

## Variation in Floral Sex Allocation in *Polygonatum odoratum* (Liliaceae)

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• **Background and aims** It is well known that resource allocation to male and female functions can be highly variable in hermaphroditic plants. The purpose of this study was to investigate variations in sexual investment at different levels (flower, plant and population) in *Polygonatum odoratum*, a plant with sequentially opening flowers.

• **Methods** Pollen and ovule production in base, middle and top flowers of *P. odoratum* flowering shoots from two natural populations were quantified. Plant measurements of phenotypic and functional gender were calculated in both populations. Total leaf number was used to investigate the relationship between gender assessments and plant size.

• **Key results** Pollen and ovule production varied depending on flower position, although the precise pattern differed between both studied populations; only investment in female floral function decreased markedly from base to top flowers in both populations. The frequency distribution of phenotypic gender and their relationship with plant size differed between populations. Phenotypic and functional gender were correlated in both populations.

• **Conclusions** Sexual investment in *P. odoratum* has shown a marked variability within plants, among plants, and between populations, which confirms the importance of analysing sex expression in plants of this type. Differences in relative investment in male and female components (phenotypic gender) are reflected in the functional gender and it would be expected that the evolution of sexual specialization in *Polygonatum odoratum* would be promoted.

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**Key words:** Sex allocation, functional gender, phenotypic gender, plant size, *Polygonatum odoratum*.

### INTRODUCTION

The capacity of plants to produce both male and female gametes over their lifespan, with variation both in time and in space, undoubtedly constitutes one of their most fascinating characteristics, and has attracted the attention of plant evolutionary biologists ever since Darwin. However, it is only in the last two decades that real efforts have been made to understand sexual investment in hermaphrodite plants, and more particularly to understand how resources available for reproduction are distributed between the male and female functions. The recognition that hermaphroditism is not equivalent to 'equisexuality', together with the conceptual distinction between phenotypic and functional gender (Horowitz, 1978; Lloyd, 1980a), has permitted the development of theoretical models of the evolution of reproductive systems, and the integration of sexual investment into general theories of the reproductive ecology of hermaphrodite species (Charlesworth and Charlesworth, 1981; Charnov, 1982; Zhang and Jiang, 2002; Delph, 2003).

Numerous authors have shown that investment in the male and female reproductive functions may vary considerably within hermaphroditic species (e.g. see Charlesworth and Charlesworth, 1981; Lloyd, 1984). Research in this area has adopted four basic approaches.

(1) Some studies have focused on a central prediction of theoretical models of sexual investment, namely that

there should be a 'trade-off' (i.e. a negative correlation) between investment in male and female function. This prediction is based on the fact that the development of male and female reproductive organs requires limited resources, so that there is 'inevitably' a negative relationship between stamen/pollen production and carpel/ovule/fruit production (e.g. see Mazer and Delesalle, 1998; Mazer *et al.*, 1999; Koelewijn and Hunscheid, 2000; and references therein).

- (2) Some studies have aimed to evaluate variations in phenotypic and functional gender within and among populations (Lloyd, 1984; Pickering and Ash, 1993; Klinkhamer *et al.*, 1997; Kudo and Maeda, 1998; Dorken and Barrett, 2003), and within individuals among seasons and years (Ashman and Baker, 1992). The extent to which differences among flowers in relative investment in male and female components (i.e. phenotypic gender) are reflected in functional gender is of interest from the evolutionary viewpoint. Little or no correspondence between the two would be expected to inhibit the selection of sexual characteristics and therefore hinder the evolution of sexual specialization in hermaphroditic plants (see Guitián *et al.*, 2003).
- (3) Some studies have focused on the relationships existing between sexual investment and plant size [the 'size allocation model' (SAM); see references in Klinkhamer *et al.*, 1997; Wright and Barrett, 1999; Sarkissian *et al.*, 2001; García, 2003]. The SAM predicts that sexual investment may depend on the plant's resource status: relative allocations to male and female functions (and

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indeed the optimal total allocation to reproduction) may differ depending on plant size and/or resource availability (Charnov, 1982; Wright and Barrett, 1999).

- (4) An important line of research has been to analyse differences in sexual investment within inflorescences of hermaphrodite plants. Such studies have focused on the importance of resource limitations and/or architectural effects (see Diggle, 1995; Medrano *et al.*, 2000), while others have focused on differences in male and female reproductive success (Devlin and Stephenson, 1987; Brunet and Eckert, 1998). This latter focus, explored in plants with inflorescences showing sequential flower opening, indicates that the different contributions of male and female functions to fitness may exert selective pressure on reproductive investment in terms of flowers (see Brunet and Charlesworth, 1995).

These four different approaches have all highlighted the marked variability of sexual investment in hermaphrodite plants, and represent different perspectives for analysing the multiple selection pressures to which plants are subjected. From the evolutionary viewpoint, the different approaches are consistent with the view that selection should favour efficient investment in resources through the male and female functions (Lloyd, 1984).

In the present study, sexual investment was investigated at different levels in two populations of *Polygonatum odoratum*, and an attempt was made to answer the following specific questions. (1) Is there variation between investment in male function (pollen) and female function (ovules) within flowering shoots and/or between populations? (2) Does phenotypic and/or functional gender differ among individuals and populations? (3) At the plant level is there some relationship between phenotypic and functional gender? (4) Does male/female reproductive investment vary depending on plant size? *Polygonatum odoratum* is well-suited to studies of this type because (a) shoot leaf number and shoot flower number are correlated, (b) sexual investment varies among flowers, and (c) flowering shoots show sequential flower opening (see Gutián *et al.*, 2001).

## MATERIALS AND METHODS

### *Study species and sites*

*Polygonatum odoratum* (Miller) Druce (Liliaceae) is a clonal rhizomatous geophyte with shoots of up to 50 cm in height, and with very short-stalked leaves arising from the stem. This species is widely distributed in Europe and also occurs in Morocco. It occurs on shady sites on a variety of substrates in both deciduous and evergreen forest. The flowering period commences in April–May. The mean number of cauline leaves on non-flowering shoots was  $6.5 \pm 1.6$  (range 2–10;  $n = 25$ ), versus  $9.4 \pm 1.6$  (range 6–14;  $n = 25$ ) on shoots that flowered. These means differ significantly ( $t = 60.7$ ;  $P < 0.0001$ ). The species is self-incompatible and pollination is largely by bumblebees (*Bombus terrestris*, *B. pratorum* and *B. pascuorum*). The mean number of flowers produced per flowering shoot is  $5.6 \pm 4.4$  (see Gutián *et al.*, 2001). Although flowers open progressively from the

bottom to the top of the flowering shoot, there is considerable overlap in the anthesis period of most of the flowers. Within a flowering shoot, axillary flowers are developed at the base of the cauline leaves, where they generally arise grouped in cymes of one, two or sometimes three. Flowering shoots produce either all male flowers, or all hermaphrodite flowers, or varying proportions of both. The yellowish-green to white perianth of male and hermaphrodite flowers is tubular (1.5–3 cm long). Hermaphrodite flowers produce six stamens, a three-locular ovary (with two to eight ovules per locule), and a well-developed slender style (approx. 1 cm long) which is ended by a small three-lobed stigma. Male flowers also have six stamens, but the style is hardly observable at simple sight (absolutely atrophied or absent), and when observed with the stereomicroscope no ovules are developed in their ovaries. The fruit is a black berry of about 1 cm in diameter, containing seven to nine seeds. Throughout this study, the unit of analysis was a flowering shoot. Different flowering shoots can belong to the same individual plant due to the clonal reproduction of the species. To sample different individuals, in all cases selected flowering shoots were always >1 m distant from each other.

The study was performed in two field populations separated by >200 km, Caurel and Santiago (hereafter CAU and SAN). The CAU population is situated at the western end of the Cantabrian Range (Lugo, north-west Spain;  $42^{\circ}36'N$ ,  $7^{\circ}19'W$ ), close to 'O alto do Couto', at 1300 m a.s.l, in a mixed deciduous woodland of *Quercus robur* L., *Betula alba* L. and *Corylus avellana* (C. Koch.) Winkl. The SAN population is situated near Santiago de Compostela (A Coruña, north-west Spain;  $42^{\circ}52'N$ ,  $8^{\circ}28'W$ ), at 400 m a.s.l, in a *Quercus robur* deciduous woodland.

### *Variation in sexual investment within flowering shoots*

*Floral sex ratio.* To investigate variation in male : hermaphrodite flower ratio within flowering shoots, and whether these patterns of variation differ between the two populations of study, 44 plants were selected in CAU and 58 in SAN. The sexual type of all flowers produced in each inflorescence on all shoots of these plants was monitored and recorded throughout the 2001 flowering period.

*Pollen and ovule production.* In each population 20 plants were randomly selected and all flowering buds produced were collected 1 or 2 d before anthesis ( $n = 137$  and  $n = 157$ , respectively, in CAU and SAN populations). Each bud position in the inflorescence was recorded. Buds were maintained in individual vials containing FAA (formol + acetic acid + 70 % ethanol, at 5 : 5 : 90 v/v) until examination in the laboratory. Ovaries from all collected flower buds were dissected to determine ovule number per flower with the aid of a stereomicroscope. Pollen was counted using a particle counter (Coulter Counter<sup>®</sup> Z2; Beckman Inc.) equipped with a 100  $\mu$ m aperture tube and a particle size channellizer accessory. For estimation of pollen production, two anthers from each flower bud (one from each stamen whorl) were examined. Anther content was released into a vial containing 0.5 mL of electrolytic solution (Isoton II<sup>®</sup>), by mechanical destruction of the theca walls and subsequent vortexing. The wall fragments were then removed and the

remaining solution poured into a beaker containing 50 mL of Isoton II. The vial was then refilled with 0.5 mL of Isoton II and shaken to collect any remaining pollen, then emptied into the beaker. The final volume was thus 51 mL (0.5 + 50 + 0.5). Pollen size in *P. odoratum* had been reported previously to vary between 16 and 46  $\mu\text{m}$  (Valdés *et al.*, 1987). However, pollen size in the present samples ranged between 19 and 29  $\mu\text{m}$ , so the counter was set to count in this range (improving accuracy). For each flower (i.e. each 51 mL pollen suspension sample) five repeat counts were performed, each in 0.5 mL of the pollen suspension sample. Counter accuracy was checked by manual counting under a light microscope: for these counts the pollen from two anthers per flower was suspended in 1 mL of a solution of detergent and safranin, from which ten 5  $\mu\text{L}$  replicates were then obtained for counting (Herrera, 1987).

#### Gender and size assessments

The basic measure of the phenotypic gender of a plant is the ratio of ovule number to pollen grain number. However, this is necessarily a destructive measure, and assessment of the relationship between phenotypic gender and functional gender requires a non-destructive measure. To non-destructively assess gender, 65 plants were tagged with metal labels in SAN and 50 plants in CAU, and these plants monitored until fruit set (about 2 months after flower opening). For each flower, the position in the inflorescence, and presence or absence of stamen whorls and carpel whorls were also recorded.

Standardized phenotypic gender ( $G_i$ ) of each plant was calculated as per Lloyd (1980a):

$$G_i = O_i / (O_i + p_i E),$$

where  $O_i$  is the number of carpel (female) whorls,  $p_i$  is the number of stamen (male) whorls, and  $E$  is mean  $O_i/p_i$  in the population.  $G_i$  may thus vary between 0 for plants producing only pollen and 1 for plants producing only ovules.

Standardized functional gender ( $G$ ) of each plant was likewise calculated as per Lloyd (1980a):

$$G = d_i / (d_i + I_i E'),$$

where  $d_i$  is the number of fruits produced,  $I_i$  is the number of stamen (male) whorls, and  $E'$  is mean  $d_i/I_i$  in the population.

The above formulae assume that stamen whorl number is proportional to pollen number, and that carpel whorl number is proportional to ovule number. To confirm the validity of this assumption, regression analysis was used, considering the 292 flowers for which destructive pollen and ovule counts had been obtained. The result of these analyses (not shown) indicated a strong relationship between a plant's number of female whorls and its number of ovules, and likewise between a plant's number of male whorls and its number of pollen grains. Thus whorl numbers can reasonably be used as indicators of gender.

To investigate relationships between the different gender assessments (phenotypic and functional) and plant size, total leaf number was used as the estimate of plant size. The leaves of *P. odoratum* develop acropetally and the shoot

must reach a certain size and bear a certain number of leaves before becoming a flowering shoot. Considering all flowering shoots, a strong significant positive correlation between leaf number and flower number was previously verified ( $r = 0.797$ ;  $P < 0.0001$ ).

#### Statistical analyses

Within-shoot among-flower variation in pollen and ovule production was explored by fitting generalized linear mixed models (SAS macro GLIMMIX; Littell *et al.*, 1996) to the data. Given that the selected flowering shoots bore varying numbers of flowers, the position of each flower on the shoot was classified in relative terms as 'base' (lowest third of inflorescence), 'middle' (middle third), or 'top' (top third). The factors 'population', 'relative position', and their interaction were considered as fixed effects in the model, while 'plant' nested within 'population' was considered as a random effect. All data were analysed using the SAS system (SAS Institute, 1999).

Total variance in pollen and ovule production was partitioned into components due to variation among populations and plants using the MIXED procedure ('restricted maximum likelihood' estimation method) in the SAS statistical package. The factors 'Population' and 'Plant' (nested within 'Population') were included in the analyses as categorical factors.

## RESULTS

#### Variation in sexual investment within flowering shoots

**Floral sex ratio.** Figure 1 shows mean male : hermaphrodite flower ratios in each position of *P. odoratum* flowering shoots with different numbers of cymes, in both populations studied. In the SAN population, male flowers were only produced in distal positions of the flowering shoot, regardless of number of cymes per shoot. In the CAU population, the distal-most positions also have a greater ratio of male to hermaphrodite flowers than the more basal ones. However, this pattern is most clearly observed in shoots with five to seven cymes, while in shoots with two to four cymes male flowers are also frequently produced in basal positions.

**Pollen and ovule production.** *Polygonatum odoratum* shows wide variation in all traits measured. The mean number of ovules per flower was  $12.5 \pm 1.9$  in SAN and  $12.7 \pm 2.3$  in CAU. The mean number of pollen grains per flower was  $42\,054 \pm 10\,683$  in SAN and  $55\,772 \pm 13\,637$  in CAU. Analysis of variance indicated that about 70 % of variance in numbers of ovules is among flowers on the same plant, and the remaining 30 % among plants (not between populations); by contrast, variance in numbers of pollen grains is rather evenly attributable to variation within plants, among plants and between populations (Table 1).

The number of ovules per flower declined from basal to top position along the flowering shoot, both in SAN and CAU, whereas the number of pollen grains per flower did not show such a clear pattern: in CAU, the number of pollen

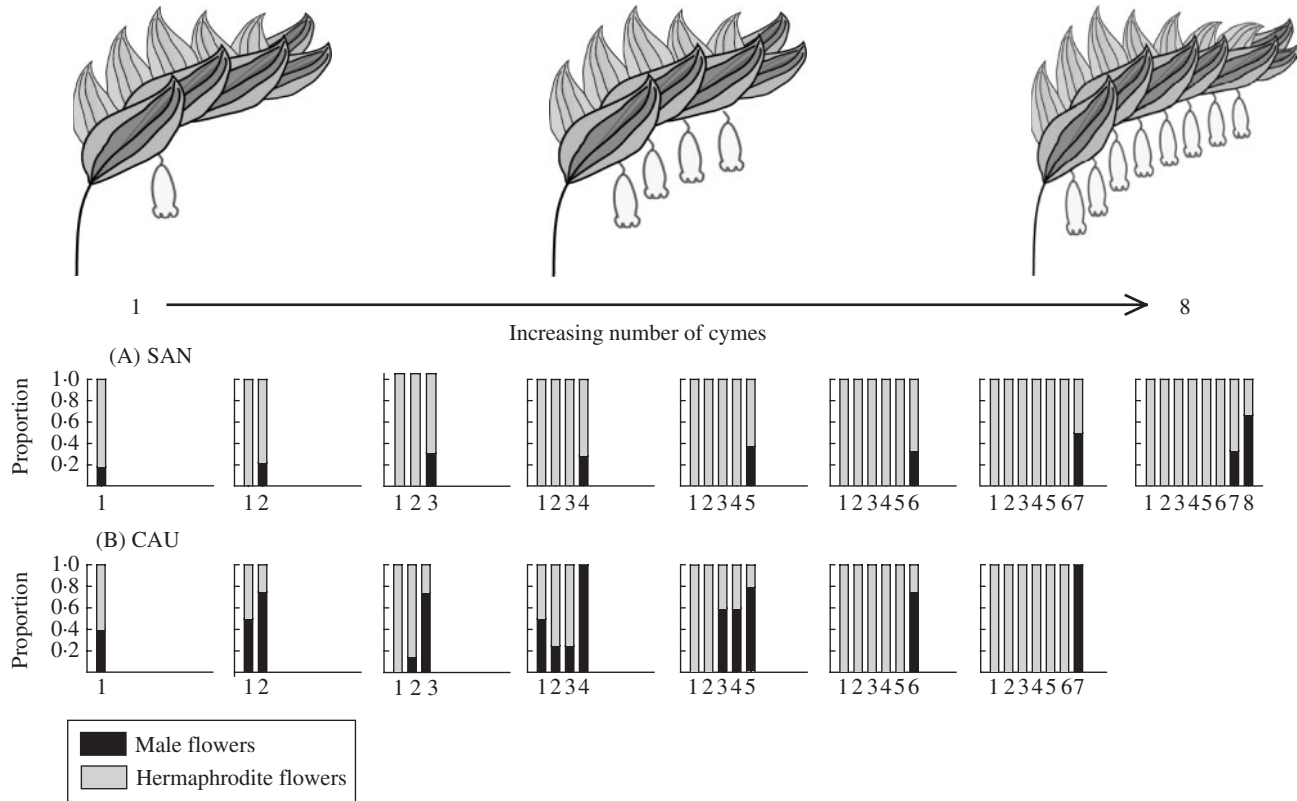


FIG. 1. Relative proportions of male and hermaphrodite flowers produced in the different cymes of *P. odoratum* flowering shoots in (A) SAN and (B) CAU populations.

TABLE 1. Percentages of variance in NO (number of ovules per flower) and NP (number of pollen grains per flower) explained by the different factors for *Polygonatum odoratum* plants in the two study populations

Factor	NO		NP	
	Variance (%)	<i>P</i>	Variance (%)	<i>P</i>
Population	0	—	41.51	0.2498
Plant (population)	30.09	0.0009	35.82	<0.0001
Residual (= within plant)	69.91	<0.0001	22.67	—

grains per flower declined from base to top, whereas in SAN there was no a clear variation along the flowering shoot (Fig. 2). The results of analysis of variance in numbers of ovules and pollen grains with position along the flowering shoot are shown in Table 2. These results show that the number of ovules per flower does not vary significantly between populations, but does vary among the three positions along the shoot; the interaction 'population × position' was not significant. Note, however, that male flowers were included in this analysis, and that this result can be affected by their presence mostly in distal positions of the flowering shoots. However, if only hermaphrodite flowers were considered, the pattern of decline in number of ovules per flower with position persisted ( $F = 4.49$ ;  $P = 0.015$ ),

but differences between populations did not exist ( $F = 1.95$ ;  $P = 0.173$ ), and the interaction 'population × position' was also not significant ( $F = 1.28$ ;  $P = 0.286$ ). The number of pollen grains per flower varied significantly both between populations and among positions, though again the interaction was not significant.

#### Gender and size

*Polygonatum odoratum* has flowering shoots with male flowers and hermaphrodite flowers. The female : male whorl ratio ( $E$ ) was 0.91 in SAN and 0.65 in CAU. Standardized phenotypic gender ( $G_i$ ) ranged from 0 (i.e. totally male plants) to 0.52 (plants with all flowers hermaphrodite) in SAN, and from 0 to 0.60 in CAU. The percentage of flowers that set fruit ranged between 0 and 100 in both populations ( $0.46 \pm 0.35$  in SAN and  $0.17 \pm 0.25$  in CAU). The fruit: stamen ratio ( $E'$ ) was 0.47 in SAN and 0.22 in CAU. Standardized functional gender ( $G$ ) ranged between 0 and 0.68 in SAN, and between 0 and 0.82 in CAU. The distribution of both  $G_i$  and  $G$  differed markedly between the two populations (Fig. 3).  $G_i$  was more homogenous among individuals in SAN than in CAU. In SAN the distribution of  $G_i$  was basically unimodal, with most plants equisexual, whereas in CAU the distribution was markedly bimodal, with large numbers of both equisexual and male-only plants.  $G_i$  and  $G$  were significantly correlated both in SAN ( $r = 0.283$ ;  $P = 0.022$ ) and CAU ( $r = 0.514$ ;  $P < 0.0001$ ).

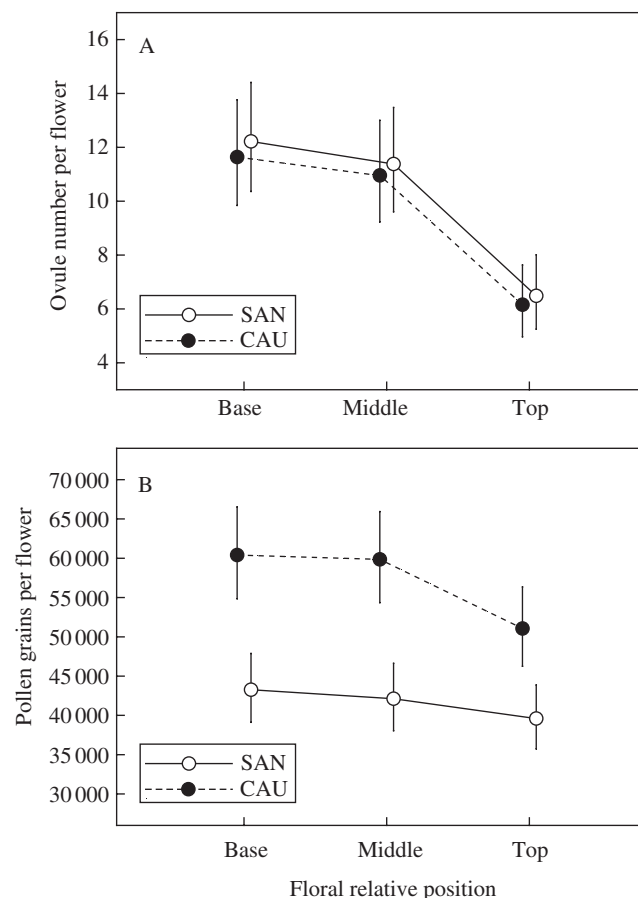


FIG. 2. Variation between relative positions (base, middle and top) in (A) number of ovules per flower and (B) number of pollen grains produced per flower for *Polygonatum odoratum* plants in the study populations. Values plotted are back-transformed model adjusted means obtained from fitting generalized linear models to the data. Vertical lines denote the 95 % parametric confidence intervals around the means.

TABLE 2. Results of analysis of variance in NO (number of ovules per flower) and NP (number of pollen grains per flower) with factors population and position in the flowering shoot (base, middle, and top), for *Polygonatum* plants in the two study populations

Dependent variable	Effect in the model	Significance test		
		d.f.	F	P
NO	Population	1,38	0.26	0.6145
	Relative position	2,76	30.58	<0.0001
	Population × relative position	2,76	0.00	0.9959
NP	Population	1,38	24.18	<0.0001
	Relative position	2,76	13.24	<0.0001
	Population × relative position	2,76	1.84	0.1658

The relationship between phenotypic gender ( $G_i$ ) and plant size, as assessed on the basis of number of leaves, is shown in Fig. 4. The line shown is a cubic spline fitted to the data for both populations. As can be seen, the relationship

between gender and plant size differs between the two populations being more predictable in the SAN population (see also Fig. 1).

## DISCUSSION

Sex allocation theory predicts that sexually plastic individuals will be able to increase their fitness by adjusting their relative investment in male and female sexual function in response to variation in environmental conditions (Charnov, 1979). In *Polygonatum odoratum*, sexual variability is seen both in the male : female gamete ratio at the flower level, and in male : hermaphrodite flower ratio at the plant level.

### Pollen and ovule production

The results of the present study indicate that *P. odoratum* shows marked sexual variability in both traits considered (ovule number, pollen grain number) and at all levels considered (flower, plant and population). Variations in sexual investment at the individual level have been documented in numerous hermaphroditic species and have been attributed to changes in age and resource status of plants and/or genetic factors (Lloyd and Bawa, 1984; Meagher, 1988; Charlesworth, 1999; Mazer and Dawson, 2001). However, there have been few studies of how this variation is distributed among different possible levels (however, see Gutián *et al.*, 2003, and references therein). The results presented here indicate that the two traits analysed show very different patterns of variation: the number of ovules per flower showed high within-plant variation, whereas the number of pollen grains per flower showed high among-plant within-population variation and high between-population variation. Consequently, the male components of gender in *P. odoratum* appear to be more stable at the individual level than the female components. This is in line with findings obtained for other species (*Rhaphanus sativus*, Mazer, 1992; *Campanula rapunculoides*, Vogler *et al.*, 1999; *Helleborus foetidus*, Gutián *et al.*, 2003).

In general, in *Polygonatum odoratum* both number of ovules and number of pollen grains declined from proximal to distal positions in the flowering shoot. A decline in pollen and/or ovule production per flower has been recorded in numerous previous studies (e.g. Ashman and Hitchens, 2000; Mazer and Dawson, 2001; Ishii and Sakai, 2002; García, 2003; Ishii, 2004); in the present study, however, the pattern observed differed between the two populations. In CAU, both ovule number and pollen grain number showed a clear decline from proximal to distal positions, whereas in SAN pollen grain number showed little change with position in the inflorescence, so that distal flowers are proportionally more male. In other words, the positional variation in ovule number in SAN leads to a variation in sex expression within the flowering shoots. More interestingly, in both populations of *P. odoratum* studied quantitative variation in gender among morphologically hermaphroditic flowers exists, because when flowers without ovules are excluded from the analysis, the decline in ovule

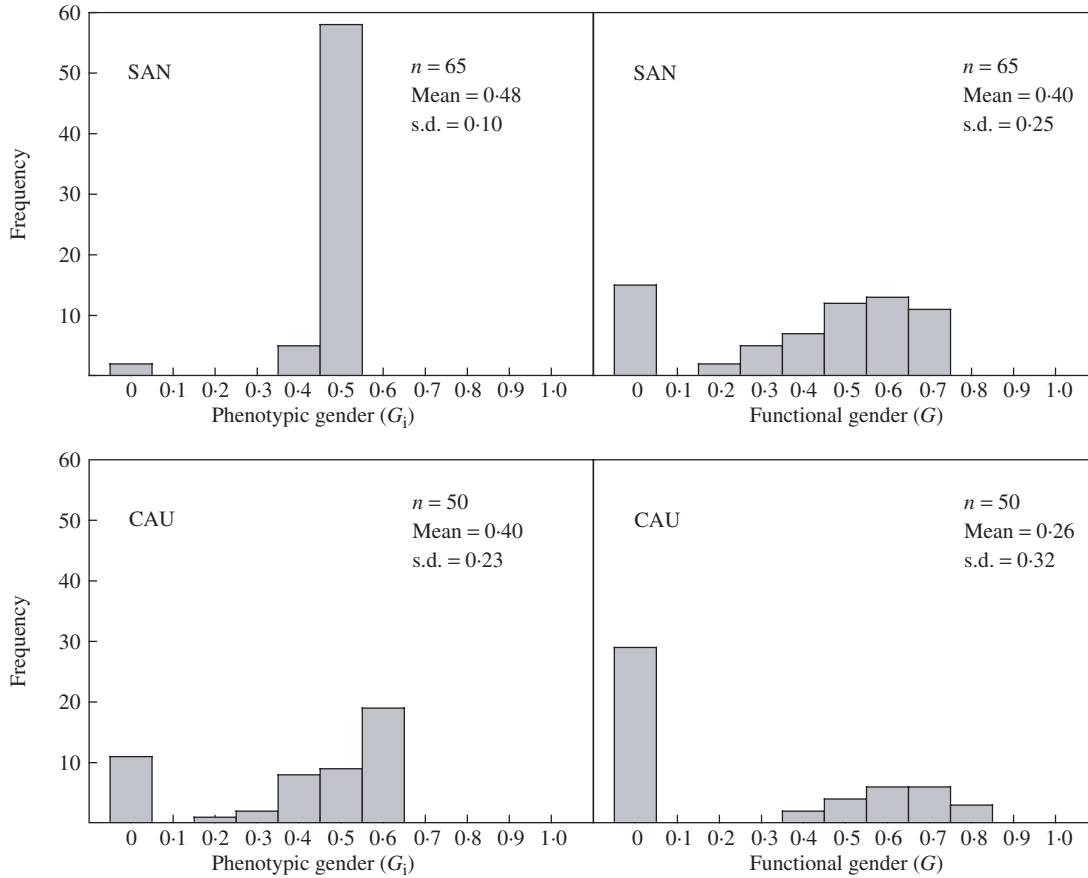


FIG. 3. Observed frequency distributions of phenotypic ( $G_i$ ) and functional gender ( $G$ ) in both *Polygonatum odoratum* populations studied.

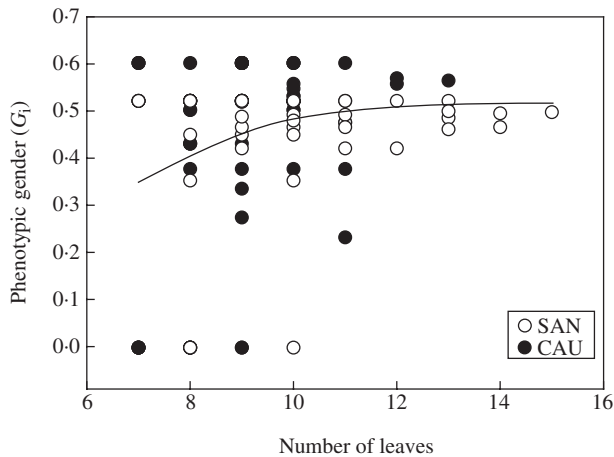


FIG. 4. Plot of phenotypic gender ( $G_i$ ) against plant size, defined as number of leaves. Open circles, plants for SAN population; filled circles, plants from the CAU population. The line is a cubic spline fitted to the data for both populations.

production from proximal to distal positions remains significant.

In hermaphroditic plants with sequentially opening inflorescences, the contribution to fitness via the male and female functions may vary among flowers in different positions, as a result of differences in the flowers' 'mating environment' (Brunet and Charlesworth, 1995). Factors

such as the spatial separation of sexual phases (dichogamy) or the spatial directionality of pollinator movement may modify the mating environment and thus alter the relative fitnesses of male and female gender. In *P. odoratum* flowering stems show sequential opening, and pollinators (basically *Bombus* species) show clear directionality, starting with proximal flowers and continuing to more distal flowers. Thus the first flower of the shoot is likely to receive xenogamous pollen only, while the remaining flowers will receive varying proportions of xenogamous and geitonogamous pollen, which will limit pollination effectiveness, since this is a self-incompatible species (for more on the negative effects of geitonogamy, see Klinkhamer and de Jong, 1993). In this situation, the probability that a flower in distal-most position will receive effective pollination is very small, except when this is the only flower remaining open (by which time the attractiveness of the inflorescence as a whole is greatly reduced). Thus the flowers in a flowering shoot of *P. odoratum* have different probabilities of 'siring' offspring: distal flowers are more likely to donate pollen to the flowers of neighbouring plants.

#### Gender variation and size

The size sex allocation model predicts an increase in female function with increasing plant size, so that large

plants will be more female in terms of both phenotypic and functional gender (de Jong and Klinkhamer, 1989; Wright and Barrett, 1999; Sarkissian *et al.*, 2001). As shown in Fig. 4, the relationship between size and gender differs between the two populations. In the SAN population the number of male flowers does not seem to be affected by the plant size (see Klinkhamer *et al.*, 1997), whereas the number of hermaphrodite flowers increased with increasing size; in CAU, by contrast, there appears to be a threshold size below which plants have only male flowers. This ‘male-only strategy’ may be an adaptative strategy to conserve resources (for additional explanations, see Huang *et al.*, 2002). Various authors have reported variations in phenotypic and/or functional gender at different levels in hermaphrodite plants, with both unimodal and bimodal population patterns generally attributable to differences in the size and resources of the plants within the population (Solomon, 1985; Eriksson, 1987; Kudo and Maeda, 1998; Huang *et al.*, 2002; Dorken and Barrett, 2003). Presumably, the distributions of phenotypic and functional gender indicated by our results reflect different degrees of control of within-shoot maternal investment (number of flowers with ovary, number of ovaries that produce fruit) in response to spatial and temporal variations in resource availability.

#### Evolutionary consequences

Andromonoecy (i.e. presence of male-only and hermaphrodite flowers within a single plant) has been suggested to be a mechanism for adjusting sexual phenotype in response to changing environmental conditions (Lloyd and Bawa, 1984). In line with this model, it would be expected that (a) it will occur in species in which the cost of producing mature fruit is high and the optimal number of male flowers is higher than the number of potentially fruit-producing flowers (see Diggle, 1993), and (b) sexual expression in andromonoecious plants may vary among individuals, among populations and among years (Diggle, 1994). The results of the present study indicate considerable variation in sex expression at the different levels considered, and previously reported results (Gutián *et al.*, 2001) have shown a decline in fruit set levels in distal flowers of *P. odoratum* attributable to competition for resources. Consequently, flowers at different positions in the inflorescence will vary as regards the quantity of resources destined for reproduction. In line with Lloyd (1980b), when there are morphological gradients along shoots or within inflorescences giving some flowers a predictable advantage over others, producing a small proportion of hermaphrodite flowers is more advantageous than hermaphroditism combined with low fruit set.

Various authors have considered the possible role of pollinator movement directionality in the evolution of sexual systems in plants with sequentially opening inflorescences, stressing the importance of differences in the probability of pollen transfer at each position in the inflorescence (e.g. Lloyd and Webb, 1986; Brunet, 1996; Kudo *et al.*, 2001; Ishii, 2004). In self-incompatible plants such as *P. odoratum*, this effect may be even greater, since pollen deposited on the stigma of another flower will not

fertilize ovules. In line with Brunet and Charlesworth (1995), the male specialization of the distal flowers of *P. odoratum*, and the consequent functional andromonoecy, may be attributable to differences in available resources and probabilities of pollen transfer at each position in the inflorescence. Transition between functional and morphological andromonoecy must require a pre-anthesis mechanism for determination of ovary development in functionally female sterile flowers (Miller and Diggle, 2003).

In conclusion, sexual investment in *P. odoratum* showed a marked variability within plants, among plants, and between populations, which confirms the importance of analysing sex expression in plants of this type. Differences in relative investment in male and female components (phenotypic gender) are reflected in the functional gender and it would be expected that the evolution of sexual specialization in *Polygonatum odoratum* be promoted.

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