

Ant Pollination of the Perennial Herb *Blandfordia grandiflora* (Liliaceae)

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Ant pollination of the perennial herb *Blandfordia grandiflora* (Liliaceae)

Mike Ramsey

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Blandfordia grandiflora plants are self-compatible, but do not self-pollinate autonomously. Worker ants of an unidentified *Iridomyrmex* sp. self-pollinated flowers. Seed-set was about 17%, which was significantly greater than autonomous self-pollination ($< 1\%$), but less than experimental self-pollination (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 seed-set; seed-sets of flowers with exerted or included stigmas were 1% for autonomous self-pollination and 21% for ant-pollination treatments. Ants possessed thoracic metapleural glands, suggesting that antibiotic secretions that inhibit pollen function are produced. 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 significantly (78.3% vs 76.3%), suggesting that, if secretions are produced, they only marginally affect pollen function. Pollen loads of ants were small; individual ants carried about 28 pollen grains, 50% of which were carried on the legs and, hence, away from potential metapleural gland secretions. Ants were observed more often on bagged flowers from which introduced honeybees were excluded than on unbagged flowers; honeybees probably removed floral nectar from unbagged flowers before ants could recruit to them. In *B. grandiflora*, avian pollinators infrequently visit flowers. By assuring reproduction, ant-mediated self-pollination may have been an important factor in the evolution of self-compatibility, and may have reduced selection for autonomous self-pollination.

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Plant breeding systems are dynamic, as evidenced by the frequent transition from predominant outcrossing to selfing in related taxa (Stebbins 1950, Lloyd 1965, 1980, Ornduff 1969, Jain 1976, Rick 1982, Barrett 1988, Wyatt 1988). The evolution of selfing depends on a number of genetic and ecological factors (reviewed by Uyenoyama et al. 1993). An important ecological factor favouring the evolution of selfing is reproductive assurance; when pollinators are scarce or absent, selection should favour traits that assure reproductive success (e.g. Lloyd 1965, Rick et al. 1979, Schoen 1982, Piper et al. 1986, Wyatt 1986, Ramsey 1995a). Rick (1982) proposed three largely independent genetic phases in the

transition of breeding systems from outcrossing to selfing that permit self-fertilization, ensure autonomous self-pollination when pollinators are absent, and allocate resources from pollinator attraction and male functions to female functions. Not all self-compatible plants display all these changes, however, and some plants have large flowers that are apparently adapted for outcrossing and exhibit mixed mating systems (Cruden and Lyon 1989, Lloyd and Schoen 1992, Motten and Antonovics 1992). Although some of these plants self-pollinate autonomously (e.g. Schoen and Brown 1991, Dole 1992), others do not (Lloyd and Schoen 1992). These latter plants are of interest because they may provide insight

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into the factors affecting the evolution of self-compatibility and autonomous self-pollination.

I have examined factors that affect the evolution of selfing in the perennial herb *Blandfordia grandiflora* R. Br. (Liliaceae) by comparing self-incompatible and self-compatible plants from coastal and tableland habitats, respectively. Floral morphology and nectar production of coastal and tableland plants are similar, and suggest pollination by nectarivorous birds (Ramsey 1993, Ramsey et al. 1994). However, whereas nectarivorous birds are abundant and important pollinators in coastal populations, such birds are found in low abundances and visit flowers infrequently in tableland populations (Ramsey 1995a, b). Sex allocation in tableland flowers is female-biased; more ovules and seeds, but less pollen, are produced than by coastal flowers. Moreover, tableland flowers and their individual parts weigh less, although proportional dry mass is similar to coastal flowers as is the timing of floral functions (Ramsey 1993, 1995a, b, Ramsey et al. 1993, 1994). These findings suggest that self-compatibility in tableland plants is a derived trait, and a consequence of selection to assure reproduction because avian pollinators are scarce. Nevertheless, tableland flowers do not self-pollinate autonomously, suggesting that flowers are self-pollinated by an alternative mechanism (Ramsey et al. 1993, Ramsey 1995c).

The most common visitors to tableland flowers, other than introduced honeybees (*Apis mellifera*, Apidae), are workers of an undescribed native ant species (*Iridomyrmex* sp., Formicidae). These ants visit *B. grandiflora* flowers for nectar, and, despite being much smaller than the flowers, occasionally contact the anthers and stigma. Early studies considered ants to be poor pollinators because they are not highly mobile, not reliably in an area, and not endowed with body parts that facilitate the pickup and delivery of pollen (e.g. Hickman 1974). A more recently proposed factor is that ants secrete an antibiotic substance which inhibits pollen function (Beattie et al. 1984, Peakall et al. 1991 and references within). Few field studies have examined ant pollination, but ants contribute substantially to seed production in at least some plants (Hickman 1974, Wyatt 1981, Peakall and Beattie 1989, 1991, Gómez and Zamora 1992).

Here I examine the importance of *Iridomyrmex* worker ants as pollinators of self-compatible *B. grandiflora*. The specific aims of this study are: 1) to test whether ants can self- and/or cross-pollinate flowers; 2) to examine whether contact with ants reduces pollen germination; and 3) to examine characteristics of the ants that enable them to pollinate flowers. Moreover, I consider whether self-pollination by ants could have been a factor in the selection of self-compatibility by assuring pollination, and whether this could have weakened selection for autonomous self-pollination.

Methods

Study site and species

I examined tableland populations of *B. grandiflora*, within Gibraltar Range National Park, in northeastern New South Wales, Australia (29°36' S, 152°16' E; 1000 m a.s.l.). *Blandfordia grandiflora* is found predominantly in coastal habitats, but a few peripheral, isolated tableland populations are located about 95 km from the nearest coastal populations (Ramsey et al. 1994). Gene flow between coastal and tableland populations is unlikely, as pollinators are unlikely to travel between populations and seeds have no apparent structures that would facilitate long-distance dispersal (Ramsey 1995a, M. Ramsey unpubl.).

Plants flower predominantly after fires, and I studied two populations 5 km apart for 1 yr each in the first year after a fire. Most flowering occurs from December to March, with peak flowering in January. Plants bloom for 1–3 wk, depending on how many flowers they have. Flowering plants consist of a corm (dry weight, ca 1.2 g), with about seven, narrow, basal leaves (length and width of the longest leaf, ca 300 mm and 4 mm, respectively), and a single terminal, racemose inflorescence (length and diameter, ca 580 mm and 3.5 mm, respectively). Most inflorescences have 1–2 pendulous, bell-shaped, red and yellow hermaphroditic flowers (Ramsey et al. 1994, Ramsey 1995a, M. Ramsey unpubl.). Corollas are about 62 mm in length and 35 mm in diameter at the widest point (Ramsey et al. 1994), and flowers produce about 50 µl of dilute nectar (concentration w:v in sucrose equivalents, 28%; Ramsey 1993), suggesting pollination by nectarivorous birds. Such birds, however, are found in low abundance and infrequently visit flowers; during peak flowering, when visitation is greatest, only about 30% of flowers are visited (Ramsey 1995a). Introduced honeybees (*Apis mellifera*) are the most important pollinators (Ramsey 1995c). Except for the *Iridomyrmex* ants examined in this study, other insects have rarely been observed visiting flowers (M. Ramsey unpubl.). Ants are present in all tableland *B. grandiflora* habitats that have been examined. Plants do not reproduce vegetatively, and are long-lived (> 10 yr). There is no persistent seed bank; most seeds germinate within 8 wk following release from the dehiscent capsules (M. Ramsey unpubl.).

Exclusion experiments

I examined the efficacy of ants as pollinators by using exclusion treatments in January and February of 1991 and 1992. I assigned plants randomly to one of the following treatments: 1) Open pollination – All pollinators had access to flowers. 2) Bagged – Bags excluded all pollinators from flowers, except ants. Seed-set resulted from self- and cross-pollination mediated by ants. 3)

Bagged + emasculated – Bags excluded all pollinators from flowers, except ants. I removed the anthers as flowers opened. Seed-set resulted from cross-pollination mediated by ants. 4) Bagged + tanglefoot – All pollinators including ants were excluded. Tanglefoot was smeared on inflorescence stalks and the vegetation immediately surrounding each plant was trimmed. Seed-set resulted from autonomous self-pollination.

Plants were bagged with fabric netting similar to bridal veil (1 mm² mesh). Sample sizes in treatments varied from 25 to 35 plants in both years, although some flowers and fruits were eaten by insects. About 6 wk after treatments, fruits were harvested, dissected and the numbers of seeds and undeveloped ovules were counted. Ovule number varied among plants and the percentage of ovules that developed into seeds was used to determine seed set. Percent seed-set of treatments was arcsine-square root transformed and compared with a two-way mixed model ANOVA (unweighted means analysis for unbalanced design: Winer et al. 1991), with treatments and years as fixed and random factors, respectively (Bennington and Thayne 1994). Pairwise comparisons were made using SNK tests (Winer et al. 1991). The bagged + emasculated treatment was excluded from analyses because too few plants produced seeds. I used a log-linear model for a three-way contingency table (BMDP4F, Dixon 1992) to examine the effects of treatment and year on the number of plants setting seeds.

Effects of stigma-anther separation on pollination by ants

Because the degree of selfing is often negatively related to the degree of stigma exertion (e.g. Schoen 1982), I examined the effect of stigma-anther separation on seed-set of plants with and without access by ants. In *B. grandiflora*, stigma-anther separation exhibits continuous variation. The mean stigma-anther separation is 6.8 ± 0.4 mm (Ramsey 1993), but about 17% of plants (N = 1006) have stigmas at the same level as anthers (M. Ramsey unpubl.). In the ant-access treatment, I bagged 25 flowers with stigmas exerted ≥ 5mm and 15 flowers with included stigmas (i.e. anthers at the same height or below the stigma). In the ant exclusion treatment, I bagged 15 flowers with either exerted or included stigmas. Ants were excluded as described previously, and percent seed-set was determined. Because the data violated the assumptions of ANOVA, I compared treatments using a non-parametric one-way ANOVA (Kruskal-Wallis test) and a posteriori tests (Zar 1984). I used a log-linear model for a three-way contingency table (BMDP4F, Dixon 1992) to examine the effects of ant access and stigma position on the number of plants setting seeds.

Effects of ants on pollen

Laboratory studies of pollen germination

I examined the effects of ant metapleural secretions by comparing the germination of pollen that had or had not been in contact with ants of *Iridomyrmex* sp. that visit *B. grandiflora* flowers. Anthers from 20 plants were stored separately in vials. In the ant-contact treatment, live ants were held by their legs with fine forceps. Fresh pollen from one anther was smeared onto the ant integument with a fine dissecting needle and left for 15 min. The pollen was then transferred to a drop of germination medium (Prakash 1986) on a microscope slide and incubated for about 3 h at room temperature, after which a drop of acetocarmine stain was applied. Control pollen from the same anther was tested similarly, but was not exposed to ants. After incubation, pollen was examined at 100× magnification and the number of germinated grains counted (ant-treated pollen grains, N = 4505; untreated grains, N = 6242). Different ants were used in each of the 20 trials. I compared percent germinated grains using a single-factor repeated measures ANOVA (Winer et al. 1991). Data were arcsine-square root transformed. Because there were only two within-subject factors per plant, correction factors to adjust F-ratios were not used (LaTour and Miniard 1983).

Field studies of seed-set

I examined the effects of metapleural secretions by pollinating flowers with pollen that had or had not been in contact with ants. I assigned 30 plants to either ant or control treatments, and emasculated and bagged the flowers. I collected two anthers from each of 10 plants. One anther from each plant was placed into one of two 50-ml plastic jars so that each jar contained one anther from each of the 10 plants. The pollen within each jar was mixed. Into one jar, I placed about 70 ants. Lids were placed on both jars. Jars were stored at ambient temperature for about 12 h, by which time the ants were covered with pollen. I then immobilised the ants by placing the jars onto crushed ice. Flowers in the ant treatment were pollinated by brushing four of the ants on the stigmas until the stigmas were covered with pollen. In the control treatment, I pollinated stigmas with pollen from the jar without ants using a dissecting needle. Percent seed-set was arcsine-square root transformed and compared using a one-way ANOVA.

Examination of ants

Pollen loads on ants

I emasculated flowers that had both pollen on their anthers and attending ants (N = 20) and placed them in 70% alcohol. I counted the number of ants per flower. From 10 flowers, I counted the number of pollen grains per ant (N = 57) using a dissecting microscope at 40× magnification.

Floral visitation by ants

I determined the extent of ant visitation to flowers by documenting their presence or absence at 30 bagged and unbagged flowers once a day for 3 d in January of 1991 and 1992. I compared the proportion of bagged and unbagged plants with ants using a 2 × 2 contingency table (G-test) for each year. In January and February of both years, I walked transects on three occasions each month and examined at least 50 unbagged flowers for the presence of ants. It was not possible to determine whether ants moved between plants, because I quickly lost sight of them after they left flowers and entered the surrounding vegetation.

Scanning electron microscopy

I examined ants using SEM to establish the presence of metapleural glands and morphological traits that may assist the adherence of pollen to their bodies.

Results

Exclusion treatments

Preliminary statistical analyses showed that the treatment × year interaction term was not significant ($F_{2,152} = 3.11$, $P > 0.05$), and it was pooled with the error term to examine the significance of the main effects (Table 1). Percent seed-set among treatments differed significantly ($F_{2,154} = 258.45$, $P < 0.001$). Seed-set also differed between years ($F_{1,154} = 6.70$, $P < 0.05$), and was due to the slightly higher seed-set in the open and bagged treatments in 1992. In both years, seed-set was greatest in the open treatment, in which all pollinators had access to flowers, and was intermediate in the bagged treatment, in which only ants had access to flowers. When all pollinators were excluded (bagged + tanglefoot), seed-set was significantly less than bagged flowers that were visited by ants only. In the bagged + emasculated treatments, seed set was very low; one plant produced two seeds in 1991 and two plants produced one seed each in

1992. This indicates that ants do not cross-pollinate flowers, and seed-set caused by ants was due to self-pollination.

The percentage of plants producing fruit varied with treatment. In both years, 100% of open plants, about 88% of bagged plants, about 34% of bagged + tanglefoot plants, and about 8% of bagged + emasculated plants produced fruit. The appropriate log-linear model of the three-way contingency table (treatments × year × fruit) included the main effects of treatments and fruit and the treatments × fruit interaction ($G_8 = 5.28$, $P > 0.50$). Inclusion of either year as a main effect or other interaction terms did not significantly improve the fit of the model (analysis of deviance: Manly 1992).

Effects of stigma-anther separation on pollination by ants

Percent seed-set among treatments differed significantly ($H_3 = 19.29$, $P < 0.001$). Seed-set was greater when ants had access to flowers than when they were excluded, but stigma-anther separation did not affect seed set in either treatment (Table 2). The percentage of plants setting seeds was dependent upon whether ants had access to flowers or were excluded, but was independent of stigma-anther separation. When ants had access, 91.6% and 86.7% of flowers with exerted and included stigmas, respectively, set seeds. When ants were excluded, only 38.5% and 45.5% of flowers with exerted and included stigmas, respectively, set seeds. The appropriate log-linear model of the three-way contingency table (ant access × stigma position × fruit) included the main effects of ant access and fruit and the ant access × fruit interaction ($G_4 = 2.48$, $P > 0.60$). Inclusion of either stigma position as a main effect or other interaction terms did not significantly improve the fit of the model (analysis of deviance: Manly 1992). These findings suggest that seed-set, whether caused by either ant pollination or autonomous self-pollination was not affected by stigma-anther separation.

Table 1. Mean (± SE) percent seed-set of plants in exclusion treatments examining ant pollination in 1991 and 1992. Treatments are described in the methods. Values with different superscripts differed significantly among treatments within years (SNK tests, $P < 0.05$). Sample sizes are in parentheses. Bagged + emasculated plants were not included in the analysis, because only 3 of 40 plants produced seeds.

	Open	Bagged only	Bagged + tanglefoot	Bagged + emasculated
1991	48.9 ^a ±3.4 (20)	12.3 ^b ±2.3 (27)	0.7 ^c ±0.3 (22)	0.1 ±0.1 (21)
1992	54.5 ^a ±3.3 (25)	20.9 ^b ±2.3 (33)	0.5 ^c ±0.2 (31)	0.1 ±0.1 (19)

Effects of ants on pollen

In the laboratory, contact with ants significantly reduced

Table 2. Mean (± SE) percent seed-set of plants with flowers with either exerted or included stigmas when ants either had access or were excluded. Sample sizes are in parentheses. Values with different superscripts differed significantly ($P < 0.05$).

Ant access		Ant exclusion	
exerted	included	exerted	included
22.2 ^a ±5.0 (24)	20.1 ^a ±4.3 (15)	0.8 ^b ±0.4 (13)	1.0 ^b ±0.5 (11)

the percentage of pollen grains that germinated (ant pollen, $72.7\% \pm 1.8$; control pollen, $78.7\% \pm 1.6$; $F_{1,19} = 20.87$, $P < 0.001$).

In the field, percent seed-set of flowers that were pollinated with pollen that had been either in contact with ants or had not, did not differ significantly (ant pollen: $N = 14$, $78.3\% \pm 3.0$; non-ant pollen: $N = 12$, $76.3\% \pm 3.6$; $F_{1,24} = 0.11$, $P > 0.50$).

Examination of ants

Pollen loads on ants

The mean number of ants counted per flower was 7.8 ± 1.1 . Of the 57 ants examined, 54 had one or more pollen grains adhering to their bodies. Overall, ants had 28.2 ± 8.2 pollen grains on their bodies. This value may underestimate the pollen load, however, as some grains were probably washed off when ants were stored in alcohol. About 50% of pollen grains (14.3 ± 3.8) were on the legs, indicating that ants carried pollen on parts of the body that were located away from the metapleural glands.

Floral visitation by ants

The proportion of flowers that were visited by ants depended upon whether flowers were bagged or unbagged (1991: $G_1 = 12.24$, $P < 0.005$; 1992: $G_1 = 16.88$, $P < 0.001$). In both years, all the bagged flowers were visited by ants, whereas 40% and 30% of unbagged flowers were visited by ants over the 3-d monitoring period in 1991 and 1992, respectively. On transects in January and February, the mean percentage of unbagged flowers with ants in 1991 was $13.4\% \pm 2.9$ and $11.3\% \pm 2.4$, respectively, and in 1992 was $14.0\% \pm 4.2$ and $13.3\% \pm 3.7$, respectively.

Scanning electron microscopy

SEM confirmed the presence of external openings of metapleural glands at the posterior end of the thorax (Fig. 1a). The tarsal segments, tibia and femur were covered with hairs to which pollen grains adhered (Fig. 1b, d). These findings suggest that ants can carry pollen grains on their legs without the pollen coming into contact with the metapleural glands or their secretions. The head, thorax, and abdomen were also covered with hairs. Except for a few long hairs, however, they appeared shorter than those on the legs.

Discussion

Pollination by ants

The findings of the present study demonstrate that ants self-pollinate *B. grandiflora* flowers. About 95% of plants from which all pollinators except ants were excluded produced seeds, and seed-set was about 17%

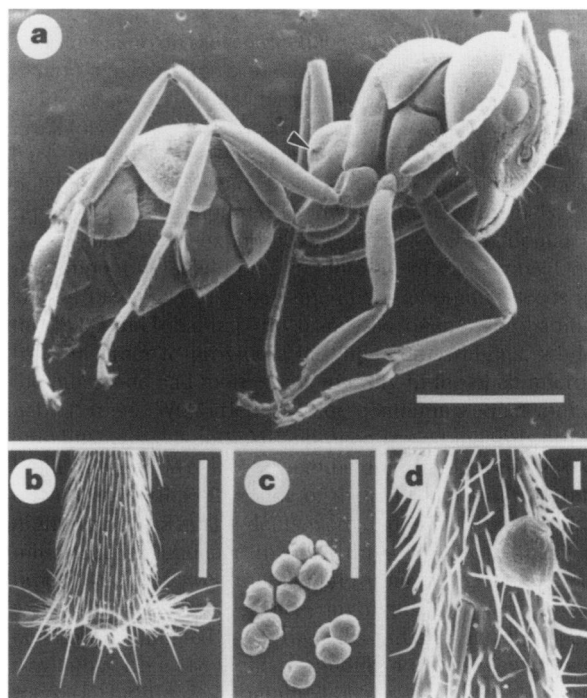


Fig. 1. SEM of a worker ant of the unidentified *Iridomyrmex* species that visits *B. grandiflora* flowers. a) An individual worker ant. The arrow shows the location of the metapleural gland. Scale bar = 1000 μ m; b) Last tarsal segment of the mid-leg. Scale bar = 100 μ m; c) *B. grandiflora* pollen grains. Scale bar = 100 μ m; d) First tarsal segment of the mid-leg with attached pollen grain. Scale bar = 10 μ m.

(about 28 seeds). While foraging for nectar, ants occasionally contact the floral reproductive parts by walking within flowers along the stamens and style. This results in the accumulation of small pollen loads on their legs and the incidental self-pollination of stigmas. Moreover, in *B. grandiflora*, pollen is viable and stigmas are receptive for a lengthy period (at least 8 d, Ramsey 1993), thus increasing the probability of ants contacting reproductive parts and self-pollinating flowers. The effectiveness of ants as pollinators of *B. grandiflora* is unusual, considering that floral morphology and nectar production are indicative of pollination by nectarivorous birds (Ramsey 1993, 1995a, Ramsey et al. 1994). Ant-pollinated flowers are typically small (e.g. *Polygonum cascadenae* (Polygonaceae), Hickman 1974; *Diamorpha smalli* (Crassulaceae), Wyatt 1981; *Scleranthus perennis* (Caryophyllaceae), Svensson 1985; *Microtis parviflora* (Orchidaceae), Peakall and Beattie 1991; *Hormathophylla spinosa* (Cruciferae), Gómez and Zamora 1992). In large flowers, such as those of *B. grandiflora*, ants are usually nectar thieves and do not contact the anthers or stigmas (e.g. Herrera et al. 1984).

Ant pollination has been demonstrated in only a few plant species (reviewed by Peakall et al. 1991). The paucity of ant pollination has been attributed to the production of antibiotic secretions from the metapleural glands

of ants, which often reduce pollen viability (Beattie et al. 1984, Peakall et al. 1991). In *B. grandiflora*, pollen function was not strongly diminished by potential secretions from the metapleural glands. Pollen germination decreased by only about 6% after contact with ants. Furthermore, there was no significant decrease in seed-set of flowers pollinated with pollen that had been in contact with ants. Because excess pollen was added to stigmas, however, it is possible that a small potential effect may have been masked. Seed-set in both treatments was similar to other cross-pollination treatments in previous studies (Ramsey et al. 1993, Ramsey 1995c).

Several factors may contribute to the effectiveness of these ants as pollinators. First, about 50% of the pollen found on ants was on their legs, away from the metapleural glands. This may prevent or reduce contact with metapleural secretions before pollen is deposited onto stigmas (Peakall and Beattie 1989). The legs also were covered with hairs, which facilitates the carrying of pollen. Second, different ant species vary in the effect of their metapleural secretions on pollen function (Hull and Beattie 1988), and ants in the present study may produce secretions that only slightly affect pollen function (Peakall and Beattie 1989). Third, plant species differ in the degree of resistance to metapleural secretions (Hull and Beattie 1988), and *B. grandiflora* pollen may be resistant to the secretions of these ants. If these ants were major pollinators of *B. grandiflora* before honeybees were introduced, selection would have favoured plants producing pollen that was not affected by such secretions.

Although ants self-pollinated *B. grandiflora* flowers, they did not effect cross-pollination. Ants were observed visiting emasculated flowers, but seed set was only 0.1%, compared to 17% for intact flowers. Seed set in the absence of pollinators is similar to that reported in a previous study (Ramsey et al. 1993). High nectar production by *B. grandiflora* (24-h production: volume = 49.1 ± 3.6 μ L, % concentration w:v in sucrose equivalents = 27.9 ± 1.7 %; Ramsey 1993) may allow ants to fill their crops without having to visit other flowers, thus promoting self-, but not cross-pollination. In other ant-pollinated plants, flowers are small and nectar production is low. During foraging, ants may visit several to many flowers, cross-pollinating some of them, despite short pollen dispersal distances (Hickman 1974, Wyatt 1981, Wyatt and Stoneburner 1981, Peakall and Beattie 1991, Gómez and Zamora 1992).

The present-day importance of ants as pollinators of *B. grandiflora* is probably minimal. Ants visited unbagged flowers infrequently compared to bagged flowers, probably because introduced honeybees remove floral nectar before ants can recruit to flowers. Furthermore, when ants do visit flowers, their effectiveness as pollinators may be reduced because honeybees remove pollen rapidly from anthers shortly after flowers open (Ramsey 1995c). Ants, however, may have been more important in the past. Honeybees have only been in Australia for about 150 yr (probably less at the tableland study site).

The effect of honeybees on *B. grandiflora* reproduction has been described elsewhere (Ramsey 1995c). Before honeybees were introduced, ants probably played an important role in *B. grandiflora* reproduction by providing reproductive assurance when avian pollinators were scarce. Because plants flower only sporadically in response to fires, do not reproduce vegetatively, and there is no persistent seed bank (Ramsey 1995a, M. Ramsey unpubl.), ant pollination may have played an important role in maintaining *B. grandiflora* populations.

Evolutionary considerations

When seed production is limited by pollinator availability, selection often favours traits that assure reproductive success independently of pollinators via self-compatibility and autonomous self-pollination (e.g. Rick et al. 1979, Schoen 1982, Piper et al. 1986, Wyatt 1986, Cruden and Lyon 1989, Vaughton and Ramsey 1995). In *B. grandiflora*, floral morphology and nectar production suggest pollination by nectarivorous birds, but observations at tableland sites over a 5-yr period indicate that birds are in low abundance and visit flowers infrequently (Ramsey 1993, 1995a). If similar patterns of abundance and visitation occurred in the past, reliable self-pollination by ants may have resulted in selection for self-compatibility by providing reproductive assurance. Although plants are self-compatible, flowers do not self-pollinate autonomously. Ant-mediated self-pollination may have provided sufficient reproductive assurance to reduce selection for floral modification(s) permitting autonomous selfing. Other factors influencing selection for autonomous selfing include the probability of outcrossing, the relative fitness of selfed progeny, the reduction of outcrossed ovules and pollen due to selfing (seed and pollen discounting), the timing of selfing relative to outcrossing, and the presence of genetic variation for traits permitting autonomous selfing (Holsinger 1992, Lloyd 1992, Uyenoyama et al. 1993).

Because seed-set from ant pollination (17%) is less than when self pollen is not limiting (40%; Ramsey et al. 1993, Ramsey 1995c), selection should favour autonomous selfing, in the absence of cross-pollination. The absence of autonomous selfing may be due to low levels of cross-pollination effected by nectarivorous birds, despite their low abundance (Ramsey 1995a, b). About 30% of flowers are visited by birds during peak flowering when most of the seeds are produced (Ramsey 1995a). Although the probability of visitation is low, the fitness advantage of cross-pollination is high. The relative fitness of selfed progeny during the first year is only 0.31, indicating a substantial outcrossing advantage (Ramsey 1995a). Although the maximum advantage would occur when cross pollen is not limiting, lower levels of cross-pollination following occasional visits by birds could still convey fitness advantages.

In *B. grandiflora*, substantial seed discounting occurs.

when experimental outcrossing occurs after selfing (Ramsey et al. 1993, Ramsey 1995c). Under natural conditions, this could prevent selection for autonomous selfing (Lloyd 1992). Although seed discounting also occurs with ant-mediated self-pollination, some ovules remain unfertilized, thus allowing opportunities for outcrossing. Compared to seed discounting, pollen discounting is unlikely to be important. Pollen : ovule ratios are high (ca 5600; Ramsey 1993), and autonomous selfing is unlikely to reduce the amount of pollen available for outcrossing, unless the floral morphology of selfing variants was modified substantially (Lloyd 1992).

Style and stamen length exhibit continuous variation (Ramsey 1993, Ramsey et al. 1994, M. Ramsey unpubl.), suggesting polygenic control, and the potential for selection to modify floral morphology allowing autonomous selfing (e.g. Shore and Barrett 1990, Carr and Fenster 1994). Such modifications would have to adjust the position of the stigma and anthers rather than just their height, since seed set was < 1% when ants were excluded from flowers with included stigmas. Modifications that allow selfing to occur before or at the same time as outcrossing (i.e. prior or competing selfing) are unlikely to be selectively favoured because they would result in seed discounting (Lloyd 1992, Ramsey et al. 1993, Ramsey 1995c). In contrast, modifications that allow delayed selfing, which occurs after outcrossing and provides reproductive assurance if outcrossing does not occur, should be favoured, providing that selfed progeny are viable and subsequent reproduction is unaffected (Lloyd 1992). The absence of delayed selfing in *B. grandiflora* suggests that the necessary floral modifications have not arisen but are possible, or that they are constrained by genetic and/or developmental factors.

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