

**Ecology and evolution of self-fertilization in a perennial herb,
Blandfordia grandiflora R. Br. (Liliaceae)**

by

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that the help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Mike Ramsey
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Preface

I have presented this thesis as a series of seven independent but interrelated papers (Chapters 2-8), a general introduction and statement of aims (Chapter 1), and a final, integrating conclusion (Chapter 9). Five of the chapters have been published or are in press (Chapters 2-6). Chapter 8 has been submitted for publication and Chapter 7 will be submitted soon after the thesis has been submitted. Minor changes have been made to "update" some of the published papers. The style of the chapters changes slightly throughout the thesis in accordance to the editorial requirements of the journals to which they were submitted. Some repetition is inevitable in a thesis presentation such as this because methods (e.g. study site and study species) and references are included in each chapter. Tables and figures are located after the references in each chapter.

Electrophoretic studies of the mating systems and the genetic diversities of the self-fertile tableland and the self-infertile coastal populations were initially planned for my doctoral project. My project would have benefitted greatly by such studies. However, these studies could not be undertaken during my PhD candidature because the necessary facilities were not available and financial resources were insufficient to undertake such studies elsewhere. Genetic studies, to be undertaken elsewhere, are currently being planned, but will depend upon the availability of and my ability to obtain a postdoctoral fellowship.

Summary

In this study, I investigated the breeding systems of tableland and coastal populations of the perennial herb *Blandfordia grandiflora* R. Br. (Liliaceae). Tableland plants were self-fertile and coastal plants were self-infertile, and I used a largely comparative approach to gain insight into the ecology and evolution of self-fertility in the tableland populations.

Specifically, for tableland and coastal populations I examined the breeding systems, floral morphology and patterns of reproductive allocation, geographic variation in reproductive and vegetative characters throughout about 90% of the species range, availability of pollinators and pollen limitation, and in the tableland populations, the expression and magnitude of inbreeding depression. This approach allowed me to quantify the differences in breeding systems and then to assess the importance of inbreeding depression and reproductive assurance as factors in the evolution of self-fertility in the tableland populations.

The breeding systems of disjunct tableland and coastal populations of *B. grandiflora* were determined using hand-pollination experiments. In both populations, 90% or more of self-pollinated plants produced seeds. Tableland plants were significantly more self-fertile than coastal plants (relative self-fertility: tableland, 0.55 ± 0.03 ; coast; 0.08 ± 0.02). Significant variation among plants for self-fertility was found in both populations. Autofertility was low in both populations ($\leq 1.6\%$ seed set), indicating that pollen vectors are necessary for seed set. Seed set by agamospermy in both populations was less than 0.1%. Seed abortion was greater in self-pollinated plants than cross-pollinated plants in both populations. In both self- and cross-pollinated plants, seed abortion was twice as great in the coastal population than in the tableland population. No evidence was found for stigmatic or stylar self-incompatibility. Self and cross pollen adhered to and germinated equally well on stigmas in both populations (72 - 77% germination), and there were no differences between pollination treatments or populations in the percentage of ovules penetrated by pollen tubes (82 - 89%). When self-pollination preceded cross-pollination by 24 h or longer, seed set was significantly reduced compared to flowers that were cross-pollinated only, suggesting ovules were pre-empted by self pollen tubes. Collectively, these results strongly suggest that self seed set was reduced by a mechanism operating at the ovule level, such as early-acting inbreeding depression due to recessive seed-aborting genes, although incomplete late-acting self-incompatibility cannot be completely ruled out. For coastal plants, this mechanism largely prevents the production of selfed seeds, indicating plants were predominantly outcrossing. For tableland plants, substantial seed set by selfing may occur under natural conditions.

Evolutionary shifts in breeding systems are often accompanied by changes in reproductive attributes such as floral morphology and biology, and pre- and postzygotic allocation patterns. The effects of breeding system variation on several such attributes were examined in self-fertile tableland and outcrossing coastal populations of *B. grandiflora*. In general, overall flower size was similar in both populations, although pedicel and pistil stipe diameters were greater in coastal plants, and pistil length and stigma-anther separation were

greater in tableland plants. Floral parts from coastal flowers weighed more, but proportional biomass allocation to floral parts was similar in both populations. Daily nectar production per flower was similar in both populations. Tableland flowers produced more ovules but fewer pollen grains than did coastal flowers and pollen : ovule ratios differed significantly (P:O ratios: coast, 11500; tableland, 5600). Open-pollinated tableland fruits produced more seeds than coastal fruits, but individual seeds weighed less. Total seed biomass of tableland fruits was greater than coastal fruits. Prezygotic relative male biomass and relative male biomass that were estimated from stamen and seed weights did not differ significantly between populations. Similarly, relative biomass allocation to pollinator attraction (i.e. corolla and nectar) did not differ between populations. The onset and duration of stigmatic receptivity and pollen longevity of flowers from the two populations were similar. The large differences in ovule, seed and pollen production supports sex allocation theory which predicts that in self-fertile plants resource allocation should be female biased whereas in outcrossing plants, allocation should be male biased. The similarity between the *B. grandiflora* populations in other aspects of floral morphology, allocation patterns and biology suggests that changes in ovule and pollen production precede changes in other traits during the evolution of autonomous selfing. Overall, these findings suggest that (a) self-fertility has evolved in tableland plants only recently and selection has had insufficient time to change floral traits, or (b) self-fertility is not recent and floral traits promoting outcrossing have been maintained by selection, imposed by inbreeding depression and/or overdominance.

For ten *B. grandiflora* populations spanning about 90% of the species' range, univariate and multivariate analyses on 14 vegetative and reproductive characters separated plants into distinct coastal and tableland groups. Distinguishing characters were number of flowers and leaves, leaf length and width, and inflorescence stalk height and diameter; coastal plants were larger than tableland plants. In transplant and common garden experiments, coastal and tableland plants retained their phenotypic distinctness, suggesting that differences in vegetative morphology were genetically determined and not the result of phenotypic plasticity due to differences in environmental factors. Coastal plants exhibited clinal variation with latitude; in general, plants from northern populations were marginally larger than plants from southern populations. Compared to tableland plants, coastal plants had greater pollen : ovule ratios, and produced fewer but heavier seeds per flower. Tableland and coastal plants are phenotypically distinct, indicating that separate subspecific status may be warranted.

Natural seed set was consistently pollen-limited for 5 yr in coastal *B. grandiflora* populations. The extent of pollen limitation varied within seasons, depending on when individual plants flowered. In all years, percent seed set was greatest in January (peak flowering, 36 - 41%) and least in March (end of flowering, 12 - 14%). Seed set of hand-pollinated fruits did not differ within flowering seasons (46 - 52%), indicating that pollination factors caused natural seed set to vary. Variation in natural seed set was positively correlated with frequency of visits to flowers by nectarivorous birds, but not their abundance in the area. Bird abundance increased during the flowering season, but floral visits decreased. *B.*

grandiflora flowers received about 2 visits in December, 3 in January, and < 1 in February and March. *Banksia serrata* plants also flowered in February and March and competed for bird pollinators with *B. grandiflora*. In these months, birds preferentially foraged at *Ba. serrata* inflorescences which produced about 10-fold more nectar per 24 h than *B. grandiflora* inflorescences. The consistently greater natural seed set in January suggests that optimizing selection would favour plants that flower in this month.

In self-fertile tableland populations of *B. grandiflora*, natural seed set was consistently pollen-limited for 2 yr. Percent seed set of open-pollinated plants (50 - 57%) did not differ within or between years, and was about 19% less than experimentally cross-pollinated plants (70 - 75%). Floral visits by introduced honeybees, the most common visitors, did not differ through the flowering season and the number of pollen grains deposited on stigmas within 12 h of flowers opening exceeded the number of ovules per flower, indicating that the quality rather than the quantity of pollination limited seed set. Pollen limitation was caused by concurrent self and cross pollination and the subsequent abortion of some selfed ovules due to inbreeding depression. Natural seed set (55%) was intermediate between selfed (43%) and crossed (75%) flowers and was not increased when flowers that had been available to pollinators for 24 h were cross-pollinated by hand, suggesting that ovules were already fertilized. Similarly, experimental pollination with both cross and self pollen within 24 h of flowers opening did not increase seed set relative to natural seed set, indicating that both cross and self fertilizations had occurred. In contrast, when selfing followed crossing by 48 h, or vice versa, seed set did not differ from crossed only or selfed only flowers, respectively, indicating that ovules were pre-empted by the first pollination. Collectively, these results indicate that under natural conditions self pollen pre-empts ovules, rendering them unavailable for cross fertilization. This selfing reduces fecundity by 50%, as estimated from the natural production of cross seeds when selfing was prevented. Consequently, selection should favour floral traits such as increased stigma-anther separation or protandry that reduce interference between male and female functions that leads to selfing.

The magnitude of inbreeding depression and the availability of avian pollinators were examined to assess their importance in the evolution of self-fertility in tableland *B. grandiflora* populations. Inbreeding depression was estimated by comparing the performance of selfed and crossed progeny from field-pollinated plants. Seed set was greater and seed abortion was less after cross pollination than after self pollination. Seed weights did not differ. In glasshouse experiments, crossed progeny outperformed selfed progeny for seed germination, speed of germination, seedling dry weight, relative growth rate and survival. Cumulative inbreeding depression, estimated from seed set, seed germination, seedling dry weight and survival, was severe (0.61 ± 0.04 to 0.80 ± 0.07) and significantly greater than 0.50, the threshold level of inbreeding depression below which increased selfing should evolve. The zygote selfing rate was high (0.62), but differed significantly from 1.0, indicating a mixed mating system. The selfing rate decreased significantly to 0.22 - 0.32 at the seedling stage, suggesting that few selfed progeny survive. Further decreases in the selfing rate at later

life-cycle stages may prevent or slow the purging of recessive deleterious alleles. Low zygote selfing rates and high genomic mutation rates may maintain high inbreeding depression in *B. grandiflora*. The availability of avian pollinators was assessed for 3 yr in five different *B. grandiflora* populations. Floral morphology and nectar production strongly suggests pollination by nectarivorous birds. Seed set of caged plants from which nectarivorous birds were excluded did not differ from uncaged plants (both about 53%), indicating that birds are not important pollinators. Natural seed set was similar through the year (48.9-58.5%), and was predominantly due to introduced honeybees. The number of birds that were observed on transects (peak flowering in January, 0.6 - 2.3; end of flowering in March, 0.3 - 1.0), and the percentage of flowers that were visited by birds (January, 21%; March, 1%) were low. The number of birds on transects was correlated with floral visitation, suggesting that visitation would increase if birds were more numerous. The paucity of avian pollinators was probably an important factor in the evolution of self-fertility in tableland *B. grandiflora*, despite high inbreeding depression.

Worker ants of an unidentified *Iridomyrmex* sp. self-pollinated flowers of tableland *B. grandiflora* plants that are self-fertile but not autonomously self-pollinating. Self seed set of flowers that were visited by ants only (17%) was significantly greater than autonomous self seed set of flowers from which all pollinators had been excluded (< 1%), but was significantly less than seed set of hand self-pollinated flowers (40%). Seed set of emasculated flowers visited by ants was only 0.1%, indicating that ants do not cross-pollinate flowers. Differences in anther-stigma separation did not affect self seed set caused autonomously or by ants; seed sets of flowers with exerted or non-exserted stigmas were 1% for the autonomous treatments and 21% for the ant treatments. Ants possessed thoracic metapleural glands, suggesting that antibiotic secretions are produced which may inhibit pollen function. Although significantly fewer pollen grains germinated after thoracic contact compared to control pollen, the difference was only 6% (72.7% vs 78.7%). Seed set of flowers that were cross-pollinated with pollen that either had been in contact with ants or had not did not differ (78.3% vs 76.3%), suggesting that if secretions are produced, they only marginally affect pollen function. Pollen loads on ants were small; individuals carried about 28 pollen grains, 50% of which were on the legs away from potential metapleural gland secretions. Ants were observed more often on bagged flowers than unbagged flowers; introduced honeybees probably removed floral nectar before ants could recruit to unbagged flowers. Self pollination by ants may have been an important factor in the evolution of self-fertility because it would have provided reproductive assurance in the absence of avian pollinators. Also, ant pollination may have reduced selection for autonomous self-pollination, thus maintaining floral traits that promote outcrossing.

The overall findings of the present study suggest that the differences in the breeding systems between tableland and coastal populations of *B. grandiflora* are related to differences in the availability of avian pollinators. Abundance of and floral visitation by nectarivorous birds were high in coastal populations compared to tableland populations where both were

sufficiently low to adversely affect reproductive success. Experimental and natural exclusion of nectarivorous birds from flowering plants in coastal populations significantly decreased seed set by about three-fold. These findings are consistent with the hypothesis that reproductive assurance was an important selective factor in the evolution of self-fertility in tableland populations, despite inbreeding depression that significantly exceeded $\delta = 0.50$, the threshold value below which increased selfing is expected to evolve. Although self-fertility has evolved, floral traits that ensure autonomous self-pollination have not. Before the importation of honeybees, ants may have self-pollinated flowers, thereby providing reproductive assurance without changes in floral morphology that cause 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. Overall, these findings suggest that reproductive assurance was an important selective factor in the evolution of self-fertility.

Plate 1. Inflorescences of *Blandfordia grandiflora* from (A) a coastal population, and (B) a tableland population. Although flower size appears to be different, corolla length is similar in both populations (ca. 61 mm).

(A)



(B)



Plate 2. Flowering populations of *Blauforata grandiflora* from a (A) a coastal site, and (B) tableland site.

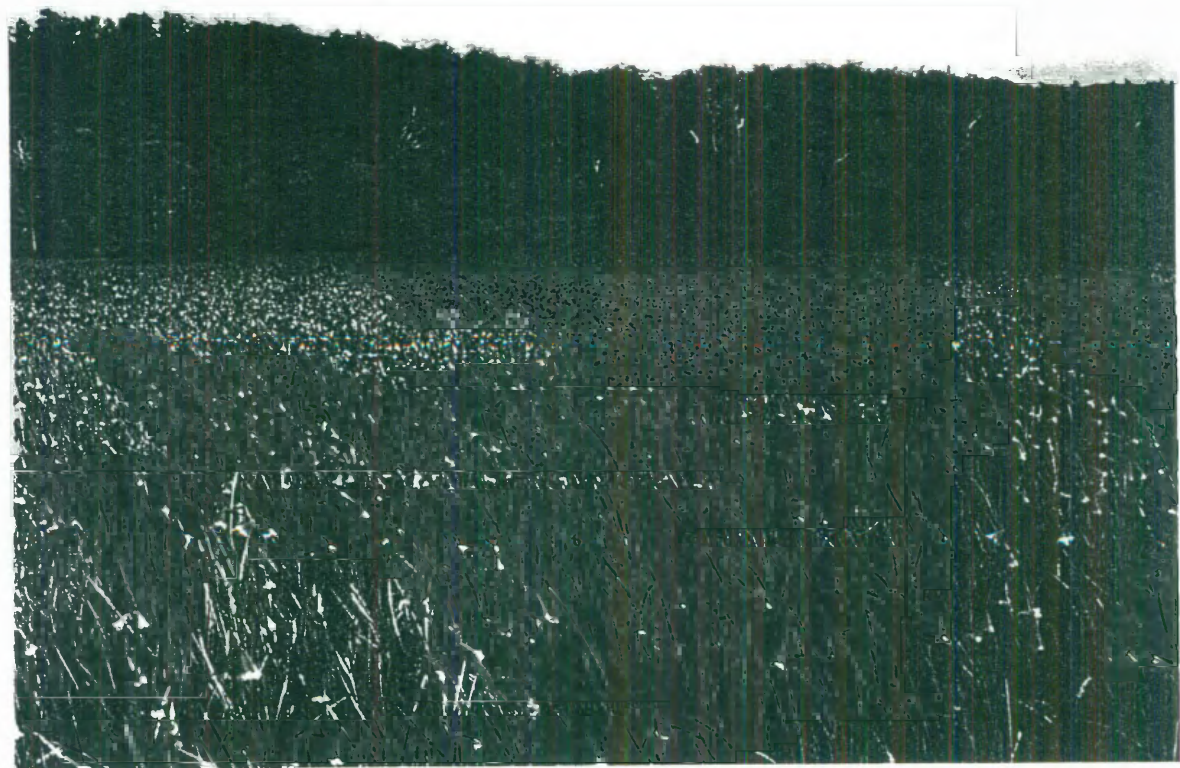


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