Variation in structural gender in the hermaphrodite *Helleborus foetidus* (Ranunculaceae): within- and among-population patterns

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Abstract. In hermaphrodite plants, variations in structural gender (defined as the ratio between male and female gametes) may occur at different levels (among flowers, plants, and populations). In this study, we investigated variation in four traits influencing structural gender (number of carpels, ovules per carpel, stamens, and pollen grains per stamen) within and among six distant populations of the hermaphrodite perennial herb *Helleborus foetidus* (Ranunculaceae) in the Iberian Peninsula. Our results show that the four traits investigated varied significantly at all levels considered. Traits influencing the female sexual component (number of carpels and ovules per carpel) showed greater variation at the lowermost levels (within flower and plant) than traits influencing the male component, which in turn varied more markedly among populations. Number of carpels per flower and number of pollen grains per anther were the most important traits affecting between-plant variation in structural gender. There was no evidence of significant plant-level trade-offs or correlations between the various male and female traits, which covaried differently across populations. The observed between-population variation in structural gender of *Helleborus foetidus* can be explained as a consequence of differences in self-pollination levels related to a flower's "mating environment".

Introduction

Since Darwin's publication of *The Different Forms of Flowers on Plants of the Same Species* (1877), there has been a continuing effort to understand the process of plant sexual reproduction and, more specifically, gender specialization. A critical step in the understanding of gender specialization was the recognition that in flowering plants, hermaphroditism does not necessarily mean equi-sexuality, i.e. a balanced gender with roughly similar contributions by male and female functions to reproduction (Horovitz 1978). Following Horovitz's (1978) and Lloyd's (1980a, b; 1984) initial insight, numerous studies have subsequently examined patterns of variation in gender and sex expression in plants with hermaphroditic flowers, both from empirical and theoretical viewpoints (e. g. Charlesworth and Charlesworth 1981; Charnov 1982; Queller 1983; Lloyd and Bawa 1984; Broyles and Wyatt 1990, 1995; Mazer and Delesalle 1998).

Although most investigations have recognized two different quantitative measures of plant gender, namely structural (= phenotypic) and functional gender (Lloyd 1980a, Lloyd and Bawa 1984), the most frequently emphasized measure has tended to be functional gender, i.e., the relationship between the proportion of seeds produced and the proportion of seeds sired by a given individual. Reliable assessment of the functional gender of a given individual requires knowledge not only of the number of pollen grains and seeds produced by that individual, but also of sex ratio, selfing levels and levels of inbreeding depression in the population (Lloyd 1980a, b). Functional gender is effectively an estimate of the relative proportions of an individual's fitness that are realized via male and female functions, a question that would appear to be critical for the evolution of many reproductive features, such as floral display and inflorescence design (Cruzan et al. 1988, Fishbein and Venable 1996, Fritz and Nilsson 1996). Relatively few studies, however, have attempted a detailed examination of patterns of variation in structural gender in hermaphroditic species. By structural gender we mean the relative number of male and female gametes produced by an individual plant. Clearly, measuring structural gender in natural populations, and assessing its patterns of variation, are considerably easier than measuring functional gender. Why, then, the relative neglect of structural gender as an objective of plant reproduction studies? One possible reason is that number of stamens, carpels and ovules generally exhibit little or no intraspecific variability, and these floral traits are commonly used to separate families and genera (Cronquist 1981), so little opportunities seems to be left for variability to occur in structural gender in most plants. Perhaps for this reason, most studies that have examined the relationship between the number of pollen grains and the number of ovules (pollen:ovule ratio) have focused on interspecific variation, and have largely ignored the possibility of significant intraspecific variation (Cruden

1977, Mazer and Hultgård 1993, Mazer and Delesalle 1998, Cruden 2000, Wyatt et al. 2000, Jürgens et al. 2002).

Patterns of intraspecific variation in structural gender are interesting in their own right for at least three reasons. First, structural gender is a critical factor in determining functional gender. All else being equal, individual variation in structural gender will be mirrored by differences in functional gender. Second, intraspecific patterns of variation in the number of male and female gametes at the individual flower level may provide insight into the relative importance of male and female functions as evolutionary driving forces for floral phenotypes. For example, the comparative extent of individual variability in male- and female-related traits may inform us about which of the two sexual functions has played a greater selective role in shaping floral structural traits (Stanton et al. 1986). Third, a conspicuous trend exists in angiosperm evolution, from basal primitive lineages with an indeterminate number of male and female gamete-bearing structures, towards more advanced lineages with extraordinarily reduced variability in the number of such structures per flower (Stebbins 1974). The study of patterns of intraspecific variation in structural gender in some of these primitive lineages may provide insight into some of the factors potentially involved in these macroevolutionary patterns characterizing angiosperm floral evolution.

This paper presents a descriptive analysis of patterns of variation in structural gender within and among six distant populations of the hermaphroditic perennial herb *Helleborus foetidus* (Ranunculaceae) in the Iberian Peninsula. This species is particularly well suited to this type of inquiry, as it exhibits broad intraspecific variation in all the four floral traits that may contribute to variation in structural gender, namely number of carpels per flower, number of ovules per carpel, number of stamens per flower, and number of pollen grains per anther. Our objectives are (1) to document patterns of within and between population variation in structural

gender of individual plants; (2) to assess the relative magnitudes of individual variability in the male- and female-related components of structural gender, and determine the relative importance of variation in each of these four components as determinants of individual variation in structural gender; and (3) to identify possible trade-offs between same-sex components (e.g. number of stamens vs. number of pollen grains per anther, number of carpels vs. number of ovules per carpel) and between different-sex components (total number of ovules vs. total number of pollen grains).

Material and methods

Study system. *Helleborus foetidus* L. is a self-compatible, perennial herb distributed over much of western, central and southwestern Europe (Werner and Ebel 1994). In the Iberian Peninsula, it typically occurs in clearings, forest edges, and the understory of deciduous and mixed forests. Flowering mainly takes place from January to March. Plants consist of one to several ramets that develop a terminal inflorescence after several seasons of vegetative growth. Flowers are protogynous, hermaphroditic, and apocarpous. The number of carpels per flower ranges between 1 and 5 (mostly 1–3), and the number of anthers between 25 and 60 (see results below). Anthers start to dehisce centripetally when flowers are 6–8 days old, and the pollen presentation period lasts for nearly two weeks. The extremely long-lived flowers (up to 20 days, Vesprini et al. 1999) are mainly pollinated by bumblebees and anthophorid bees (Herrera et al. 2001).

Methods. Flowers were collected in February-April 1998–1999 from six *H. foetidus* populations all over the Iberian Peninsula (Fig. 1). The nearest populations were ~70 km apart, while the most distant ones were ~850 km away. Plants were growing in a variety of habitat types, including evergreen oak forests, mixed pine oak forests, chestnut groves, and open successional shrublands. In CAU and MAG (see Fig. 1) flowers were collected in the two years of study, while in CAZ flowers were collected only in 1998 and in MON, GRA and PIR only in 1999. In CAU, CAZ, and MAG, sampled plants were living in two different populations (~2–4 km apart) but for the present

study these plants were pooled into a single data set, because preliminary analyses showed almost no variation between populations in a given region in any of the four traits analyzed. Five flowers were collected from 26–67 plants in each population, and preserved in 2.5–2.5–95% formaldehyde-acetic acid-ethyl alcohol (FAA). The number of stamens and carpels, and the number of ovules per carpel, were determined for all collected flowers. To account for possible differences in the number of carpels and stamens between early- and late-opening flowers, we attempted to sample flowers in each locality as closely as possible to the local flowering peak.

A subset of the flowers collected in 1999 (from CAU, MAG, MON, GRA and PIR; Fig. 1) was used for pollen counting. Pollen was counted using a particle counter (Coulter Counter® Z2, Beckman Coulter Inc.) equipped with a 100 μm aperture tube and a particle-size channelizer accessory. From each flower we removed a subsample of completely closed anthers and separately counted the number of pollen grains in each (N = 1380 anthers, 1–5 anthers per flower; N = 314 flowers, 1–5 flowers per plant; N = 76 plants, 10–25 plants per population). Each anther was placed in a clean 1.5 ml polypropylene centrifuge tube filled with 1 ml of saline solution (IsothonII®), and gently squashed until the pollen grains were released into the solution. Each of these samples was then prepared for counting by suspension and shaking in 75 ml of IsothonII®. We counted the number of grains in five 500 μl aliquots (particle size channels 18–40 μm), and the average number was used to calculate the total number of grains per anther.

Statistical analyses. Total variance in the four traits considered (number of carpels per flower, number of ovules per carpel, number of stamens per flower, and pollen grains per anther) was partitioned into components due to variation among populations, plants and flowers, using the MIXED procedure ("restricted maximum likelihood" estimation method) in the SAS statistical package (SAS Institute 1996). Univariate analyses were performed separately for each study year, and included "population", "plant" and "flower" as categorical random factors in a completely nested design.

To assess among-individual variability in each component of structural gender within populations, we calculated the mean values of all traits for each individual plant and the corresponding
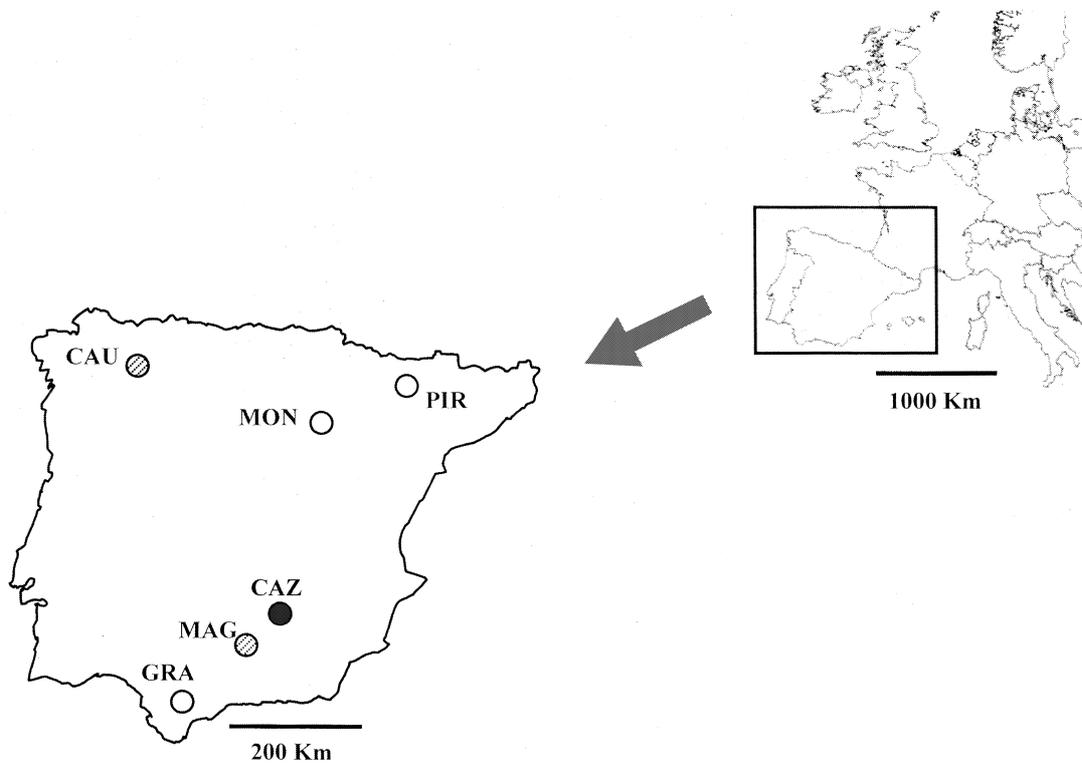
Fig. 1. Locations in the Iberian Peninsula of the six populations of *Helleborus foetidus* studied. Dashed dots represent populations from which data were obtained for both years of study (1998 and 1999). Black and white dots represent populations from which data were obtained only for 1998 or 1999, respectively. Number of plants (N_p) and flowers (N_f) sampled at each site and year are in parentheses. Sites were: CAU, Sierra del Caurel (1998: $N_p = 64$, $N_f = 294$; 1999: $N_p = 54$, $N_f = 270$); PIR, Pirineos (1999: $N_p = 30$, $N_f = 150$); MON, Sierra del Moncayo (1999: $N_p = 32$, $N_f = 159$); CAZ, Sierra de Cazorla (1998: $N_p = 67$, $N_f = 229$); MAG, Sierra Mágina (1998: $N_p = 60$, $N_f = 297$; 1999: $N_p = 26$, $N_f = 129$); and GRA, Sierra de Grazalema (1999: $N_p = 35$, $N_f = 158$)

coefficients of variation (CV) for each population. Heterogeneity among populations for within-population variability was assessed using Levene's test for relative variability (Van Valen 1978).

The relative influence of each of the four traits on variation in structural gender at the plant level was determined by conducting a multiple regression analysis and using the resulting standardized partial regression coefficients as indicators of the relative importance of each trait in explaining between-plant variation in structural gender. The dependent variable was the log-transformed ratio of mean number of pollen grains per flower to mean number of ovules per flower (i.e. plant-level structural gender). The independent variables were the number of carpels per flower, the number of ovules per carpel, the number of stamens per flower, and the number of pollen grains per anther. This analysis was performed with the data for 1999 only.

To identify possible trade-offs between traits influencing the number of gametes of the same sex, and between traits of different sex (number of carpels per flower vs. number of ovules per carpel; number of stamens per flower vs. number of pollen grains; and number of pollen grains per flower vs. number of ovules per flower), we performed analyses of covariance by fitting generalized linear models to the data, considering mean values for each plant (procedure GENMOD, error = Poisson, link = logarithm; SAS Institute 1996). Additionally, we investigated whether the relationships detected were consistent among populations. We performed separate analyses with number of ovules per carpel, number of pollen grains per anther, or number of pollen grains per flower as dependent variables (mean values for each plant), and number of carpels per flower, number of stamens per flower, or number of

ovules per flower, respectively, as independent covariates. The models also included population (as a categorical variable) and the interaction between population and each of the independent variables (carpel number × population, stamen number × population and ovule number × population). Statistically significant interaction terms indicate that the slopes of the regression of the dependent variable on the covariate vary among populations.

Results

Within-population variation in mean trait values. Figure 2 summarizes the variation in structural-gender traits (mean values for

individual plants) among populations and between years. The number of carpels per flower (mean ± SD) ranged from 1.9 ± 0.5 (population GRA, 1999) to 2.9 ± 0.4 (MAG, 1999); the number of ovules per carpel from 10.2 ± 0.9 (PIR, 1999) to 11.4 ± 0.6 (MON, 1999). The number of stamens per flower ranged from 33.3 ± 4.6 (MAG, 1999) to 41.9 ± 3.5 (PIR, 1999); and the number of pollen grains per anther from 13,600 ± 1,633 (MON, 1999) to 23,201 ± 3,427 (CAU, 1999).

Population-level coefficients of variation were generally less than 15%: the number of carpels per flower was the most variable trait (CV 12.4–27.1%, MON 1999 and GRA 1999,
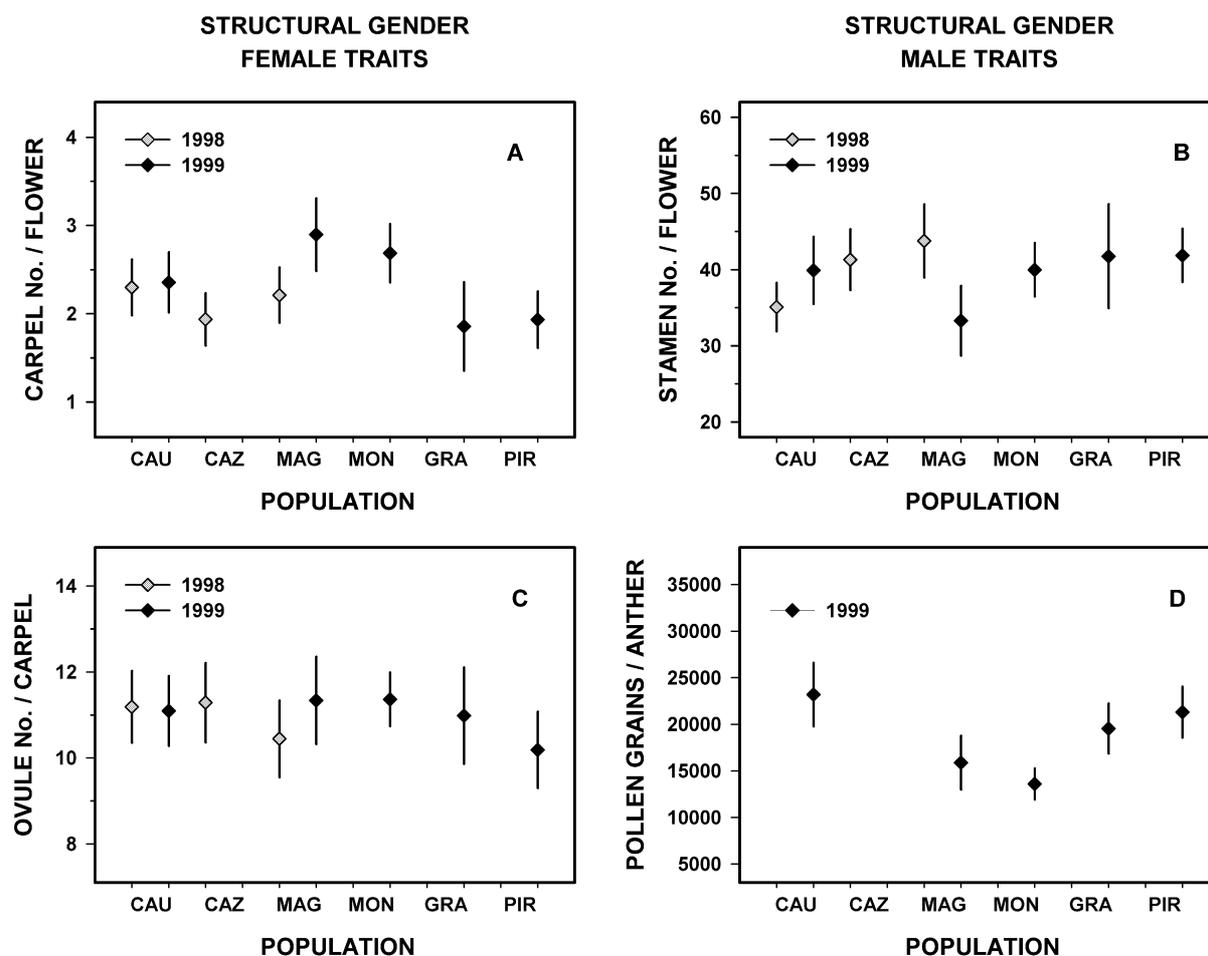
Fig. 2. Variation among *Helleborus foetidus* populations for the two study seasons in individual-plant means of the different structural gender components considered in this study: **A** carpels per flower, **B** stamens per flower, **C** ovules per carpel, and **D** pollen grains per anther. Values are means ± standard deviations. See Materials and Methods and Fig. 1 for details on sample sizes

respectively), and the number of ovules per carpel the least variable (CV 5.5–10.2% MON 1999 and GRA 1999, respectively). The GRA population showed the highest coefficients of variation, and the MON population the lowest (Table 1). Considering all populations together, the number of ovules per carpel and the number of stamens per flower showed lower CVs than the number of carpels per flower and the number of pollen grains per anther.

The results of Levene's test for relative variability (Table 1) indicated that in 1998 there was no significant heterogeneity among populations in the within-population variability of any of the traits analyzed. Nevertheless, we detected in 1999 significant heterogeneity for the within-population variability of the number of ovules per carpel and the number of stamens per flower.

Patterns of variation. Figure 3 shows the proportion of the variance in structural gender traits explained by each of the sources of variation considered (flower, plant, and population) in the two study seasons. All traits varied significantly among flowers within plants, and among plants within populations. The number of pollen grains per anther and the number of ovules per carpel varied significantly within flowers. None of the traits varied significantly among populations. On average,

the sources accounting for the largest proportion of variance in traits affecting structural gender were "plant" and "flower", although their relative contributions varied considerably among traits and seasons.

In both study seasons, the two traits influencing the number of female gametes (carpels per flower and ovules per carpel) showed greater within-plant and within-flower variability than the corresponding male components (stamens per flower, pollen grains per anther). The proportion of the variance explained by population was small for both female traits. Of the male components, the number of stamens per flower behaved differently in the two seasons: "population" was the factor that explained the largest proportion of variance in 1998, but "plant" explained the largest proportion in 1999. The trait that showed the most marked variation among populations was the number of pollen grains per anther although, as stated earlier, population was not a significant source of variation in any of the male-related components.

Relative contribution to structural gender. The plant-level structural-gender value (i.e. the ratio of mean number of pollen grains per flower to mean number of ovules per flower) showed high variability among populations (Fig. 4), ranging from 15,919 ± 2,699 in MON and

Table 1. Coefficients of variation (%) for each of the four components of structural gender in *Helleborus foetidus*. Values are shown for each population and each year. Also shown are the results of Levene tests to assess whether CVs vary significantly among populations (Van Valen 1978)

Population	Carpels/flower		Ovules/carpel		Stamens/flower		Pollen grains/anther	
	1998	1999	1998	1999	1998	1999	1998	1999
Caurel	13.8	14.5	7.5	7.4	9.2	11.1	–	14.8
Cazorla	15.4	–	8.2	–	9.7	–	–	–
Mágina	14.2	14.3	8.6	9.0	11.0	13.8	–	18.3
Moncayo	–	12.4	–	5.5	–	8.8	–	12.2
Grazalema	–	27.1	–	10.2	–	16.4	–	13.8
Pirineo	–	16.6	–	8.8	–	8.4	–	12.9
ALL	16.1	23.1	8.7	8.9	13.6	13.8	–	23.9
Levene Test								
F	0.64	6.80	0.16	10.94	1.03	12.43	–	2.17
P	0.728	0.147	0.925	**0.027**	0.596	**0.014**	–	0.704
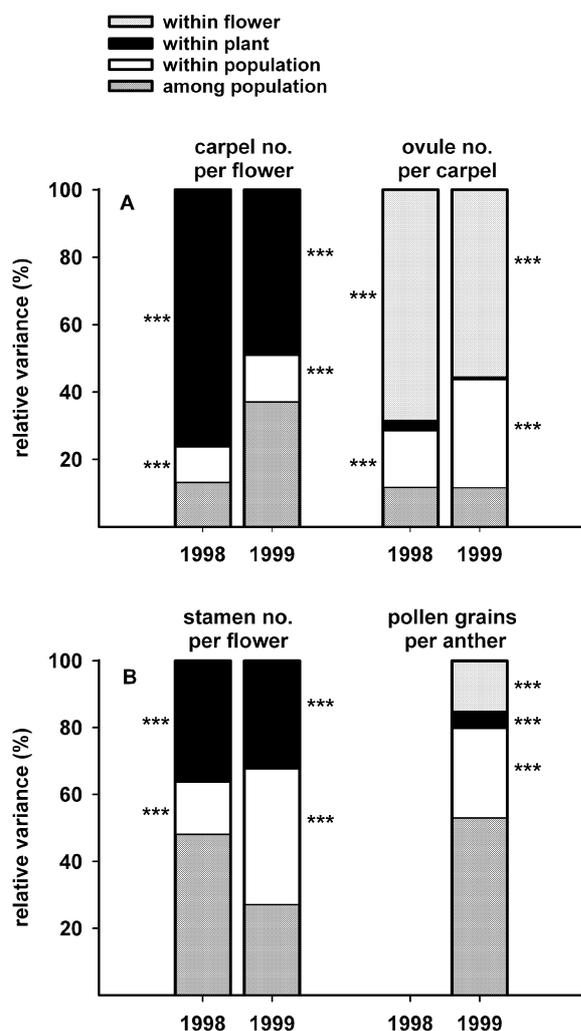
Fig. 3. Proportions of variance in the structural-gender traits (**A** female traits; **B** male traits; considering those traits evaluated in both years of this study) accounted for by variation among populations, among plants within populations, among flowers within plants, and within flowers. Asterisks indicate statistically significant sources of variation ($p < 0.0001$)

17,146 ± 5,957 in MAG populations to 47,254 ± 14,145 in PIR population. The results of the multiple regression analysis (Table 2) indicated that the number of carpels per flower and number of pollen grains per anther were the most important predictors of the variation in plant-level structural gender. Number of stamens per flower and number of ovules per carpel made less important but still statistically
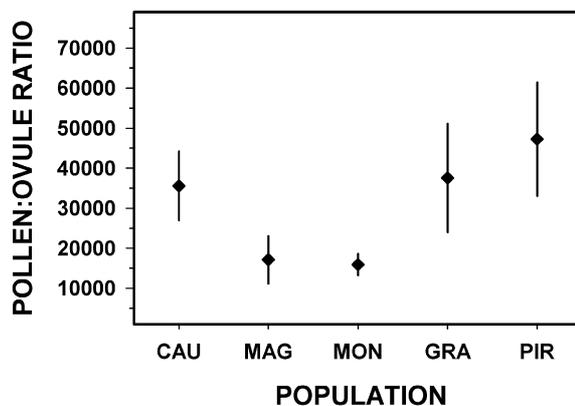
Fig. 4. Variation among *Helleborus foetidus* populations in individual-plant means of pollen:ovule ratio. Values are means ± standard deviations. See materials and methods and Fig. 1 for details on sample sizes

significant contributions to plant-level structural gender.

Trade-offs between variables. The data for 1998 revealed a marginally significant relationship between the mean number of ovules per carpel and the mean number of carpels per flower at the plant level. These traits covaried differently across populations (Table 3). In 1999, we did not detect any compensatory (i. e. negative) relationship between the number of ovules per carpel and the number of carpels per flower at the plant level (Fig. 5a, b), although the results of the analysis indicated significant differences among populations, as indicated by the significant interaction. In the MAG population, the nature of the relationship between the two traits differed between 1998 and 1999: in 1998 there was a significant negative correlation between the number of carpels per flower and the number of ovules per carpel, while in 1999 there was a significant positive correlation between these two variables (Fig. 5a, b).

We did not detect any compensatory response between the number of pollen grains per anther and the number of stamens per flower (Table 3). In fact, there was a significant positive correlation between these two variables in the GRA and CAU populations (Fig. 5c). Similarly, we did not detect a

Table 2. Partial regression coefficients and significance levels obtained from multiple regression analysis applied to log-transformed pollen:ovule ratios of *Helleborus foetidus* plants (see text for details). Four predictor variables were introduced: carpel number per flower, ovule number per carpel, stamen number per flower and pollen grain number per anther. b = standardized partial regression coefficient. t = Student's t. Based on $N = 76$ plants, seven populations combined

Predictor variable	b	t	P
Carpels per flower	−0.512	−32.11	0.0001
Ovules per carpel	−0.150	−10.63	0.0001
Stamens per flower	0.275	17.09	0.0001
Pollen grains per anther	0.453	28.26	0.0001

Table 3. Effects of population (categorical variable) and number of carpels per flower, number of stamens per flower, or number of ovules per flower (independent covariates) respectively on (a) number of ovules per carpel, (b) number of pollen grains per anther, and (c) number of pollen grains per flower estimated by fitting generalized linear models to the data, (procedure GENMOD, error = Poisson, link = logarithm). The models also included the interaction between population and each of the independent variables (carpel number × population, stamen number × population and ovule number × population, respectively). Separate analyses for each dependent variable (mean values for each plant) and year were preformed

| Variable	Source of variation	1998			1999		
		df	Chi-square	P	df	Chi-square	P
a) Ovules per carpel	Carpels per flower	1	3.48	0.062	1	2.38	0.123
	Population	2	5.43	0.062	4	12.30	0.015
	Carpels*Population	2	9.75	0.008	4	12.66	0.013
b) Pollen grains per anther	Stamens per flower	–	–	–	1	0.52	0.472
	Population	–	–	–	4	2.41	0.661
	Stamens*Population	–	–	–	4	3.20	0.525
c) Pollen grains per flower	Ovules per flower	–	–	–	1	0.18	0.669
	Population	–	–	–	4	1.02	0.907
	Ovules*Population	–	–	–	4	6.17	0.187

consistent negative relationship between the number of pollen grains per flower and the number of ovules per flower (Table 3): these two variables were negatively correlated in the MAG population but positively correlated in the CAU population (Fig. 5d).

Discussion

We have analyzed the variation in structural gender in *Helleborus foetidus*, in several distant populations, using a study design with three novel approaches. First, we investigated the variation in the female and male components of structural gender at three different levels (flower, plant, and population). To our knowledge, this is the first time that an approach of this type has been used. Second, the study plant has hermaphroditic flowers; thus, unlike monoecious species, the "decisions" regarding sexual assignment may be made during floral development (Mazer and Dawson 2001). Third, *H. foetidus* – like other Ranunculaceae – shows a variable number of stamens and carpels: thus, modifications in structural gender can be achieved not only by varying pollen and ovule number, but also stamen and carpel number.

Patterns of gender variation. Our results reveal marked variation in all components of
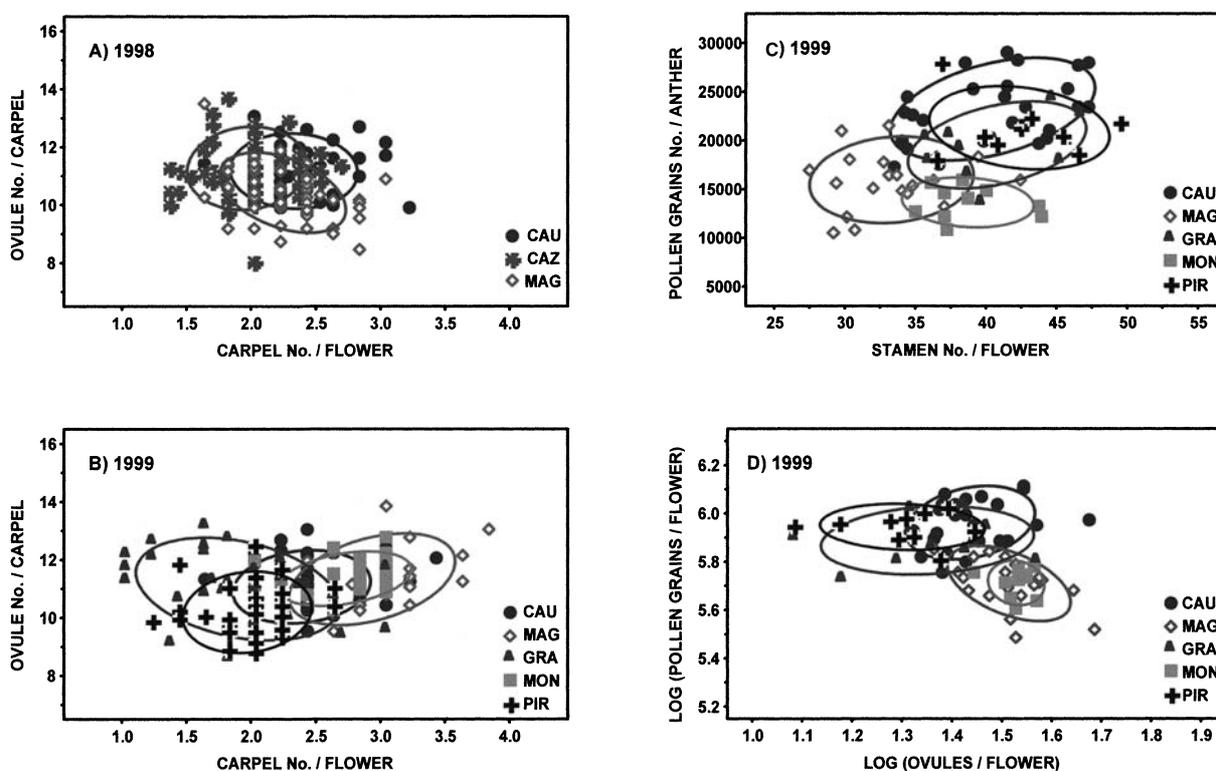
Fig. 5. Scatterplots of individual plants on axes representing (**A**) mean number of ovules per carpel versus mean number of carpels per flower in 1998, (**B**) mean number of ovules per carpel versus mean number of carpels per flower in 1999, (**C**) mean number of pollen grains per anther versus mean number of stamens per flower in 1999, and (**D**) mean number of pollen grains per flower versus mean number of ovules per flower in 1999 (log transformed). Ellipses show 95% confidence limits for each population

structural gender, at all levels considered (flower, plant, and population). Previous studies have documented variations in sexual investment in different species at various levels (see Mazer and Dawson 2001), and a number of these studies have detected variation specifically in structural gender (Schoen 1982, Campbell 1992, Pickering and Ash 1993, Kudo and Maeda 1998, Wright and Barrett 1999). These variations have been related to changes in the age and/or resource status of plants or individual flowers (Mazer 1992, Ashman and Baker 1992, Klinkhamer and de Jong 1993, Kudo and Maeda 1998), and/or to genetic causes (Mazer and Delesalle 1996, Vogler et al. 1999, Koelewijn and Hunscheid 2000).

The different components of structural gender showed very different patterns of variation among populations. Considering the female components, particularly high variances were detected within flowers in the number of ovules per carpel, and within plants among flowers in the number of carpels per flower. Considering the male components, particularly high variances were detected among populations in the number of pollen grains per anther and the number of stamens per flower.

In general, the female components of structural gender showed most variation within flower and plants, while the male components showed most variation among populations. Previous studies have detected higher stability in male components, including pollen grain size and number (Stanton et al. 1991, Willis 1993). Mazer (1992), and more recently Vogler et al. (1999) found that the male components of gender in *Raphanus sat-*

ivus and *Campanula ranunculoides* were less sensitive to environmental conditions than the female components. In contrast, Delph et al. (1997) have shown that pollen characteristics may vary with environmental conditions, and Delesalle and Mazer (1995) found that male investment in *Spergularia marina* was no more stable than female investment in response to variations in resource availability. According to this view, the male components of gender in *H. foetidus* (number of stamens and number of pollen grains per anther) appear to be more stable within individuals than the female components.

Variation in structural gender. The present results show marked variation in the structural gender of *H. foetidus*, with all four traits considered contributing to this variation. We have used pollen:ovule ratio as a measure of a plant's structural gender. In recent decades, this variable has often been viewed as more or less consistent within species, and has been considered to reflect species-level reproductive mechanisms. In a number of species, however, P:O ratio has been shown to vary considerably among populations, among plants, within plants, and during a flowering season (Devlin 1989, Damgaard and Loeschcke 1994, Ramsey et al. 1994, Affre et al. 1995, Wright and Barrett 1999). Our results for *H. foetidus* likewise reveal marked variability in P:O ratio, which was three times higher in the PIR population than in the MON and MAG populations. Recently, Cruden (2000) has reported high variability in P:O ratio in xenogamous species, with values of up to 200,000 in some Boraginaceae.

A recent study has postulated (a) that P:O ratio may show greater intraspecific variation in xenogamous species than in phylogenetically related obligate selfers, and (b) that in facultative selfers in which particular periods or populations show high levels of spontaneous self-pollination, lower P:O ratios may be selectively favored (Mazer and Delesalle 1998). In line with these predictions, Schoen (1982) found increased ovule production in hermaphrodite populations, with higher levels of self-pollination under natural conditions. In a

within-population study, Damgaard and Abbot (1995) found a positive correlation between selfing level and P:O ratio, in contrast with the negative correlations reported in previous between-population and between-taxa studies. In a previous study, Herrera et al. (2001) found that the relative frequency of spontaneous autogamy was higher in northwestern Spain (the Caurel populations) than in southern Spain (the Mágina populations). In view of the predictions of Mazer and Delasalle (1998), we would expect lower P:O ratios in northwestern Spain. However, our results indicate higher P:O ratios in northwestern Spain (Caurel) than in southern Spain (Mágina). In fact, in Caurel both ovule number and pollen number were relatively high, while in Mágina ovule number was relatively high and pollen number relatively low.

Trade-off between female and male allocation. A central assumption of the models of sexual investment is that there is a negative correlation between male and female investment at the flower level, since both types of investment are assumed to come from a single and limited resource stock (Charlesworth and Charlesworth 1981, Charnov 1982, Campbell 2000). Under these assumptions, we would expect a negative relationship between the number of stamens and the number of carpels, between the number of pollen grains and the number of ovules, between pollen size and ovule size, and between flower production and fruit production. Nevertheless, our data for *H. foetidus* do not meet these predictions: at the flower level, we did not detect any trade-off between the investment in male and female components of structural gender. A lack of trade-off has been reported frequently both at the flower and at the plant level, and has been attributed to factors including the difficulty of eliminating positive covariances induced by environmental heterogeneity in single-generation studies (Mazer et al. 1999), and the possibility that resources for the expression of female and male traits may come from different pools (Delesalle and Mazer 1995). However, Mazer and Delesalle (1998) have

pointed out that mating system itself should have a strong influence on whether or not a negative (vs. positive) correlation evolves between pollen and ovule production per flower. They argue that in predominantly selfing species there is no a priori reason to expect a negative correlation between male and female investment.

Evolutionary consequences. The most obvious conclusion of the present study is that structural gender in the hermaphrodite *H. foetidus* shows marked variation at various levels (within plants, within populations, and among populations). Variations in structural gender among individual hermaphrodite plants have been attributed to genotype, environmental factors and/or plant physiological status. Variations in structural gender among populations may result from natural selection, phenotypic plasticity and/or genetic drift (see Mazer and Dawson 2001, and references therein). Interpretation of observed variations in evolutionary terms however is difficult, and our data do not directly address this question; nevertheless, our results permit some comments in this regard.

In plants like *H. foetidus* that present a sequential flowering pattern, pollen from flowers at different stages will have different opportunities for siring offspring. The contribution of male and female components to fitness may vary depending on the flowers' "mating environment", and this variation may exert selective pressure on the relative investment in male and female components (see Brunet and Charlesworth 1995). Certain combinations of flowering phenology, together with the temporal separation of the sexual phases, may lead to different degrees of geitonogamy, and this again may exert selective pressure on relative investment in male and female components. Previous studies (Herrera et al. 2001) have suggested marked differences in the mating environment of flowers of *H. foetidus*, due to (1) sequential opening associated with protogyny, and (2) marked differences in the degree of overlap between the male and female phases in

different regions of the Iberian Peninsula. This regional variation in the degree of intrafloral overlap between the male and female stages provides a proximate explanation for the observed pattern of differences in self-pollination levels in *H. foetidus* (see Herrera et al. 2001), and it seems reasonable to assume that this may imply differences among flowers in relative investment in male and female components.

The extent to which differences among flowers in the relative investment in male and female components (i.e. in structural gender) are reflected in the *functional* gender is important and currently under study. Little or no correspondence between the two would be expected to inhibit the selection of sexual characteristics and therefore prevent the evolution of sexual specialization in hermaphroditic plants like *Helleborus foetidus*.

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