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Sexual allocation in single-flowered hermaphroditic individuals in relation to plant and flower size

Abstract Gender expression in hermaphroditic plant species usually departs from strict equisexuality. Study of those departures can aid understanding of non hermaphroditic breeding systems and prevalence of hermaphroditism within angiosperms. Plant size is one of the most studied factors in relation to gender modification. We studied variation in gender expression in the hermaphroditic, mostly single-flowered *Paeonia cambessedesii*. We separately studied gender modification with increasing plant and flower size using a variety of currencies: number of ovules and stamens, dry mass, N and P. Flower size and number of floral structures (petals, stamens, carpels, and ovules) increased with plant size. Number of ovules increased more rapidly with increasing plant size than number of stamens, indicating a bias towards femaleness with increasing plant size. A similar pattern was found when regressing number of stamens and number of seeds against plant size. Number of floral structures increased with increasing flower mass, but no significant difference was found between stamens and ovules in their rate of increase. Thus, gender modification at plant level was not consistent with patterns at flower level. No differential allocation to stamens vs gynoecium, or sexual structures vs petals was found when using dry mass, N or P as currencies. However, a disproportionate increase in female allocation was found when number of structures was utilised as currency. Study of size-dependent gender expression will benefit from contrast of results obtained using several analysis levels and allocation currencies.

Keywords Allocation currency · Ovule packaging · Size-dependent sex allocation

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Introduction

Patterns of sexual expression in hermaphroditic plants often depart from strict equisexuality (Bawa and Webb 1983; Lloyd and Bawa 1984; Ross 1990; Pickering and Ash 1993). Study of such patterns of sexual expression, and the potential causes behind them, is needed for a better understanding of the evolution of non-hermaphroditic breeding systems and the prevalence of hermaphroditism within angiosperms.

Ultimately, emphasis on male or female gender is dependent on each gender's fitness gain curve, i.e. the fitness returns of relative investment into male and female functions (Charnov 1982). Among the factors influencing fitness gain curves, plant size—either directly or by means of the so called "budget effect" (Klinkhamer et al. 1997)—has received much attention (de Jong and Klinkhamer 1989, 1994; Bickel and Freeman 1993). Practical problems in measuring fitness gain curves make it difficult to predict which gender will be emphasised (Klinkhamer et al. 1997). However, there is some consensus that increasing plant size will entail a bias towards femaleness in entomophilous species (Klinkhamer et al. 1997), while the opposite will generally occur in anemophilous plants (Bickel and Freeman 1993).

At a proximate level, modification of phenotypic sexual expression depends on a plant's ability to modify its number of flowers, floral organs (stamens, carpels) per flower, or "gametes" (pollen, ovules) per floral organ. Number of floral organs is more—both intra- and interspecifically—variable in primitive angiosperms, while it is relatively fixed in more advanced angiosperms (Endress 1990). Gender modification has been shown for species in which number of flowers, floral organs, and

gametes per floral organ are not fixed (Bawa and Webb 1983). Gender modifications by altering gamete number per floral organ (Vogler et al. 1999) or biomass of floral organs (Kudo et al. 2001) are also known. Finally, functional gender modification can occur by altering patterns of seed production and seed-ovule ratio (Stöcklin and Favre 1994), even in species with a fixed number of floral organs per flower (Klinkhamer and de Jong 1993), i.e. a constant phenotypic gender expression.

Although possibilities of phenotypic or functional gender modification seem a priori limited for single-flowered species, they have been shown to occur (e.g. Wright and Barrett 1999). Furthermore, single-flowered species provide convenient, simple systems to study size-dependent gender expression and their underlying causes. First, only number or size of floral organs can be modified with plant size. Second, as floral display is equivalent to flower size, connections among floral display, importance of attractive structures and seed-ovule ratio are easier to establish, without the confounding effect of flower number.

Those changes in gender expression can be mediated by plant size-dependent changes in flower size (Sakai 1998; Wright and Barrett 1999) entailing a differential increase in the amount or size of some organs (Koelewijn and Hunscheid 2000; Méndez 2001; Ushimaru and Nakata 2001). It is usually considered that constraints due to requirements of fine tuning to pollinator body (Cresswell 1998; Ushimaru and Nakata 2001) or to maintenance cost of bigger flower portions (Galen 1999) will keep flowers or floral organs within a limited range of variation or below a certain size threshold. It is thus interesting to explore the potential of single-flowered species to increase flower size and its bearing on gender expression. Such increase can be a result of purely ontogenetic processes without any functional meaning (Kudoh et al. 2001). But it could also be a functionally meaningful variation related to cost-benefit considerations, i.e. an increase in flower size could involve differences in expected gains through female, male or attractive structures (Méndez 2001).

The relationship between plant and flower sizes in single-flowered plants can be tight (Wright and Barrett 1999) or loose (Sánchez-Lafuente 2002) and thus both plant and flower levels are relevant in the study of size-dependent gender modification. Usually, the allocation at the flower level has been measured as metric estimations of size (Ushimaru and Nakata 2001), number of gametes (Wright and Barrett 1999) or dry mass (Wright and Barrett 1999; Méndez 2001). Other potentially relevant currencies, such as N or P (Ashman and Baker 1992), have been seldom quantified despite repeated warnings that they could give a different view of allocation patterns (Goldman and Willson 1986). Thus, consistency in sexual expression across levels and currencies should be tested.

We studied the ability of the single-flowered hermaphroditic *Paeonia cambessedesii* to modify its sex expression with plant and flower size. Our specific questions were: (1) How variable is flower size and number of floral organs? (2) Is that variation related to differences in plant and/or flower size? (3) Do differences in plant and/or flower size entail a change in phenotypic or functional sexual expression? (4) Are patterns of sexual allocation consistent across levels (ramet, flower) and currencies (number of structures, dry mass, N, P)?

Materials and methods

Study species

P. cambessedesii (Paeoniaceae) is a herbaceous perennial plant endemic to the Balearic Islands. The perennating organ is a tuber from which the aerial part develops during winter. Stems bear imparipinnate leaves with a variable number of folioles. Most individuals in our study population produced a single stem; the frequency of multistemmed individuals, i.e. stems probably connected to the same underground rhizome, was 15.6% (n=231). In other populations the frequency of multistemmed individuals can be higher, depending on growing conditions. On flowering stems a single terminal flower bud develops in late March to middle April. The flower has a variable number of dark pink petals, with many stamens and several carpels. Despite the presence of a disc around the carpels, no nectar is secreted. The species is self-compatible (A. Traveset, in preparation). Flowers are mainly visited by bees and small beetles (A. Traveset, in preparation). Fruits ripen in June and aerial portions dry out shortly after.

The study was carried out at Es Fondall de Ses Basses, between Andraxt and Estellencs, a locality at around 100 m above sea level in the mountains north-west of Mallorca. The plants were growing on a north-facing slope on calcareous soil. Vegetation was dominated by the palm *Chamaerops humilis* and the exotic tussock grass *Ampelodesmos mauritanica*.

Flower size, number of floral organs and sexual expression in relation to plant size

Between 10 and 14 April 2000, a total of 53 ramets with flower buds were haphazardly chosen and labelled. For each ramet, leaves were measured to the nearest millimetre from its attachment point at the stem to the tip of the apical leaflet; plant size was estimated as the sum of the length of all its leaves. This estimate of plant size was well correlated to vegetative dry mass (r =0.82, n =33, P <0.0001). As there is a gradual transition from leaves to bracts to sepals, we considered as leaves those being at least 2 mm apart from the floral receptacle.

For all labelled ramets, number of petals, stamens and carpels were counted and length to the nearest millimetre of 3–4 outer petals was measured. After fruit maturation, fruits were collected and both seeds and undeveloped ovules were counted. Herbivory and fruit abortion, as well as use of a subsample for other purposes (see next section), caused missing values for some of the variables. Final sample sizes are shown in Table 1. Sexual expression was assessed by comparing stamen number vs ovules or seeds (see below). Although the more proper comparison would be pollen amount, instead of stamen number, this was not feasible in this study. A preliminary assessment of pollen amount per stamen yielded an average of 20,636 pollen grains per anther (range: 15,366–29,299; n =4 flowers, 5 anthers per flower, 5 replicates per anther). Variation between replicates of the same anther accounted for 15.1% of the total variance, while anthers of the same flower accounted for an additional 30.2% and differences between flowers for 54.7%.

Table 1 Mean ± SD, range, and sample size for vegetative and reproductive variables in *Paeonia cambessedesii*. Results of correlation analysis testing the relationship between reproductive variables and plant size index are also indicated

Variable	Mean ± SD	Range	n	r	P
Leaf number	6.0±1.7	4–10	53		
Total leaf length (mm; plant size index)	820.5±369.0	266–1,719	53		
Flower dry mass (mg)	486.1±123.0	239.7–739.2	21	0.49	0.0234
Sepal number	4.0±0.9	3–6	21		
Petal number	7.6±1.4	5–11	51	0.78	0.0000
Average outer petal length (mm)	40.9±8.6	28.0–61.8	46	0.56	0.0001
Stamen number	164.9±60.3	81–323	41	0.78	0.0000
Carpel number	5.1±1.6	2–9	53	0.69	0.0000
Ovule number per flower	85.4±41.0	34–198	41	0.64	0.0000
Average ovule number per carpel	16.4±4.0	8.5–25.2	41		
Seed number	21.2±21.0	4–92	28	0.66	0.0001
Seed set (%)	23.8±10.9	4.6–48.7	26	0.32	0.1074

Sexual expression at flower level and resource allocation to different floral structures

From the labelled ramets, a subsample of 15 flowers in female (petals open and stigmas receptive but anthers not dehiscent) or early hermaphrodite (<20% of the anthers dehiscent) stage were harvested on 10 and 12 April 2000. Six additional flowers were harvested on 1 April 2001. For this subsample, additional data were collected to address sexual expression at flower level and to examine resource (dry mass, N, P) allocation to floral structures.

In the lab, each flower was divided into petiole, bracts, sepals (including bracts just partially transformed in sepals), accessory leaves (i.e. leaves directly borne on the floral receptacle), receptacle, carpels, stamens and petals. The number of sepals, petals, stamens, carpels and ovules per carpel was counted. Length of every petal was measured to the nearest 0.5 mm. All the portions were subsequently oven-dried for 2 days at 60°C and weighed to the nearest 0.1 mg. For N and P content analysis, petiole, receptacle and sepals were pooled. Each structure was digested in sulphuric acid and analysed for total N and P by means of a Flow Injection Analyser (Tecator, Höganäs, Sweden).

Statistical analyses

At the plant level, relationships between size of flowers or number of floral organs and plant size were studied by correlation analysis. Similar statistical methods were used to analyse the increase of floral organ number with flower size.

In addition, rate of increase in allocation to stamens vs ovules or seeds was investigated by comparing the slopes of the relationships between the above-mentioned structures and plant size, after $\log_{10}$-transformation (Ushimaru and Nakata 2001). In these cases regression analysis was utilised, as we were specifically interested in the slope of the relationships. Reduced major axis (RMA) regression slopes were calculated, as recommended by Green (1999). Comparisons between slopes were carried out by a *t*-test (Zar 1999).

Allometric variation between pairs of floral organs (e.g. stamen vs ovule number) was studied according to the procedures explained in Méndez (2001). Briefly, $\log_{10}$-transformed variables were regressed against each other and the RMA slope value was obtained. Departure of the slope from 1, indicating a disproportionate increase in one of the floral organs with an increase in the other floral organ's amount, was tested by means of a *t*-test (Zar 1999). Comparisons were made using four kinds of allocation currencies: number of floral structures and dry mass, N or P allocated to floral fractions.

Patterns of ovule packaging within flowers were studied as follows. Number of ovules per flower (OVU) is determined by two components: number of carpels per flower (CAR) and average number of ovules per carpel (OVUCAR). The contribution of variation in both of these components to variation in number of ovules per flower was analysed according to procedures described in Méndez (1997) and Sánchez-Lafuente et al. (1999). Variance in

$\log_{10}$-transformed values of OVU, CAR and OVUCAR was calculated, as well as 2 times the covariance between log-transformed values of CAR and OVUCAR. Variances and covariances were divided by Var (log OVU) and expressed as percentages.

Comparisons between stamens and carpels within a flower were done by *t*-tests for paired samples, after testing normality of the difference between pairs of values (Zar 1999). When this assumption was not met, Wilcoxon matched-pairs test was used.

Results

Flower size, number of floral organs and sexual expression in relation to plant size

All reproductive ramets studied were single-flowered, as typical for this species. Leaf number and plant size index, as well as flower size, showed a broad variation (Table 1). Flower dry mass as well as average length of outer petals significantly increased with plant size index (Table 1). Flowers had a variable number of petals (5 to 11), stamens (81 to 323), carpels (2 to 9) and ovules (34 to 198; Table 1). Number of petals, stamens, carpels and ovules per flower increased with increasing plant size index (Table 1). Stamen number increased at a significantly lower rate than ovule number with increasing plant size index (Fig. 1). Seed number significantly increased with increasing plant size index, but not seed set (Table 1). Seed number increased at a higher rate than stamen number with increasing plant size index (Fig. 1).

Number of floral organs and sexual expression in relation to flower size

Number and average length of petals, and number of stamens, carpels and ovules significantly increased with increasing flower mass (Table 2). Number of sepals and average number of ovules per carpel showed no significant relationship with flower mass (Table 2). None of the relationships above improved when plant size was added to the regression model (results not shown). Stamen and ovule number increased at a similar rate with increasing flower mass (b_{RMA}±SE =1.364±0.194 for stamens, 1.246±0.230 for ovules; t_{38} =0.952; P >0.1).
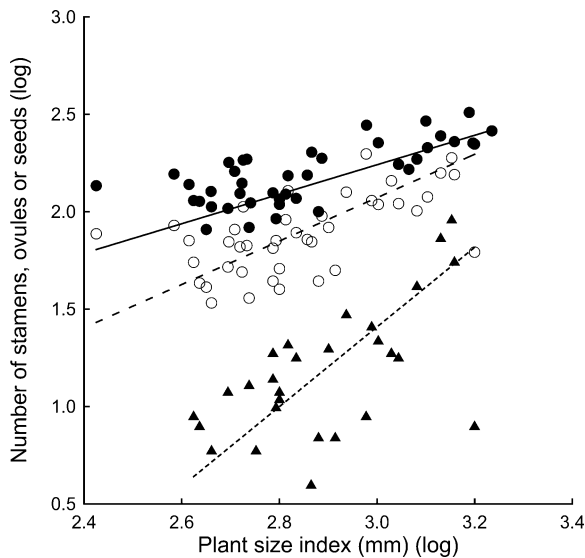
Fig. 1 Increase in the number of stamens (*closed dots*), ovules (*open dots*), and seeds (*closed triangles*) with increasing plant size index. Slopes presented are based on reduced major axis (RMA) regression: $b\pm SE = 0.757\pm0.084$ for stamens, 1.114 ± 0.143 for ovules, 2.046 ± 0.316 for seeds. RMA slopes for stamens and ovules were significantly different ($t_{78} =2.198$; $P <0.05$). RMA slopes for stamens and seeds were significantly different ($t_{65} =4.828$; $P <0.001$)

Table 2 Results of correlation analysis testing the relationship between the amount or length of floral organs and flower mass in *P. cambessedesii*

Variable	r	n	P
Sepal number	0.21	21	0.3638
Petal number	0.47	21	0.0303
Average petal length (mm)	0.58	15	0.0224
Stamen number	0.74	21	0.0001
Carpel number	0.59	21	0.0046
Ovule number per flower	0.61	21	0.0035
Average ovule number per carpel	0.16	21	0.4880

Log_{10} ovule number per flower increased with increasing log_{10} carpel number per flower with a slope significantly greater than 1 ($b_{RMA} =1.583\pm0.120$ SE, $t_{39} =4.858$, $P <0.001$). This suggests that flowers with more carpels contained more ovules per carpel. Indeed, mean number of ovules per carpel increased with increasing carpel number per flower ($r =0.48$, $n =41$, $P =0.002$). Variation in CAR accounted for 39.5% of the variation in OVU while the covariance between CAR and mean ovule number per carpel (OVUCAR) accounted for an additional 29.6% and variation in OVUCAR 29.4%.

Resource allocation to floral structures

Petals were the floral structure receiving the highest allocation in terms of dry mass (Table 3). Overall, sexual structures comprised around 35% of floral dry mass, compared to around 34% for green accessory structures and 31% for attractive structures (Table 3). Patterns of resource allocation to different flower portions in terms of nutrients showed some differences with respect to dry mass allocation (Table 3). Both in terms of N and P, stamens were the floral structure receiving the highest allocation (Table 3). Overall, sexual structures comprised around 52% of total N or P, compared to 30–27% for green accessory structures and 19–21% for attractive structures (Table 3). [N] (Wilcoxon test: $Z =-3.146$, $n =21$, $P =0.017$) and [P] (Wilcoxon test: $Z =-3.563$, $n =21$, $P =0.004$) were significantly higher in carpels than in stamens. [N] (*t*-test for paired samples: $t_{20} =21.30$, $P =0.000$) and [P] (*t*-test for paired samples: $t_{20} =11.97$, $P <0.001$) were higher in sexual structures than in attractive structures.

The structure of correlations among floral portions differed across allocation currencies (Table 4). Correlations in terms of P qualitatively agreed with those in terms of dry mass, although correlation between stamens and gynoecium was stronger, while correlation between stamens and petals was weaker (Table 4A, C). On the other hand, correlations in terms of N showed inverse patterns of significance to the ones in terms of dry mass or P (Table 4B), except for the correlation between petals and gynoecium.

Table 3 Mean ± SD (%) allocation of resources to different floral structures in *P. cambessedesii* in terms of dry mass, nitrogen and phosphorus ($n =21$ flowers). Concentrations are given in mmol/g dry mass

Structure	Mass (mg)	Nitrogen (µg)	Phosphorus (µg)	[Nitrogen]	[Phosphorus]
Green accessory structures	163.1±41.7 (34.2)	3,247.8±1,043.3 (30.0)	422.0±322.1 (26.7)	141.9±29.6	8.7±8.3
Petiole	25.7±14.4	2,440.9±625.2	348.1±310.5	135.8±29.2	9.1±10.1
Receptacle	45.2±12.6				
Sepals	58.7±16.8				
Accessory leaf	37.0±21.4[a]	891.8±563.1[a]	81.7±45.0[a]	166.0±50.6[a]	7.2±1.6[a]
Petals	153.1±53.9 (31.1)	2,021.4±576.3 (18.8)	316.8±114.5 (21.4)	97.9±21.4	6.8±2.1
Sexual structures	169.8±51.8 (34.7)	5,599.5±1,750.7 (51.2)	799.2±343.7 (52.0)	235.4±30.7	14.9±3.7
Stamens	114.2±36.5	3,545.1±1,310.1	501.8±258.8	220.9±41.4	13.8±4.6
Gynoecium	55.6±24.2	2,054.4±849.7	297.4±118.7	277.8±121.9	17.9±5.3

[a] $n =19$ flowers

Table 4 Pearson correlation coefficients (significance) between dry mass, nitrogen or phosphorus of different floral portions in *P. cambessedesii*. $n = 21$ in all cases

	Petals	Stamens	Gynoecium
A—Dry mass			
Green accessory structures	0.345 (*P* =0.126)	0.352 (*P* =0.117)	0.435 (*P* =0.049)
Petals		0.681 (*P* =0.001)	0.613 (*P* =0.003)
Stamens			0.435 (*P* =0.049)
B—Nitrogen			
Green accessory structures	0.537 (*P* =0.012)	0.502 (*P* =0.021)	0.339 (*P* =0.133)
Petals		0.360 (*P* =0.109)	0.683 (*P* =0.001)
Stamens			0.281 (*P* =0.217)
C—Phosphorus			
Green accessory structures	0.218 (*P* =0.343)	0.372 (*P* =0.097)	0.195 (*P* =0.396)
Petals		0.496 (*P* =0.022)	0.667 (*P* =0.001)
Stamens			0.604 (*P* =0.004)

Table 5 Results of the RMA regression testing allometry (significant departure from a slope of 1) in the comparison of selected floral portions, using three different allocation currencies ($n = 21$ in all cases)

Comparison/currency	r	b ±SE	t_{19}	P
Androecium vs gynoecium				
Dry mass	0.362	0.876±0.187	−0.663	>0.5
Nitrogen	0.344	1.044±0.225	0.196	>0.5
Phosphorus	0.521	1.386±0.272	1.419	>0.05
Attractive vs sexual structures				
Dry mass	0.772	1.179±0.172	0.685	>0.5
Nitrogen	0.656	0.883±0.153	−0.765	>0.2
Phosphorus	0.657	0.956±0.166	−0.265	>0.5

Sexual expression in terms of number of floral structures, dry mass, N and P

Number of stamens and ovules per flower were positively correlated ($r = 0.76$, $n = 33$, $P < 0.0001$). An allometric analysis of stamen vs ovule number revealed a slope significantly lower than 1 ($b_{RMA} = 0.759 \pm 0.087$ SE, $t_{31} = -2.762$, $P < 0.01$), indicating a disproportionate increase in ovule number with increasing stamen number. Number of stamens and seeds per flower were also positively correlated ($r = 0.72$, $n = 18$, $P = 0.0007$). An allometric analysis of stamen vs seed number revealed a slope significantly lower than 1 ($b_{RMA} = 0.494 \pm 0.089$ SE, $t_{16} = -5.555$, $P < 0.001$), indicating a disproportionate increase in seed number with increasing stamen number.

When using dry mass, N or P as allocation currency, increase in resource allocation to all fractions tested (androecium vs gynoecium, attractive vs sexual structures) was isometric (Table 5). Only in terms of P, was the departure from 1 in the comparison androecium vs gynoecium close to significance (Table 5).

Discussion

Flower size, number of floral organs and sexual expression in relation to plant size

Our study showed substantial intraspecific variation for number of floral structures in *P. cambessedesii*. Similar variation has been documented for other species of herbaceous *Paeonia* (Schlising 1976; Sánchez-Lafuente et al. 1999; Sánchez-Lafuente 2002). In *P. cambessedesii* both flower size and amount of floral organs increased with increasing plant size. In single-flowered *P. broteroi* individuals, however, Sánchez-Lafuente (2002; Sánchez-Lafuente et al. 1999) found weak or non significant correlations between plant height and flower size. In a broader context, positive relationships between plant and flower size have been found for other single-flowered species (*Erythronium*, Sakai 1998; *Trillium*, Wright and Barrett 1999) and are generally expected to hold for plants, on a mechanical and natural history basis (Primack 1987). Finding out whether this intraspecific variation in *P. cambessedesii* represents a fixed trait of each genotype or varies in a plastic way with changes in the size of a given individual will require following the same individuals along several reproductive events. Notwithstanding, in individuals with several ramets, flower size and number of floral structures was not fixed but dependent on ramet size (M. Méndez and A. Traveset, personal observation). Thus, that variation and its relationship with plant size is plastic to some extent.

In entomophilous plants, previous studies have predominantly found increased femaleness with increasing plant size (de Jong and Klinkhamer 1989; Klinkhamer and de Jong 1993; Pickering and Ash 1993; see Wright and Barrett 1999 for two single-flowered *Trillium* species). However, no change in sex expression with plant size (Pickering and Ash 1993) and a few cases of increased maleness (Méndez 2001; Klinkhamer et al. 1997) are known. It is interesting to note that in our study increased femaleness was found both as a change in phenotypic (stamens vs ovules) and functional (stamens vs seeds) gender expression. This means that allocation patterns at flowering had actual consequences for female

fitness. Providing that variation in number of floral structures and its purported plastic relationship with plant size has a genetic basis, this opens the interesting possibility that those fitness consequences could feedback into the allocation to floral structures.

As the present study was purely descriptive, the causal mechanisms underlying the relationship between plant size, flower size and gender modification remain unknown. This is, however, true for most previous studies of size-dependent gender (SDG) modification in plants (e.g. de Jong and Klinkhamer 1989; Wright and Barrett 1999). The reason could be that, despite a well established theory for the "size advantage model" of Ghiselin (1969; Charnov 1982), the generality of SDG modification in cosexual plants remains contentious (Klinkhamer et al. 1997). While further observational evidence for SDG modification is needed, we acknowledge that experimental studies should be conducted for those species in which the patterns have already been documented. As the distinction by Klinkhamer et al. (1997) between "direct" and "budget" effects of plant size reveals, plant size integrates very diverse factors potentially affecting gender expression. Local mate competition (LMC), local resource competition (LRC), geitonogamy, resource level and increased attractiveness to pollinators are the most commonly considered (Ghiselin 1969; de Jong and Klinkhamer 1994; Klinkhamer et al. 1997). Estimates of LMC and LRC have rarely been made in plants in relation to sexual allocation (see however Rademaker and de Jong 1999; Greef et al. 2001) and should be considered in future studies. Experimental manipulations or resource level and floral display will also be necessary in order to ascertain which mechanisms underlie the relationship between plant size and sexual expression in *P. cambessedesii*. In addition, experimental manipulation of phenotypic gender expression will be needed in order to show the adaptive value of the trends in gender modification.

Number of floral organs and sexual expression in relation to flower size

For single-flowered plants, we hypothesized that a larger plant could modify its gender expression by means of associated changes in flower size. As flower size influences visitation rate by pollinators (Bell 1985), as well as increased maintenance costs (Galen 1999), fitness gain curves could also be affected, resulting in modification of gender expression. Flower size-dependent changes in allocation to floral structures have been reported for multi-flowered species (Ashman and Baker 1992). If flowers mainly have a male function (Bell 1985), one would expect for single-flowered species a disproportionate increase in petal number or size, or stamen number or mass, with increasing plant size. In *P. cambessedesii*, petal size and number of floral structures increased with increasing flower mass but no change in gender expression was associated to that increase. Our results show that changes in gender expression at the plant level were at

least partially uncoupled from flower size due to: (1) loose correlation between plant and flower size, and (2) rather loose phenotypic correlations among floral portions (see also Sánchez-Lafuente 2002 for *P. broteroi*). This looseness could be related to the generalist pollination syndrome in *Paeonia* (Bernhardt 2000; see however Sánchez-Lafuente 2002 for intraspecific spatial variation in correlation between floral characters and pollinator fauna in a generalist *Paeonia*). In addition, the lack of agreement between patterns at plant and flower levels points to adaptive modification of gender expression and not to mere changes in allocation as a result of ontogenetic constraints related to increased flower size.

Resource allocation to floral structures and consistency among currencies

Only a few studies have directly analysed the scaling of male allocation with increased female allocation (Koelewijn and Hunscheid 2000; Méndez 2001). This kind of scaling analysis cannot be directly interpreted in the light of plant or flower size because no independent measurement of plant of flower size is utilised in the *x* axis. However, it is warranted as long as the relationship between floral portions compared and plant (or flower) size is positive and not very weak, as in our study (see Sarkissian et al. 2001, Fig. 1c for a similar caveat). It also allows us to explore allometric relationships using different allocation currencies, as well as other ranges of relationships (e.g. attractive structures vs sexual structures, Méndez 2001).

In the present study, a disproportionate increase in ovule and seed number was found with increasing number of stamens (cf. Koelewijn and Hunscheid 2000; Méndez 2001). This result was consistent with the analyses at plant level. However, no change in sex expression was found when using dry mass, N or P as allocation currency. Several non mutually exclusive factors could account for those inconsistencies: (1) partial uncoupling of plant and flower size, (2) loose covariation among floral structures, (3) smaller size of individual floral structures with an increase in their numbers, and (4) varying concentration of N and P in each structure.

Study of N and P allocation to floral structures provided information about how costly those structures were for the plant. The increased femaleness detected in some of our analyses is remarkable when considering that female structures had high concentration of both N and P. This supports the view that the trends detected are functional and not a mere allometric change along "lines of minor resistance" (e.g. disproportionate allocation to inexpensive—in terms of N and P—attractive structures).

In conclusion, gender expression in *P. cambesedesii* became biased towards femaleness with increasing plant size when comparing amount of structures. These results make sense with what is known about the natural history of the species and general predictions of sexual allocation theory. However, inconsistencies between levels of anal-

ysis (flower, ramet), between methods of analysis (direct comparison of structures vs regression against plant/ flower size) and among currencies (dry mass, nutrients, number of structures) warn against reliance in one single approach, study level or currency. Study of size-dependence of gender expression will benefit from the contrast of results obtained using all the above-mentioned factors.

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