

Chapter 1

General Introduction

Introduction

Flowering plants exhibit a great diversity of floral form and modes of pollination (reviewed by Fryxell 1957, Richards 1986). Underlying this diversity are several modal breeding systems: predominant outcrossing, predominant selfing, mixed outcrossing and selfing, obligate apomixis (agamospermy), and facultative apomixis (usually with outbreeding) (Jain 1976, Brown 1990). Predominant outcrossing is generally controlled by genetically determined self-incompatibility systems operating pre-zygotically (de Nettancourt 1977, but see Seavey and Bawa 1986, Ramsey et al. 1993). Self-incompatibility systems are widely distributed throughout the Angiospermae and have been reported from at least 71 families in 17 orders. Self-incompatibility is considered to have arisen early in the evolution of the Angiospermae, although whether it is of monophyletic or polyphyletic origin is unknown. Self-compatibility (i.e. self-fertility) is generally considered to be a secondarily derived trait, caused by the genetic breakdown of a self-incompatibility systems, although once a plant has become self-fertile other factors such as inbreeding depression may cause low levels of selfed seed set. In this study, self-incompatibility systems are not reviewed. Recent reviews of the major classes of self-incompatibility and their general properties can be found in de Nettancourt (1977), Richards (1986), Willson (1983) and Barrett (1988a).

One of the major pathways of breeding system evolution in flowering plants is the change from predominant outcrossing, enforced by self-incompatibility, to predominant or partial self-fertilization. The evolution of self-fertilization in plants that are closely related to primarily outcrossing species has occurred in numerous taxa and has been the subject of several theoretical and empirical studies (Darwin, 1877, Baker 1955, Fryxell 1957, Stebbins 1970, Jain 1976, Charlesworth and Charlesworth 1979, 1987, Lloyd 1979, 1980, 1992, Lande and Schemske 1985, Schemske and Lande 1985, Richards 1986, Rick 1988, Barrett 1988a,b, Wyatt 1983, 1988, Barrett and Eckert 1990, Holsinger 1988, 1992, Jarne and Charlesworth 1993, Uyenoyama et al. 1993). The various hypotheses for the evolution of self-fertility can be partitioned into two broad classes: positive and negative (Stebbins 1957, Jain 1976, Uyenoyama 1986, Holsinger 1992). Common to both classes of hypotheses, however, is the potential importance of inbreeding depression in the evolution of self-fertility.

Inbreeding depression is the lowered fitness of inbred as compared with outbred progeny and is a common phenomenon in plants occurring throughout the life cycle (Darwin 1877, 1888, Lande and Schemske 1985, Charlesworth and Charlesworth 1987, Holsinger 1992, Johnston 1992, Lloyd 1992, Uyenoyama et al. 1993, Husband and Schemske 1995). Inbreeding depression has long been considered to be the major factor in preventing the evolution of self-fertility and/or maintaining outcrossing (Knight 1799, Darwin 1888). Despite its importance, the underlying genetic basis of inbreeding depression remains unclear and is a matter of debate (Crow 1993, Mitton 1993). Two genetic causes have been proposed, and suggest that outcrossed progeny are fitter because they have a greater proportion of heterozygous loci, many of which are overdominant (overdominance hypothesis) or because their greater heterozygosity masks their largely recessive, deleterious genes (partial

dominance hypothesis). Theoretical and empirical studies suggest that although overdominance may be important in some cases, the expression of recessive or nearly recessive deleterious genes is a more important factor (Lande and Schemske 1985, Charlesworth and Charlesworth 1987, 1990, Ågren and Schemske 1993, Charlesworth et al. 1994). The magnitude of inbreeding depression is expected to evolve with the breeding system, such that with increased or prolonged selfing, inbreeding depression decreases because recessive deleterious genes are purged. However, although purging occurs in some species (e.g. Barrett and Charlesworth 1991), high genomic mutation rates, fixation of mildly deleterious genes, overdominance, intermediate zygote selfing rates and low survival of selfed progeny can act to maintain high levels of inbreeding depression (Lande and Schmeske 1985, Charlesworth and Charlesworth 1987, B. Charlesworth et al. 1990, Charlesworth et al. 1994, Lande et al. 1994). Several recent studies have examined inbreeding depression in natural plant populations (reviewed by Johnston 1992, Ågren and Schemske 1993, see Chapter 7), and it is becoming apparent that the role of inbreeding depression in the evolution of self-fertility is more complex than previously considered (e.g. Fisher 1941).

The positive hypotheses for the evolution of self-fertility suggest that genes promoting selfing increase in a population of outcrossing plants as a result of greater genetic transmission by selfing plants to succeeding generations (automatic selection, Jain 1976). The reduced genetic contribution by outcrossing plants has been termed the "cost of outcrossing." In an outcrossing population, a gene that promotes self-fertilization, but does not affect pollen production or pollen export, is expected to spread to fixation, because plants that self have a two-fold advantage of gene transmission, contributing twice as many gametes to each of their seeds as do outcrossers (Fisher 1941, Nagylaki 1976, Maynard Smith 1977, Lloyd 1979). Inbreeding depression is generally considered as the major selective factor opposing the gene transmission advantage of selfing individuals and the evolution of self-fertilization in outcrossing plants. When the fitness of selfed progeny is less than one half that of crossed progeny, outcrossing is favoured because the transmission advantage of selfers is negated. When the fitness of selfed progeny is greater than one half that of crossed progeny, increased selfing is automatically selected (Fisher 1941, Lande and Schemske 1985, Charlesworth and Charlesworth 1987, Uyenoyama et al. 1993). Recently, more complex models have shown that other factors may interact with inbreeding depression, changing the threshold at which self fertilization is selective, although most models suggest that inbreeding depression is important in the evolution of selfing (reviewed by Uyenoyama and Waller 1991a, b, c, Holsinger 1988, 1992, D. Charlesworth et al. 1990, Uyenoyama et al. 1993).

The negative hypotheses for the evolution of self-fertility suggest that selfing must provide some adaptive benefit to overcome the fitness disadvantages of inbreeding depression. Although adaptive benefits can take many forms (reviewed by Jain 1976), reproductive assurance has frequently been proposed as playing a major role in the evolution of self-fertilization. Selfing may have selective advantages over outcrossing if reproduction is ensured during colonization or population bottlenecks when access to mates is limited or

when outcrossed pollen is limited because pollinators are scarce or unreliable (e.g. Darwin 1877, Baker 1955, Levin 1972, Jain 1976, Lloyd 1980, 1992, Piper et al. 1986, Barrett 1988b, Wyatt 1986, 1988). Even with strong inbreeding depression, selfing is expected to evolve if most ovules remain unfertilized (i.e. strong pollen limitation). Theoretical models have shown that if externally mediated pollination is limiting, then the inbreeding depression threshold below which selfing is automatically favoured is shifted upwards, and self-fertility is favoured despite strong inbreeding depression, providing that selfed progeny are viable and reproduction in subsequent seasons is unaffected (Lloyd 1979, 1992). As Darwin (1877) suggested, "... for it would manifestly be more advantageous to a plant to produce self-fertilized seeds rather than none at all or extremely few seeds."

Numerous empirical studies suggest that self-fertility has evolved to ensure reproduction when pollinator activity is reduced, implying that selection is sufficient to counteract the effects of inbreeding depression (reviewed by Jain 1976, Lloyd 1980, Barrett 1988a, Wyatt 1988). However, despite the logic of the reproductive assurance hypothesis, there is little direct evidence of the importance of pollinator limitation in the evolution of self-fertilization. Many studies have been content to accept an explanation for change in mating system based on the need for reproductive assurance without examining pollinator activity. Because ecological, genetic and historical factors, interact in a complex fashion, a strictly correlative approach is unlikely to enable isolation of the specific selective forces governing changes in mating system. Studies proposing reproductive assurance as a factor in the evolution of self-fertility often lack quantitative estimates of pollinator abundance, floral visitation, pollen limitation, inbreeding depression and selfing rates, and a sound knowledge of floral biology, all of which would seem necessary to demonstrate that reproductive assurance may have been important in the evolution of self-fertility (but see Lloyd 1965, Rick et al. 1978, Piper et al. 1986, Wyatt 1986, Barrett 1988b, Husband and Barrett 1992, and references within). For example, the reproductive assurance hypothesis implies that the major selective force favouring selfing is strong pollen limitation. However, self-fertility may be advantageous with weak pollen limitation and strong inbreeding depression, depending on the timing of self-pollination relative to cross-pollination (i.e. delayed selfing, Lloyd 1979, 1992).

The theoretical literature on the evolution of plant mating systems is substantial, and has examined many genetic and ecological factors influencing the selection of self-fertility (reviewed by Lande and Schemske 1985, Holsinger 1992, D. Charlesworth et al. 1990, Lloyd 1992, Uyenoyama et al. 1993, Lande et al. 1994). However, there is now a plethora of models but too few data are yet available from natural populations to test such models.

In this study, I examine aspects of the ecology and evolution of self-fertility in natural populations of *Blandfordia grandiflora* R. Br. (Liliaceae). The overall aims were to determine the factors that may have been important in the evolution of self-fertility in tableland populations of *B. grandiflora*. In particular, I was interested in determining the potential importance of inbreeding depression and reproductive assurance. This study of the breeding systems of tableland and coastal populations of *B. grandiflora* was motivated by my

observations of greater seed set per fruit for tableland plants than coastal plants, despite the apparent paucity of avian pollinators in the tableland habitats. I used a largely comparative approach and examined aspects of the present-day reproductive ecology of self-fertile tableland and self-infertile coastal populations of *B. grandiflora* to gain insight into the evolution of self-fertility. Intraspecific studies, such as the present study, are desirable because interpopulation variation in the genetic background of the populations is minimized. The more genetically similar the populations being compared, the greater the confidence that the observed differences are the result of the breeding system and historical factors rather than other unmeasured genetic factors (Holtsford and Ellstrand 1990).

I have presented seven data chapters in this thesis. The first three of these provide background data that address questions concerning differences in the breeding systems, floral morphology and sex allocation patterns between tableland and coastal populations. The remaining chapters address the questions of pollinator availability, inbreeding depression and reproductive assurance. This overall approach allowed me to quantify the differences in breeding systems and then to assess the importance of inbreeding depression and reproductive assurance in the evolution of self-fertility in the tableland populations.

To accomplish the aims of the present study, I examined the following aspects of plant reproductive biology and ecology.

(1) I examined the breeding systems of tableland and coastal populations in Chapter 2 and addressed the following questions:

- (a) Are tableland plants self-fertile and, if so, to what extent?
- (b) Are tableland plants autonomously self-pollinating?
- (c) Are coastal plants self-infertile?
- (d) What is the mechanism that prevents self-fertilization, and how does it break down to allow self-fertilization and selfed seed set?

(2) Changes in breeding systems are often associated with changes in floral morphology, floral biology and resource allocation to sexual functions (e.g. Lloyd 1965, Ornduff 1969, Wyatt 1988). In Chapter 3, I compared tableland and coastal populations and addressed the following questions:

- (a) Do floral morphologies differ?
- (b) Do the allocation patterns to male and female functions differ and do they conform to patterns proposed by sex allocation theory (e.g. Charnov 1987)?
- (c) Do pollen longevity and onset and duration of stigmatic receptivity differ?

(3) In Chapter 3, I compared tableland and coastal populations at similar latitudes.

Tableland populations, however, are restricted to a relatively small area compared to the main range of *B. grandiflora* which is predominantly coastal. In Chapter 4, I compare ten tableland and coastal populations spanning about 90% of the species' range to address the following questions:

- (a) What is the extent of differentiation in reproductive and vegetative characters (i) among coastal populations,

- (ii) among tableland populations, and
- (iii) between tableland and coastal populations?

(4) Many self-infertile plants depend upon pollinators for successful reproduction, and consequently the abundance and behaviour of pollen vectors can become an important ecological factor influencing seed production. If the paucity of avian pollinators was a factor promoting selfing in the tableland population, the exclusion of these animals should have a major effect on the reproduction of self-infertile coastal plants. In Chapter 5, I examined pollen limitation of seed set and availability of avian pollinators in self-infertile coastal populations, and addressed the following questions:

- (a) Is seed production pollen-limited?
- (b) Does pollen limitation and availability of avian pollinators vary within and/or among flowering seasons?
- (c) What are the causes and possible consequences of pollen-limitation?

(5) Self-fertile plants may be pollen-limited. Although the quantity of pollen deposited on stigmas may be important, pollen quality may also be important if some self-fertilized ovules are aborted thereby preventing cross-fertilization. In Chapter 6, I examined pollen limitation of seed set in self-fertile tableland populations, and addressed the following questions:

- (a) Is seed production pollen-limited?
- (b) Does pollen limitation vary within and/or among flowering seasons?
- (c) Is pollen limitation caused by the quantity or quality of pollen deposited onto stigmas?
- (d) What are the possible consequences of pollen-limitation?

(6) A major factor thought to prevent the evolution of self-fertility is inbreeding depression. However, self-fertility can be advantageous despite high inbreeding depression if it ensures reproduction. In Chapter 7, I examined the magnitude of inbreeding depression, the selfing rate (average frequency of self-fertilization, Lloyd 1980) and the availability and activity of avian pollinators in self-fertile tableland populations, and addressed the following questions:

- (a) What is the magnitude of inbreeding depression at different life-cycle stages and the magnitude of the cumulative inbreeding depression?
- (b) Is the magnitude of the cumulative inbreeding depression sufficient to prevent the automatic selection of self-fertility?
- (c) What is the selfing rate at different stages in the life cycle?
- (d) Are avian pollinator abundance and floral visitation sufficiently low that reproductive assurance was a major factor promoting self-fertility?

(7) An important consideration in the evolution of self-fertility is how and when self-pollination occurs (Lloyd 1979, 1992). Self-fertile tableland plants do not self-pollinate autonomously (Ramsey et al. 1993, Chapter 2), suggesting that if

reproductive assurance was an important factor in the evolution of self-fertility, self-pollination must have been mediated by other means. When other pollinators are excluded from plants, worker ants of an unidentified *Iridomyrmex* species visit flowers for nectar. However, ant pollination is rare and some ants are known to produce antibiotic substances that inhibit pollen function (Beattie et al. 1984, Peakall et al. 1991). In Chapter 8, I examined the possible role of ants in the evolution of self-fertility, and addressed the following questions:

- (a) Is pollen function inhibited if it has been in contact with ants?
- (b) Do ants pollinate flowers and, if so, do they effect self-pollination or cross-pollination, or both?
- (c) What are the possible evolutionary consequences of ant pollination?

Throughout this thesis I use the terms self-fertility and self-infertility instead of self-compatibility and self-incompatibility, respectively. These latter terms imply that the genetic mechanism preventing selfing is known (e.g. gametophytic SI and sporophytic SI). In *B. grandiflora*, the mechanism is unknown and, although embryonic lethality is a strong possibility (see Ramsey et al. 1993, Chapter 2), until it is determined I prefer the use of the less specific terms. I define self-infertility as the inability of a fertile hermaphroditic plant to produce viable seeds following self-pollination when the genetic mechanism preventing selfing is not known. I use self-infertility instead of self-sterility to be consistent with the use of self-fertile. Also, self-sterility has a wider meaning and can also imply that a failure to produce viable seed is caused by either chromosomal abnormalities, or some form of physiological problem affecting gamete formation or embryo development (Williams 1964).

Dahlgren et al. (1985) propose that *Blandfordia* spp. warrant separate family status, the Blandfordiaceae. Consistent with Henderson (1987), however, I have considered *Blandfordia* as a member of the family Liliaceae.

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Chapter 2

Breeding Systems of Disjunct Populations of Christmas Bells (*Blandfordia grandiflora* R.Br., Liliaceae) : Variation in Self-fertility and an Ovular Mechanism Regulating Self-fertilisation.

(Australian Journal of Botany 41 : 35-47)

Chapter 3

Floral morphology, biology and sex allocation in disjunct populations of Christmas bells (*Blandfordia grandiflora*, Liliaceae) with different breeding systems.

(Australian Journal of Botany 41: 749-762)

Chapter 4

Geographic variation in morphological and reproductive characters of coastal and tableland populations of *Blandfordia grandiflora*, Liliaceae.

(Plant Systematics and Evolution 192: 215-30)

Chapter 5

Causes and consequences of seasonal variation in pollen limitation of seed production in *Blandfordia grandiflora* (Liliaceae)

(Oikos 72, in press)

Chapter 6

Ovule pre-emption and pollen limitation in a self-fertile perennial herb
(Blandfordia grandiflora, Liliaceae)

(Oecologia, in press)

Chapter 7

Inbreeding depression, selfing rates and pollinator availability in a partially self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae).

Chapter 8

Ant pollination, reproductive assurance and evolution of self-fertility in the perennial herb *Blandfordia grandiflora*, Liliaceae.

(Oikos: in review)

Chapter 9

General Conclusion

Conclusion

One of the major pathways of breeding system evolution in flowering plants is the change from predominant outcrossing, enforced by physiological self-incompatibility, to predominant or partial self-fertilization (Stebbins 1957, 1974, Grant 1975, Jain 1976, de Nettancourt 1977, Lloyd 1980, Wyatt 1983, 1988, Richards 1986, Barrett 1988, Rick 1988). This type of evolutionary change in breeding system has probably occurred in *Blandfordia grandiflora* R. Br. (Liliaceae); tableland populations of *B. grandiflora* are partially self-fertile whereas coastal populations, the nearest of which are only about 95 km distant, are self-infertile (Ramsey et al. 1993, Chapter 2). In this study, I quantified the differences in the breeding systems and examined the potential consequences with regard to morphology, allocation patterns and seed production (Ramsey et al. 1993, 1994, Ramsey 1993, Chapters 2, 3, & 4). I then investigated ecological and genetic factors that may have influenced the evolution of self-fertility (Ramsey 1995a, b, c, Chapters 5, 6, 7, & 8). The major findings of this study suggest that pollinator limitation due to the paucity of avian pollinators and subsequent selective pressure for reproductive assurance were the most likely factors favouring the evolution of self-fertility in the tableland populations of *B. grandiflora*. Inbreeding depression is too high to facilitate the automatic selection of self-fertility (e.g. Fisher 1941), suggesting that an adaptive explanation is required. At coastal sites, *B. grandiflora* plants are self-infertile, and nectarivorous birds frequently visit flowers and are the major pollinators. In contrast, at tableland sites, plants are self-fertile, and nectarivorous birds are in low abundance and infrequently visit flowers. However, although self-fertile, tableland flowers do not self-pollinate autonomously. Worker ants of an undescribed *Iridomyrmex* species may have self-pollinated flowers in the past. Such self-pollination would have favoured self-fertile plants, without changes in floral morphology that permit autonomous self-pollination but may prevent occasional outcrossing and the associated reproductive benefits.

Self-fertility is generally considered to be a trait derived from a condition of predominantly outcrossing that is enforced by physiological self-incompatibility (Stebbins 1957, 1974, Grant 1975, Jain 1976, de Nettancourt 1977, Lloyd 1980, Wyatt 1983, 1988, Richards 1986, Barrett 1988, Rick 1988). In the present study, the mechanism preventing the production of selfed progeny in self-infertile coastal populations could not be determined. In these populations, self pollen tubes enter ovules at a rate that is indistinguishable from cross pollen tubes (Ramsey et al. 1993, Chapter 2). One explanation of these findings is that late-acting self-incompatibility allows ovules to be penetrated by self pollen tubes but prevents fertilization (Seavey and Bawa 1986). For example, in *Acacia retinodes*, self pollen tubes are arrested in the nucellus (Kenrick et al. 1986). Another explanation is that self-fertilization occurs, but selfed ovules are aborted due to high genetic load that is expressed when recessive lethal genes become homozygous (i.e. mutational load not segregational load, cf. Weins et al. 1997; Bawa et al. 1989, Charlesworth 1989). Critics of this hypothesis argue that the genetic load would have to be unrealistically high to maintain self-infertility such that most selfed zygotes are aborted (Kenrick and Knox 1986, Waser and Price 1991). However, such

criticisms are often based on methods of calculating the genetic load that assume that all embryonic deaths are caused by genetic factors (e.g. Koski 1971, Waser and Price 1991). Consequently, they overestimate the number of lethal equivalents (Savolainen et al. 1992) that are required to maintain high levels of self-infertility. Recent models that account for both genetic and environmental mortality provide substantially lower estimates (Savolainen et al. 1992), suggesting that the genetic load required to maintain self-infertility may not have to be unrealistically large. Lower estimates of lethal equivalents do not confirm the role of embryonic lethals in determining self-infertility (see Charlesworth 1989), although it does render such a hypothesis plausible and thus warrants further study of the relationship between inbreeding depression and self-infertility (e.g. Burbidge and James 1991). In self-infertile populations of *B. grandiflora*, cytological studies will be needed to determine whether late-acting self-incompatibility or early-acting inbreeding depression prevents the production of selfed seeds (e.g. Kenrick et al. 1986, Manasse and Pinney 1991; see Ramsey et al. 1993, Chapter 2). Experiments examining heritability and inheritance patterns of self-fertility (e.g. Antonovics 1968, Robacker and Ascher 1982) would also provide useful information, although long-term studies are required because plants grown from seed take 3-4 yr to flower.

In self-fertile populations, I have proposed that the reduction in selfed seed set compared to crossed seed set is due to inbreeding depression based on the criteria proposed by Seavey and Bawa (1986) (Ramsey et al. 1993, Chapters 2 & 7). However, even if reduced selfed seed set is due to "partial" late-acting self-incompatibility, the main conclusions of the study would not be affected. I recalculated the relative fitness of selfing excluding seed set, but including zygote survival which was determined as the proportion of sound seeds divided by the number of sound seeds plus the number of detected aborted seeds. Thus, zygote survival omits unfertilized ovules and aborted embryos that could not be detected. Relative self performance for zygote survival was 0.89 ± 0.02 , and is thus a conservative estimate of fitness compared to relative self performance for seed set (i.e. 0.56 ± 0.04 , Table 1, Chapter 7). The mean cumulative relative fitness of selfing of the plants described in Chapter 7 was calculated as the product of the relative self performances for percent zygote survival, percent seed germination, seedling dry weight at 365 d and percent seedling survival, and was 0.50 ± 0.03 ($N = 20$). Mean cumulative inbreeding depression was 0.49 ± 0.03 . Details of how relative fitness and inbreeding depression were calculated are provided in Chapter 7. This estimate of inbreeding depression was compared to 0.50, the threshold value of inbreeding depression below which increased selfing should evolve (i.e. $\delta = 0.50$; Fisher 1941, Lande and Schemske 1985, Charlesworth and Charlesworth 1987), and was found not to differ significantly ($t_{18} < 1.0$, $P > 0.50$; t-test for the comparison of a single observation with the mean of a sample, Sokal and Rohlf 1981). Also, this value of inbreeding depression is most certainly an underestimate as discussed in detail in Chapter 7. Actual inbreeding depression would exceed $\delta = 0.50$, and thus, substantiate the conclusions of this study concerning the evolution of self-fertility (see Chapter 7).

Evolutionary shifts in breeding systems from self-infertility to self-fertility are often accompanied by changes in reproductive attributes such as floral morphology and biology, and allocation patterns to pollinator attraction (corolla and floral nectar) and male (pollen) and female (ovules and seeds) functions. In general, flowers become smaller, herkogamy and dichogamy become less pronounced, allocation to structures or substances attracting pollinators is reduced, and allocation to male and female functions is decreased and increased, respectively. Such changes are most pronounced in plants that are autonomously self-pollinating (reviewed by Ornduff 1969, Cruden 1977, Charnov 1987, Richards 1986, Wyatt 1988, Charlesworth and Morgan 1991). Variation in the breeding system between self-fertile tableland and self-infertile coastal populations of *B. grandiflora* was not associated with changes in flower size and morphology, floral nectar production, or changes in herkogamy and dichogamy. Of particular interest, self-fertile plants did not exhibit changes to floral traits permitting autonomous self-pollination (Ramsey 1993, 1995c, Ramsey et al. 1993, 1994, Chapters 2, 3, 4 & 8). In contrast, breeding system differences were associated with differences in patterns of investment to reproduction, although these patterns were not clear-cut (Ramsey 1993, Ramsey et al. 1994, Chapters 3 & 4). There were no differences between self-fertile and self-infertile populations in relative biomass of individual floral parts, relative male biomass and relative biomass of corollas and floral nectar. However, absolute biomass of flowers and floral parts was greater for coastal plants. These biomass differences, but the lack of differences in flower size, may reflect selection for robust flowers in the coastal populations. Flowers may have to be more robust to withstand the increased handling by nectarivorous birds since birds visit flowers much more frequently in coastal than in tableland populations (Fig 1 this chapter, Ramsey 1995a, b, Chapters 5, 6 & 7).

Consistent with sex allocation theory (e.g. Charnov 1987), self-fertile tableland flowers allocated more resources to female function (numbers of ovules and seeds) and less to male function (pollen) than did self-infertile coastal flowers (Ramsey 1993, Ramsey et al. 1994, Chapter 3 & 4). These trends may represent an early (or intermediary) evolutionary stage between self-fertility and autonomous selfing in the tableland plants. This hypothesis suggests that once self-fertility evolves, it inevitably results in autonomously self-pollinating plants that are independent of pollinators for successful seed production (Stebbins 1974, Bull and Charnov 1985, Lande and Schemske 1985, Richards 1986). Alternatively, these trends may be a consequence of stabilizing selection for reproductive characters. Flower size and morphology in the self-fertile tableland populations may be maintained due to the reproductive advantages caused by occasional outcrossing effected by nectarivorous birds (Chapter 7) and reproductive assurance caused by ants self-pollinating flowers (Ramsey 1995c, Chapter 8). The differences in pollen and ovule production per flower may be the consequence of selection to optimize male and female reproductive success under the different pollination conditions that are found in tableland and coastal populations. The similarities of flower size and morphology, but the differences in pollen and ovule production, suggest that these traits are genetically independent, and thus able to respond to different

selective factors. To examine this hypothesis, interpopulation crossing studies are required to determine the quantitative genetics of the floral characters, and to estimate the phenotypic and genetic correlations between these floral traits (e.g. Shore and Barrett 1990, Conner and Via 1993). Such studies, however, will require patience because of the lengthy period between seed production and first flowering. Electrophoretic analyses may provide useful information concerning the extent of genetic divergence and the phylogenetic relationships between tableland and coastal populations (e.g. Olmstead 1990, Allen et al. 1991), although they will be less useful in examining specific selective hypotheses.

Coastal *B. grandiflora* plants were larger, produced more flowers and allocated more dry matter to reproduction than tableland plants (Ramsey et al. 1994, Chapter 4). This is consistent with predictions that outcrossing plants are larger than selfing plants because selection favours larger floral displays to attract pollinators, and the size and weight of vegetative structures increases as a correlate of increased reproductive biomass allocation (Wyatt 1984b). However, other factors may also influence plant size. The smaller size of self-fertile tableland plants may reflect increased levels of homozygosity (i.e. inbreeding depression) due to enforced inbreeding (Falconer 1989). As with most native plants, the history of inbreeding and hence the importance of this factor in self-fertile tableland populations of *B. grandiflora* is not known. Another explanation is that size differences are not causally related to differences in breeding systems, but are related to local environmental conditions. For example, in populations of the *Arenaria uniflora* complex, vegetative characters of populations did not reflect differences in breeding system, but reflected the geographic proximity of populations to each other (Wyatt 1984a). In *B. grandiflora*, the observed plant size differences may reflect either local adaptation to, or physiological constraints imposed by the different environmental conditions at the coastal and tableland sites. However, plant size probably has a genetic basis as evidenced by the results of common garden and reciprocal transplant experiments (Ramsey et al. 1994, Chapter 4), suggesting that local adaptation or the negative effects of inbreeding in the tableland populations may be the major factors affecting plant size. Heritability studies of plant size would provide useful data to decide between these alternatives; the difficulties of such studies have been previously mentioned. Electrophoretic analyses determining the extent of genetic variation in the different populations and large scale reciprocal transplant experiments would provide insight into the importance of past inbreeding and local adaptation, respectively.

The major conclusion of this study is that pollinator limitation and subsequent selective pressure for reproductive assurance were the most likely factors favouring the evolution of self-fertility in the tableland populations. Inbreeding depression is too high to facilitate the automatic selection of self-fertility (e.g. Fisher 1941), and hence an adaptive explanation is required (Chapter 7). The prevention of gene flow to preserve local adaptation (e.g. Antonovics 1968, Lefebvre 1970) is an unlikely factor in *B. grandiflora* because populations are disjunct and gene flow between them is unlikely (Ramsey et al. 1994, Chapter 4). Floral morphology and nectar production in tableland and coastal populations indicate

that nectarivorous birds should be the major pollinators (Ramsey 1993, Ramsey et al. 1994, Chapters 3 & 4). However, although such birds are important pollinators of *B. grandiflora* plants at coastal sites, they are in low abundance and infrequently visit flowers at tableland sites (Ramsey 1995a, Chapters 5 & 7). The differences during the flowering season in bird abundance, floral visitation and seed set from Chapters 5 and 7 are summarized in Fig. 1. Differences in bird abundance and floral visits were significantly greater in coastal than tableland populations. Depending on the month within the flowering season, abundance ranged from 6-37 times greater and visitation ranged from being the same to 24 times greater in the coastal populations (Fig. 1A, B). In self-infertile coastal populations, seed set was variable during the flowering season (Fig. 1C). In these populations, seed set and floral visits by birds were positively correlated, and when birds switched their foraging from *B. grandiflora* in February and March or when birds were experimentally excluded from flowers in January, seed set become severely pollen-limited (Fig. 1C; Ramsey 1995a, Chapter 5). These findings indicate the importance of nectarivorous birds to the reproductive success of self-infertile plants. In contrast, seed set of self-fertile tableland plants did not differ during the flowering season (Fig. 1C). The benefit of self-fertility is apparent by comparing the seed set of tableland and coastal plants during February and March. During these months, introduced honeybees are the major floral visitors and floral visitation is similar in both populations (M. Ramsey unpubl.). Seed set in the self-fertile tableland populations was almost 4-fold greater, indicating that self-fertility increases seed set and provides reproductive assurance under present-day natural conditions (Fig. 1C).

As discussed above, self-fertility is considered a derived trait (Stebbins 1957, 1974. Grant 1975, Jain 1976, de Nettancourt 1977, Lloyd 1980, Wyatt 1983, 1988, Barrett 1988. Rick 1988), indicating that ancestral *B. grandiflora* plants of present-day tableland populations would have been self-infertile. As demonstrated by the natural and experimental exclusion of nectarivorous birds from self-infertile coastal plants (Ramsey 1995a, Chapter 5), the paucity of nectarivorous birds could cause strong selection for reproductive assurance. These findings suggest that in ancestral self-infertile tableland populations, pollen limitation caused by the paucity of nectarivorous birds would favour self-fertile individuals. Such selection would depend on the presence of genetic variation among individuals for self-fertility. Present-day coastal populations of *B. grandiflora* are self-infertile, although variation among plants for low levels of self-fertility is present (Ramsey et al. 1993, Chapter 2). If ancestral self-infertile tableland populations exhibited similar variation and such variation was genetically determined, self-fertile individuals would have been favoured by the paucity of avian pollinators and selection for reproductive assurance, providing the flowers of such plants were self-pollinated. Present-day tableland flowers do not self-pollinate autonomously (Ramsey et al. 1993. Ramsey 1995c, Chapter 2 & 8). However, worker ants of an unidentified *Iridomyrmex* species are capable of self-pollinating flowers, and in the absence of other pollen vectors in the ancestral self-infertile tableland populations, they may have been the major floral visitors and pollinators (Ramsey 1995c, Chapter 8). In the absence of nectarivorous

birds and cross-pollination, self-pollination effected by ants would have favoured self-fertile individuals by providing reproductive assurance. Such self-pollination may have also prevented selection for changes in floral morphology allowing autonomous self-pollination. Morphological changes allowing autonomous self-pollination may not have been selectively favoured because they may reduce or prevent occasional floral visits by nectarivorous birds, thereby reducing the reproductive benefits that accrue from outcrossing. The lack of mechanisms that allow delayed autonomous self-pollination is surprising, since this mode of selfing is always advantageous, providing some selfed progeny survive and reproduction in subsequent seasons is unaffected (Lloyd 1979, 1992). As suggested by Lloyd (1992), such mechanisms may not be present due to genetic constraints and the lack of such selfing variants in the populations. Presumably, the high levels of inbreeding depression experienced by present-day selfed progeny were present in the past. This may have hindered, but would not have prevented, the selection of self-fertility, providing that selfing rates were high and some progeny survived to reproduce (Chapter 7; Lloyd 1979, 1992).

I have proposed that past selection has favoured self-fertility in tableland populations of *B. grandiflora*. However, the present-day selective regime is probably quite different, due to the presence of the introduced European honeybee. At tableland sites, honeybees are now the major pollinators of *B. grandiflora* (Ramsey 1995b, Chapter 6). Honeybees forage at flowers for either pollen or nectar, but rarely both. Pollen-collecting bees visit newly open flowers and deposit both self and cross pollen, resulting in self- and cross-fertilizations. Self-fertilization reduces fitness because some of the selfed ovules are aborted due to inbreeding depression and selfing pre-empts ovules which could be fertilized by later arriving cross pollen. Such cross pollen is deposited by nectar-collecting bees which visit older flowers after they have been visited by pollen-collecting honeybees. These findings suggest that selection should favour floral traits that promote outcrossing since, if self-pollination can be reduced, seed set and seed quality can be increased (Ramsey 1995b, Chapter 6 & 7). Increased outcrossing should lead to mating patterns that are more random, resulting in lower rates of selfing and purging of deleterious genes, which, in turn and over time, will result in higher levels of inbreeding depression and lower levels of self-fertility (Chapter 7; Barrett and Charlesworth 1991, Lande et al. 1994).

The main conclusion of the present study, as with most evolutionary hypotheses, is speculative. One of the problems associated with studies of adaptation is whether the particular trait under consideration evolved under selective pressures and ecological conditions that are similar to those observed at present, or whether it evolved under different circumstances. While recognizing this caveat, the overall findings of the present study suggest that pollinator limitation due to the paucity of avian pollinators and subsequent selective pressure for reproductive assurance were the most likely factors favouring the evolution of self-fertility in the tableland populations of *B. grandiflora*. Future research examining this hypothesis should concentrate on the genetic aspects of the study that I have referred to throughout this thesis. Crossing experiments to determine the heritabilities of reproductive

traits and the genetic correlations between these traits will provide interesting data on the availability of variation on which selection can act and whether selection will act independently on different traits. Unfortunately, the life-cycle of *B. grandiflora* is not conducive to such experiments, and long-term studies will be required. Reciprocal transplant experiments could be used to consider adaptive hypotheses concerning the geographic variation found within the species for reproductive and vegetative traits. In the present study, electrophoretic studies examining the mating systems and the genetic diversities of the self-fertile and self-infertile populations could not be undertaken because the necessary facilities were unavailable and financial resources were insufficient. Such studies should be undertaken because they provide important baseline genetic information and play an integral role in elucidating the genetic differences between self-fertile and self-infertile plants.

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Fig. 1. Comparison between self-fertile tableland and self-infertile coastal populations of *Blandfordia grandiflora* for (A) number of nectarivorous birds on transects, (B) number of visits per flower by birds, and (C) percent seed set, over the 1989-1990 flowering season from December to March. Data are means (\pm SE), and are adapted from Fig. 4 in Chapters 5 & 7. Specific methods of data collection are provided in these chapters. Each month, transects and flower patches were monitored for 3 d. and percent seed set was assessed from 41-52 fruits. The populations shown (SC, tableland; SUB, coast) are representative of the other populations that were examined. The numbers of birds per transect and visits per flower were compared between populations and within months using nonparametric H-tests (Meddis 1984), since the data did not conform to the assumptions of parametric statistics. No visits to flowers occurred in March. For (A) and (B), bars within months with different letters differed significantly ($P < 0.05$). Percent seed set was compared using a two-way, model 1 ANOVA (unweighted means analysis; Winer et al. 1991) with month and populations as factors. Because the population \times month interaction was significant ($F_{3,375} = 13.77$, $P < 0.001$), I tested the significance of the simple main effects rather than the overall main effects (Winer et al. 1991). In this analysis, seed set of populations differed within all months (all $P < 0.001$). Seed set among months within populations did not differ in the tableland population ($P = 0.25$), but differed significantly in the coastal population ($P < 0.001$). In the coastal population, differences among the months were then determined with SNK tests. For (C), bars with different letters differed significantly ($P < 0.05$).

