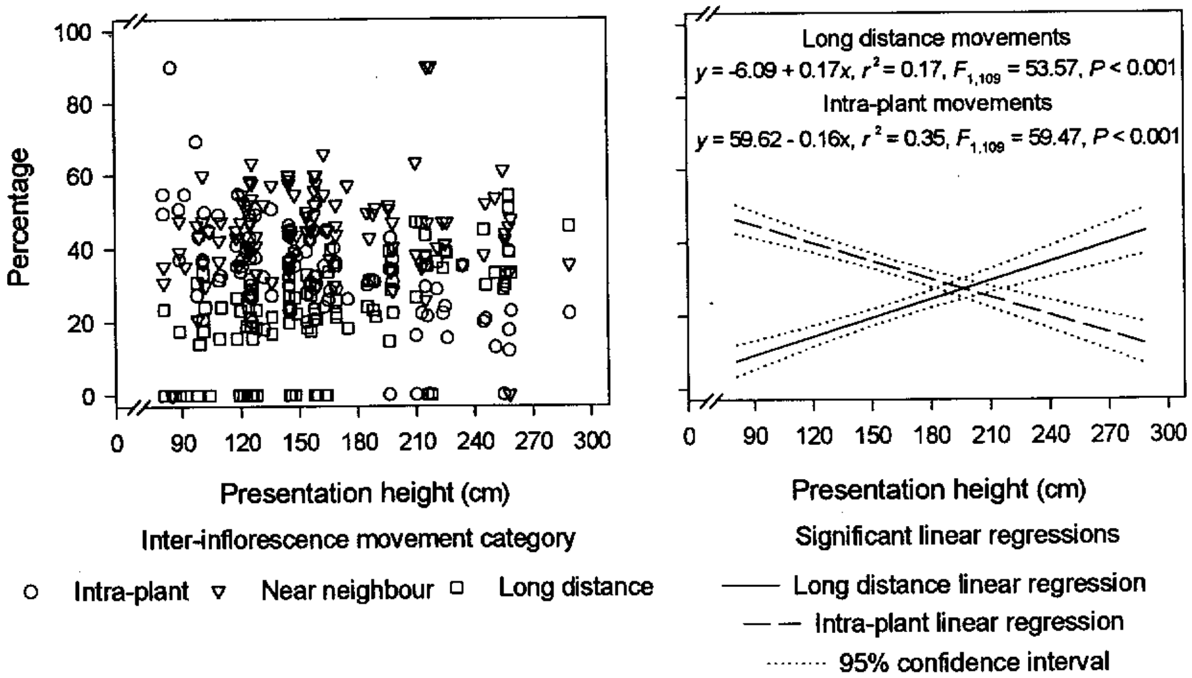

Figure 7.8 Relationship between presentation height and the proportion of inter-inflorescence movement categories received by inflorescences of *Banksia tricuspis*.

Table 7.10 Corrected ANOVA results of significance and deviation from linearity of regression models for the distance of each inter-inflorescence movement category (y) and presentation height in *Banksia tricuspis*. (Identical letters after significant linear regressions indicate no significant difference between correlation coefficients, $P < 0.05$.)

Source of variance	Inter-inflorescence movement category (y)								
	Intra-plant			Near neighbour			Long distance		
	F	*P*	df	*F*	*P*	df	*F*	*P*	df
Between groups of y	1.577	0.006	59	1.656	0.002	60	2.117	0.000	53
Linear regression	12.939	0.000^{a}	1	7.214	0.007^{a}	1	26.558	0.000^{a}	1
Deviation from linearity	1.373	0.041	58	1.549	0.006	59	1.612	0.006	52

Spearman's rank correlation results indicated that the frequency of inflorescence visits, both overall and for each of the inter-inflorescence movement categories were mostly significantly correlated with display height (Table 7.7). The exception was for intra-plant movements which displayed a weakly negative association. Average estimates of visitation time and inter-inflorescence movement distance for each inflorescence were also significantly correlated with presentation height (Table 7.7). These significant correlations were verified by Kendall's coefficient of concordance and marked agreement in the ranking of foraging attributes and inter-inflorescence movement categories with presentation height (Table 7.7).

Comparisons between correlation coefficients for the association between each foraging attribute and the two display traits indicated that most coefficients were homogeneous (Table 7.11). However, significant incongruity was detected between correlations involving the distance between consecutive inter-inflorescence visits, the number of long distance inter-inflorescence movements and the proportion of intra-plant and long distance visits received at an inflorescence. In all instances, relationships with presentation height were stronger. A similar result was obtained from the rank correlation coefficient results (Table 7.7).

Table 7.11 Significance of difference (*Z*) between correlation coefficients for the relationship between foraging attributes and each display traits in *Banksia tricuspis*.

Foraging attribute	Correlation coefficients for relationship with		$Z_{0.05,(2)}$
	Inflorescence length	Presentation height	
Number of visits	0.334	0.474	1.234
Duration of visit	0.249	0.277	0.954
Distance between visits	0.158	0.282	4.139***
Number of inter-inflorescence movements			
Intra-plant	0.091	-0.037	0.942
Near neighbour	0.291	0.385	0.781
Long distance	0.352	0.648	2.969**
Proportion of inter-inflorescence visits			
Intra-plant	0.180	-0.594	6.362***
Near neighbour	0.067	0.087	0.638
Long distance	0.335	0.574	2.241*
Distance of inter-inflorescence movement			
Intra-plant	0.106	0.149	0.321
Near neighbour	0.027	0.085	1.846
Long distance	0.144	0.236	0.702

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

7.4 Discussion

The inflorescence display of *Banksia tricuspis* had a significant affect on the foraging activities of avian pollen vectors. The position of open florets, size and presentation height of the inflorescence act as predictors for overall inflorescence attractiveness, and are correlated with the number of visits an inflorescence receives, the duration of these visits, and the distance over which consecutive visits are made. As this species has a zoophilous reproductive system (van Leeuwen and Lamont 1986), these independent inflorescence traits may directly affect maternal fecundity and progeny fitness through their influence on pollen-limiting constraints, male function and mate choice. Previous investigations (Chapters 5 and 6) have indicated that these inflorescence traits may also influence female function which conforms with the expectation that the attractiveness of the floral displays enhances overall plant fitness through its effects on both male and female function (Wilson *et al.* 1994).

The foraging activities of the five inflorescence visiting bird species were dependent on inflorescence display with high visitation to an inflorescence being associated with longer foraging durations and greater inter-inflorescence movement distances. Near neighbour, followed by intra-plant inter-inflorescence movements, were the most frequent and together accounted for 77.5% of all foraging movements. The opportunity for geitonogamous and consanguineous pollen deposition and the deleterious impacts such pollination promotes through possible inbreeding depression and biparental inbreeding, was therefore high. Vaughton (1990) observed similar frequencies in the number of inter-inflorescence movements by the Eastern Spinebill (*Acanthorhynchus tenuirostris*) while foraging on *B. spinulosa*.

7.4.1 *Influence of anthesis position on pollinator foraging*

The position of the 'advancing front' (Collins and Spice 1986) had a marked correlation with all foraging attributes examined. It appeared that the least favourable positions along the inflorescence were in apical and peduncular regions where the number of visits, their duration and the distance between consecutive visits were the lowest. This pattern concurs with similar results obtained by Collins and Spice (1986) and suggests that pollen transfer and deposition were greatest in middle to middle-peduncular regions of the inflorescence. The preference of pollinators to forage on inflorescences at particular developmental stages has previously been reported (Lamont and Collins 1988, Ramsey 1988b, Collins *et al.* 1990) and may be attributed to morphological (Collins and Rebelo 1987) and colour (Lamont and Collins 1988) cues. In *B. tricuspis*, morphological cues are probably associated with shape modifications to the inflorescence as dehisced florets increase the width of the inflorescence. Similarly, a greater availability of nectar and pollen in middle to middle-peduncular positions along the inflorescence may promote the assortative foraging behaviour observed during this investigation. Previous studies in *B. tricuspis* (Chapter 5) have demonstrated that middle to middle-peduncular inflorescence positions contain significantly more florets than more apical or peduncular positions, which implies that these regions produce more nectar and pollen. Variation in source-sink interactions and the vascular supply network along the axis of the inflorescence, as has been suggested previously in this species (Chapter 5) and in other banksias

(Vaughton 1993), may also affect nectar production along the inflorescence and promote the pattern of foraging observed in this investigation.

The lower visitation frequency of pollinators to peduncular positions cannot adequately be explained by nectar and pollen supply constraints associated with reduced floret number and inadequacies in the vascular supply, as such positions contain similar numbers of florets to adjacent, more apical positions (Chapter 5) and are spatially closer to the nutrient source. Alternatively, the marked decrease in visitation to peduncular positions may be attributed to the perching requirements of pollinators when foraging. Most observations of pollinators indicated that the inflorescence visitor perched on undehisced florets below the 'advancing front' and probed upwards into and immediately above this front. Inflorescences on which anthesis has progressed to peduncular positions may therefore prevent pollinators from perching due to a lack of alighting space along the inflorescence, as dehisced florets do not provide a stable perching platform. Pollinators where observed on occasions to perch on the inflorescence's supporting branch and probe the inflorescence, however, crowding of leaves on the supporting branches often prohibited perching.

The pattern of pollinator foraging observed during this investigation closely resembled the non-random distribution of follicles and viable seeds along the infructescence (Chapter 5) and suggests that fecundity is dependent on pollinator foraging behaviour. McFarland (1985) attributed differences in follicle distribution along the infructescences of sympatric *B. integrifolia* and *B. spinulosa* to differences in pollinator foraging, promoted through contrasting patterns of nectar availability and differences in the availability of suitable perching platforms. Pollinators have also been cited as the principal control governing the preponderant distribution of fruit in the top third of waratah (*Telopea speciosissima*) inflorescences. Goldingay and Whelan (1993) attributed this fruiting pattern to an increase in the frequency of pollinator visits to the upper portion of the waratah inflorescence.

The importance of the 'advancing front' as an attractiveness cue was also highlighted by the significant association with the frequency of each type of inter-inflorescence movement. Generally, the frequency of visits in each inter-inflorescence movement category peaked in the middle to middle peduncular positions and declined notably towards both the apex and peduncle. The proportion of inter-inflorescence movements received at each inflorescence position suggests that the diversity of the pollen load received by an inflorescence was greatest in middle positions. In these positions, the difference between the three inter-inflorescence movement categories was the smallest and in many cases inter-plant movements were more frequent than intra-plant movements. I suggest that the opportunity for geitonogamous pollination would probably decrease in the attractive middle inflorescence positions as a consequence of an increase in the number of visits and their duration, which would dilute the volume of self pollen present. Geitonogamous pollen deposition would, however, remain high as the number of intra-plant movements also increase in inflorescences on which the 'advancing front' is located in middle positions. Similarly, the opportunity for biparental inbreeding as a consequence of consanguineous pollination would increase in attractive inflorescence positions, as near-neighbour pollinator movements were proportionally more abundant in these positions (Figure 7.3). Substantial variation in the average distance of inter-inflorescence movements and the positively skewed distribution of these movements would also sustain the opportunity for geitonogamous and consanguineous pollination, despite increases in the number of long distance movements.

Suggested increases in the diversity of the pollen load received in middle inflorescence positions would enhance the opportunity for mate choice through female function. Previously reported non-random patterns in the distribution of aborted seeds along the infructescence(Chapter 5), which peaked in middle positions, supports the proposition. Similarly, the tendency for estimates of outcrossing to be subdued in middle to middle peduncular positions (Chapter 5) in the facultatively xenogamous species (Chapter 2) also supports the proposition that pollen diversity was high.

Fluctuation in the proportion of long distance inter-inflorescence movements with progression of the 'advancing front' along the inflorescence promoted an increase in the opportunity for xenogamous pollen deposition in middle to middle peduncular positions. Previously demonstrated increases in zygote survival (Chapter 5), and thus the number of viable seeds in middle to middle peduncular inflorescence positions, may be attributed to pollinator foraging and the deposition of acceptable pollen types.

Similarly, previously reported high levels of zygote abortion in apical positions may in part be attributed to the poor quality of pollen deposited in these positions, as long-distance inter-inflorescence movements were minimal and the least frequent visits were observed here.

7.4.2 *Influence of inflorescence size and presentation height on pollinator foraging*

The size and presentation height of a *B. tricuspis* inflorescence has a discernible influence on pollinator foraging. As either size, through the addition of florets, or presentation height increased, inflorescences enticed more pollinators which foraged for a longer duration on the inflorescence and moved greater distances between consecutive inflorescence visits. I conclude that variations in size and presentation height of inflorescences in *B. tricuspis* augments the conspicuousness of an inflorescence which enhances it attractiveness to pollinators. The relative importance of either inflorescence size or presentation height was not readily determined as collinearity, which degrades multiple regressions and prohibits the identification of the most important predictor variable (Kirby 1993), was diagnosed between both display traits. Correlation coefficient (Table 7.11), Spearman's rank correlation and nonparametric association test (Table 7.7) results, however, imply that presentation height exerted a stronger influence over pollinator foraging than inflorescence size. Previous investigations have indicated that presentation height also strongly influences maternal fecundity and progeny fitness (Chapter 6).

Increases in pollinator visits with increasing inflorescence size have been reported in other species (Zimmerman 1987, Klinkhamer *et al.* 1989, Eckhart 1991, Rodríguez-Robles *et al.* 1992, Ohara and Higashi 1994) including some banksias (Paton and Turner 1985, McFarland 1985, Goldingay and Whelan 1990). Generally, the increase in inflorescence size and its effect on pollinator foraging has been attributed to an augmentation in the availability of nectar and pollen rewards, which in most instances increases both the frequency of visits and their duration (McFarland 1985, Burd 1994, Ohara and Higashi 1994). Changes in the conspicuousness of an inflorescence through variation in its location within the crown have also been reported for other species (Burd 1994) including other banksias. Ramsey (1989) reported that most pollinators preferentially forage on conspicuous inflorescences in *B. menziesii*, although aggressive interaction between species biased the foraging activities of some pollinators which preferentially foraged on inflorescences lower in the canopy. Similarly, pollinators were reported to preferentially visit higher inflorescences of the waratah in typically sparse populations of this species (Pyke 1981). The duration of an inflorescence visit may also increase with presentation height in the waratah (Pyke and Paton 1983).

Results from this investigation suggest that the size and presentation height of a *B. tricuspis* inflorescence have the greatest influence on the number of visits received and the proportion of visits which are the consequence of long distance inter-inflorescence foraging movements. Presentation height also significantly influenced the proportion of visits which were of an intra-plant nature, with the number of visits decreasing as presentation height increased relative to the other inter-inflorescence movement categories. Increases in the number of visits to an inflorescence as a consequence of these two display traits would enhance the opportunity for pollination through both pollen export and deposition. Similarly, increases in the frequency of long distance inter-inflorescence movements would enhance the opportunity for xenogamous pollination and increase the diversity of an inflorescence's pollen load, thus promoting mate choice. The probability of geitonogamous and consanguineous pollen deposition appears to remain largely unchanged with variations in these display traits, although the opportunity for geitonogamy would decrease with presentation height, as long-distance movements increase at the expense of intra-plant movements.

My previous investigations have demonstrated that surrogates for progeny fitness, especially estimates of outcrossing, are significantly correlated with inflorescence size and presentation height (Chapter 6). I suggest that the increased opportunity for xenogamous pollination through a larger proportion of long distance inter-inflorescence movements is primarily responsible for this progeny fitness-inflorescence attractiveness association. Similarly, apparent concordance in the proportion of near-neighbour inter-inflorescence movements for both display traits and intra-plant movements for inflorescence size, would enhance the opportunity for mate choice through the receipt of a more diverse pollen load. A reduction in the probability of zygote survival with presentation height, which is accompanied by escalating viable seed production and increasing outcrossing (Chapter 6), concurs with the above proposition that higher inflorescence displays receive a more diverse pollen load facilitating selection based on mate choice.

7.4.3 *Inflorescence attractiveness, pollinator foraging and plant fitness*

I suggest that the attractiveness of a *B. tricuspis* inflorescence to pollinators is enhanced by increasing the conspicuousness of the display. Conspicuousness was augmented by changes in the pattern of floret dehiscence, floret number and location of the inflorescence within the crown. Pyke (1987) has also suggested that conspicuousness enhanced the attractiveness of the inflorescence display in the waratah. Changes in the availability and quantity of nectar and pollen rewards with progression of the 'advancing front' along the inflorescence and an increase in the number of nectaries on an inflorescence may also function to enhance attractiveness.

Attractiveness of a *B. tricuspis* inflorescence appeared to most significantly influence the number of visits which an inflorescence received and the proportion of visits which were from non-related conspecifics. Attractiveness also enhanced the duration of inflorescence visits. Therefore, I propose that inflorescence attractiveness in *B. tricuspis* has important consequences for plant fitness which are primarily promoted through improvements in progeny fitness as a consequence of increases in the opportunity for xenogamous pollination. The documented increase in the proportion of long distance inter-inflorescence movements with attractiveness enhanced the opportunity for xenogamous pollination, and agrees with the positive association between attractiveness and reproductive success (Chapters 5 and 6). Concurrently, I also propose that attractive displays enhance progeny fitness through increasing the opportunity for mate choice as levels of geitonogamy and consanguineous pollen deposition increase with attractiveness, as inferred through intra-plant and near neighbour movements. Previously documented relationships between zygote survival and attractiveness, which were postulated to be influenced by maternal choice selective decisions based on genetic quality considerations (Chapters 5 and 6), agree with this proposition.

The dilemma of attractive inflorescence displays (Hessing 1988, Klinkhamer and de Jong 1993, Harder and Barrett 1996) appears to be moderated in this *Banksia*. Previous investigations (Chapters 5 and 6) demonstrating: (i) a positive relationship between maternal fecundity and attractiveness; and (ii) estimates of outcrossing which approach panmixia in attractive displays, both suggest that geitonogamy and insufficient pollen transfer are not limiting constraints on male and female functional roles in the reproductive success of this *Banksia*. I suggest that the moderation of this dilemma of attraction occurs as a consequence of the greater number of visits received by attractive displays, which enhanced the opportunity for pollen deposition, especially pollen from non-related conspecifics, and reduced the volume of self pollen present through increased pollen export. Similarly, the increased duration of pollinator foraging with increases in inflorescence attractiveness also augments the opportunity for pollen deposition while reducing the volume of self pollen present.

It follows therefore that, attractive displays enhance both male and female functional roles in reproductive success and thus plant fitness in this species. Male function is enhanced through pollen export and increases in the opportunity for outcrossed seed siring thereby advancing an inflorescence as a pollen parent. Heterogeneity and positive correlations between pollinator foraging attributes and inflorescence attractiveness indicate that the male functional role in reproduction may be enhanced by 'surplus' flowers. Previous investigations (Chapters 5 and 6) have suggested that 'surplus' flowers also enhance female function, which I suggest is reinforced by inflorescence displays that promote pollinator foraging behaviour which appreciate the opportunity for mate choice. The concomitant augmentation of both male and female functional roles in reproductive success has previously been reported in other plant species and may be favoured selectively (Campbell *et al.* 1991, Wilson *et al.* 1994). I propose that the production of 'surplus' flowers, which enhances attractiveness and reduces the randomness of pollination, provides a means of optimising outcrossing and enhancing progeny fitness which may be considered adaptive in this long-lived resprouting species (Carpenter and Recher 1979, Jarne and Charlesworth 1993). Optimisation of outcrossing has also been suggested as an explanation for the production of 'surplus' flowers in other *Banksia* species which feature panmictic outcrossing estimates, despite considerable opportunity for geitonogamous pollen receipt (Scott 1980, Carthew *et al.* 1988, Vaughton and Carthew 1993).

Maximising plant fitness through increasing inflorescence attractiveness may be advantageous as moderating the randomness of pollinator behaviour should improve female reproductive success (Campbell 1989, Podolsky 1993). Results from this investigation suggest that the floral display of *B. tricuspis* influences pollinator foraging behaviour, which enhances male reproductive success and increases the opportunity for female reproductive success through outcrossed pollen receipt and mate choice. The evolutionary advantages of attractive displays, however, must be assessed with caution in this species as large attractive displays also experience considerable floral damage (Chapter 3) which significantly impinges on reproductive output. As noted by Wyatt (1982) caution should be exercised when attributing floral displays to selection propelled by sex function and reproductive fitness alone as other selective forces may be operating simultaneously and could even represent a stronger selective influence.

7.4.4 *Conclusions*

The inflorescence display of *B. tricuspis* imposes considerable influence over the pattern of pollination. This influence was illustrated by variation in the number of visits an inflorescence received, the duration of these visits and the distance over which consecutive visits were made with changes in the attractiveness of the inflorescence display. The principal influence of inflorescence attractiveness, which I suggest was primarily a function of conspicuousness and the availability of nectar/pollen rewards, appears to be on the number of visits which an inflorescence received and the proportion of these visits which were likely to promote xenogamous pollination. I submit that heterogeneity in attractiveness among and within an inflorescence influences both male and female sex function through the type, volume and pattern of pollen transferred. Attractive displays enhance the opportunity for male functional success through pollen export and outcrossed seed siring, while female functional success is enhanced through pollen import and mate choice promoted by pollen of contrasting genetic quality. I propose that the mating costs of attractive displays are moderated as a consequence of numerous visits of extended duration, which dilute the volume of self pollen and increase the opportunity for pollen export to conspecifics. Nevertheless caution in attributing the evolutionary advantage of attractive inflorescence displays to selection that moderates the randomness of pollinator visits and reproductive success is suggested.

Future research to clarify the influence of inflorescence attractiveness on pollinator foraging behaviour and thus the type, volume and pattern of pollen transfer should attempt to document the rates of pollen removal from inflorescences of contrasting attractiveness. Pollen analogues could also be employed to examine how inflorescence attractiveness effects pollen dilution and carryover by honeyeaters between differentially attractive inflorescences. Such experiments would require meticulous observation and the rigorous application and assessment of dispersal patterns for the pollen analogous along and among inflorescences with regards to their attractiveness.

The use of sophisticated genetic techniques may also be an illuminating approach for assessing the effects of inflorescence attractiveness on pollinator behaviour, especially in populations which contain rare genetic markers (e.g. alleles, genotypes) . In such populations it would be possible to compare the frequency of widespread and rare genetic markers in the progeny from inflorescences which vary in attractiveness. Such data would highlight those inflorescences which receive numerous pollinator visits and would permit intra- and inter-inflorescence comparisons with respect to the genetic composition of their progeny. Verification of propositions in this chapter would be achieved if the frequency of rare genetic markers were highest among the progeny from attractive inflorescences. This type of investigation inherently would provide useful information on pollen flow patterns within populations. However, it would be contingent on uniform genetic selection, especially the non-expression of mechanisms which impinge on progeny with rare genetic markers.

7.5 References

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CHAPTER 8

CONCLUSIONS

Optimising strategies for the conservation of a species requires an appreciation of the diverse genetic and ecological factors which influence demographic change (Pavlik and Manning 1993, Schemske *et al.* 1994). These factors primarily affect vital rates - birth, growth and death (Caswell 1989), which determine species resilience and long-term population persistence. An understanding of the biological processes and causes of variation which affect these vital rates, particularly for life history stages which contribute most to resilience and population persistence, is critical to the design and implementation of effective management and recovery programs.

This investigation was designed to identify and examine the biological processes and causes of variation that limited reproductive success in *Banksia tricuspis.* Previous and synchronous investigations (van Leeuwen and Lamont 1986, Lamont and van Leeuwen 1988, van Leeuwen *et al.* 1990) suggest that genetic and ecological factors influencing reproductive effort and output (maternal fecundity) were having major demographic impacts. Environmental heterogeneity, insignificant seedling survivorship and sensitivity of juvenile plants to fire have also been cited as factors influencing the demographics of this species (Lamont and van Leewuen 1988, van Leeuwen *et al.* 1990), so were not addressed in the current thesis.

Genetic factors considered to influence the reproductive success of *B. tricuspis* were primarily associated with the mating system, its variation and the mechanisms which enhanced outcrossing, such as mate choice. Like many other banksias (Scott, 1981, Carthew *et al.* 1988, Coates and Sokolowski 1992, Vaughton and Carthew 1993, Sampson *et al.* 1994), the mating system of this species approached panmixia in most instances. These substantial levels of outcrossing were obtained despite considerable opportunity for substantial geitonogamous and consanguineous pollination. I propose that mechanisms of selective abortion, propelled through mate choice, operate to ensure high quality progeny in this resprouting species. I also propose that the quantity of progeny produced during a reproductive bout is maximised through bet-hedging opportunities. Therefore, the commensurate operation of selective abortion and bet-hedging in *B. tricuspis* operates to ensure reproductive potency and minimisation of the effects of pollinator randomness.

The variation between populations in levels of outbreeding in this species conforms with the notion that mating systems are not fixed but vary in space and time (Hamrick 1989, Barrett and Eckert 1990). This heterogeneity was also apparent in the incongruity in fidelity of mate choice within and between inflorescences, as inferred through the patterns of zygote survival. Inter-population mating system heterogeneity was primarily attributed to incongruity in the disturbance (fire) history of sampled populations. I attribute these differences to inconsistencies in the ability of individual populations to attract and sustain pollinator assemblages which achieve the required pollen transfer. Population size may also influence mating system heterogeneity as outcrossing estimates tend to increase with population size. Presumably larger populations can support a more abundant pollinator assemblage which affects the incidence of acceptable pollination (Lamont *et al.* 1993, Sampson *et al.* 1996). However, incongruity in this population size - outcrossing relationship was evident, with small populations maintaining elevated outcrossing estimates despite considerable opportunity for geitonogamy and consanguineous matings. In one disturbed population, the maintenance of high outcrossing was attributed to a change in the pollinator assemblage, with the disturbance regime removing pollinators which promoted geitonogamous and consanguineous matings. In two other undisturbed populations, the maintenance of high outcrossing was attributed to the actions of a selective mechanism favouring heterozygotes, thus limiting the opportunity for inbreeding.

I propose that the heterogeneity in outcrossing within and between inflorescences was attributable primarily to mechanisms which promoted progeny quality and minimised the randomness of pollination. These mechanisms were controlled by genetic considerations and principally involved mate choice and the genetic relatedness of the pollen load to the maternal plant. Within the inflorescence I maintain that these genetic mechanisms operate synergistically with maternal resource consideration. For example, progeny originating from middle to peduncular positions along the inflorescence rachis were better provisioned and endured less genetic selection than progeny arising from more apical positions where the demand for resources among developing progeny was less. This position-based differentiation occurred despite middle to peduncular positions receiving considerable inter-plant and near neighbour pollinator visits. I suggest that differential provisioning and moderation in selection with inflorescence position is attributable to variations in maternal resource availability along the inflorescence rachis, which influences the fidelity of the genetic selection mechanisms.

Similarly, between inflorescence heterogeneity was attributed to differences in inflorescence attractiveness, particularly presentation height and its influence on pollinator behaviour. Generally, attractive inflorescence displays produced the most adequately provisioned progeny, which were also of high genetic quality. Such progeny was produced despite these inflorescences being remote to maternal resource reservoirs and receiving considerable inter-plant and near neighbour pollinator visits. Results indicated that attractive displays were better provisioned with maternal resources and enticed proportionally more xenogamous pollinator visits which I propose reduced the opportunity for selection among zygotes bases on genetic load considerations. I suggest that the inclusion of maternal resource considerations with genetic processes, which select among zygotes, ensures the maintenance of high progeny quality and maximises the opportunity to increase progeny quantity in this *Banksia*. James and Kennington (1993) also propose that maternal resource considerations are important in the intensity and determination of genetic selection processes in many *Eucalyptus* species.

Ecological factors identified as impinging on the reproductive success of *B. tricuspis* were associated with constraints imposed by floral damage and pollinator behaviour. Floral damage had the most significant impact on reproductive success, severely reducing potential reproductive output in this species. Nevertheless, this impact was somewhat mitigated by a partial recovery compensatory response where progeny production increased with the magnitude of floral damage. I argue that this recovery response occurred as a consequence of increases in pollination success, as a reduction in nectivorous resources may compel pollinators to raise inter-inflorescence movement distances thereby increasing the opportunity for xenogamous pollination. Similarly, a reduction in nectivorous resources may also increase the duration of inflorescence foraging bouts which increases the opportunity for pollen export and deposition (Paton and Ford 1983). Increased augmentation in the availability of maternal resources was rejected as a reason for this recovery response as no association was detected with the probability of zygote survival, which remained constant.

Pollinator behaviour significantly influences the reproductive success of *B. tricuspis*, as demonstrated in previous investigations which showed that animal vectors are essential for reproduction (van Leeuwen and Lamont 1986). Variation in reproductive success with inflorescence display traits and the non-random distribution of progeny along the rachis, as measured both through progeny quality and quantity attributes, were attributed to pollinator foraging and pollination uncertainty. Inflorescence displays which enticed pollinators paradoxically also increased the opportunity for unsuitable matings, as illustrated by elevated levels of zygote abortion. However, I propose that genetic selective processes which vary in intensity with maternal resource status mitigated the deleterious impacts of unsuitable matings.

Traits which influenced the attractiveness of the floral display in *B. tricuspis*, such as inflorescence size, presentation height and the position of the 'advancing front' along the rachis, markedly influenced the agents of both floral damage and pollination. The relationship between floral damage and inflorescence attractiveness was affirmed by all three damaging agents. Port Lincoln Ringneck Parrots preferentially visited and removed floret parts from the largest inflorescences, *Arthrophora* larvae abundance increased with inflorescence size and Carnaby's Black Cockatoo preferentially visited insect-occupied inflorescences. Similarly, both insect-occupied inflorescences and damage to inflorescences by cockatoos were most frequent on plants with large floral displays. Pollinators, mostly honeyeaters, also discriminated between floral displays on the premise of attractiveness. Large conspicuous displays received more pollinator visits, received longer foraging bouts and enticed pollinators over greater distances. I suggest that attractive displays enhance the opportunity for xenogamous matings as the probability of pollen receipt from genetically unrelated plants increased and the likelihood of unsuitable mating was reduced through increased pollen export to conspecifics.

The floral display traits which influenced the agents of floral damage and pollination appeared to vary. Inflorescence length exerted significant control over the actions of damaging agents, while presentation height and the position of the 'advancing front' appeared to determine pollinator behaviour. I propose that the influence of inflorescence length on the agents of floral damage can be attributed to the greater nutrient resource afforded by larger inflorescences, firstly with regards to nectar for parrots, secondly, in terms of plant tissue for rachis-boring larvae and finally, in terms of the number of larvae present in large inflorescences for cockatoos. The governing influence of presentation height on pollinators may be attributed to inflorescence conspicuousness and accessibility. This pollinator-presentation height relationship may also be reinforced by an optimal foraging strategy, whereby height based selection of inflorescences may confer greater rewards compared with size based selection. I propose that selection based on inflorescence size is unrewarding as the impacts of floral damaging agents, particularly rachis-boring larvae, reduce the availability of potential nectar rewards.

The impacts of the associations between attractiveness and the opportunity for both floral damage and pollination on reproductive success were converse. Floral damage reduced fecundity while pollination increased the opportunity for reproductive success. The association between floral damage and attractiveness conforms with the proposition that unattractive, inconspicuous displays mitigate predation (Scott 1982). However, the selective advantage of producing unassuming displays is questionable, as these displays also experienced the least pollinator visitation and produced progeny of the lowest genetic quality. Small inflorescence displays also were the poorest at provisioning progeny. This ambiguity in the selective advantage of attractive displays reinforces Wyatt's (1982) cautionary remarks regarding the ascribing of selection for particular floral traits to evolutionary forces in isolation of other selection propelling processes.

Maternal resource considerations are among the ecological factors cited as impinging on reproductive success in many species (Lee 1988, Stephenson 1992, Lawrence 1993). Throughout this thesis I argue that maternal resources were not limiting. This premise is founded on the lignotuberous habit of this *Banksia*, which should confer a nutrient resource in excess of reproductive demands. However, constraints on the availability of maternal resources, fostered through postulated inadequacies in an inflorescence's vascular network and source-sink relationships, were submitted as influencing the pattern of fecundity and fitness within and between infructescences. Notwithstanding the effects of such anatomical and physiologically imposed constraints, I suggest that maternal resources were allocated in a strategic manner to maximise fitness.

Evidence supporting the moderation of maternal resource constraints and the strategic allocation between successive reproductive developmental stages was forthcoming on numerous occasions. Firstly, the successful conversion of all undamaged inflorescences to infructescences indicated that resources were not limiting at this stage in the reproductive cycle. Interestingly, this successful conversion also implied that pollination was not limiting at this developmental stage. Secondly, consistency between seasons in reproductive success, as measured by a number of fecundity attributes, suggested that resources were not limiting and indicated inter-year predictability in supply. Thirdly, the partial compensatory response to floral damage, as highlighted by inflated fecundity among undamaged inflorescences with increasing overall damage, endorses the strategic allocation of resources between successive developmental stages. Fourthly, the failure to detect any quality-quantity trade-offs between successive developmental stages for most reproductive attributes also implies that the availability of maternal resources was not constraining.

Throughout the thesis I maintain that, after the obvious impacts of floral damage, pollinator/pollen limitation considerations were foremost in constraining and influencing reproductive success. This assertion was collectively supported by numerous propositions which emphasise increases in fecundity and fitness with a moderation in pollinator/pollen limitation. For example, increases in the opportunity for xenogamous matings, following alteration in the pollinator assemblage induced by habitat disturbance, enhanced progeny fitness. Similarly, increased pollinator visitation, increasing the opportunity for self pollen export and xenogamous pollen receipt, enhanced both fecundity and fitness of attractive inflorescence displays.

Augmentation of pollinator attraction effort, through the inherent allocation of additional maternal resources with increased floret number, also elevated progeny fitness and fecundity. Throughout the thesis I suggest that selection processes which mitigate the impacts of pollinator/pollen limitation on reproductive success in *B. tricuspis* were favoured as they confer the opportunity to increase both progeny fitness and fecundity.

The most obvious adaptation which operates to mitigate pollinator/pollen limitation in this species was the production of 'surplus' flowers. A reduction in pollinator/pollen limitation through the inherent increase in attractiveness afforded by 'surplus' flowers has been proposed as one of the ultimate functional explanations for this phenomenon (Sutherland and Delph 1984, Stephenson 1992, Morgan 1993, Goldingay and Whelan 1993). I argue that the increased attractiveness of 'surplus' flowers affects pollinator behaviour which augmented pollination success in this *Banksia*. The effect of this increase in pollination success was most apparent with respect to progeny fitness, although fecundity also increased in many instances.

As submitted by other authors (Ayre and Whelan 1989, Ehrlén 1991, 1993, Guitián 1993, Siemens 1994), I also argue that 'surplus' flowers serve other functional roles which may not necessarily be mutually exclusive and operate synergistically to maximise reproductive success. I propose that 'surplus' flowers also increased the opportunity for selective abortion and bet-hedging. Selective abortion was principally advanced by the attainment of high outcrossing estimates, despite the considerable opportunity for promiscuous pollen receipt - especially among attractive inflorescence displays. Commensurate high zygote abortion among attractive displays also supported the notion of selective abortion. Similarly, explanations advanced for the pattern of within infructescence fecundity and progeny quality also promoted selective abortion. I suggested that the observed patterns were attributable to the abandonment of zygotes with low fitness prospects in favour of those with higher fitness expectations, especially in the presence of intra-inflorescence maternal resource provisioning constraints. Such zygotes were located towards the apical end of infructescences, where the opportunity for non-xenogamous pollination was high. I suggest that discrimination between zygotes with low and high fitness expectations is under resource ordained maternal control rather than a zygote's competitive ability.

The uniform character of aborted zygotes (Lamont and van Leeuwen 1988, van Leeuwen, unpublished data) denotes that they are discarded at an early developmental stage, an outcome which conserves maternal resources through minimising the allocation to indigent zygotes.

Bet-hedging was supported through the 'reserve ovule' hypothesis (Ehrlén 1991) where excess ovules act as a reserve supply compensating for ovule mortality. The partial recovery compensatory response to floral damage corroborated the notion that 'surplus' flowers act as a reserve ovule supply. The 'resource available' hypothesis (Guitián 1993) was also partially invoked to explain the overproduction of ovules. This hypothesis was supported by the upward adjustment to fecundity in response to the postulated increase in maternal resources with progression towards the peduncular end of the inflorescence and the commensurate relaxation in genetic selective forces. Consistency and low variability in fecundity between years also promoted bet-hedging as an explanation for 'surplus' flowers.

I propose that in *B. tricuspis* the ultimate functional role of 'surplus' flowers was to enhance the opportunity to maximise progeny fitness. This was achieved primarily by alleviating pollinator/pollen limiting constraints through increasing the attractiveness of floral displays and modifying pollinator behaviour to enhance the opportunity for pollination. Paradoxically, the attractive role for 'surplus' flowers was challenged by the increasing probability of floral damage. However, predictability with respect to this deleterious impact may mitigate its significance as a selective force. I also propose that 'surplus' flowers provide an opportunity for selective abortion and bet-hedging. Sympatrically, these roles serve to reduce the effects of the randomness of pollination through assortative seed maturation and elevate fecundity when the opportunity arises. These roles for 'surplus' flowers and the associated complementary genetic and ecological factors affecting reproductive success in *B. tricuspis* conform with the expectation that long-lived resprouting species indulge in selection of superior quality progeny (Carpenter and Recher 1979, Jarne and Charlesworth 1993). The cost of this progeny quality/provisioning advantage is reduced maternal fecundity. I suggest that increased fecundity would not be favoured selectively in *B. tricuspis* as no demographic advantage would be forthcoming. Previous studies indicating considerable erosion of seed viability and canopy storage within four years of accession, negligible inter-fire establishment, high seedling mortality in the first five years post-germination (98%) and meagre recruitment of seedlings into the adult population over an 18 year period (0.2% increase) challenge the functional imperative to maximise fecundity (Lamont and van Leeuwen 1988, van Leeuwen *et al.* 1990, van Leeuwen, unpublished data).

In summation, results and propositions presented on the factors which affect reproductive success in *B. tricuspis* imply that considerable selection operates to enhance progeny fitness and maximise fecundity. The processes which propel these selective forces are not mutually exclusive and operate synergistically to maintain the selective advantage of this species. Both internal and external processes were invoked to explain and justify the patterns of reproductive success observed and elucidate their selective significance. Floral damage and the randomness of pollination influenced reproductive success to the greatest extent. However, the effects of these extrinsic processes were moderated and buffered through intrinsic maternal processes which facilitated assortative seed maturation and progeny provisioning.

Many challenges for future research are raised by this thesis. Verifying the role of habitat modification in altering a species mating systems, especially for endangered taxa, is paramount for any conservation effort. This is particularly relevant in instances where fire is the perturbation, as this agent of modification is an important and frequently used land management tool (Whelan 1995, Bond and van Wilgen 1996). Quantifying the role and value of all pollinating agents in reproduction and determining their effects on mating system estimates should be investigated. Determining the strength and fidelity of the relationship between this *Banksia*, the moth larvae and the cockatoo over a larger number of consecutive seasons would also be of interest. Such continued monitoring of reproductive effort and output would also confirm temporal consistency and synchrony in reproductive success as implied here.

Investigating the validity of the fitness-fecundity advantages bestowed on resprouters is of considerable interest and requires further attention. Quantification of any selective fitness advantage will require a sympatric investigation of conspecific resprouting and nonsprouting species pairs. Verification of progeny quality-quantity trade-offs with the type of disturbance life history response would have major implications for the management of many endangered species, especially those which are candidates for re-introductions, population translocations and intensive habitat management and restoration.

Experimental investigations and characterisation of the processes during selective abortion, particularly with regards to the time of abandonment and mode of assortment between developing zygotes would also be worthwhile. In parallel with this investigation it would be beneficial to determine if sink strength (ovule size) is uniform over the inflorescence rachis. Similarly, delineating the functionality of an inflorescence's vascular network would provide information on the presence of a maternal resource provisions gradient. Unfortunately, I cannot recommend undertaking this type of work on species like *B. tricuspis* where experimental manipulation of florets, inflorescences and plants is extremely difficult and the rewards are minimal due to high inflorescence mortality and their low fecundity.

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