

Reproductive allocation in a gender dimorphic shrub: anomalous female investment in *Gynatrix pulchella*?

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Summary

1 In gender dimorphic species, reproductive allocation (RA, the ratio of reproductive to vegetative biomass) is predicted to be greater in female plants than in male plants. Empirical research on dimorphic plant species supports this hypothesis. To date, of 44 dimorphic angiosperms for which RA has been reported in the literature, RA is greater in females than males in 40 species, is equal in four, and in no species is it greater in males.

2 In many instances where differential RA occurs, sexual dimorphism in morphological or physiological traits has been reported. This dimorphism is often attributed to the differing costs of reproduction or to selection to counteract such costs.

3 We investigated RA and other morphological and physiological characters in *Gynatrix pulchella*, a dimorphic species that we found ranges from dioecious to subdioecious or gynodioecious, depending on season and locality. Our results showed that contrary to our predictions functionally male plants allocated significantly more biomass to reproduction than female plants across three populations. Greater male RA was due to a combination of larger, more numerous flowers and lower leaf biomass per branch than females.

4 There were no detectable costs of greater RA in males in terms of decreased overall growth or increased mortality. Additionally, leaf nitrogen content was greater in males than in females and there were no between-sex differences in gas exchange.

5 The finding that male plants allocate significantly more resources to reproduction than females in *G. pulchella* is apparently a unique case. However, equivalent RA for the sexes does occur in gynodioecious species and we suggest that higher male RA in gynodioecious plants may be more common than predictions based on sexual systems involving pure males.

6 Our results challenge accepted theory that allocation to reproduction should be greater in female plants than in males. In light of these findings, predictions regarding which sex should allocate more to reproduction need to be revised to accommodate the diversity of plant sexual systems.

Key-words: Dioecy, *Gynatrix pulchella*, gynodioecy, reproductive allocation, reproductive costs, resource allocation, sexual dimorphism, sexual system, subdioecy

Journal of Ecology (2006)

doi: 10.1111/j.1365-2745.2006.01164.x

Introduction

Patterns of resource allocation to male vs. female reproductive output are predicted to differ because fitness is achieved differently for male and female functions. Specifically, male fitness is determined by access to mates, whereas female fitness is driven by resources needed for the production of offspring (Bateman

1948). In dimorphic species (different sexual functions in separate genets), individuals are said to 'specialize' in either male or female function (Maynard Smith 1978; Bawa 1980) and generally differ in the ways in which they allocate resources. Dimorphic sexual systems in plants represent a continuum from dioecy (male and female flowers on separate individuals) to gyno- or androdioecy, where individuals are either hermaphroditic or unisexual (female or male, respectively). Subdioecy is potentially an evolutionarily intermediate phase between gynodioecy and dioecy, in which male and female flowers

are borne on separate plants, each of which may additionally bear perfect (hermaphroditic) flowers (Sakai & Weller 1999). Despite the continual variation among these systems, relative fitness achieved via male or female function is quantitative in nature. For example, a plant that bears both male and female flowers may set a small number of seeds but achieve the majority of its fitness via pollen export. Thus, in most cases individuals are thought to be generally functionally either male or female (Lloyd 1976; Delph & Wolf 2005).

Individuals that achieve fitness primarily through male function (hereafter, males) are predicted to optimize the *quantity* of mating opportunities by increasing investment in pollen production and pollinator attractiveness. These pressures generally result in enhanced floral display through an increase in flower number (Bawa 1980). Individuals that achieve fitness primarily through female function (hereafter, females) must clearly also produce flowers but these are generally fewer and without pollen. Instead, females specialize by increasing allocation to fruit and seed maturation, thereby optimizing the *quality* of offspring (Bawa 1980). While males must maximize access to mates, a small increase in pollen production is expected to effect large increases in fertilization, thus creating a situation of diminishing returns for allocation to male function (Lloyd & Webb 1977; Bawa 1980). By contrast, provided pollen receipt does not decrease, a continued increase in allocation to ovule production should continue to increase seed set until resource limitation prevents further fruit production (Lloyd & Webb 1977; Bawa 1980). Not only are the energetic requirements for producing fruit generally greater than for flower production but also the maturation of fruit usually extends for a considerable period after flowering. Therefore, unless exceptionally large amounts of pollen are produced, optimal resource allocation to female function is predicted to be greater than that to male function per reproductive bout (Lloyd & Webb 1977).

The theoretical prediction of greater female allocation is strongly supported by empirical evidence from studies on reproductive allocation (RA, the ratio of reproductive to vegetative biomass) in dimorphic plant species. In dioecious species (separate male and female individuals), RA 'per bout' is nearly always greater in females than in males (Delph 1999). Allocation to reproduction is occasionally equal for both sexes, but this is generally among wind-pollinated species, in which male pollen production is high relative to insect-pollinated species (Cruden 1977; Delph 1999; Obeso 2002). Of 41 dioecious angiosperm species (including three subdioecious: separate male, female and inconstant hermaphrodite individuals), female RA exceeds that of males in 39 cases (Delph 1990; Barrett *et al.* 1999; Delph 1999; Nicotra 1999a; Obeso 2002; Leigh & Nicotra 2003; Pickering & Arthur 2003; Pickering *et al.* 2003). The remaining two dioecious species are wind-pollinated and in these, RA does not differ between males and females (references in Delph 1999). Of three

gynodioecious species (separate female and hermaphrodite individuals) for which RA has been reported, female allocation is greater than male allocation in one (Poot 1997), slightly greater (though not significantly) in the second (Kohn 1989), and does not differ significantly between males and females in the third (Ashman 1994).

Given the different energy requirements associated with the respective RA strategies of each sex, we might expect secondary sex characteristics to differ accordingly (Lloyd & Webb 1977). Although not widely researched, morphological and physiological secondary sex characteristics are known to vary between the sexes of dimorphic species. For example, sex-based differences have been found in leaf size (Wallace & Rundel 1980; Agren 1988), growth (Nicotra 1999b) and photosynthetic rates (Dawson & Bliss 1993; Laporte & Delph 1996). Sexual dimorphism in secondary sex characteristics may be the result of between-sex differences in reproductive costs (Lloyd & Webb 1977; Delph 1999) or might arise through selection for traits that help ameliorate these costs (Cipollini & Stiles 1991; Nicotra 1999b; Nicotra *et al.* 2003). Here we define cost as a 'decrease in future reproduction, growth, or survival caused by investing in current reproduction' (Delph 1999, p. 150).

In this study, we aimed to test the hypotheses that allocation to reproduction by females of a dimorphic species will be greater than by males, and that females accordingly incur greater costs than males. Further, we asked whether females possess morphological or physiological traits that might offset reproductive costs or enhance their reproductive function. We addressed these questions in *Gynatrix pulchella* (Malvaceae), a reportedly dioecious species with inconspicuous fruits and no obvious dimorphic characters (Harden 1990–93).

The outcomes of our work were surprising for two reasons: first, we discovered that many of the male plants of this species bear fruit, with populations ranging from almost completely dioecious to subdioecious or gynodioecious. Both the quality and quantity of the fruits and seeds of these inconstant males were inferior to those of female plants. This finding, coupled with their role as the sole pollen donors, leads us to conclude that even the male plants with perfect flowers are functionally male (Lloyd 1976; Lloyd 1980). Secondly, contrary to predictions and the available empirical evidence, we found female RA to be significantly lower than male RA. We discuss the possible causes and implications of this finding.

Methods and materials

STUDY SPECIES

Gynatrix pulchella (Willd.) Alef. (Malvaceae), or hemp bush, is a spreading, reportedly dioecious, evergreen shrub growing up to 4 m high. Male flowers are stalked and borne in loose inflorescences, whereas female flowers are mostly sessile or shortly stalked. Male flowers

mostly possess non-functional ovaries, while female flowers have some staminodes (Harden 1990–93). The fruits are dry and separate into five mericarps when mature. Insects are the likely pollinators as the flowers have a strong chocolate scent, presumably a pollinator attractant, and small flies, bees and butterflies were observed on flowering plants.

FIELD SITES

Three populations of *G. pulchella* were studied in the Australian Capital Territory, Australia: Tidbinbilla Nature Reserve; Orroral Valley in Namadgi National Park; and Googong Dam Foreshores. At Tidbinbilla, a large population grew in low-lying, drainage areas and along creek beds in wet sclerophyll forest. Here, *G. pulchella* plants existed as tall understorey shrubs, somewhat shaded by the taller eucalypt canopy, with a small number growing in minor clearings. The plants growing at Orroral Valley also occurred along a creek line, shaded by a eucalypt overstorey, but were generally smaller and had thinner canopies. This population was scattered across a broader area than Tidbinbilla. The Googong Dam site contrasted with the other two by being exposed to full sun and wind. This population existed as a single-species stand with a few outlying individuals growing on the edge of a narrow gorge containing a small creek.

In the spring/summer of 2000, we conducted a study on 20 plants (10 males, 10 females) at Tidbinbilla. This study involved morphological, physiological and allocation measurements. In 2002, we repeated the allocation measurements on the 20 original plants plus 107 additional plants at Tidbinbilla, and on the populations at Googong and Orroral Valley. At Tidbinbilla, we sampled 127 plants (70 males, 57 females) in an area running approximately 200 m along a creek bed and 30 m either side of the water's edge. At Orroral Valley, we sampled 68 plants (40 males, 28 females) within an area running approximately 300 m along a creek and 10 m either side. At Googong, 103 plants (55 males, 48 females) were sampled. This population was clustered along approximately 30 m of the gorge, with the majority of plants not more than 3 m from the edge, and a few scattered individuals up to 80 m from the main group. At each site, all reproductively active individuals within the defined areas were sampled. Although resprouting occurs in this species, clonality was ruled out by tracing each leading stem back to a discreet root system and single individuals were identified among closely spaced plants in all three locations.

PLANT AGE

For every tagged plant at each site in 2002, we measured the basal diameter at soil surface. To determine the extent to which stem diameter represents plant age, we took sections through sample dead stems collected from Googong. We measured the girth under bark and

counted growth rings for each section. We found that girth increased with the number of rings (ANOVA, $F_{1,10} = 7.30$, $P = 0.022$), indicating that basal diameter is a reasonable predictor of plant age for this species.

REPRODUCTIVE ALLOCATION

Reproductive allocation was measured in 2000 on the 20 plants at Tidbinbilla and in 2002 on all sample plants at the three sites. We estimated RA using reproductive biomass, including peduncles, flowers and fruits, as a proportion of vegetative biomass (Reekie & Bazzaz 1987a; Obeso 2002). Because *G. pulchella* is a rare and large woody species, we did not destructively sample, hence we measured RA on a modular basis. We calculated reproductive biomass allocated to a branch, standardized as a ratio of reproductive biomass to leaf, stem or total vegetative biomass for a branch, RA_{leaf} , RA_{stem} , and $RA_{\text{leaf+stem}}$, respectively. Note that the sampling regime was designed based on the assumption that *G. pulchella* was dioecious and thus only females should set fruit, as was the case in 2000.

Through a visual assessment of the plants during flowering, we ascertained that males and females did not appear to differ in the proportion of branches that bore flowers. We selected a representative flowering branch from each plant. A branch was selected if the number of flowers it bore was typical of the majority of flowering branches on that plant. Each branch was cut at a node representing that season's growth, usually where it joined a larger branch. The size of the selected branch varied from site to site as the size and growth habit of the plants varied among sites. At Tidbinbilla, where plants were larger than the other two sites, average branch length was $20.4 \pm \text{SD } 7.33$ cm. At Orroral, most plants were considerably smaller than at Tidbinbilla, and branch length averaged 14.5 ± 6.26 cm. At Googong, where most plants consisted of a single stem with a small number of side branches, branch length averaged 14.5 ± 5.42 cm. The number of leaves and flowers was recorded for each branch. Male branches were removed at the peak of flowering and brought back to the laboratory where flowers, peduncles, leaves and stems were separated, oven-dried and weighed. Female branches were left on the plant until fruit had matured. Five flowers were removed from each study plant, oven-dried and weighed to calculate the mean single flower mass for each plant. An estimate of total flower mass for each branch was obtained by multiplying the mean flower mass on the individual plant by the initial number of flowers on the sample branch plus the total peduncle mass for that branch. Reproductive allocation (RA_{leaf} , RA_{stem} , $RA_{\text{leaf+stem}}$) for each male plant was calculated as flower mass per unit mass of vegetation. For each female plant, RA was the mass of non-fruiting flowers plus the total mass of fruits per unit mass of vegetation. To determine whether the leaf biomass estimate for females changed between flowering and fruiting, the number of leaves on female branches

was counted at both stages. We detected virtually no new leaf growth or leaf drop in female plants during the period between when they flowered and when we collected fruiting branches.

Estimates of the relative reproductive investment made by each sex can vary, depending on the currency used (Reekie & Bazzaz 1987b). Nevertheless, calculations of RA based on carbon are thought to reflect allocation of other resources, particularly if carbon is a limiting resource, for example in the shaded understorey (Goldman & Willson 1986; Reekie & Bazzaz 1987b). To assess the effect of differing currency, we calculated RA as a function of nitrogen content for Tidbinbilla plants in 2000 (RA_N , grams of nitrogen in reproductive structures per gram of leaf tissue). Nitrogen content was determined by a semi-micro Kjeldahl technique (AOAC 1984). Tissue samples were digested at 250 °C for 15 min, then 460 °C for 1 hour in a Tectator 2012 heating block with 6 mL sulphuric acid and a selenium catalyst. Cooled samples were then distilled and titrated with a Gerhardt Vapodest-5 apparatus. Flowers and fruits were small enough to be digested entire (some pollen was lost from male flowers during handling). Due to limited reproductive tissue being available for individual plant assays, flowers from several plants of each sex were pooled. The same procedure was used for fruits. The results of these analyses were used to estimate nitrogen allocation to reproduction.

FEMALE FUNCTION IN INCONSTANT MALE PLANTS

Because we discovered that some male plants produced fruit in 2002 after we had collected male branches for RA measurements, we do not know how many fruit each branch would have set or what proportion of the flowers had functional ovaries. We did, however, record the number of males that set fruit at each site. To investigate the quality and quantity of propagules produced via female function on inconstant males, we assessed the number and mass of fruit and seed. We collected and weighed fruits from sample fruiting branches of the males that fruited at Orroral Valley and Googong. We later collected additional fruits from a subset of plants at Googong (30 males, 30 females) and Tidbinbilla (23 males, all the fruit-bearing males we could find, and 30 females) to obtain fruit masses for Tidbinbilla, additional fruit masses for Googong, and the number of fruits per branch, relative to females for these two sites. For these additional fruits we recorded the number of seeds that were pale and round (fully developed) vs. dark and shrivelled (most likely aborted or non-fertilized ovules), and weighed the fully developed seed to obtain an average seed mass for each sex.

LEAF TRAITS

To assess patterns of biomass allocation within leaves we measured specific leaf area (SLA $m^2 g^{-1}$ leaf tissue)

at Tidbinbilla in 2000 on the 20 study plants plus 10 additional plants (five of each sex). All leaves on a single flowering branch were collected and scanned in the laboratory on a flatbed scanner and analysed using the public domain NIH Image program (<http://rsb.info.nih.gov/nih-image/>) to obtain a total leaf area for the branch. Leaves were oven-dried and weighed and the total leaf area on the branch was divided by the total dry mass of the leaves for that branch to obtain an estimate of SLA for each plant.

Leaf nitrogen and chlorophyll content were analysed for the 20 study plants at Tidbinbilla in 2000. Leaves of both sexes were collected during flowering. Leaf nitrogen analysis was carried out as described for flowers and fruits except that leaf tissue was ground for analysis to obtain homogenous samples and individual analyses were carried out for each plant. For chlorophyll analysis, two sections of a young, healthy leaf from each of 20 plants were cut from either side of the mid-vein. These sections were scanned to obtain an area and immediately frozen in liquid nitrogen and stored at -80 °C until used for chlorophyll extraction. Chlorophyll was extracted with buffered acetone and concentration was determined using a DU 640 Spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) (Porra *et al.* 1989). Chlorophyll concentration was divided by the area of the sample discs to obtain an estimate of leaf chlorophyll content ($chl\ a + chl\ b$, $mol\ m^{-2}$) for each plant.

GAS EXCHANGE

Gas exchange measurements were conducted on the 20 original study plants at Tidbinbilla at three periods: in November 2000 during flowering; when females were fruiting in December; and in January after most fruits had dehisced. Measurements were made on three young, fully expanded leaves per plant. All leaves were fully expanded and of similar age (three to five nodes down from the apical meristem). Selected leaves were those that received maximum direct sunlight given the position of the plant, i.e. generally facing north-east. In nearly all cases, the same leaf was used for all sampling periods. In the final sampling period when most fruits had dehisced, three of the 60 sample leaves had disappeared; in these cases a new leaf was selected, adjacent to the original leaf, and facing a similar angle.

Measurements were made using a LI-COR 6400 portable photosynthesis system (LI-COR Inc.). Light was provided with the LI-COR chamber-mounted Red/Blue light source. Saturating light level for *G. pulchella* was determined via a series of preliminary light response curves and set for measurements at a photon flux density of 1800 $mol\ m^{-2}\ s^{-1}$. Relative humidity ranged between 50 and 70%. We conducted measurements between the hours of 08.30 and 14.30 and ceased when leaf temperatures reached 36 °C. During November and December, maximum air temperatures ranged from 25 to 37 °C, and reached

Table 1 Results of analysis of covariance for differences in reproductive and vegetative allocation parameters on each branch in *Gynatrix pulchella* plants in 2002 with respect to sex and site effects and adjusted for basal diameter

Trait	d.f.	Site		Sex		Site × Sex		Basal diameter		Model R ²
		F-value	Probability	F-value	Probability	F-value	Probability	F-value	Probability	
Mean flower biomass	(6291)	322.63	< 0.0001	716.77	< 0.0001	41.15	< 0.0001	4.81	0.0291	0.86
Mean fruit biomass	(6291)	1.33	0.2665	13.92	0.0002	3.01	0.0515	1.00	0.3188	0.11
Peduncle biomass	(6291)	40.86	< 0.0001	12.97	0.0004	0.84	0.4311	0.10	0.7503	0.27
Flower biomass	(6291)	42.04	< 0.0001	80.42	< 0.0001	9.76	< 0.0001	1.70	0.1937	0.42
Number of flowers	(6291)	33.57	< 0.0001	15.69	< 0.0001	0.09	0.9167	13.26	0.0003	0.28
Leaf biomass	(6291)	205.75	< 0.0001	11.79	0.0007	0.00	0.9963	3.68	0.0560	0.61
Stem biomass	(6291)	57.27	< 0.0001	3.13	0.0779	0.57	0.5659	0.73	0.3935	0.30
Leaf + stem biomass	(6291)	9.42	< 0.0001	33.56	< 0.0001	6.65	0.0015	3.75	0.0537	0.19
Reproductive biomass	(6291)	39.06	< 0.0001	2.88	0.0908	1.89	0.1530	2.16	0.1427	0.23
RA _{leaf}	(6291)	16.79	< 0.0001	16.00	< 0.0001	4.18	0.0163	4.01	0.0461	0.19
RA _{stem}	(6291)	0.86	0.4242	0.02	0.8815	2.02	0.1341	7.74	0.0057	0.05
RA _{leaf+stem}	(6291)	36.88	< 0.0001	9.24	0.0026	3.34	0.0367	14.05	0.0002	0.30
Basal diameter	(5292)	19.76	< 0.0001	0.02	0.8907	0.32	0.7318	—	—	0.12

42 C in January. We tested for variation in gas exchange among leaves from a single flush by taking measurements on a number of sample leaves at various nodes along a branch. We found the variation among leaves from different nodes on the same branch to be similar to the variation among leaves from the same nodes on different branches, suggesting that within a season, the effects of leaf age on gas exchange were negligible.

DATA ANALYSIS

Male fruit data were analysed using ANOVA, with site and sex as the main effects. Reproductive allocation data were analysed using analysis of covariance, with sex and site as discrete factors. Plant basal diameter, a measure of plant age, was included as a covariate because reproductive allocation can be influenced by plant size (Pino *et al.* 2002). Measures of gas exchange through time were analysed using repeated measures MANOVA. All linear model analyses were done in JMP (SAS Institute Inc., Cary, NC, USA). Sex ratios at the three sites were analysed using a replicated *G*-test (Sokal & Rohlf 1995).

Results

FEMALE FUNCTION IN INCONSTANT MALE PLANTS

The discovery of fruit production among 'male' plants came as a surprise to us in 2002. Here we begin by presenting our results on inconstancy among males. At Googong, male fruit production was heavy and 35 of the 55 males fruited. At Orroral Valley, 27 of the 40 male plants produced fruit. At Tidbinbilla in 2000, no fruits were observed on male plants, whereas in 2002 we noticed the occasional male plant bearing up to five fruits, none of which were borne on our study plants. Male fruit was smaller than female fruit at Tidbinbilla and Orroral Valley and did not differ from female fruit mass at Googong (Tables 1 and 2). Females bore more fruits and seeds per branch than males at Googong and Tidbinbilla, the two sites for which this was recorded (Fig. 1). Additionally, both the proportion of seed that was fully developed and the seed mean mass was greater in females (Fig. 1).

Because only a proportion of males fruited across sites and those that did produced fewer, smaller fruits and seeds, we will refer to our non-female study plants as male. We feel that this is justified for two reasons: first, seed counts of gynodioecious 'males' are known to overestimate the genetic contribution of these individuals via ovules; and secondly, these inconstant males differ from true hermaphrodites by passing on the majority of their genes via pollen, rendering them functionally male (Lloyd 1976). Considering all functional males in our populations as male, sex ratios did not differ significantly from equality on a site-by-site basis or when all data were pooled (Table 3).

Table 2 Measures of mean individual flower and fruit mass, total number of flowers per branch, total biomass per branch and basal diameter in 2002. Values are the mean \pm standard error in milligrams (unless stated) for males and females at each site. The last column shows the ratio of male : female pooled for the three sites. For results of statistical analyses see Table 1

Trait	Tidbinbilla		Orroral Valley		Googong		Ratio M:F
	Males	Females	Males	Females	Males	Females	
Mean flower biomass	4.65 \pm 0.09	2.37 \pm 0.05	2.68 \pm 0.09	1.52 \pm 0.05	2.58 \pm 0.05	1.31 \pm 0.02	1 : 0.5
Mean fruit biomass	9.44 \pm 0.48	11.32 \pm 0.31	8.82 \pm 0.57	11.27 \pm 1.02	9.66 \pm 0.38	9.85 \pm 0.39	1 : 1.2
Peduncle biomass	55.84 \pm 4.95	38.89 \pm 3.88	25.54 \pm 2.76	17.82 \pm 3.33	22.03 \pm 2.10	12.09 \pm 1.48	1 : 0.7
Flower biomass	479.51 \pm 32.75	201.11 \pm 14.98	223.57 \pm 19.25	101.13 \pm 13.01	233.90 \pm 12.97	122.09 \pm 8.99	1 : 0.5
Number of flowers	105.50 \pm 7.55	83.96 \pm 5.64	83.95 \pm 7.01	67.89 \pm 9.64	92.11 \pm 5.08	94.29 \pm 7.20	1 : 0.9
Leaf biomass	806.32 \pm 50.14	920.81 \pm 51.75	145.72 \pm 17.92	264.98 \pm 26.72	98.72 \pm 8.83	210.28 \pm 19.89	1 : 1.3
Stem biomass	389.44 \pm 28.04	445.03 \pm 29.80	178.42 \pm 16.71	182.36 \pm 24.38	184.26 \pm 12.01	234.00 \pm 16.07	1 : 1.2
Leaf + stem biomass	1195.77 \pm 73.33	1365.84 \pm 70.89	324.14 \pm 31.06	447.34 \pm 43.31	282.98 \pm 17.92	444.28 \pm 32.80	1 : 1.2
Reproductive biomass	535.34 \pm 37.09	547.26 \pm 41.78	249.11 \pm 20.60	261.13 \pm 49.09	255.92 \pm 14.62	383.58 \pm 31.24	1 : 1.1
Basal diameter (cm)	4.55 \pm 0.27	5.00 \pm 0.28	4.48 \pm 0.40	4.12 \pm 0.48	7.15 \pm 0.67	7.22 \pm 0.67	

Table 3 Results of replicated *G*-test for sex ratio bias of *Gynatrix pulchella* at Tidbinbilla, Orroral Valley and Googong

Site	Proportion		d.f.	<i>G</i>	Probability
	male	<i>n</i>			
Tidbinbilla	0.55	127	1	1.333	NS
Orroral	0.59	68	1	2.129	NS
Googong	0.53	103	1	0.476	NS
<i>G</i> -total			3	3.938	NS
<i>G</i> -pooled			1	3.443	NS
<i>G</i> -heterogeneity			2	0.495	NS

REPRODUCTIVE ALLOCATION

Contrary to our expectations that female RA would exceed that of males, female RA at Tidbinbilla in 2000 never exceeded male RA, irrespective of the method used to calculate it. Furthermore, when RA was calculated as a ratio of reproductive biomass to a combined leaf and stem biomass per branch, RA_{l+s} , female plants had significantly lower RA than male plants (ANOVA: males, mean 0.18 ± 0.03 SE; females, mean 0.09 ± 0.02 SE; $F_{(1,18)} = 7.14$, $P = 0.0156$). The same pattern was found when reproductive allocation was calculated as a ratio of reproductive biomass to leaf biomass alone, RA_{leaf} , to stem biomass alone, RA_{stem} , or as a function of nitrogen, RA_N , although the pattern was not statistically significant in these cases (data not shown).

These unexpected RA results prompted further field investigation to confirm that the findings were not due to small sample size or an atypical year in terms of lower fruit production. In 2002, we found average female RA_{leaf} and RA_{l+s} to be significantly lower than that of males, though the effect of sex on RA_{leaf} and RA_{l+s} differed among sites (Fig. 2, Table 1). On an individual site basis, female RA_{leaf} was significantly lower than that of males at Googong and Orroral Valley, whereas at Tidbinbilla the difference was not significant (Fig. 2a). Female RA_{l+s} was lower than that of males at Orroral Valley and Tidbinbilla, though not significantly so at the latter site, whereas no between-sex difference in RA_{l+s} could be detected at Googong (Fig. 2c). At no site did RA_{stem} differ between the sexes (Fig. 2b). Basal diameter was a significant predictor of RA_{leaf} , RA_{stem} , and RA_{l+s} (Table 1) but did not differ between the sexes (Tables 1 and 2).

Females allocated less biomass to the production of flowers than males. Individual female flowers weighed approximately half as much as male flowers, although this difference varied among sites (Tables 1 and 2). As expected, females also invested less biomass in peduncles than males (Tables 1 and 2). On average, the number of flowers and total flower biomass per branch was lower for female plants than male plants (Tables 1 and 2).

Reproductive allocation is a ratio of reproductive biomass to vegetative biomass, therefore between-sex differences in RA can result from variation in either

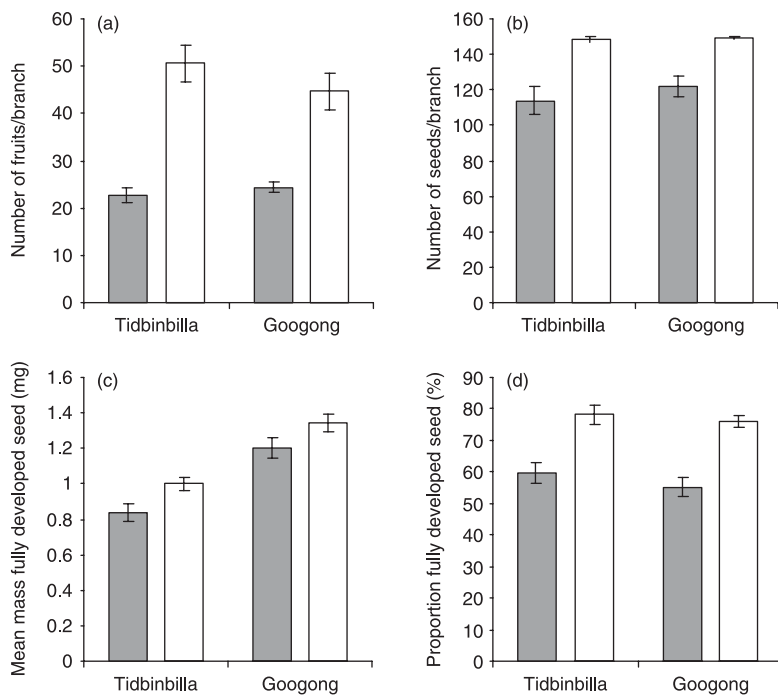


Fig. 1 Measures of mean fruit and seed numbers and masses of *Gynatrix pulchella* inconstant males (shaded bars) and females (open bars) at Tidbinbilla and Googong. Error bars represent the standard error.

one or both factors. In order to determine the relevant importance of reproductive vs. vegetative allocation in influencing RA, we investigated between-sex differences in the individual components of RA. Across sites, average total reproductive biomass per branch did not differ between sexes (Tables 1 and 2), although male reproductive biomass would have been higher had we included male fruit in our RA calculations. By contrast, total leaf and leaf + stem biomass was significantly greater in females than in males, though no between-sex difference was found when stem biomass was considered alone (Tables 1 and 2).

LEAF TRAITS AND PHYSIOLOGY

Male plants at Tidbinbilla had a significantly greater concentration of nitrogen (%) in their leaves than female plants when compared at the height of flowering (ANOVA: males, mean 4.3 ± 0.07 SE; females, mean 4.0 ± 0.09 SE; $F_{1,18} = 4.80$, $P < 0.0404$). No difference in leaf chlorophyll content ($\mu\text{mol m}^{-2}$) between male and female plants could be detected (males, mean 118.1 ± 19.97 SE; females, mean 126.9 ± 14.92 SE; $F_{1,18} = 0.12$, $P > 0.7391$). Specific leaf area ($\text{m}^2 \text{g}^{-1}$) did not differ between the sexes (males, mean 0.016 ± 0.0005 SE, females, mean 0.016 ± 0.0007 , $F_{1,28} = 0.03$, $P > 0.8617$).

Photosynthetic rate declined through time, with significant differences among the flowering, fruiting and post-fruiting periods; however, no differences between the sexes could be detected (Fig. 3, Table 4). Similar results were obtained for stomatal conductance

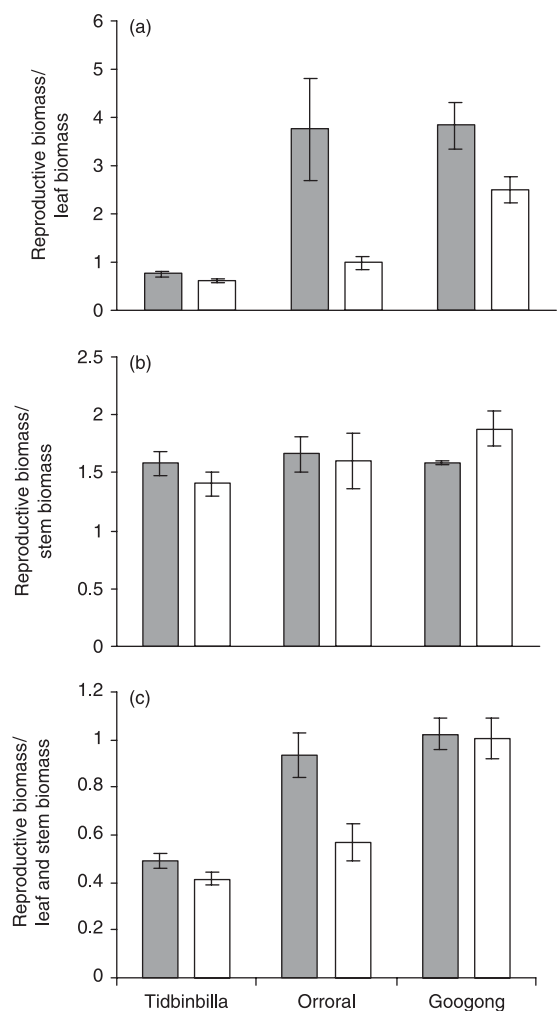


Fig. 2 Reproductive allocation in *Gynatrix pulchella* plants (males = shaded bars, females = open bars) at Tidbinbilla, Orroral Valey and Googong. Graphs a–c represent RA_{leaf} , RA_{stem} and $RA_{\text{leaf+stem}}$, respectively.

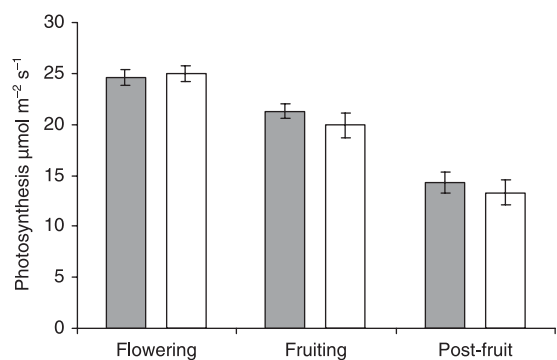


Fig. 3 Photosynthetic rate in male (shaded bars) and female (unshaded bars) *Gynatrix pulchella* plants during three reproductive periods.

and transpiration (Table 4). Such a decline in gas exchange through time could be due to leaves ageing, the increasing heat in summer, or the effects of reproduction.

Table 4 Repeated measures MANOVA tests for differences in gas exchange ($\text{mol m}^{-2} \text{s}^{-1}$) between male and female *Gynatrix pulchella* plants over three sampling periods: flowering, fruiting and post-fruiting

Trait	Time		Sex		Time \times Sex	
	F-value	Probability	F-value	Probability	F-value	Probability
Photosynthesis	135.96 _(2,17)	< 0.0001	0.38 _(1,18)	0.5461	1.41 _(2,17)	0.2717
Transpiration	36.48 _(2,17)	< 0.0001	0.52 _(1,18)	0.4788	0.32 _(2,17)	0.7292
Conductance	52.16 _(2,17)	< 0.0001	2.79 _(1,18)	0.1119	0.04 _(2,17)	0.9633

Discussion

Our finding of lower female RA_{leaf} and RA_{1+3} for *G. pulchella* when all sites were pooled, and at two individual sites, is unexpected. Of the recorded 44 studies documenting RA in dimorphic species, female RA is greater than male RA in 40 cases and in the remaining four male and female RA is equal (see References in Introduction). In no case is female RA lower than that of males. Hence, the occurrence of females with lower RA than males in *G. pulchella* represents the first such documented case. These results exclude allocation to fruit in inconstant males, inclusion of which would amplify the difference in RA. We argue that the staminate individuals of this species, irrespective of inconstancy, should be regarded as males. However, even if we consider inconstant males to be hermaphroditic morphs, we are still faced with anomalous patterns of RA. Here we will examine the relative importance of flower number and size, fruit number and size, and vegetative allocation to our RA results.

REPRODUCTIVE ALLOCATION: FLOWERS

Male plants of dimorphic species frequently produce more flowers than females, thought to be an adaptation to increase attractiveness to pollinators (Bawa 1980; Bond & Midgley 1988; Maki 1996). In *G. pulchella*, male plants bore up to 25% more flowers than females. Males also invested more biomass in inflorescence architecture, having much greater peduncle mass than females, which had either small or no peduncles. Additionally, male individual flower mass in *G. pulchella* was up to twice that of females, due to the larger petals and a staminal column with many pollen-bearing anthers.

REPRODUCTIVE ALLOCATION: FRUIT

Relative to hermaphroditism, dimorphism is generally associated with large, fleshy, animal-dispersed seeds, which often demand a high resource input (Bawa 1980; Givnish 1980). Thus, while heavy male flower investment often leads to higher or equivalent male RA at flowering, subsequent fruit production by females results in their having a greater total RA per reproductive bout than males (Bawa 1980; Wallace & Rundel 1980; Gross & Soule 1981; Lovett Doust & Lovett Doust 1988; Popp & Reinartz 1988; Kohn 1989; Delph 1990; Antos & Allen 1994; Delph & Meagher 1995;

Obeso 1997; Hogan *et al.* 1998; Rocheleau & Houle 2001). Even among gynodioecious species, the combined allocation to flower and fruit production has not been associated with greater male than female RA (Kohn 1989; Ashman 1994). One explanation for this pattern is that dioecious species are often animal dispersed and greater allocation to fruit production is the result of selection for increased attractiveness to seed dispersers (Bawa 1980; Givnish 1980). Individual fruit mass of dimorphic species can be up to 30 times greater than that of flowers (Antos & Allen 1994). The fruits of *G. pulchella* apparently disperse ballistically and are relatively small, averaging 11 mg, making them only six times the mass of female flowers and three times the mass of male flowers. Thus, the small *G. pulchella* fruits may restrict the extent of female reproductive biomass such that, coupled with the high flower investment by males, total female reproductive biomass does not exceed that of males.

In this study, we did not incorporate male fruit production into the RA measurements. As hermaphroditic males of gynodioecious species produce both flowers and fruits, it is reasonable to expect that their total RA might be higher than that of pure males. This expectation is partly borne out in the literature: relative to dioecious species, where female RA is nearly always higher, gynodioecious species appear more likely to have equivalent RA (see References in Introduction). For this reason, in the present study where *G. pulchella* populations included gynodioecy, we might also expect an equal RA for the sexes. However, in the two sites where populations tended towards gynodioecy, male RA was significantly greater than female RA even without the inclusion of male fruit in the calculations. Therefore, our results do not fit expectations for any dimorphic species and, based on the available literature, cannot necessarily be explained by sexual system alone.

VEGETATIVE ALLOCATION: REPRODUCTIVE COSTS

In a dimorphic plant species, costs of reproduction should be more evident in the sex with higher RA (Lloyd & Webb 1977; Delph 1999). Thus, we might expect *G. pulchella* males to compensate for high reproductive investment by having less frequent reproductive bouts, a shorter lifespan, reduced growth rate or reduced vegetative growth. In our study, *G. pulchella*

males did not appear to reproduce less frequently than females, as all individuals bar a few young seedlings (less than 1 year old) in our populations were reproductively active. Increased mortality by one sex might be indicated if a population had a biased sex ratio or was age-structured according to sex. We found no evidence of female-biased sex ratios in any of our populations. Similarly, we found no between-sex difference in basal diameter, which correlated with age for this species. A similar average plant size between the sexes also suggests that there is no negative effect of male reproduction on overall growth rate. Thus, at the whole plant level, we find little evidence of indirect costs of reproduction.

In dimorphic species where high male flower output leads to an equivalent total reproductive biomass per module for each sex, female RA calculated as a proportion of total allocation is nearly always greater than male RA (Delph 1990; Pickering *et al.* 2003). In *G. pulchella*, the average reproductive biomass on a module (excluding fruit from inconstant males) for the sexes was equivalent, whereas average male vegetative biomass was lower than female vegetative biomass. High reproductive demand can reduce vegetative growth in dioecious species and is assumed to represent a resource trade-off by females between reproductive and vegetative allocation (Allen & Antos 1993; Cipollini & Whigham 1994; Houssard *et al.* 1994; Rocheleau & Houle 2001). For *G. pulchella* at Googong and Orroral Valley, where the majority of male plants fruited, males had significantly less vegetative biomass than females, whereas at Tidbinbilla, where virtually no males fruited, this difference was not significant. This association may suggest that fruit production by males at Googong and Orroral Valley negatively affects their vegetative growth, but without directly manipulating RA we cannot be certain (Nicotra 1999a). Nor can we comment on whether the variation in fruit production by males between and among populations is triggered by resource availability (see Delph & Wolf 2005). However, we note that female plants produced larger and more numerous fruits yet had higher vegetative biomass than males; thus any effect of fruit on vegetative allocation is unlikely to be general to the species. Other possible explanations for a lower male leaf biomass are that males can produce more reproductive biomass with a relatively lower local vegetative investment or that male RA is subsidized by long-distance transport of resources.

The high nitrogen content of pollen means that heavy flower production could incur considerable costs to males (Goldman & Willson 1986). For example, nitrogen might be translocated from vegetative to reproductive function (Karlsson 1994; Saulnier & Reekie 1995), which could ultimately lead to a decrease in male photosynthetic rates. In this study, male *G. pulchella* plants had more nitrogen in their leaves than females at flowering and showed no difference in photosynthetic traits relative to females. Thus, males had

relatively lower photosynthetic nitrogen use efficiency, but not a lower per unit area carbon gain potential. Higher leaf nitrogen in males could mean that they have the capacity to up-regulate their photosynthetic rate to ameliorate reproductive costs, though we found no evidence of this as not only gas exchange rates but also leaf chlorophyll content were equal for the sexes. Thus, while reproductive demands apparently do not negatively affect male gas exchange any more than in females, nor does male reproduction induce photosynthetic up-regulation.

Conclusions

The present results suggest several avenues of further investigation. As this species is rare (and relatively large), our work was restricted to minimal destruction and thus a modular focus was taken. When examining the populations for study, we inspected dozens of plants to determine whether males and females appeared to differ in the proportion of branches devoted to reproduction. Although we did not assess this statistically, our impression was that they did not differ. Because of this approach we cannot comment on patterns of root allocation or whole plant RA. However, *G. pulchella* is suitable for propagation and, as such, could be the focus of further study on: whole plant (including root) allocation patterns; indirect costs associated with reproduction; and correlates (for example resource availability) of variation in RA and constancy in males.

Why do *G. pulchella* males allocate more resources to reproduction than females and why hasn't such an occurrence been reported before? As with dioecious species, functional males of gynodioecious species can invest significantly more resources in flowers than females, for example in gynodioecious *Cucurbita foetidissima* (Kohn 1989). In the case of *Cucurbita*, where fruits are large and fleshy, allocation to female fruit is so high that female RA equals that of hermaphrodite males (Kohn 1989). By contrast, not only do *G. pulchella* males produce much larger and more numerous flowers than females but also fruits of this species are small and potentially relatively inexpensive to produce. Heavy flower production by *G. pulchella* males is apparently sufficient to elevate their RA above that of females. It is worth noting that of the many studies examining RA in dimorphic species, only a handful of these concern gynodioecious and subdioecious species. Further, unlike dioecious insect-pollinated species where female RA is always higher, equivalent RA can occur in insect-pollinated gynodioecious species (see References in Introduction). This trend suggests that RA of functional males is likely to be higher in gynodioecious than dioecious species. Future research on a greater number of gynodioecious and subdioecious species may uncover more cases of higher male RA. If so, predictions as to which sex we should expect to allocate more to reproduction will need to be modified to accommodate the diversity of plant sexual systems.

Acknowledgements

This research was supported by an Australian Research Council large grant to A.B.N. A.L. was supported by an Australian National University Research School of Biological Sciences Honours Scholarship. Thanks to the Canberra Nature Parks for access to plants at Tidbinbilla Nature Reserve, Googong Foreshores and Orroral Valley. We are grateful to Spencer Barrett and two anonymous reviewers for helpful comments on an earlier version of this manuscript.

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Received 11 April 2006

revision accepted 5 June 2006

Handling Editor: Honor Prentice