

Floral Morphology, Biology and Sex Allocation in Disjunct Populations of Christmas Bells (*Blandfordia grandiflora*, Liliaceae) with Different Breeding Systems

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Abstract

Evolutionary shifts in breeding systems are often accompanied by changes in reproductive attributes such as floral morphology and biology, and pre- and post-zygotic allocation patterns. The effects of breeding system variation on several such attributes were examined in self-fertile tableland and outcrossing coastal populations of *Blandfordia grandiflora* R. Br. 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. Although all floral parts from coastal flowers weighed more, 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. Pollen–ovule ratios were 11 500 in coastal flowers and 5600 in tableland flowers. 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 did not differ significantly between populations. Relative male biomass, estimated from stamen and seed weights, was 5% greater in coastal plants, although populations did not differ significantly. 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 lack of differences between the Christmas bell populations in other aspects of floral morphology, allocation patterns and biology suggests that changes in ovule and pollen production precedes changes in other traits during the evolution of autonomous selfing. Overall, these findings suggest that tableland plants may have evolved self-fertility only recently and selection has had insufficient time to change floral traits. Alternatively, self-fertility may not have evolved recently and floral traits promoting outcrossing have been maintained by selection, imposed by inbreeding depression and/or overdominance.

Introduction

The most frequent evolutionary change in breeding systems of flowering plants is the shift from predominant outcrossing to selfing (Stebbins 1970). Such shifts in breeding systems are often associated with changes in floral morphology, floral biology and resource allocation to sexual function (Ornduff 1969; Jain 1976; Schoen 1982*a, b*; Cruden and Lyon 1985; Wyatt 1988; Morgan and Barrett 1989). Frequently, floral adaptations of outcrossing plants that prevent selfing or attract pollinators are absent or reduced in related selfing plants. For example, outcrossing plants are often protandrous whereas selfing plants are not (Schoen 1982*a*; Wyatt 1984*a*; Holtsford and Ellstrand 1992). Further, plants from selfing populations are often characterised by smaller petals, stamens and styles, by lower

nectar and pollen production, and by lower pollen-ovule ratios than plants from outcrossing populations (Lloyd 1965; Ornduff 1969; Cruden 1977; Schoen 1977, 1982*a, b*; Rick 1982; Wyatt 1984*a, b, c*, 1988; Barrett 1988; Ritland and Ritland 1989).

Sex allocation theory predicts that selection should favour individuals that devote more resources to the sex function that conveys the greatest fitness. Selfing plants are expected to allocate less to male functions because fewer ovules are available for cross-fertilisation by the pollen pool and, consequently, resources expended on male functions gain less in terms of fitness (Goldman and Willson 1986; Charlesworth and Charlesworth 1987*a*; Charnov 1987; Lloyd 1987; Charlesworth and Morgan 1991; Brunet 1992; Morgan 1992). If structures and rewards that attract pollinators, such as corollas and floral nectar, benefit male fitness more than female fitness (Bell 1985), resource allocation to such functions should decrease also with increasing selfing rate. However, the decrease in resource allocation to attractive structures will depend upon the extent to which female fitness benefits by attracting pollinators (Charlesworth and Charlesworth 1987*a*; Lloyd 1987). Resources that are saved by reducing male functions can be redirected into female functions (e.g. ovules and seeds) or into growth and maintenance of vegetative structures (Schoen 1982*a*; Lloyd 1987).

Much of the empirical work examining how changes in breeding system have affected reproductive traits has involved interspecific comparisons (e.g. Cruden 1977; Cruden and Lyon 1985; Vasek and Weng 1988). Such studies have provided valuable information on the characters that distinguish selfing and outcrossing species. However, intraspecific studies that compare selfing and outcrossing populations provide greater insight into the evolution of changes in breeding system, since such changes are unlikely to be overly confounded by taxonomic differences. Most previous studies have compared autonomously selfing plants with outcrossing plants. Differences between such plants are usually pronounced (reviewed in Ornduff 1969; Jain 1976; Wyatt 1988). Few studies have compared self-fertile plants that are not autonomously selfing and depend upon pollinators with obligately outcrossing plants. Such comparisons are of interest as they may provide information concerning the intermediary evolutionary steps between outcrossing and autonomous selfing. Christmas bells (*Blandfordia grandiflora* R.Br., Liliaceae) are suitable plants to investigate the reproductive consequences of breeding system variation. Tableland populations of Christmas bells are self-fertile, but not autonomously selfing, whereas coastal populations are outcrossing. Relative self-fertilities, determined as the ratio of self seed set to cross seed set following controlled pollinations, are 0.55 and 0.08 for tableland and coastal plants, respectively. Pollinators are required for seed set in both populations (Ramsey *et al.* 1993). In this study, I compared several attributes of flowers and fruits that may be expected to change with breeding system for tableland and coastal populations of Christmas bells. Specifically, I examined: (1) floral morphology and biomass-allocation patterns; (2) pollen, ovule and nectar production per flower; (3) seed set and seed and fruit biomass; (4) the onset and duration of stigma receptivity; and (5) pollen longevity.

Methods

Study Species and Areas

Christmas bells are erect, perennial herbs with basal leaves and a single terminal, racemose inflorescence. Flowers are hermaphroditic. Populations occur in wet heathland and sedgeland communities on damp sand or peaty soils of low pH. Populations are found predominantly in coastal and near coastal areas of north-eastern New South Wales and south-eastern Queensland although a few populations also occur in restricted tableland areas of north-eastern New South Wales, at the western periphery of the species' range (Elliot and Jones 1982; Henderson 1987). Plants flower *en masse* between November and April following wildfires.

Study sites were located in north-eastern New South Wales, at Gibraltar Range National Park (29°36' S, 152°11' E; 1000 m a.s.l.) on the New England tablelands and Yuraygir National Park (29°51' S, 153°16' E; 5 m a.s.l.) on the coast. Gibraltar Range has cold dry winters, mild dry

springs and warm wet summers and autumns. At Yuraygir, temperatures throughout the year are higher than at Gibraltar Range, particularly during the winter. At both sites the average annual rainfall is similar. The study was conducted during the flowering seasons of 1989, 1990 and 1991.

Floral Morphology and Biomass

In both populations, floral measurements and dry weights were obtained from 50 flowers, each from a different plant. Racemes were bagged with fabric netting bags (1 mm² mesh) to allow prezygotic measurement of flowers. Flowers were measured after they had been open for 24–36 h. Eight characters were measured: (1) pedicel length; (2) pedicel diameter at the mid-point; (3) corolla length; (4) corolla width at the base of the corolla lobes; (5) pistil and stipe length; (6) length of the longest stamen; (7) stipe diameter at the mid-point. Stigma–anther separation was determined by subtracting the pistil lengths from the stamen lengths or vice versa. After measurements were completed, flowers were dissected in four parts: (1) pedicel; (2) corolla; (3) pistil (stigma, style, ovary, stipe); (4) stamens (anthers, filaments). These parts were dried to a constant weight and weighed to the nearest 0.01 mg. For each population, the proportion of the total biomass allocated to pedicels, corollas, pistils and stamens was determined. To determine whether biomass allocation to the corolla, the pistil or stamens were correlated with morphological floral traits I calculated Pearson correlation coefficients.

Pollen and Ovule Production per Flower

In each population, undehisced anthers were collected from 30 flowers and were placed in separate dry, plastic vials. When anthers had dehisced, acetocarmine (5 mL) was added to each vial. Vials were vortexed and samples were immediately placed into a haemocytometer and pollen grains counted using a compound microscope ($\times 100$ magnification). Four replicates per vial were counted and the mean of these was multiplied by the dilution factor. To determine the number of ovules per flower for each population, 100 fruits each from different plants were selected, dissected and the number of seeds and ovules counted. Ovule number per fruit was determined by adding the number of seeds and ovules together. Pollen–ovule ratios were estimated for each population.

Nectar Production

Nectar production over a 24 h period was assessed for 50 flowers on different plants in each population. Flowers that had been open for at least 24 h but less than 72 h were used. In the morning, floral nectar was removed using capillary tubes. Flowers were then bagged. In the tableland population, a clear sticky substance (Bird-Off®, Rentokil Pty Ltd) was coated on flower stalks to exclude ants from flowers (Ramsey *et al.* 1993). After 24 h, nectar was removed from flowers using 50 μ L capillary tubes and volumes determined. Nectar concentrations were measured in sucrose equivalents using a hand-held refractometer and converted to mg sucrose per volume (Bolten *et al.* 1979).

Seed Set and Seed and Fruit Biomass

To examine biomass allocation pattern after fruit set, fruits and seeds were collected from open-pollinated plants that flowered during January, when natural levels of seed set were greatest. In each population, 50 green, full-sized fruits from different plants that had been marked previously were collected and the number of seeds in each were counted. Mean seed weight per population was determined by weighing 1000 seeds from bulk seed collections. Before weighing, seeds were stored in paper bags for 4 months. Weights of stored and oven dried seeds did not differ significantly (M. Ramsey, unpublished data), indicating that stored seeds could be used in lieu of dried seed. In each population, 100 mature fruit capsules without seeds were collected, dried at 60°C for 7 days and weighed to the nearest 0.01 mg. Mean fruit weights for each population were estimated as the product of the seed set per fruit and the mean seed weight for the population, plus the fruit capsule weight.

Relative Allocation to Male and Attraction Functions

To examine the predictions of sex allocation theory that fewer resources should be allocated to male and attraction functions in selfing plants, I estimated the following relative biomass

allocations for each population. Prezygotic relative male biomass was estimated as $M_1 = a/(a+g)$, where a and g are the dry weights of stamens and pistils, respectively. Relative male biomass after fruit set, using stamens and seeds, was estimated as $M_2 = a/(s+a)$, where a is as defined above and s is the total seed weight per fruit. Relative biomass of the corolla was estimated as $C = cf$, where c is the dry weight of the corolla and f is the total flower biomass. Relative biomass of sucrose was estimated as $N = n/(n+f)$, where n is the product of 24 h sucrose production and the length of time corollas remain attractive (6 days; M. Ramsey, unpublished data) and f is as defined above. Total allocation to attraction, including both nectar and corolla biomasses, was estimated as $A = (n+c)/(n+f)$, where c , n and f are as defined above. Because mean population values were used to calculate the relative male biomass (M_2), and the relative biomasses of nectar (N), and total attraction (A), binomial tests were used to estimate standard errors and compare populations (Snedecor and Cochran 1989).

Onset and Duration of Stigma Receptivity

The onset of stigmatic receptivity was examined for 20 flowers on different plants in each population. Inflorescences were bagged to exclude pollinators. As flowers opened, they were emasculated and cross-pollinated either 8 h or 24 h later with a mixture of fresh pollen from five plants. Flowers were harvested 4 h after pollination and were stored in 70% alcohol. The tips of styles with stigmas were placed in a drop of safranin O and aniline blue stain (Dionne and Spicer 1958) for 15 minutes. They were then placed in a drop of glycerol on a microscope slide and squashed under a coverslip. The number of grains on stigmas and the percentage that had germinated were then counted.

To examine the length of time that stigmas remained receptive, flowers of different ages on different plants were cross-pollinated with a mixture of fresh pollen from five plants. Flowers were covered with mesh bags to exclude pollinators. When flowers opened they were emasculated and were cross-pollinated either right away (day 0) or on the third, sixth, seventh or eighth day following opening. In each population, 15 flowers were pollinated in the 0 and 3 day treatments, although subsequently, some were eaten by insects. In the other treatments, seven and eight flowers were used in the tableland and coastal populations, respectively. Fruits were harvested about 6 weeks after pollination. Seed set was assessed as the proportion of ovules that developed into seeds.

Pollen Longevity

Field studies. Pollen longevity in the field was examined by cross-pollinating different recipient flowers with pollen of different ages. Pollen age was determined as the number of days since flowers opened. Six flowers on different plants that opened at the same time were chosen as pollen donors. When donor flowers opened, one of the six anthers was removed from each and placed in a dry, plastic vial. Pollen from the donors was thoroughly mixed and then used to pollinate six recipient flowers. Recipient flowers (one per plant) were emasculated and bagged as they opened, and then pollinated 24 h later. Donor plants were bagged except for when anthers were being removed. In the tableland population, this procedure was followed for 6 days. In the coastal population, this procedure was used except that 12 recipient flowers were cross-pollinated with pollen that was either 0, 3 or 5 days of age. Fruits of the recipient flowers were harvested and seed set assessed as described previously.

Laboratory studies. A potential problem using seed set as an indicator of pollen viability is that seed set may be high even if only a low proportion of the pollen is viable; a low proportion of viable grains may fertilise most ovules if the overall pollen load exceeds the number of ovules per flower. Here I examine the decline in pollen viability with time by germinating pollen in the laboratory. Pollen age was determined as the number of days since flower opening. For each population, two anthers from each of 10 newly opened flowers on different plants were collected and their pollen mixed in a dry, plastic vial. Vials were left open and were stored on a laboratory bench. Mean (\pm s.e.) maximal and minimal temperatures were 22.5 ± 0.4 and 15.6 ± 0.4 , respectively, during the period that germination trials were conducted.

Pollen from vials was subsampled using a dissecting needle and placed onto a drop of germination medium (Prakash 1986) on a microscope slide. Pollen was incubated at room temperature for 3 hours after which time a drop of acetocarmine stain and a coverslip were applied.

Transects across the slides were viewed at $\times 40$ magnification and the numbers of germinated and ungerminated grains were counted. On each slide, at least 500 pollen grains were examined. For each pollen age, two slides per population were viewed. After pollen collection, germination trials were conducted every day for 14 days and then every second day until the pollen was 44 days old. As a control, pollen was killed by heating in a microwave oven for 6 min, and then placed in germination medium and examined for germination.

Statistical Analyses

Most traits were compared using either one- or two-way analyses of variance (ANOVA). If variances were heterogeneous, counted and proportional data were square-root and arcsine transformed, respectively. Measurement and biomass data were transformed using natural logarithms (Sokal and Rohlf 1981). Student-Newman-Keuls (SNK) *a posteriori* multiple range tests were used if one-way ANOVAs were significant. For all biomass and morphological estimates, coefficients of variation were calculated to compare the relative variability for each trait between populations. In the text, means are presented with standard errors (\pm s.e.).

Results

Floral Morphology and Biomass Allocation

Of the eight metric characters, four were significantly different between tableland and coastal flowers (Table 1). There were no differences in pedicel length, corolla length and width or stamen length. Pedicel and stipe diameters were significantly greater for coastal flowers whereas pistil length and stigma-anther separation were significantly greater for tableland flowers (Table 1).

Table 1. Floral morphology of tableland and coastal Christmas bells

Mean \pm s.e. measurements are given. In each population, 50 flowers on different plants were measured. One-way ANOVAs were used to compare populations. Degrees of freedom for all ANOVAs were 1,98. Significance of *F* ratios are denoted by superscripts; ^{ns} not significant, * $P < 0.05$, *** $P < 0.001$. Coefficients of variation are given in parentheses

Popu- lation	Floral measurements (mm)							
	pedicel length	pedicel diameter	corolla length	corolla width	pistil length	stamen length	stigma-stamen separation	stipe diameter
Table- land	27.1 ± 1.1 (28.9)	2.0 ± 0.03 (10.8)	60.0 ± 0.7 (8.0)	22.9 ± 0.3 (9.6)	60.6 ± 0.8 (9.6)	53.7 ± 0.7 (8.8)	6.8 ± 0.4 (36.5)	2.1 ± 0.1 (40.2)
Coast	25.8 ± 0.8 (21.9)	2.2 ± 0.04 (12.9)	57.8 ± 0.7 (8.8)	22.6 ± 0.3 (10.6)	57.5 ± 0.7 (8.6)	53.7 ± 0.7 (8.9)	3.7 ± 0.4 (81.7)	2.7 ± 0.1 (10.4)
<i>F</i>	0.88 ^{ns}	23.39***	5.01 ^{ns}	0.41 ^{ns}	8.11*	0.10 ^{ns}	30.11***	20.94***

Dry weights of flowers and the four dissected floral parts are summarised in Table 2. All floral parts of coastal flowers weighed significantly more than those of tableland flowers. However, the proportional contribution of each floral part to the total floral biomass did not differ between tableland and coastal populations. In both populations, proportional biomass allocation was significantly greater to pistils than to stamens (Table 2; one-way ANOVAs; tableland, $F_{1,98} = 39.71$, $P < 0.001$; coast, $F_{1,98} = 15.38$, $P < 0.001$). In both populations, a large proportion (0.65) of total floral biomass was allocated to corolla.

All correlations among corolla, pistil and stamen morphological and biomass traits were positive, significant and exceeded 0.50 (all $P < 0.001$) and were of similar magnitudes for both populations. The highest correlations were between corolla, pistil and stamen lengths (all $r \geq 0.83$). Correlations between dry weights of corollas, pistils and stamens exceeded 0.70. The lack of negative correlations suggests that there were no phenotypic

Table 2. Floral biomass allocation of tableland and coastal Christmas bells

Mean \pm s.e. dry weights of floral parts and proportional dry weight of floral parts to total flower dry weight are given. In each population, 50 flowers from different plants were weighed. One-way ANOVAs were used to compare populations. Degrees of freedom for all ANOVAs were 1,98. Significance of *F* ratios are denoted by superscripts; ^{ns} not significant, *** *P* < 0.001.

Coefficients of variation are given in parentheses

Population	Dry weight (mg)					Proportion of total flower dry weight			
	total flower	pedicel	corolla	pistil	stamens	pedicel	corolla	pistil	stamens
Tableland	174.1 ± 4.3 (17.4)	16.1 ± 0.7 (31.5)	112.9 ± 2.9 (18.5)	24.2 ± 0.7 (19.3)	20.9 ± 0.5 (15.8)	0.092 ± 0.003 (23.1)	0.647 ± 0.004 (4.4)	0.139 ± 0.002 (10.2)	0.121 ± 0.002 (11.7)
Coast	214.2 ± 4.5 (16.2)	20.8 ± 0.8 (27.2)	138.6 ± 3.1 (15.9)	28.7 ± 0.9 (23.3)	26.1 ± 0.6 (14.9)	0.097 ± 0.003 (21.8)	0.647 ± 0.003 (3.3)	0.133 ± 0.002 (10.6)	0.123 ± 0.002 (11.5)
<i>F</i>	37.71***	18.61***	35.55***	15.39***	52.69***	1.54 ^{ns}	0.92 ^{ns}	4.51 ^{ns}	0.42 ^{ns}

tradeoffs between biomass allocations to male, female or attraction functions. These results may reflect developmental and pleiotropic or linkage relationships among traits and may have more allometric than evolutionary significance.

Pollen and Ovule Production per Flower

Pollen counts, ovule counts and pollen-ovule ratios are given in Table 3. Tableland flowers produced significantly more ovules but significantly fewer pollen grains than coastal flowers. Overall, the pollen-ovule ratio of coastal flowers was almost twice as great as that of tableland flowers.

Table 3. Pollen and ovule production per flower in tableland and coastal Christmas bells

Means \pm s.e. are given. For pollen and ovule production, 30 and 100 flowers, respectively, were examined in each population. One-way ANOVAs were used to compare populations. Degrees of freedom are in parentheses. All *F* ratios were significant (*P* < 0.001)

Population	Number of pollen grains ($\times 10^6$)	Number of ovules	Pollen-ovule ratio
Tableland	0.93 ± 0.03	169.2 ± 2.8	5590 ± 179
Coast	1.41 ± 0.04	123.5 ± 1.7	11449 ± 294
<i>F</i>	116.86 (1,58)	197.34 (1,198)	295.50 (1,58)

Nectar Production

Volumes, concentrations and weight (mg) of sucrose produced by flowers over a 24 h period are given in Table 4. None of these differed between populations. In both populations, flowers produced about 50 μ L of nectar of concentration 25%–28% sucrose equivalents.

Seed Set and Seed and Fruit Biomass

Seed sets and seed and fruit weights are given in Table 5. Tableland fruits produced more seeds than coastal fruits, although individual seeds weighed less. Total fruit biomass (capsule+seeds) of tableland fruits was significantly greater than coastal fruits. Total seed biomass per fruit was greater for tableland fruits whereas capsule biomass was greater for coastal fruits.

Table 4. Floral nectar production in tableland and coastal Christmas bells

Means \pm s.e. are given. Nectar production was measured from flowers that had been bagged for 24 h. In each population, 50 flowers on different plants were examined. One-way ANOVAs were used to compare populations; none was significant ($P > 0.20$). Degrees of freedom for all ANOVAs were 1,98

Population	Volume (μ L)	Concentration (w:v) (% sucrose equivalents)	Sucrose (mg)
Tableland	49.1 ± 3.6	27.9 ± 1.7	13.2 ± 1.0
Coast	49.0 ± 3.2	24.6 ± 1.1	11.6 ± 0.7
<i>F</i>	0.01	2.10	0.40

Table 5. Seed set and seed and fruit biomass of open-pollinated tableland and coastal Christmas bells

Means \pm s.e. are given. All weights are in mg. One-way ANOVAs were used to compare populations. Significance of *F* ratios are denoted by superscripts; ^{ns} not significant, ** $P < 0.02$, *** $P < 0.001$. Sample sizes are given in parentheses

Population	Number of seeds per fruit (<i>n</i> = 50)	Individual seed wt (<i>n</i> = 1000)	Seed wt per fruit (<i>n</i> = 50)	Fruit capsule wt (<i>n</i> = 100)	Total fruit wt (<i>n</i> = 100)
Tableland	96.4 ± 4.7	2.13 ± 0.01	205.8 ± 10.3	199.9 ± 4.2	405.7 ± 4.2
Coast	48.7 ± 2.6	3.41 ± 0.01	166.1 ± 9.0	227.7 ± 4.8	393.8 ± 4.8
<i>F</i>	66.55***	2690.4***	8.22**	19.02***	6.89**
degrees of freedom	(1,98)	(1,998)	(1,98)	(1,198)	(1,198)

Relative Allocation to Male and Attraction Functions

Estimates of the relative allocations to male and attraction functions are given in Table 6. Contrary to predictions of sex allocation theory, relative allocations to male and attraction functions were similar for both populations. Prezygotic relative male biomass (M_1) of both populations was about 0.47. Although relative male biomass after fruit set (M_2) was 5% greater for the coastal population, it did not differ significantly from the tableland population. Allocations to attraction were high in both populations and did not differ significantly. Relative corolla biomasses (*C*) were about 0.65. Relative biomasses of nectar (*N*) and total attraction (*A*) for both populations were about 0.28 and 0.75, respectively.

Onset and Duration of Stigma Receptivity

Stigmas of flowers that had been open for 8 h appeared to be drier and smaller than flowers that had been open for 24 h, although these differences were not quantified. There were no differences between the populations in the number of grains adhering to stigmas at the two pollination times following flower opening (Table 7; two-way ANOVA; $F_{1,16} = 0.13$, $P > 0.50$), although significantly more grains adhered to stigmas of flowers that had been open for 24 h ($F_{1,36} = 343.02$, $P < 0.001$). The population \times pollination time interaction was not significant ($F_{1,36} = 0.89$, $P < 0.50$). In the 8 h treatment, it is likely that grains were washed off when placed in storage vials. In the 24 h treatment,

Table 6. Relative biomass allocation to male function and pollinator attraction

Means \pm s.e. are given. Relative male biomass, M_1 was estimated from stamens and pistils, whereas M_2 was estimated from stamens and seeds. Methods of calculating the other estimates are given in the text. For M_1 and C , populations were compared with one-way ANOVAs. The other estimates were compared with binomial tests, with $n = 50$. Degrees of freedom are in parentheses. All comparisons were not significant ($P > 0.05$).

Population	Relative male biomass	Relative male biomass	Relative biomass allocation to pollinator attraction		
	M_1	M_2	corolla C	nectar N	corolla+nectar A
Tableland	0.47 ± 0.01	0.09 ± 0.04	0.65 ± 0.01	0.31 ± 0.07	0.76 ± 0.06
Coast	0.48 ± 0.01	0.14 ± 0.05	0.65 ± 0.01	0.25 ± 0.06	0.73 ± 0.06
F^a or $X^{2,b}$	3.54 ^a (1,98)	0.63 ^b (1)	0.92 ^a (1,98)	0.58 ^b (1)	0.39 ^b (1)

Table 7. Onset of stigmatic receptivity for tableland and coastal Christmas bells

Means \pm s.e. number of pollen grains adhering and percentage germinating on stigmas are given. Flowers were cross-pollinated at either 8 h or 24 h after flowers had opened. At each time, 10 flowers were pollinated in each population. The number of pollen grains adhering to stigmas did not differ between populations at the two times ($P > 0.50$), although significantly more grains were on the 24 h cross-pollinated stigmas ($P < 0.001$). The percentage of germinated grains at 24 h did not differ between populations ($P > 0.50$)

Population		Time since flower opening	
		8 h	24 h
Tableland	Number of pollen grains	20.6 ± 2.8	288.0 ± 37.1
	Percentage germination	0.0 ± 2.0	74.1 ± 2.0
	Number of pollen grains	27.2 ± 3.3	272.3 ± 42.5
Coast	Percentage germination	0.0 ± 2.6	76.1 ± 2.6

grains were more firmly attached because they had germinated and tubes had penetrated the stigmatic papillae. In both populations, no grains germinated on stigmas from the 8 h treatment, indicating that stigmas were not yet receptive. In contrast, about 75% of grains had germinated on stigmas in the 24 h treatment in both populations (Table 7; one-way ANOVA; $F_{1,18} = 0.45$, $P > 0.50$).

In the tableland population, stigmas were fully receptive for 8 days following flower opening. Seed set of flowers that had been open for 9 days before they were pollinated was reduced significantly by almost half (Table 8; one-way ANOVA; $F_{5,50} = 7.60$; $P < 0.001$). In the coastal population, stigmas remained fully receptive for 7 days following flower opening. Seed sets of flowers that had been cross-pollinated either upon opening or after 3, 6 or 7 days did not differ significantly (Table 8). Seed sets of flowers that had been open for either 8 or 9 days before they were pollinated were significantly lower than earlier pollinated flowers (Table 8; one-way ANOVA; $F_{5,50} = 22.29$, $P < 0.001$).

Table 8. Duration of stigmatic receptivity for tableland and coastal Christmas bells

Mean \pm s.e. percentage seed set per fruit is given. Different flowers were cross-pollinated at different ages which was determined as the number of days since flower opening. Sample sizes are given in parentheses. Within a population, treatments were compared with one-way ANOVAs and SNK tests. Values with different superscripts differed significantly ($P < 0.05$)

0	3	Days since flower opening			8	9
		6	7			
Tableland						
77.5 ^a	75.7 ^a	79.5 ^a	76.2 ^a	79.3 ^a	41.1 ^b	
± 2.9	± 2.1	± 2.9	± 5.5	± 5.1	± 10.7	
(13)	(15)	(7)	(7)	(7)	(7)	
Coast						
44.2 ^a	46.2 ^a	36.7 ^a	32.9 ^a	8.5 ^b	1.9 ^b	
± 5.1	± 3.6	± 7.1	± 7.1	± 5.1	± 1.4	
(12)	(10)	(8)	(8)	(8)	(8)	

Pollen Longevity

Field studies

In both populations, the age of pollen, assessed as the number of days since flower opening, had no effect upon seed set. Percentage seed sets of tableland flowers that were pollinated with pollen from newly opened flowers or pollen 1–5 days old did not differ significantly (range, 75.2 ± 5.4 – 84.5 ± 3.0 ; one-way ANOVA; $F_{5,30} = 0.65$, $P > 0.50$). Similarly, percentage seed sets of coastal flowers that were pollinated with pollen either 0, 1 or 5 days old did not differ significantly (range, 44.2 ± 5.1 – 53.6 ± 6.2 ; one-way ANOVA; $F_{5,30} = 0.70$, $P > 0.50$).

Laboratory studies

Decreases in viability over time, as determined by percentage germination, were similar for pollen collected from tableland and coastal populations (Fig. 1). Slopes and y-intercepts of least square linear regressions of pollen germination versus pollen age did not differ significantly between populations (Fig. 1; t -tests; slopes, $t_{54} = 1.95$, $P > 0.05$; y-intercepts, $t_{55} = 0.55$, $P > 0.50$). In both populations, about 80% of pollen germinated on the day after it was collected. Percentage germination decreased slowly to about 50% by 10 days after collection. Percentage germination remained at about 50% until 20 days after collection when it started to decline. By day 44, only 3% of pollen germinated (Fig. 1). In the heat-treated controls, no pollen grains germinated.

Discussion

Floral Morphology and Biology

In comparison with the outcrossing coastal Christmas bells, the floral attributes of the self-fertile tableland plants showed no evidence of adaptation for autonomous selfing such as reduced flower size, simultaneous presentation of pollen and receptive stigma, decreased pollen longevity or decreased nectar production. Three largely independent genetic phases in the evolution of breeding systems from obligate outcrossing to autonomous selfing have been recognised. These include changes that: (1) permit self-fertilisation; (2) ensure automatic self-pollination when pollinators are absent; and (3) reduce the size of floral structures and the amount of rewards that attract pollinators (Rick 1982). Tableland Christmas bells are self-fertile whereas coastal plants are outcrossing as determined by controlled hand

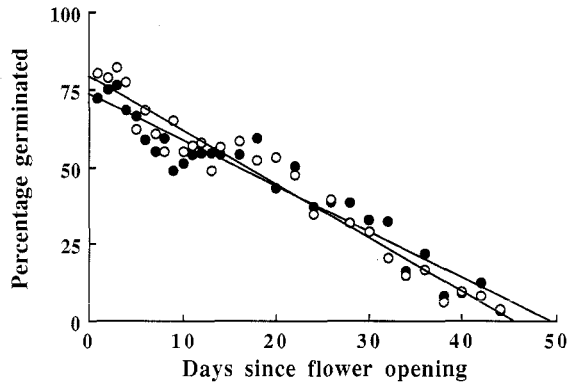


Fig. 1. Changes in the percentage of germinated pollen grains with time since flower opening for tableland (○) and coastal (●) Christmas bells. Least squares linear regressions for the populations were: tableland, $y = 79.1 - 1.7x$, $F_{1,27} = 538.66$, $P < 0.001$, $r^2 = 0.95$; coast, $y = 73.6 - 1.5x$, $F_{1,27} = 323.70$, $P < 0.001$, $r^2 = 0.92$. Slopes and y -intercepts of the two regressions did not differ significantly ($P > 0.05$). Each point is the mean of two replicates. At least 500 pollen grains were examined for each replicate.

pollination experiments. Although the genetic mechanism preventing selfing is not yet fully known, it is late-acting, occurring at the level of the ovule (Ramsey *et al.* 1993).

The floral attributes of the self-fertile tableland plants showed no evidence that automatic self pollination occurs. These results concur with those of Ramsey *et al.* (1993), who found that few or no seeds were produced when pollinators were excluded from flowers. In other self-fertile plants, lack of protandry or decreased stigma-anther separation or both ensure self pollination (Schoen 1982*b*; Wyatt 1984*a*; Ritland and Ritland 1989; Holtsford and Ellstrand 1992). In the present study, flowers of both populations were protandrous. Interestingly, stigma-anther separation was greater in self-fertile tableland flowers than in coastal flowers. Increased separation often increases the level of outcrossing (Holtsford and Ellstrand 1992), and large separation in tableland flowers may have this effect. Traits increasing outcrossing in the tableland plants would be advantageous because self seeds and seedlings suffer inbreeding depression (M. Ramsey, unpublished data). In addition, low levels of within-flower self pollination are mediated by ants irrespective of the separation distance (M. Ramsey, unpublished data). Such pollination would ensure reproduction in the absence of other pollinators and may have prevented selection for automatic selfing, and maintained or promoted traits that increase outcrossing. Genetic studies of the mating system are now required to examine the relationship between stigma-anther separation and outcrossing rate.

Corolla size and floral nectar production were similar for both populations. Because the self-fertile tableland plants require pollinators to self-pollinate, the structures and rewards that attract pollinators should be retained by selection (Lloyd 1987). In addition, if outcrossing occurs and increases plant fitness, selection for pollinator attraction would not be independent of the influence of pollinators (Cumaraswamy and Bawa 1989).

Overall, these findings suggest that selection has favoured self-fertility in tableland plants, but genetic changes to ensure automatic self pollination and to reduce the attractiveness of flowers have not occurred. At present, the reasons for the lack of differences between plants from the two populations is unclear. Perhaps self-fertility has evolved only recently in the tableland population and selection has had insufficient time to change floral traits.

Alternatively, self-fertility may not have evolved recently, but there has been strong selection for outcrossing, imposed by inbreeding depression and/or overdominance.

Floral Biomass and Sex Allocation

Although overall flower size was similar, coastal flowers weighed more than tableland flowers. Self-fertile plants often produce lighter flowers, but this is usually associated with a decrease in flower size (e.g. Ritland and Ritland 1989). In the present study, the weight differences were not due to any particular floral part, since all parts of coastal flowers weighed more. The reasons for the differences in overall flower dry weight are unclear at present.

Although the floral parts of coastal flowers weighed more than those of tableland flowers, the proportional biomass of stamens, pistil and corolla did not differ between populations. Sex allocation theory predicts that selection should favour the sex function that conveys the highest fitness and, consequently, selfing plants should allocate fewer resources to male function and more resources to female function, compared to outcrossing plants. Allocation to pollinator attraction in selfing plants will depend upon the need for pollinators for self-pollination and the level of inbreeding depression following selfing (Charnov 1982, 1987; Goldman and Willson 1986; Charlesworth and Charlesworth 1987a; Lloyd 1987; Charlesworth and Morgan 1991; Brunet 1992; Morgan 1992). Five other studies have examined the relationship between sex allocation patterns and selfing at the intraspecific level (Schoen 1982a, *Gilia achilleifolia* Benth. (Polemoniaceae); Charnov 1987, *Oryza perennis* Monench. (Poaceae); Cumaraswamy and Bawa 1989, *Cajanus cajan* L. (Fabaceae); Morgan and Barrett 1989, *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae); Ritland and Ritland 1989, *Mimulus guttus* Fischer complex (Scrophulariaceae)). All used dry weight as the currency of allocation. Three studies examined prezygotic sex allocation (Cumaraswamy and Bawa 1989; Morgan and Barrett 1989; Ritland and Ritland 1989). Although all found a decrease in relative male biomass with increased selfing, only the findings of Cumaraswamy and Bawa (1989) were significant. In the present study, relative male biomass (M_1), did not differ between populations. Three studies examined relative male biomass after fruit set using stamen and seed biomasses; all found a significant decrease with increased selfing (Schoen 1982a; Charnov 1987; Morgan and Barrett 1989). In the present study, similar relative male biomass estimates (M_2) showed a trend in the predicted direction, although populations did not differ significantly. Examining allocation to attraction, Morgan and Barrett (1989) and Ritland and Ritland (1989) reported non-significant decreases in the proportional corolla biomass with increasing selfing rate. Interestingly, in *G. achilleifolia*, corolla and calyx biomass did not decrease with increased selfing (Schoen 1982b). In the present study, there were no differences between populations in relative biomass of attraction when values were estimated from either corolla biomass or nectar biomass or both combined. However, because selfing in tableland plants depends upon pollinators (Ramsey *et al.* 1993), the high allocation to pollinator attraction in both populations is not unexpected (Lloyd 1987).

The lack of correspondence in the present study between relative male biomass and predictions of sex allocation theory may be due to the fact that the two populations do not exhibit large enough differences in their mating systems. Although plants in the two populations differ greatly in their relative self-fertilities (0.55 and 0.08 for tableland and coastal plants, respectively; Ramsey *et al.* 1993), there may be fewer differences in actual outcrossing rates. Biomass may not be a character appropriate for the examination of sex allocation unless extremes of the potential selfing-outcrossing gradient are exhibited, such as found in populations of *G. achilleifolia* where selfing rates ranged from 0.21 to 0.83 (Schoen 1982a). Genetic studies of the mating systems of both Christmas bell populations are needed to examine the relationship between relative male biomass and outcrossing rate.

In contrast to the lack of differences in the proportional biomass of flower parts, differences in pollen, ovule and seed production were pronounced. Tableland flowers produced significantly fewer pollen grains, but significantly more ovules and seeds than did coastal flowers. The pollen-ovule ratio of tableland flowers was less than half that of coastal flowers. These findings are consistent with theoretical predictions of reduced male function and increased female function in self-fertilising plants (e.g. Charlesworth and Charlesworth 1987*a*; Lloyd 1987). In addition, the findings suggest that changes to traits that are directly related to opportunities of fertility gain, such as pollen and ovules, precede changes to other traits that affect fertility indirectly by attracting pollinators (e.g. corolla size, nectar production).

Reduced pollen production and pollen-ovule ratios in selfing plants have been reported frequently in both interspecific and intraspecific comparisons (Cruden 1977; Schoen 1977; Wyatt 1984*b*; Vasek and Wang 1988). In contrast, the numbers of ovules per flower does not always increase in selfing plants (Wyatt 1984*b*; Vasek and Wang 1988; Ritland and Ritland 1989), suggesting that, at the flower level, pollen grain number and ovule number respond separately to selection. Selfing plants can increase female function without altering the number of ovules per flower by increasing the number of flowers that produce fruit (Lloyd 1987). Also, an increase in ovule and seed production may not always increase female fitness. For example, if seed dispersal is limited and sib-competition occurs among seedlings, the production of a large number of genetically similar seeds may result in frequency dependent selection and high seedling mortality (Charnov 1987). Nevertheless, as has been found in the present study, some selfing plants produce more ovules and seeds per flower than outcrossing plants (Schoen 1982*a*; Vasek and Wang 1988; Lyons and Antonovics 1991). These findings are of relevance to evolutionary models of self-fertility and sex allocation. In selfing plants, self seeds may be of lower fitness than outcross seeds because of inbreeding depression (Charlesworth and Charlesworth 1987*b*). Furthermore, because the probability of outcrossing is decreased in selfing plants the genetic value of pollen is reduced. Consequently, the production of more ovules and seeds, but not pollen, may enable a self-fertile plant to compensate for the reduced probability of survival of selfed progeny, thereby increasing its contribution of genes to the next generation. The redirection of resources saved by decreased pollen production may play an important role in the production of these extra ovules and seeds.

Conclusions

Variation in the breeding system between self-fertile tableland and outcrossing coastal Christmas bells is associated with differences in patterns of investment to reproduction. These patterns, however, are not clear cut and may represent either an early or intermediary evolutionary stage between self-fertility and autonomous selfing. There were no differences in the onset and duration of stigmatic receptivity, pollen longevity and nectar production of flowers from the two populations. Biomass of individual flowers was greater for coastal plants, although flower size, proportional biomass of floral parts and relative male biomass did not differ between populations. However, as predicted by sex allocation theory and evidenced by the production of more ovules and seeds but less pollen, tableland flowers allocated more resources to female function and less to male function than coastal flowers.

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