

Phenotypic gender in *Hormathophylla spinosa* (Brassicaceae), a perfect hermaphrodite with tetradynamous flowers, is variable

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Abstract. Angiosperms show an evolutionary trend from an indefinite to a fixed number of floral organs. When floral formula inconstancy in recent angiosperms is reported, it is often considered as a byproduct of stress and its fitness consequences remain mostly unexplored. We report substantial nonhomeotic meristic variation in stamen number (0–10 stamens per flower) in two populations of *Hormathophylla spinosa* during four years. This variation was plastic, suggesting its functional role in the adjustment of phenotypic gender. However, no correlations were found between phenotypic gender and plant size, pollinator or herbivore abundance. Effects on female reproductive success were inconsistent on a per-flower and on a per-plant basis, rendering adaptive explanations in terms of selfing or resource adjustment unsatisfactory with the data available. Nevertheless, individuals showing larger interannual variation in phenotypic gender showed higher female reproductive success, suggesting an advantage for gender modification. Although our results do not easily conform to any adaptive explanation, this remarkable example of breakdown of trait canalization should stimulate the study of the mechanisms and ultimate causes responsible for the maintenance of fixed floral traits.

Key words: Brassicaceae, floral formula, *Hormathophylla*, meristic variation, reproductive

success, sexual expression, Sierra Nevada, stamen number.

Introduction

Floral structure in angiosperms is usually represented by means of a floral formula, which summarizes the number and arrangement of floral organs (sepals, petals, stamens and carpels). Angiosperms show a well established evolutionary trend from an indefinite and variable (usually large) number of floral organs in ancient families to a reduced and fixed number of floral whorls and organs in recent families (Endress 1990, Takhtajan 1991). This trend culminates with the constancy of the floral formula at a family level in recent angiosperms. The evolutionary advantages of this reduction and fixation in floral parts are supposed to be a higher structural and functional integration of the flower (Takhtajan 1991). Despite all the above, cases of floral formula inconstancy (meristic variation) involving bract (Wilson and Stapp 1979), sepal (Ellstrand 1983, Levy 1997), petal (Huether 1969, Ellstrand 1983, Inoue and Takahashi 1991, Levy 1997), stamen (Ellstrand 1983, Ellstrand et al. 1984, Inoue and Takahashi

1991, Levy 1997) and carpel number (Saunders 1923, Arber 1931, Ellstrand 1983, Ellstrand and Mitchell 1988, Levy 1997) have been reported. In some cases, it has been shown that those 'abnormal' flowers were not anecdotal in populations (over 50% of flowers, Wilson and Stapp 1979; 10% of flowers, Ellstrand 1983). Reports have been mostly descriptive (Huether 1969, Ellstrand 1983, Ellstrand and Mitchell 1988, Inoue and Takahashi 1991) or have explored the environmental or genetic basis of the inconstancy (Huether 1968, Wilson and Stapp 1979, Levy 1997). Usually no function has been attributed to 'abnormal flowers', which have often been considered as a byproduct of stress (Huether 1968, Wilson and Stapp 1979). Fitness consequences of meristic variation remain mostly unexplored.

Sex allocation theory (Charnov 1982) provides a suitable framework to analyze the functionality of meristic variation. Modification of phenotypic gender, i.e. relative allocation to male vs. female structures, has been documented for a number of species in which the number of floral structures is not fixed (*Muntingia calabura*, Bawa and Webb 1983, *Potentilla anserina*, Eriksson 1987, *Ranunculus*, Pickering and Ash 1993, *Paeonia cambessedesii*, Méndez and Traveset 2003). Floral formula constancy entails that phenotypic gender becomes more fixed in recent compared to more basal angiosperms. Taxa with a constant floral formula can modify their phenotypic gender only by means of subtle variability in the number of pollen grains and/or ovules per flower (Orchidaceae: Piper and Waite 1988, Malvaceae: Havens et al. 1995) or in the relative size of sexual organs (Campbell 1992, Kudo et al. 2001, Ishii and Sakai 2002). Nevertheless, many recent families lack this variability-particularly, in the number of ovules per flower (Takhtajan 1991) - and are able to modify mostly their functional gender, i.e. their fitness gains through male vs. female gender (Mazer et al. 1989, Klinkhamer and de Jong 1993). Meristic variation reintroduces the possibility of modification of phenotypic gender in these taxa.

Brassicaceae is a recent family that shows a remarkably constant floral ground plan, with four sepals, four petals, two carpels and six stamens (Hedge 1976, Hall et al. 2002; see Endress 1992 for exceptions). Tetrastamens emerge as a synapomorphy in phylogenetic reconstructions of Brassicaceae (Hall et al. 2002). *Hormathophylla spinosa* (L.) K pfer is a long-lived Brassicaceae shrub inhabiting the high mountains of the Western Mediterranean. As all crucifers, this shrub is described as having a tetrastamens androecium with six stamens (the perfect hermaphrodite flower) (Castroviejo et al. 1993). However, in the Sierra Nevada mountains (SE Spain) we have observed that the number of stamens per flower highly varies from 0 to 10. This entails a remarkable violation of floral formula constancy within a family traditionally characterized by a fixed floral formula. In this communication, by studying more than 4000 flowers from 50 plants in two populations during four years, we 1) describe the pattern of gender variation at several spatial and temporal scales, 2) analyze the factors, both intrinsic (such as plant size, flower production or flower size) and extrinsic (such as herbivory and previous-year gender expression) that affect gender expression, 3) investigate the functional role of the phenotypic gender expressed by each plant, in terms of pollinator attraction and efficiency, as well as floral herbivores and pre-dispersal seed predator incidence, and finally 4) determine the effect of the phenotypic gender on the female reproductive success of the plants.

Materials and methods

Plant natural history and study sites. *Hormathophylla spinosa* is a long-lived shrub inhabiting the high mountains of the Western Mediterranean. In Sierra Nevada mountains (SE Spain), this species occurs from 1600 to 3300 m above sea level (a.s.l.). In these mountains, this species is pollinated by a wide and diverse pollinator assemblage, outstanding the endemic ant *Proformica*

longiseta (Formicidae), several species of flies, hoverflies and solitary bees (Gómez and Zamora 1999). Although *H. spinosa* is self-compatible, it requires pollinators for full seed set. At highest elevations *H. spinosa* is also wind-pollinated (Gómez and Zamora 1996). This species invariably has four ovules per flower (Gómez and Zamora 1999). Many flowers and fruits are consumed by ungulates, mainly Spanish ibex *Capra pyrenaica* and domestic sheep (Gómez and Zamora 2000, Gómez and González-Megías 2002). Ripe seeds in surviving fruits are depredated within the fruit by a weevil seed predator, *Ceutorhynchus* sp. nov. (Curculionidae; Gómez and Zamora 1994).

This study was conducted in two populations from the high mountains of the Sierra Nevada (Spain). The first population (population B hereafter) is located at Cabecera del Río San Juan (2500 m a.s.l.). The second population (population C hereafter) is located at Cabecera del Río Veleta (3130 m a.s.l.). Both populations had *H. spinosa* as dominant species, in similar densities (87.5 ± 13.6 and 74.2 ± 15.1 individuals/100 m² in populations B and C respectively) and were located on dry slopes with rocky substrate in which more than 60% of surface was open sites.

Estimation of phenotypic gender. Phenotypic gender of individual *i* can be quantified by means of the 'femaleness' index, *G*, developed by Lloyd and Bawa (1984). This index varies from 0 (complete female) to 1 (complete male) and is calculated by means of the formula:

$$G = \frac{f_i}{f_i + E \cdot m_i}$$

where f_i is a measure of investment in female function (the sum of carpels in the flowers sampled in our case), m_i is a measure of investment in male function (the sum of stamens in the flowers sampled in our case), and *E* is an equivalence factor which measures the ratio of investment in female and male structures in the whole population. *E* is calculated as

$$E = \frac{\sum f_i}{\sum m_i}$$

and it accounts for the uneven production of female and male structures per flower.

When *G* is calculated using the number of floral structures and is applied to hermaphroditic

species with a variable number of sexual structures per flower, *E* must be empirically obtained for each data set (e.g. Pickering and Ash 1993). For hermaphroditic species with a constant floral formula, as in Brassicaceae, both *E* and *G* are constant by definition. In fact, *G* is 0.5 (meaning equal relative investment in male and female structures) and *E* is simply the ratio of carpels to stamens in a flower ($E = (2 \text{ carpels}) / (6 \text{ stamens}) = 0.333$). To explore the sexual expression of individuals showing meristic variation in stamen number, we calculated a modified index of femaleness, *G'*, which indicates the departure in phenotypic gender with respect to the 'ideal of Brassicaceae', not with respect to the average phenotypic gender of the population. The formula for *G'* is the same as the one for *G* but utilises $E = 0.333$ instead of the empirical *E* for our data set. As ovule number is not variable in our study species, variation in *G'* exclusively depends on the variation in stamen number. *G'* is a useful measure of gender for our purposes in this study; plants having six stamens in all their flowers have a $G' = 0.5$ and those having more or less stamens than usual in Brassicaceae show values of *G'* lower or higher than 0.5, respectively. The only practical modification with respect to the traditional use of *G* is that average *G'* of the data set is not 0.5.

Calculations of *G'* were based on 19–33 flowers per individual in population B and 21–30 flowers in population C. *G'* was not calculated for a few individuals in which less than ten flowers were available. During 1988–91, a total of 20 and 30 individuals were studied in populations B and C, respectively, although not all individuals were sampled in all the years.

Sampling of ecological and fitness variables. We also quantified the following variables for each plant (see Gómez 1993, Gómez and Zamora 1999, Gómez and González-Megías 2002, for methodological details): 1) plant size (cm³) estimated by measuring the two diameters and the height and fitting to a hemisphere; 2) flower production (number), estimated by counting the number of flowers in ten inflorescences per plant and the total number of inflorescences beared by that plant; 3) flower size (mm), quantified with a digital caliper as the length between the tips of two opposite petals; 4) flowering duration (days) quantified as the days lapsed from first to last flower production; 5)

flowering synchrony (index) defined as the number of days that a plant overlapped as an average with co-occurring plants; 6) pollinator abundance (insects 10 min^{-1}), estimated by counting during 1-min censuses all the insects visiting flowers and contacting sexual organs per plant; 7) floral herbivores (*Timarcha lugens*, Chrysomelidae) abundance quantified as for the previous variable; 8) seed predator abundance (*Ceutorhynchus* sp., Curculionidae), quantified by counting the number of weevils per year in a sample of 50 fruits per plant; 9) fruit set, an estimate of female reproductive success, and quantified by dividing the number of ripe fruits per plant by the number of flowers produced; 10) seed set, another estimate of reproductive success, and quantified by dividing seed number by ovule number (= number of flowers \times 4) produced per plant.

Statistical analyses. Kendall's tau was utilised to calculate year-to-year gender correlation, or concordance, for individual plants. Relationships between G' and ecological variables were studied by means of Pearson correlations. To analyze the effect of phenotypic gender on plant reproductive success, we fitted a Generalized Linear Model. In these models, we also introduced as covariates plant area and flower production, two traits affecting *H. spinosa* reproductive success (Gómez and Zamora 2000).

Results

Patterns of variation in phenotypic gender. *Variation in stamen number at a flower level.* Flowers of *H. spinosa* in Sierra Nevada showed a remarkable variation in stamen number, contrary to the expectations for Brassicaceae (Fig. 1; Table 1). Percent of "typical" flowers, i.e. those with six stamens, ranged between 40 and 89% across years and populations (Table 1). We found flowers showing higher or lower stamen number with respect to the "typical" crucifer flower. Variability towards a decrease in stamen number was more obvious, both in extent (0–5 vs. 7–10) as in numbers (Table 1). Flowers with more than six stamens were almost exclusively present in population C, while flowers with less than six stamens were present in both studied populations and, in general, overnum-

bered those with more than six stamens (Table 1).

Variation in sexual expression at an individual level. Individuals showing flowers with a number of stamens other than six ranged 66.7 to 100% in population B and 60 to 86.7% in population C (Table 1). In population B, a very broad range of G' values was found and many individuals substantially departed from a G' of 0.5 (Fig. 2). G' ranged 0.501–0.963 in 1988, 0.5–0.807 in 1989, 0.5–0.833 in 1990 and 0.5–1 in 1991 (Fig. 2). In population C, variation in G' was more limited and most individuals showed little departure from the G' value of 0.5 (Fig. 2). G' ranged 0.447–0.514 in 1988, 0.481–0.615 in 1989, 0.499–0.627 in 1990 and 0.472–0.726 in 1991 (Fig. 2). Consequently, there were between-population differences in G' ($P < 0.001$, one-way ANOVA).

Temporal variation in phenotypic gender. There was variability in sexual expression of the plants across different years, especially in population B (Fig. 2). From 14 plants followed for four years at population B, 50% showed a G' different from 0.5 in the four years, while the other 50% showed a G' different from 0.5 in three years. From 20 individuals followed for four years at population C, 50% departed from $G' = 0.5$ during the four years, 25% departed from $G' = 0.5$ during three years, 15% showed a G' different from 0.5 during two years and 5% departed from $G' = 0.5$ in one study year only. Just one individual kept $G' = 0.5$ during the four years. There was no strong correlation between phenotypic gender showed by plants in one year and their gender in the next year. In fact, the Kendall's tau comparing G' between years proved significant only in two year-by-year comparisons (1988 vs 1989 in population B: tau = -0.36 , $P = 0.04$; 1990 vs 1991 in population C: tau = 0.29 , $P = 0.03$), and in both cases significance disappeared after Bonferroni corrections.

We quantified interannual variation in phenotypic gender by means of the CV of G' across years (CV was calculated only for those individuals with 3 or more years of data).

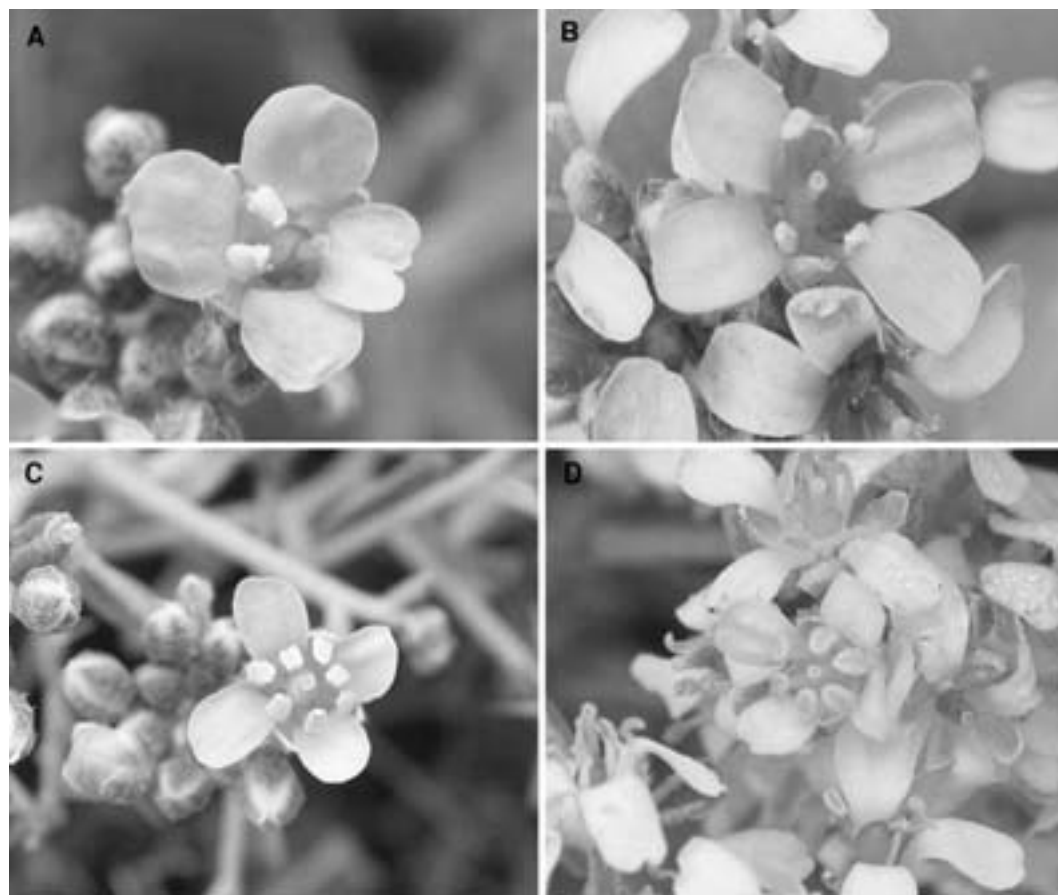


Fig. 1. Flowers of *Hormathophylla spinosa*: A 2 stamens, B 4 stamens, C 7 stamens, D 6 stamens

Distribution of CVs was clearly bimodal (Fig. 3) and allowed to distinguish between individuals of constant gender ($CV < 0.05$, average $CV = 0.016$) from those of variable gender ($CV \geq 0.089$, average $CV = 0.187$). Fifteen individuals (79.0%) in population B and four (14.8%) in population C were considered as variable (Fig. 2). These frequencies were significantly different ($G_1 = 19.473$, $P < 0.0001$).

There was a significant positive correlation between CV and G' both in population B ($r = 0.730$, $P = 0.001$, $N = 19$) and population C ($r = 0.858$, $P < 0.001$, $N = 27$). This means that those individuals being more variable in sexual expression were also those departing most from the expected value of $G' = 0.5$.

Relationship between phenotypic gender and ecological variables. *Correlation with average G' .* The phenotypic gender G' correlated significantly only to flowering synchrony ($r = 0.459$, $P = 0.048$, $N = 19$) and flower size ($r = -0.821$, $P = 0.001$, $N = 19$) and only for population B. No significant correlation was found in population C. In addition, we did not find any apparent functional role of the phenotypic gender, since no relationship was found between G' and the abundance of pollinators, floral herbivores or seed predators in any of the study years and plant populations ($P > 0.1$ in all cases).

Correlation with CV in G' . Labile individuals, those with higher CV in G' , did not differ in size from constant individuals ($P > 0.49$ for both populations). In population B the indi-

Table 1. Variability in stamen number of *Hormathophylla spinosa* at flower and individual levels. Figures are percentage of flowers or individuals per population (B, C) and year (1988–1991) showing the number of stamens indicated in the first column

No. stamens	1988		1989		1990		1991	
	B	C	B	C	B	C	B	C
0	19.7	0.2	9.5	0.8	3.1	1.2	9.4	0.8
1	10.7	0	2.9	0.2	9.8	0.4	5.0	2.3
2	5.3	0	5.2	0.4	3.6	1.1	5.5	1.0
3	5.2	0.2	5.8	2.0	4.2	1.7	3.9	2.0
4	8.8	0.8	5.4	1.6	4.9	3.6	6.4	3.3
5	9.6	3.2	9.5	3.8	3.3	3.1	4.6	3.3
6	40.5	87.8	61.5	86.0	71.0	88.8	65.2	80.7
7	0.2	4.3	0.0	3.9	0.0	0.1	0.0	4.5
8	0.0	3.0	0.0	0.8	0.0	0.0	0.0	1.8
9	0.0	0.5	0.0	0.2	0.0	0.0	0.0	0.3
10	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
No. Flowers	543	599	515	891	548	811	564	891
≠ 6	100	60.0	88.2	83.3	66.7	81.5	94.7	86.7
No. Individuals	19	20	17	30	18	27	19	30

viduals with high CV had shorter ($r = -0.645$, $P = 0.004$) and more synchronic flowering period ($r = 0.768$, $P = 0.0001$), and larger flowers ($r = 0.474$, $P = 0.046$).

Effect of phenotypic gender on female reproductive success. *Per-flower basis.* There was a significant positive correlation between flower size and G' ($r = 0.91$, $P = 0.0001$, $N = 88$ flowers). Furthermore, G' significantly affected fruit production (Table 2). Thus, aborted fruits had on average 2.31 ± 0.41 stamens ($N = 26$ fruits, 10 plants), whereas ripe fruits had 3.18 ± 0.28 stamens ($N = 51$ fruits, 10 plants). By contrast, no effect was found on number of seeds per fruit or on seed length (Table 2).

Per-plant basis. G' affected seed set in a negative way in population B (Table 3), suggesting that plants with higher G' produced fewer seeds per fruit than plants with lower G' . Moreover, CV in G' positively affected both fruit and seed set (Table 3), indicating that plants with higher between-year variability in G' had higher fruit and seed production during the four years of study. No apparent effect of CV of G' was found on seed size (Table 3).

Discussion

Brassicaceae is a family with a constant floral formula (Hedge 1976). Very rarely, four carpels have been documented instead of the usual two (Saunders 1923, Arber 1931, Crone and Lord 1993). Until now, the few departures from the usual pattern of six tetradynamous stamens within this family have involved interspecific variation within a genus (e.g. *Cardamine*, *Coronopus*, *Draba*, *Lepidium*, *Rorippa*, *Teesdalia*; Hewson 1982; see Endress 1992 for a review) while keeping stamen constancy within species. Lee et al. (2002) have related the interspecific changes in stamen number in *Lepidium* to changes in ploidy level. The only cases of intraspecific variation in stamen number we have been able to find are *Iti lacustris*, which has mostly four stamens, rarely six (Garnock-Jones and Johnson 1987), *clavata* mutants of *Arabidopsis thaliana* (e.g. Crone and Lord 1993), and *Megacarpaea polyandra*, with up to 24 stamens (Cheo et al. 2001).

Our results extend this variation and show that *H. spinosa* has substantial intra-

specific variation in stamen number, ranging from 0 to 10 stamens per flower in the Sierra Nevada. As in *clavata* mutants, this is a case of nonhomeotic meristic variation (sensu Levy 1997), because no loss of parts of adjacent whorls or addition of whorls was

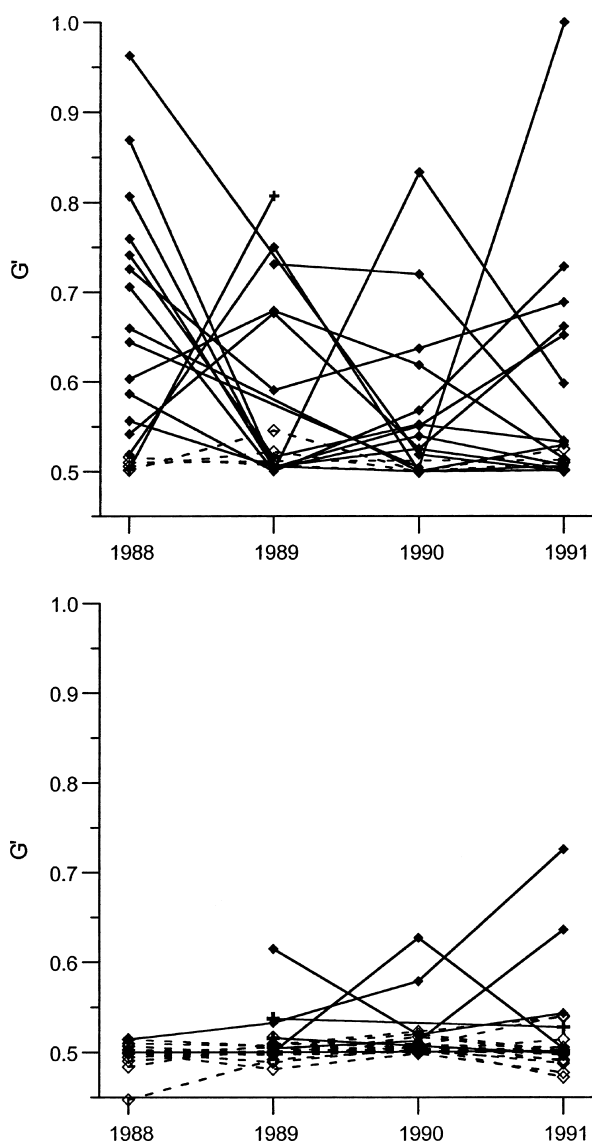


Fig. 2. Temporal variation in G' in populations B (upper panel) and C (lower panel). Continuous line and filled diamonds indicate variable individuals; dashed line and open diamonds indicate constant individuals; continuous line and crosses indicate non-classified individuals (less than 3 years of observations)

involved. By contrast to the case of *clavata* mutants, variability in *H. spinosa* was limited to stamens and no other floral whorl was modified. In addition, this variation cannot be considered as anecdotal; it was present in a high proportion of individuals in the two populations studied (and in 18 additional populations surveyed in 2004; J. M. Gómez and M. Méndez, unpublished data) and reappeared consistently in all study years. Variation limited to the staminal whorl and widespread presence of this variation make it difficult to consider it as mere noise arising from developmental instability and beg the question of its functional meaning.

Variation in stamen number allows *H. spinosa* a broader range of sexual expression than otherwise possible in Brassicaceae. Preliminary pollen counts indicate that pollen production per flower range from 384 in single-staminate flowers to 4555 in six-staminate flowers (J. M. Gómez, unpublished data), with no change in pollen number per anther or pollen viability. Pollen-ovule ratio in flowers thus changes from 96 to 1139 (from facultative autogamy to facultative xenogamy sensu Cruden 1977), obviously influencing the phenotypic gender of the plants and, thus, it has potentially an adaptive value. Variation in stamen number also has other potential influences on male and female fitness, by influencing pollen removal and deposition per visit, as shown by Kudo (2003) for *Brassica rapa*.

H. spinosa inhabits stressful Mediterranean high-mountain environments. Increased selfing could be favoured in such scenario and it agrees with a higher seed set on a per-plant basis with decreasing G' in population B. In other species, loss of stamens (Svensson 1988, Rodríguez-Riaño et al. 1999) or reduced pollen-ovule ratio (Damgaard and Loeschcke 1994) have been related to increased dependence on selfing, as predicted by models of evolution of mating systems (Charlesworth and Charlesworth 1981, Lloyd 1987). This does not seem a likely explana-

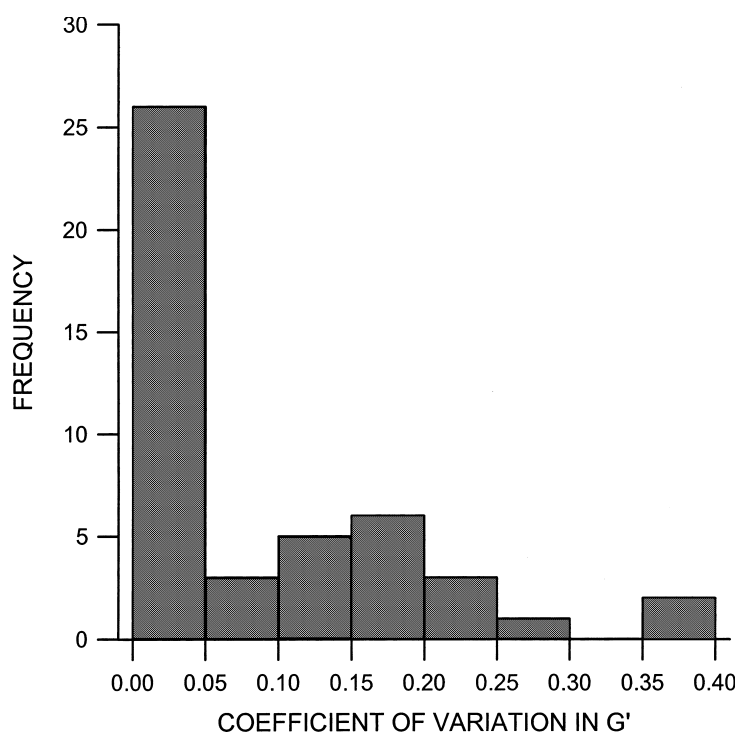


Fig. 3. Frequency distribution of the CV in G'. CV is calculated using G' of the same individual in different years

Table 2. Generalized Linear Models showing the effect of number of stamens on female reproductive success on a per-flower basis

Source of variation	df	Fruit production		Seeds per fruit		Seed length	
		Wald χ^2	P	Wald χ^2	P	t	P
Whole model	4	20.99	0.0003	6.504	0.165	3.888	0.0003
Flower size	2	1.34	0.511	2.560	0.278	-1.234	0.224
No. stamens	2	6.35	0.042	3.786	0.151	-1.302	0.200
Residuals	77/45						

tion in our case, for several reasons. First, *H. spinosa* is not pollen limited in the studied populations (Gómez and Zamora 1996). Second, this hypothesis does not account for the increase in stamen number in the high altitude population C. Third, seed set on a per-flower basis was higher in flowers with higher number of stamens, which is the opposite pattern to the one predicted if

selfing would increase with decreasing stamen number. A more comprehensive assessment of the selfing hypothesis, however, is warranted and should include direct measures of self-pollination in flowers differing in stamen number, as well as inbreeding depression in the resulting seeds.

Another possibility is that variation in stamen number is a way to adjust phenotypic

Table 3. Generalized Linear Model showing the effects of phenotypic gender (G') and interannual variation in G' ($CV_{G'}$) on female reproductive success on a per-plant basis. G' , Fruit set, Seed set and Seed length are average figures for the four study years

	Population B			Population C		
	Coefficient	<i>t</i>	P	Coefficient	<i>t</i>	P
Fruit set						
Plant area	-0.03 ± 0.01	-2.63	0.02	0.01 ± 0.01	0.85	0.41
Flower production	0.04 ± 0.01	2.90	0.01	-0.01 ± 0.01	-2.28	0.04
G'	-169.79 ± 86.06	-1.97	0.07	101.13 ± 182.66	0.55	0.59
$CV_{G'}$	1.21 ± 0.57	2.12	0.05	-0.46 ± 0.97	-0.47	0.64
Seed set						
Plant area	-0.00 ± 0.02	-1.49	0.16	0.00 ± 0.00	0.71	0.49
Flower production	0.00 ± 0.00	1.55	0.15	0.00 ± 0.00	0.41	0.68
G'	-4.35 ± 1.47	-2.96	0.01	1.09 ± 9.70	0.11	0.91
$CV_{G'}$	0.03 ± 0.01	2.67	0.02	0.03 ± 0.05	0.54	0.60
Seed length						
Plant area	0.01 ± 0.01	1.05	0.31	0.00 ± 0.00	0.02	0.98
Flower production	0.00 ± 0.00	-0.82	0.43	-0.00 ± 0.00	-0.81	0.43
G'	20.10 ± 24.61	0.82	0.43	55.00 ± 36.82	1.49	0.16
$CV_{G'}$	-0.07 ± 0.15	-0.46	0.65	-0.09 ± 0.19	-0.46	0.65

gender to fitness perspectives unrelated to selfing, such as resource level, herbivore or pollinator abundance (Freeman et al. 1980, Lloyd and Bawa 1984, Parra-Tabla et al. 2004). Interannual variation in stamen number indicated that this is a plastic trait and, in principle, susceptible to be adjusted according to resources available for reproduction. Adjustment to resource levels faces the difficulty that no correlation was found between G' and plant size, our estimate of resource availability, or to herbivory, an evident source of stress which has been proposed to affect gender expression (Ashman, 2002). G' in a given year was not correlated to fruit set in the previous year, either (results not shown). Therefore, at present it is difficult to assess if interplant and interannual variability in G' corresponded to changes in stress levels, and whether those adjustments were adaptive. Nevertheless, more accurate estimates of resource levels, such as carbohydrate or nutrient levels in plant tissues, could be responsible for interplant and interannual

variation and will require attention in the future.

Temporal variation in G' in the two populations studied indicated the existence of two kinds of individual trajectories: (a) trajectories showing a sexual expression with G' consistently equal or very close to 0.5, and (b) trajectories showing sporadic significant departures from $G' = 0.5$. With the present data it is not possible to know whether those trajectories indicate intrinsic differences or whether, given enough time, each individual in a population would eventually show an episode of significant modification of G' . In either case, slight departure from $G' = 0.5$ could probably be interpreted as mere “noise” with no adaptive function while significant departures from $G' = 0.5$ could reflect the opportunities of high gain in female fitness due to individual circumstances (accumulation of resources, etc.). Interestingly, individuals with a higher CV in G' showed a higher average fitness across the four study years. This could be interpreted as

an advantage in modifying gender expression, compared to a non-flexible sexual expression.

The lack of relationship between G' and female reproductive output can be partially explained because there was no effect of phenotypic gender on pollinator attraction. Most studies reporting variability in gender have also reported that plants allocating more to pollen production usually attract more pollinators, mostly mediated by an increase in flower size or advertisement (*Raphanus sativus*, Stanton and Preston 1988, *Ipomopsis aggregata*, Campbell 1992). In our case, although plants producing more stamens were also those producing larger flowers, we did not find any effect on pollinator visitation rate. *H. spinosa* is pollinated in the Sierra Nevada by a very diverse assemblage of very generalist pollinators (Gómez and Zamora 1999). Many of these pollinators, like ants or small flies, visit the flowers looking for nectar. Thus, any change in pollen production, if not followed by changes in nectar, would be irrelevant for pollinator attraction.

Pollinators could have mediated differential reproductive success on a per-flower basis in line with the results of Kudo (2003). In *Brassica rapa*, removal of long stamens decreased pollen deposition and increased removal of pollen per visit. If applicable to *H. spinosa*, few-stamined flowers would be more efficient in pollen donation and less efficient in pollen receipt, at least per visit. This decreased efficiency in pollen receipt could explain the positive relationship between stamen number and seed set per flower. Applicability of Kudo's results to *H. spinosa* is dependent on the extent to which those results hold with changes in flower size (smaller in *H. spinosa* compared to *B. rapa*) and pollinators (*Bombus occidentalis* in Kudo's experiments; a diverse array of visitors in *H. spinosa*).

Between-population differences in stamen number variation could be related to differences in the local pollinator assemblage. The

main pollinators in population B are nectar-eating ants and flies, whereas in population C flowers are mostly visited by pollen-gathering bees (Gómez and Zamora 1999). Wind is also a pollen dispersal vector in the higher altitude populations of *H. spinosa* (Gómez and Zamora 1996) and this could provide fitness advantage to plants with excess stamens in their flowers. However, although those are plausible hypotheses, broader population sampling will be necessary to test their validity.

In conclusion, we documented an unusual amount of variation in stamen number in *H. spinosa*. This meristic variation was widespread and plastic, suggesting its functional role in the adjustment of phenotypic gender. Lack of correlation between phenotypic gender and plant size, pollinator or herbivore abundance, as well as inconsistent effects on female reproductive success on a per-flower basis vs. on a per-plant basis, rendered adaptive explanations in terms of selfing or resource adjustment unsatisfactory with the data available. However, averaged across four study years, individuals showing more variation in phenotypic gender had higher female reproductive success than those with a constant phenotypic gender, suggesting an advantage for labile sexual expression. Although our results do not easily conform to any adaptive explanation, this remarkable example of breakdown of trait canalization offers the possibility of exploring an undeveloped field in evolutionary biology, i.e. the mechanisms and ultimate causes responsible for the maintenance of fixed characteristics (cf. Conner and Agrawal 2005, for cotyledon number). Detailed studies of resource economy, inbreeding depression and male reproductive success could help to clarify the fitness consequences of meristic variation in stamen number.

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