

Variation in Sexual Expression in the Monoecious Shrub *Buxus balearica* at Different Scales

A. Lázaro^{1,2} and M. Méndez^{3,4}

¹ Institut Mediterrani d'Estudis Avançats (C.S.I.C.-U.I.B.), c/Miquel Marqués 21, 07190 Esporles, Mallorca, Spain

² Present address: Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway

³ Botaniska Institutionen, Stockholm Universitet, 106 91 Stockholm, Sweden

⁴ Present address: Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, c/Tulipán s/n., 28933 Móstoles, Madrid, Spain

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Abstract: Monoecy allows high plasticity in gender expression because the production of separate female and male flowers increases the ability to respond to specific environmental circumstances. We studied variation in sexual expression and its correlates in the monoecious shrub *Buxus balearica*, for two years, in six populations in the Balearic Islands and four in the Iberian Peninsula. Phenotypic gender varied among populations; while island populations showed slight variations around an average gender, mainland populations showed a broad range of variation in gender among individuals, always biased towards increasing maleness compared with the other populations. Within populations, gender was not related to plant size. Between-year changes were slight and mainly consisted of an increase in relative maleness in the last year. Reproduction did not affect gender in the next year, as assessed by either observational or experimental methods. Most variation in gender expression occurred among individuals within populations (83.6%), followed by variation among populations (13.6%) and years (2.8%). Our results suggest that male-biased gender at population and plant levels was related to stressful conditions and resource limitation, because: (1) maleness was higher in mainland populations, where summer drought was stronger; (2) maleness increased with elevation; (3) fruit set was positively correlated with femaleness; (4) the percentage of male inflorescences increased over the flowering period; and (5) male inflorescences were preferentially in lower parts of the branch nodes. Higher maleness in last years, however, could be related to increased male success under synchronic flowering.

Key words: Balearic Islands, Iberian Peninsula, monoecy, phenotypic gender, plant size, reproductive costs, spatio-temporal variation, stress.

Introduction

Monoecy is one of the most common non-hermaphroditic breeding systems, occurring in approximately 5% of angiosperm species (Richards, 1997). The hormonal mechanism re-

sponsible for monoecy (Yin and Quinn, 1992) provides plants with the ability to modify gender expression in response to environmental and developmental (architecture, age; e.g., Freeman et al., 1981; Dorken and Barrett, 2003) influences, representing a potentially important selective advantage in the evolution of this breeding system (Richards, 1997). Variability in sexual expression in monoecious species has been found in every monoecious species studied (e.g., Freeman et al., 1981, 1984; Ackerly and Jasienski, 1990; Costich, 1995) and is considered to be adaptive, in the sense of producing phenotypes which optimise resource allocation to male and female functions according to plant resource levels or external conditions (Charnov, 1982; Klinkhamer et al., 1997).

The study of gender variation in monoecious plants has mainly focused on among-individual variation within populations (Freeman et al., 1981; Ackerly and Jasienski, 1990; Parra-Tabla et al., 2004). Among-individual variation has been found to be related to environmental factors such as soil water (Freeman et al., 1981; McKone and Tonkyn, 1986), nutrients (Ackerly and Jasienski, 1990), microtopography (Aizen and Kenigsten, 1990), density (Smith, 1981) and herbivory (Parra-Tabla et al., 2004). Among-individual variation has also been considered under the "size advantage model" (Klinkhamer et al., 1997) or its derivation, the "height advantage model" (Fox, 1993). In both models, increases in size (or height) are predicted to entail a bias towards the sexual function which benefits most from that increase. Usually, female function is favoured, with increases in size, because bigger size means more resources and seed production is more costly than pollen production (Delph, 1999). Increases in height favour male function in anemophilous plants, because pollen dispersal is disproportionately increased with plant height (Bickel and Freeman, 1993). The predictions of these models have often been tested in monoecious taxa (Burd and Allen, 1988; Allison, 1991; Jordano, 1991; Bickel and Freeman, 1993; Fox, 1993; Arista and Talavera, 1997; Méndez, 1998; Dorken and Barrett, 2003).

However, among-individual variation within populations is not the only interesting scale of gender variation. First, gender comparisons among populations, especially those at a large spatial scale, may be revealing since they encompass broader environmental gradients than comparisons within populations. Empirical studies with monoecious species have infrequently focused on the comparison of sexual expression among populations (but see e.g., Allison, 1991; Costich, 1995;

Arista and Talavera, 1997). A maleness increase with altitude (Jordano, 1991) and greater gender differentiation (always towards maleness) in drier sites (Costich, 1995), have been some of the geographical patterns found, and have been attributed to increased maleness under limited resource availability, since pollen production is less costly than seed production (Delph, 1999). Second, little emphasis has been given to within-individual (i.e., plastic) variation. Some studies have shown seasonal gender modification (Emms, 1993; Méndez, 1998) that could be related to resource levels (Lloyd, 1980a) or to changing mating opportunities derived from seasonal changes in the population floral gender ratio (Brunet and Charlesworth, 1995). Other studies have reported between-year variation in gender, mostly drastic gender modifications (male to monoecious: Arista and Talavera, 1997; sex choice: Clay, 1993). More information on between-year variation in gender expression and its correlates (Delesalle, 1989) is needed. In particular, a better understanding of between-year variation in the gender of masting species (i.e., those species alternating years of high fruit production with others of very low or almost no production) is especially interesting. The reason is that evolutionary explanations of masting involve between-year variation in resources or in pollen limitation (see Kelly, 1994 for details) which could differentially affect female success and optimal allocation to female function.

The present study addresses variation in gender expression at the three above-mentioned levels (among populations, among individuals within a population, and within individuals) in the monoecious, ambophilous (i.e., pollinated by wind and insects), masting, woody shrub *Buxus balearica*. We also investigate the potential environmental and reproductive causes for these gender variations. First, we describe among-population variability in gender and its relation to environmental conditions (elevation, mean precipitation). Second, we study within-population among-individual variability in gender in relation to plant variables (plant size, fruit set). Third, we describe within-individual variability in gender in relation to the position of inflorescences on the plant, as well as seasonal and between-year changes in gender. Fourth, we investigate the variance partitioning of gender expression among the above-mentioned levels.

Materials and Methods

Study species

Buxus balearica (Buxaceae) is a woody shrub up to 6 m tall, although under harsh growing conditions it adopts a creeping habit (mean \pm SD: 1.0 \pm 0.65 m). It is a tertiary endemic species of the Mediterranean Basin, distributed from Turkey to the Iberian Peninsula. It grows on limestone soil, often on rocks, under subhumid conditions, between sea level and 1000 m (Benedí, 1997).

B. balearica is monoecious and its inflorescences usually consist of one central female flower often surrounded by four male flowers. However, in some cases, the central female flower is lacking, the inflorescences thus becoming male, whereas in others, the male flowers are lacking, the inflorescences thus becoming female. Inflorescences are axillary and usually in pairs at branch nodes. Inflorescences last for a week and were considered to be protogynous (von Balthazar and Endress,

2002), although we found both protogynous and protandrous inflorescences on the same plants in all studied populations; the percentage of protogynous inflorescences varying between 40% and 95% at the beginning of the flowering period and decreasing throughout the season (Lázaro and Méndez, unpublished data). This species is self-compatible and shows inbreeding depression (Lázaro and Traveset, 2006). Although wind is the main pollen vector, ambophily has recently been reported; the importance of entomophily strongly varying among populations (Lázaro and Traveset, 2005). It is a masting species, alternating years of high and very low fruit production (Lázaro et al., 2006).

Study sites

Ten Spanish sites were included in the present study, four in the south of the Iberian Peninsula and six in Mallorca, in the Balearic Islands (Table 1). Two subpopulations were considered at Rágol (I and II) in the Iberian Peninsula, with differences in elevation, fragmentation and plant physiognomy; and at Sant Vicenç in Mallorca, with differences in exposure to sea wind (the lowest population [I], which was more protected than the highest population [II]). At all 10 sites, vegetation consisted of Mediterranean shrubs and a variable number of *Pinus halepensis* trees. These study sites were selected to encompass the range of elevation (0 to 900 m) and rainfall variability (258 to 1264 mm/year) in which the study species occurs (Table 1). In order to estimate hydric stress, we measured the relative water content (RWC) in leaves of 10 individuals in each of five populations (Cerro Gordo and Rágol I, Sant Vicenç I, Lluc and Galatzó), once per season, from spring 2002 to summer 2003, following the procedures in Koide et al. (1989). The average values for the whole year and for summer in these populations are shown in Table 1. Higher values of RWC indicate lower hydric stress.

Phenotypic gender

In spring 2003, during the flowering peak, we randomly labelled 10–45 flowering individuals per population. In each individual, 70–130 inflorescences distributed throughout the canopy were scored for gender (male, female or cosexual) and number of male flowers (for cosexual and male inflorescences). The same individuals were scored again in 2004. While 2003 was a masting year, flowering was much reduced in 2004, therefore, additional individuals were scored in some populations in 2004 to increase sample size.

Phenotypic gender is the relative investment of an individual in male vs. female function (Lloyd, 1980b). We used the ratio between female and total flowers produced per individual as an estimate of its phenotypic gender (%F hereafter), as also previously used for other monoecious species (e.g., Delesalle, 1989). We preferred this estimate of phenotypic gender to the often utilised standardised “phenotypic femaleness” (G; Lloyd and Bawa, 1984) for two related reasons. First, in multi-year studies, no consensus seems to exist on whether a single correction term E should be calculated for all the years or whether separate E values should be calculated for each year. Calculation of separate E values for different populations and years, as performed by Clay (1993), does not allow a direct comparison of phenotypic gender between samples as required in our study. Second, the alternative of calculating a common E value

Table 1 Name, location, habitat, elevation (m) and rainfall (mean annual precipitation in mm, using data for the last 10 years from the nearest meteorological stations) of the 10 studied sites of *Buxus balearica*. RWC: Mean and standard deviation of relative water content in leaves of *B. balearica* (year: whole year average; summer: summer average)

Site	Area	Location	Habitat	UTM	Elevation	Rainfall	RWC Year	Summer
Cerro Gordo	Iberian Peninsula	Coast of Granada	creek	30SVF3068	0	460.52	81.8 ± 19.2	57.1 ± 3.3
Frigiliana	Iberian Peninsula	Almijara Mountains, Málaga	wash	30SVF2170	300	462.35		
Rágol I	Iberian Peninsula	Gádor Mountains, Almería	canyon	30SWF2792	440	258.24	83.6 ± 13.6	67.1 ± 10.0
Rágol II	Iberian Peninsula	Gádor Mountains, Almería	canyon	30SWF2793	720	328.71		
Andratx	Balearic Islands	Tramuntana Mountains, south-facing cliff, Mallorca	hollow	31SDD4875	5	443.55		
Sant Vicenç I	Balearic Islands	Tramuntana Mountains, north-facing cliff, Mallorca	limestone cliff	31SEE0420	40	318.13	81.8 ± 19.2	84.4 ± 9.8
Sant Vicenç II	Balearic Islands	Tramuntana Mountains, north-facing cliff, Mallorca	rocky plateau	31SEE0420	60	318.13		
Lluc	Balearic Islands	Tramuntana Mountains, Mallorca	Aleppo pine forest	31SDE9106	320	1 253.65	87.4 ± 5.9	85.3 ± 5.9
Cúber	Balearic Islands	Tramuntana Mountains, Mallorca	limestone cliffs	31SDE8202	660	1 079.00		
Galatzó	Balearic Islands	Tramuntana Mountains, Mallorca	limestone cliffs	31SDD5687	900	746.05	90.1 ± 2.3	89.4 ± 0.5

for all populations and years could introduce autocorrelation in estimates of G values in different years and problems in statistical analyses when comparing those G values.

Among-population variability in gender

In order to study the variability in gender among populations, we compared the frequency distribution of %F across populations in each year. In addition, we explored the relationship of population elevation and mean rainfall (last 10 years) with average %F₂₀₀₃ or coefficient of variation (CV) of %F₂₀₀₃. We limited our analyses to 2003 data because this was a mast year with profuse flower production. In 2004, flowering, flower production and, consequently, sample size were much reduced.

Among-individual variability in gender

Within each population, we explored the relationships between individual %F₂₀₀₃ and plant size, flower and fruit production. Plant size was estimated in two ways, as individual height and as volume. Volume was estimated as that of a hemispheroid using the formula: $Vol = 2/3 \pi (h \cdot D/2 \cdot d/2)$, where h is height, and D and d are the longest and shortest canopy diameters, respectively, all measured to the nearest cm. Total flower and fruit production were estimated by counting the number of inflorescences or fruits in a sector of the canopy and extrapolating to the whole canopy. Fruit set was calculated as the percentage of fruits with respect to cosexual plus female inflorescences.

Within-individual variability in gender

Position effect

In spring 2003, we assessed whether inflorescences differing in gender were segregated along the branches. At three sites (Rágol I, Sant Vicenç I and Sant Vicenç II), we randomly chose 10–20 individuals per population and 10 branches per individual, and recorded the gender and position of each inflorescence on the branch. The gender of the inflorescences was scored for each pair of inflorescences at each node, from the most basal to the most apical. Branches differed in the number of nodes, with up to seven nodes per branch. Position effects were separately studied for branches having a different number of nodes.

Seasonal variation in gender

In spring 2003, we studied variation in gender of inflorescences among those flowering early, at blooming peak and late in the flowering season. We labelled 3–5 branches (30 to 60 inflorescences) from 10–20 individuals at six sites (Cerro Gordo, Rágol I, Sant Vicenç I, Sant Vicenç II, Lluc and Galatzó) and monitored them weekly, from the beginning to the end of the flowering period. At each recording date, we noted the gender (i.e., male, cosexual or female) of any newly-open inflorescence.

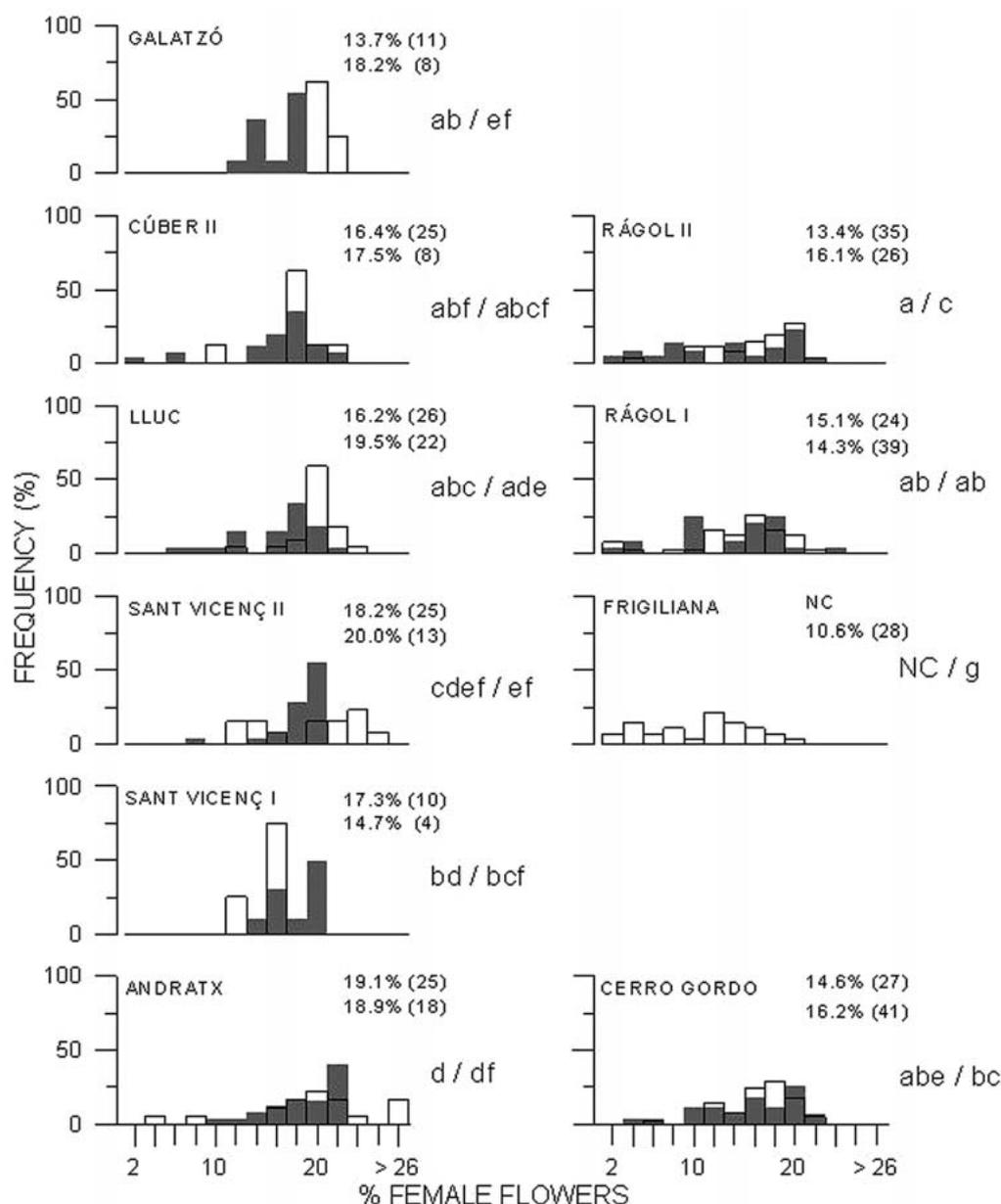


Fig. 1 Frequency distribution of %F values in different Balearic (left column) and S Iberian (right column) populations of *Buxus balearica*. Within each column, populations have been arranged from bottom to top by increasing elevation. 2003: gray; 2004: white. Figures on the upper right corner of each plot indicate median values of %F per population and year (number of individuals in brackets). Upper value is for 2003 and lower one for 2004. Letters to the right side of each histogram indicate significant differences according to the a posteriori test (2003/2004); populations sharing letters were not significantly different in their distribution of %F. NC: not calculated.

Between-year variation in gender

We studied whether there was significant between-year individual variation in gender. In addition, in order to ascertain the factors influencing between-year changes in %F, we regressed %F₂₀₀₄ against %F₂₀₀₃, fruit set in 2003 and inflorescence production in 2003. A similar analysis using %F₂₀₀₄ - %F₂₀₀₃ as the dependent variable yielded identical conclusions and is not reported here.

In a masting species, a high investment in reproduction in a given year could deplete resources for subsequent reproductive events. In order to test experimentally if previous reproduction affected sexual expression in the following year, we carried out an inflorescence bud removal experiment in three populations: Sant Vicenç I, Sant Vicenç II and Cúber. In spring 2003, two branches were labelled on 15 individuals per popu-

lation. One branch was left untouched whereas all inflorescence buds on the other one were removed. Inflorescence gender in the control branch was recorded as explained above (see "Phenotypic gender"). In spring 2004, we recorded the gender of the inflorescences in both control and treatment branches.

Statistical analyses

We used generalised linear modelling to compare the frequency distribution of %F across populations and years (Fig. 1), using a multinomial ordinal distribution (an extension of a binomial distribution to include more than two classes) with cumulative logit as a link function. For this analysis, the %F data were grouped in 14 categories, with one category for every 2% of increase in %F, from 0% to 26%, while the last category included all those individuals with %F higher than 26%.

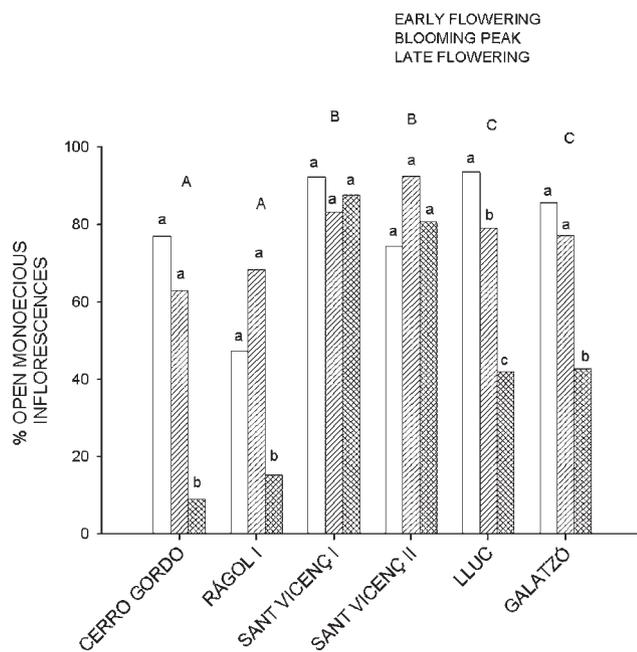


Fig. 2 Seasonal variation in the percentage of cosexual inflorescences in each population. Three flowering times were considered: early, peak and late blooming. Different uppercase letters indicate significant differences among populations; different lowercase letters indicate significant differences among flowering times within each population.

Multiple regressions were conducted by entering all independent variables at once. Correlations between individual %F and plant size, flower and fruit production were assessed using Spearman correlations.

Seasonal variations in inflorescence gender (Fig. 2) were tested by means of a generalised linear model (GLIM) with a binomial distribution and logit link function. Position effects on inflorescence gender were tested by means of G tests, using the Williams correction when expected frequencies were < 5 (Sokal and Rohlf, 1995).

All other statistical analyses involved repeated measures models in which time (year) or bud removal were considered as fixed treatments (within-subject factor) and individual plant was considered as a random factor (subject). When population was considered in the analysis, it was included as a fixed factor. We used the error distributions and link functions that fitted the observed data best (based on the whole model deviations): 1) gamma distribution and log link function, using inflorescence number as a covariate, for the comparison of %F across populations and years; and 2) gamma distribution and log link function, for the analyses with the flower bud removal experiment (only branches that flowered in 2004 were considered). In these analyses, as the population effect was not significant in a preliminary analysis, all populations were pooled. Inflorescence number per branch was used as covariate.

We evaluated gender variation at different levels by means of general linear modelling (GLM), considering year and population (nested within year) as the main factors. Deviance quotients provided the percentage of relative variance accounted for by each level, with error deviance being considered as

Table 2 Results for multiple regression analyses relating (a) mean population phenotypic gender (%F) and (b) CV of %F for each population to population elevation and mean annual rainfall (see Table 1)

Variable	B	SE	t_6	p
a) Mean %F				
Elevation	-4.6×10^{-5}	1.6×10^{-5}	-2.881	0.028
Rainfall	1.4×10^{-5}	1.5×10^{-5}	0.946	0.380
Intercept	0.159	0.010	15.915	<0.0001
Whole model: $R^2 = 0.762$, $n = 9$, $F_{2,6} = 4.15$, $p = 0.074$				
b) CV %F				
Elevation	17.4×10^{-5}	15.5×10^{-5}	1.127	0.303
Rainfall	-9.6×10^{-5}	14.7×10^{-5}	-0.650	0.540
Intercept	0.270	0.096	2.808	0.031
Whole model: $R^2 = 0.428$, $n = 9$, $F_{2,6} = 0.67$, $p = 0.544$				

among-individual within-population variance (García et al., 2005).

Repeated measures analyses and variance components analysis were performed using the GENMOD procedure of SAS 9.0 and posteriori tests after these analyses were corrected by sequential Bonferroni tests (Rice, 1989). All other statistical analyses were performed with STATISTICA 6.0 and SPSS 4.0. Except where otherwise indicated, all results are shown as mean \pm SD.

Results

Among-population variability in gender

The frequency of individuals belonging to different %F classes differed among populations in both years (2002: $\chi^2_8 = 38.99$, $p < 0.0001$ and 2003: $\chi^2_9 = 88.70$, $p < 0.0001$). Some populations showed a wide variation in %F, particularly those of the Iberian Peninsula (Fig. 1).

Mean population %F was negatively correlated with elevation (Table 2a), whereas it was not correlated with mean rainfall (Table 2a). The coefficient of variation (SD/mean) of %F was not correlated either with elevation or mean rainfall (Table 2b).

Among-individual variability in gender

%F was positively correlated with fruit set, when considering all populations together ($n = 194$, $r_s = 0.261$, $p = 0.0002$). However, when considering each population separately, we only found this relationship to be significant in Rágo II ($n = 35$, $r_s = 0.45$, $p = 0.006$) and Sant Vicenç I ($n = 10$, $r_s = 0.63$, $p = 0.048$). Inflorescence number was not related to %F except in Sant Vicenç II, where the relationship was significantly negative ($n = 21$, $r_s = -0.51$, $p = 0.018$). Neither volume nor plant height were related to %F, with the exception of plant height at Cúber ($n = 25$, $r_s = -0.55$, $p = 0.005$) and volume at Sant Vicenç II ($n = 25$, $r_s = -0.46$, $p = 0.021$).

Within-individual variability in gender

Position effect

At Ràgol I, sampled branches had 2–7 nodes. Male inflorescences were predominantly located at basal positions in branches having 3–6 nodes (G test: all $p < 0.027$). The trend was marginally significant for branches with two nodes ($p = 0.078$) and it was not significant for branches with 7 nodes. At Sant Vicenç II, sampled branches had 2–4 nodes. Male inflorescences were predominantly basal only for branches with 3 nodes ($p = 0.016$). At Sant Vicenç I, where sampled branches had 2–5 nodes, no position effect was found.

Seasonal variation in gender

Percentage of cosexual inflorescences decreased significantly over the flowering period ($\chi^2_2 = 46.67$, $p < 0.0001$), except in the two subpopulations of Sant Vicenç (interaction between inflorescence gender and flowering period: $\chi^2_{10} = 60.09$, $p < 0.0001$) where no seasonal change occurred (Fig. 2). The percentage of cosexual inflorescences was lower in Ràgol I than in the other populations ($\chi^2_5 = 94.57$, $p < 0.0001$; Fig. 2).

Between-year variation in gender

Individuals became more female-biased in the second year (non-masting year) consistently among populations (Table 3). Inflorescence production was negatively related to %F (Table 3). Despite the statistical significance of between-year variation in gender, the individual between-year change in %F was limited. For most individuals (84.6%), the between-year change in the percentage of female flowers was equal to or lower than 7%, which is the SD of %F from pooling all populations. Only 11 individuals (8.4%) showed changes between 7% and 10%. Only two individuals (1.5%) showed changes in %F higher than +20% (ca. 40% and ca. 80%, respectively). These two individuals belonged to the Andratx population and produced fewer than 10 (8 and 1) flowers in 2004.

%F₂₀₀₄ was positively related to %F₂₀₀₃ (Table 4), but not to plant volume, flower production in 2003 or fruit set in 2003, when we considered all populations together. The same trend was found in the analyses for each population (results not shown), although the positive relationship between %F₂₀₀₄ and %F₂₀₀₃ was only significant for Cerro Gordo ($p = 0.0008$) and marginally significant for Ràgol I ($p = 0.059$). In addition, a significant negative relationship between fruit set in 2003 and %F₂₀₀₄ appeared both in Cerro Gordo ($p = 0.047$) and Cúber ($p = 0.027$).

In 2004, only 12–50% of the labelled branches in the bud removal experiment flowered, depending upon the population. In the analysis for control branches, %F decreased marginally in the second year (0.55 ± 0.03 vs. 0.51 ± 0.16 in 2003 and 2004 respectively; $\chi^2 = 2.76$, $p = 0.097$) and the number of inflorescences per branch decreased significantly with %F ($\chi^2 = 5.02$, $p = 0.025$). In the analysis of the effects of bud removal on %F, neither treatment ($\chi^2 = 0.10$, $p = 0.752$) nor the number of inflorescences per branch ($\chi^2 = 2.00$, $p = 0.158$) had a significant effect.

Table 3 Results from the generalised linear model comparing %F across populations and years. Inflorescence production was included in the model as a covariate

Variable	χ^2	df	p
Year	5.23	1	0.022
Population	28.11	8	0.0005
Year \times population	10.38	6	0.109
Inflorescence production	5.05	1	0.025

Table 4 Results from the multiple regression analysis relating phenotypic gender in 2004 (%F₂₀₀₄) to number of inflorescences in 2003, fruit set in 2003, plant volume and phenotypic gender in 2003 (%F₂₀₀₃), pooling for all the populations

Variable	B	SE	t_{121}	p
Inflorescences 2003	$0.1 \cdot 10^{-5}$	$0.2 \cdot 10^{-5}$	0.488	0.626
Fruit set 2003	$0.6 \cdot 10^{-5}$	$40.5 \cdot 10^{-5}$	0.015	0.988
Plant volume	$-13.7 \cdot 10^{-5}$	$22.3 \cdot 10^{-5}$	-0.614	0.541
%F ₂₀₀₃	0.606	0.173	3.499	0.0007
Intercept	0.086	0.027	3.143	0.002

Whole model: $R^2 = 0.099$, $n = 126$, $F_{4,121} = 3.32$, $p = 0.013$

Variance partitioning in gender expression

The percentage of variance in gender expression explained by populations was 13.6% ($F_{12,216} = 2.79$, $p = 0.002$), whereas year explained a lower percentage of variance (2.8%; $F_{1,216} = 8.53$, $p = 0.004$). Most variation, however, occurred among individuals within populations (83.6%).

Discussion

Monoecy is considered to be associated with high plasticity in gender expression (Richards, 1997) because the production of separate female and male flowers provides higher ability to respond to specific environmental circumstances (Fox, 1993; Renner and Ricklefs, 1995). Variability in gender expression has been found in every monoecious species studied (e.g., Freeman et al., 1981, 1984; Ackerly and Jasienski, 1990; Costich, 1995; this study). In *B. balearica*, we found significant gender variation at every scale studied: among-populations, among-individuals within populations and within individuals (including position effects, as well as seasonal and between-year changes). Nevertheless, most variation in gender expression occurred among individuals within populations (83.6%). Below, we discuss variability at each studied level.

Among-population variability in gender in *Buxus balearica*

We found a broad range of variation in gender in a few populations, and this was always biased towards increasing maleness compared with the other populations. Gender distributions were mainly unimodal, and “flat” distribution frequencies with the presence of pure male or almost pure male individuals were only occasionally found.

A host of environmental factors has been shown to induce a modification in gender (Freeman et al., 1980). In many plants, favourable environmental conditions promote femaleness,

while environmental stress increases maleness (Freeman et al., 1980). This has been found for, among other factors, xeric conditions (Freeman et al., 1981, 1984; Costich, 1995; El-Keblawy et al., 1995). We suggest that the higher maleness found for *B. balearica* in the Iberian Peninsula populations and the increase in maleness with elevation could be related to increasingly harsh environmental conditions. Iberian populations have lower relative leaf water content in summer than Balearic populations and greater gender differentiation (always towards maleness). This result agrees with that reported by Costich (1995) for populations of monoecious plants of *Ecballium*, where greater gender differentiation (always towards maleness) was found in drier sites, reflecting gender allocation “decisions” enforced by limited resource availability. Our results for *B. balearica*, showing increased maleness at increasing elevations, agree with those of Jordano (1991) for another monoecious woody species in southern Spain, and could be related to season length and temperature decrease with elevation in mountain areas (Körner, 1999).

Among-individual variability in gender

The extent of among-individual gender variation in *B. balearica* was lower than that found in other woody, wind-pollinated monoecious species (Smith, 1981; Allison, 1991; Jordano, 1991; Sato, 2002), where a notable number of “pure males” and “pure” females were also found in the same population. Nevertheless, we found a broad range of gender variation in some populations of *B. balearica*, although we could not relate such variation to the variables measured. We did not find any support for the “height advantage” hypothesis (Fox, 1993), which predicts an increase in male allocation with increasing height for wind-pollinated plants, as pollen dispersal increases with plant height. An increase in male allocation with increasing height has been found for some wind-pollinated monoecious plants (Fox, 1993; Arista and Talavera, 1997) but not in others (Allison, 1991 – only cosexual individuals considered; Jordano, 1991; Fox, 1993). In *B. balearica*, we only found such a relationship between plant height and increasing maleness in one of ten populations. A possible reason for the lack of fit to the hypothesis is the proposal of Bickel and Freeman (1993) that this prediction is dependent on plant geometry and only applies to cylindrical growth forms. This is consistent with the observation that *B. balearica* individuals in most populations were rounded in shape. On the other hand, we similarly failed to find any evidence for the “size-advantage hypothesis” (Klinkhamer et al., 1997). Volume was also poorly related to femaleness in our dataset and the only significant relationship was contrary to the predictions of Klinkhamer et al. (1997).

We found no relationship between flower number and phenotypic gender, though there was a positive correlation between fruit set and femaleness. Similar relationships have been found in other monoecious species (Goldman, 1991; Jordano, 1991). Although this correlation could indicate a link between femaleness and plant vigour, lower fruit set with increasing maleness could also be due, at least partially, to inbreeding depression because a low female to male flower ratio may increase geitonogamy (see Jordano, 1991, for a similar argument).

Within-individual variability in gender

We found several sources of within-individual variability in gender in the study species. First, we found a position effect, that is, male inflorescences were located preferentially in the basal parts of the branches, in contrast with position effects described in many hermaphroditic species, for which an increase in male allocation occurs in apical positions (Diggle, 1995). We attribute this to apical dominance, as blooming order proceeded basipetally in the branches. A “reversed” position effect associated with basipetal flowering has been described in other monoecious species (e.g., Kawakita and Kato, 2002). Second, we observed an increase in male inflorescences over the flowering season (Fig. 2), contributing to an increase in maleness at the end of the season. Increasing maleness over the flowering season has been reported by other authors (Ashman and Baker, 1992; Emms, 1993; El-Keblawy et al., 1995) and has been attributed to seasonal resource depletion (sequential resource adjustment *sensu* Lloyd, 1980a).

All the above-mentioned changes suggest that resource depletion induced maleness in *B. balearica*. This makes it difficult to interpret the between-year gender changes, mainly towards an increase in relative femaleness in the non-mast year. Masting hypotheses related to resource depletion (Kelly, 1994) are not consistent with such a gender change. One interesting possibility is that masting could affect the balance between local mate competition (LCM) and local resource competition (LRC) (Lloyd and Bawa, 1984) in favour of male allocation. Mechanisms behind such modification could be related to the relative importance of the “pollination efficiency” and the “satiation of predator” hypotheses, which would influence LCM and LRC, respectively. The extent to which this is valid for *B. balearica* is, however, unknown because the available evidence (Lázaro et al., 2006) does not give strong support either for pollination or seed predation advantages of masting.

Individual changes in phenotypic gender between years were modest (less or equal to 7% in most individuals). These findings are consistent with those reported for other woody species (Allison, 1991; Jordano, 1991; López-Almansa et al., 2003). The few cases of stronger individual between-year changes in gender were not related to any particular phenotypic gender. By contrast, stronger changes in gender have been documented for female-biased individuals by Freeman et al. (1984) and El-Keblawy et al. (1995).

Prior fruit production is known to affect subsequent sexual expression in monoecious species, either in the same (Delesalle and Mooreside, 1995) or in the next season (Delesalle, 1989). We found very limited evidence for such an influence in *B. balearica*. First, %F₂₀₀₃ was the main variable significantly influencing %F₂₀₀₄, although prior fruit production significantly and negatively influenced %F₂₀₀₄ in Cerro Gordo and Cúber. Second, we found no influence of plant size on gender change, a pattern that has been found in other species (Allison, 1991). Other studies have also shown that plant size may have no effect on gender allocation (e.g., Elle, 1999). Third, flower bud removal did not affect gender expression or flower bud initiation in the next year. However, the lack of an effect of bud removal on gender expression could arise because bud removal at the branch level may not affect resource availability (and thus flower production) at the individual level, or could be related

to the assumption in the experimental design that different branches would act as independent physiological units (Watson and Casper, 1984). Possible resource sharing between branches would have diluted the differences between treatments.

Conclusions

Phenotypic gender in *B. balearica* varied substantially among populations, and the highest departures from modal gender values were always towards maleness. Most variation in phenotypic gender occurred among individuals within populations (83.6%), followed by variation among populations (13.6%). Overall, patterns of gender variation among populations and elevations, as well as seasonal and architectural gender modifications within individuals, suggest that male-biased gender was related to stressful conditions and low resource availability for reproduction. In contrast, gender variation among individuals of the same populations could not be related to the variables considered. Finally, the increased relative maleness in the masting year could be related to better reproductive opportunities under synchronic flowering. Studies of between-year variation in sexual expression in masting species are needed in order to further explore this hypothesis.

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A. Lázaro

Department of Ecology and Natural Resource Management
Norwegian University of Life Sciences
P.O. Box 5003
1432 Ås
Norway
E-mail: amparo.lazaro@umb.no

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