

RESEARCH PAPER

Influence of inflorescence size on sexual expression and female reproductive success in a monoecious species

R. Torices^{1,2} & M. Méndez¹¹ Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles (Madrid), Spain² Present address: Área de Botánica, Departamento de Ciencias Agroforestales, Universidad de Valladolid, Soria, Spain**Keywords**

Allocation currency; allometry; inflorescence size; monoecy; sexual allocation; sexual expression; *Tussilago farfara*.

Correspondence

R. Torices, Área de Botánica. Departamento de Ciencias Agroforestales, Universidad de Valladolid, Campus de los Pajaritos s/n., E-42002 Soria, Spain.
E-mail: rubentorices@gmail.com

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ABSTRACT

Sex allocation theory forecasts that larger plant size may modify the balance in fitness gain in both genders, leading to uneven optimal male and female allocation. This reasoning can be applied to flowers and inflorescences, because the increase in flower or inflorescence size can differentially benefit different gender functions, and thus favour preferential allocation to specific floral structures. We investigated how inflorescence size influenced sexual expression and female reproductive success in the monoecious *Tussilago farfara*, by measuring patterns of biomass, and N and P allocation. Inflorescences of *T. farfara* showed broad variation in sex expression and, according to expectations, allocation to different sexual structures showed an allometric pattern. Unexpectedly, two studied populations had a contrasting pattern of sex allocation with an increase in inflorescence size. In a shaded site, larger inflorescences were female-biased and had disproportionately more allocation to attraction structures; while in an open site, larger inflorescences were male-biased. Female reproductive success was higher in larger, showier inflorescences. Surprisingly, male flowers positively influenced female reproductive success. These allometric patterns were not easily interpretable as a result of pollen limitation when naïvely assuming an unequivocal relationship between structure and function for the inflorescence structures. In this and other Asteraceae, where inflorescences are the pollination unit, both male and female flowers can play a role in pollinator attraction.

INTRODUCTION

For out-crossing co-sexual plants, sex allocation theory forecasts that allocation of resources to male and female functions should be equal if the fitness returns through both functions are also equal (Charnov 1982). However, some circumstances may modify the balance in fitness gain through both genders, leading to uneven optimal allocations (Freeman *et al.* 1981; Brunet 1992). For example, the theory of sex allocation predicts that in favourable conditions – with larger size or with more resources – animal-pollinated plants should allocate more resources to the female than to the male function (the size advantage hypothesis) (Freeman *et al.* 1981; Charnov 1982; Lloyd & Bawa 1984). In wind-pollinated plants, maleness is expected to increase with increasing plant size (Bickel & Freeman 1993).

This reasoning has recently been applied to flowers (Méndez & Traveset 2003) or to inflorescences (Méndez 2001) for plants producing one or a few flowers/inflorescences. If a plant obtains additional resources, which allow it to produce a bigger flower/inflorescence, should it simply scale up the flower or the inflorescence, or should it modify its allocation to different floral or inflorescence parts? Méndez (2001) and Méndez & Traveset (2003) hypothesised that increases in flower or inflorescence size can differentially benefit different gender functions, thus favouring preferential allocation to

specific floral structures. This question is relevant in the light of current empirical evidence. For example, it has been shown that larger flowers or inflorescences have higher probability of attracting pollinators (Schmid-Hempel & Speiser 1988; Thomson 1988; Ohara & Higashi 1994; Conner & Rush 1996; Arroyo *et al.* 2007; Gómez *et al.* 2008), ripening seeds (Ohara & Higashi 1994; Andersson 1996; Kawarasaki & Hori 1999), or dispersing pollen (Cruzan *et al.* 1988; Rodríguez-Robles *et al.* 1992; Ishii & Sakai 2002; but see Trapnell & Hamrick 2006). Therefore, it can be advantageous to allocate disproportionately more resources to attractive, female or male structures.

Which structure obtains a disproportionate allocation can be predicted based on theoretical grounds. The hypothesis of pollen donation states that more attractive floral displays, like larger flowers or inflorescences, are the result of enhancing male, rather than female reproductive success (Sutherland & Delph 1984; Bell 1985; Stanton *et al.* 1986). Therefore, if pollen donation were the main driver of floral display, one would expect increases in male allocation with an increase in floral or inflorescence size. By contrast, the male fitness gain per unit of investment is expected to decelerate because (i) increasing pollen production leads to more competition for ovules by pollen grains from the same parent (local mate competition; Lloyd & Bawa 1984) and (ii) large flower numbers result in increasing geitonogamy and, in turn, reduce

pollen available for out-crossing (Harder & Barrett 1995; de Jong 2000). However, female fitness gain per unit of allocation is assumed to be linear (Brunet 1992). Indeed, the sex allocation theory predicts a disproportionate increase of female allocation with increasing display size for out-crossers (Klinkhamer *et al.* 1997). Mating system will also affect the pattern of sex allocation in relation to inflorescence size because it will affect fitness gain differentially in each gender. In selfing plants, less allocation to male function and to attraction structures is expected (Charlesworth & Morgan 1991). Nevertheless, allocation to attraction structures should be high if selfing is mediated by pollinator visits (Lloyd 1987).

One potential problem in determining reproductive allocation to flowers is the choice of the most appropriate allocation currency (Goldman & Willson 1986; Stanton & Galloway 1990; Charlesworth & Morgan 1991). Different currencies may yield different allocation patterns (Lovett Doust & Harper 1980; Ashman & Baker 1992; Carroll & Delph 1996; Méndez & Traveset 2003). To the greatest degree possible, sexual allocation should be assessed in several currencies (Goldman & Willson 1986).

Previous studies (Méndez 2001; Méndez & Traveset 2003) have confirmed the occurrence of changes in allocation to floral or inflorescence structures with changes in flower or inflorescence size. In this paper, we investigate the effects of inflorescence size on sexual expression and female reproductive success in the monoecious species *Tussilago farfara*, by measuring patterns of biomass, and N and P allocation to inflorescence structures. Monoecious species, where female and male unisexual flowers are on the same plant, are suitable models to test the predictions of sex allocation theory because gender can be continually modified and easily measured with female and male relative flower production (Fox 1993; Méndez 1998; Sarkissian *et al.* 2001). We addressed the following specific questions: (i) How much variation in sexual expression is present at the inflorescence level? (ii) Do sexual expression and allocation to attractive *versus* primary sexual structures change with increasing inflorescence size? (iii) Do allocation patterns differ when different allocation currencies (biomass, N or P) are utilised? and (iv) How are allometric variations in sexual expression related to female reproductive success?

MATERIAL AND METHODS

Species and study sites

Tussilago farfara L. (Asteraceae) is a perennial rhizomatous herb frequent on stony soils, but preferring clayey and loamy soils. Each ramet bears a rosette of leaves and, in reproductive individuals, one to more than 10 inflorescences. In our populations, the number of inflorescences per ramet ranged from one to 14 (modes: 1 and 7–9; Noreña) and one to 12 (mode: 3; Saús). Inflorescences are solitary heads (capitula) on scapes with achlorophyllous scales. Heads produce two kinds of flower: inner, tubular flowers that are functionally male, and four or five rows of outer, female ray flowers, which have a narrow ray (Nordenstam 2007). Fruits are unicellular, one-seeded and indehiscent, as it is common in Asteraceae (Nordenstam 2007).

Flowering in northern Spain occurs between January and May, before the leaves expand. Heads open centripetally, from outer to inner rows, so they are protogynous (Burt 1961). The species is mainly visited by Hymenoptera (Wild *et al.* 2003). It has been considered to be strictly xenogamous (Fryxell 1957; Bostock & Benton 1979; Wild *et al.* 2003) but contradictory data exist (Wild *et al.* 2003). Pollen–ovule ratio per head averaged 434 ± 304 ($n = 20$) in Noreña and 397 ± 212 ($n = 20$) in Saús, suggesting facultative autogamy to facultative xenogamy (Cruden 1977). A pilot study (results not shown) in our study sites indicated both self-compatibility and the possibility of autogamy.

After seed dispersal, the inflorescence dies, and thereafter the rhizome system becomes fragmented (Ogden 1974). Rhizomes can grow 1 m or more from their initiation point before developing the first aerial structures (Ogden 1974). Recently, it was shown that in two studied plots in Germany there were only two and three genets, although each of them was split into morphologically independent clonal fragments (Pfeiffer *et al.* 2008). Although the inflorescences sampled for the present study were at least 1.5-m apart, they probably contained a small number of genets (see Pfeiffer *et al.* 2008).

Two populations were sampled in northern Spain in 2004: Noreña (UTM coordinates: 30TTP71, 200 m asl) and Saús (30TTP80, 420 m asl). Plants grow on the verge of local roads. Substrate in Noreña has more gravel and a low plant cover, with *Salix atrocinerea* and *Viburnum opulus* as dominant species. In Saús, the verge had a closed canopy of *Acer pseudoplatanus* and an understorey with 100% plant cover, dominated by grasses and two herbs, *Primula vulgaris* and *Helleborus viridis*.

Variation in phenotypic gender and its relationship with resource availability

In each population, from 22 January to 17 March 2004, flower number was counted in 80 flowering heads, belonging to different ramets. Phenotypic gender, *i.e.*, the relative investment of an individual in male *versus* female function (Lloyd 1980), was estimated by the index G of femaleness using the formula (Lloyd & Bawa 1984):

$$G = d_i / (d_i + l_i \cdot E); E = \sum d_i / \sum l_i$$

where d_i is a measurement of female investment (number of female flowers, in our case), l_i is a measure of male investment (number of male flowers, in our case), and E is an equivalence factor, which measures the ratio of investment in female and male structures in the whole population. G varies from 1 (complete femaleness) to 0 (complete maleness) (Lloyd 1980; Lloyd & Bawa 1984).

We studied whether phenotypic gender was related to resource availability, estimated as scape dry mass and as percentage of total non-structural carbohydrates (TNC) in rhizome samples. At each site, we harvested 40 inflorescences approaching anthesis, from 22 January to 7 March 2004, together with a portion of 4 cm of the rhizome immediately below each inflorescence. Inflorescences (heads and their scapes) were oven-dried as described in the next section. Rhizomes were oven-dried at 60 °C for 72 h and then finely

ground. Carbohydrates were extracted from 20 mg of dried rhizome. We analysed the soluble and non-soluble fraction of TNC using the perchloric acid/anthrone method (Morris 1948). The anthrone technique has been frequently used to analyse carbohydrates in storage organs (*i.e.*, Salsman *et al.* 1999; Olano *et al.* 2006). Results were expressed as percentage (w/w) carbohydrates. Relationships between carbohydrate percentage or scape dry mass and the femaleness index G were studied using correlation analysis.

Allometric variation in allocation to primary sexual and attraction structures

The inflorescences harvested as described in the previous section were also utilised to study variation in allocation to primary sexual and attraction structures in relation to inflorescence size. We used scape dry mass as a surrogate of inflorescence size to investigate the allometric variation in sex allocation. Scape dry mass was chosen as our estimate of inflorescence size because, in general, it showed strong correlations with dry mass of the rest of inflorescence structures in both populations (Torices R. & Méndez M., unpublished data). We measured the following inflorescence traits: (i) length, including both aerial and subterranean parts, to the nearest 0.1 mm; (ii) number of female and male florets; and (iii) dry mass of ovaries and rays in 50 female flowers per head. All structures were oven-dried for 2 days at 60 °C and weighed to the nearest 0.1 mg. Total N and P were determined, after micro-Kjeldahl digestion, in a Skalar segmented-flow nutrient autoanalyser (Skalar Analytics, Breda, The Netherlands).

Following Méndez (2001), allometric variation was studied for two components of sexual expression: (i) allocation to male *versus* female structures and (ii) primary sexual (male flowers plus ovaries) *versus* attraction (rays of female flowers) structures. The comparisons were made using regression of the two variables: female and male structures or primary sexual and attraction structures, against each other (Méndez 2001; Méndez & Traveset 2003). In each comparison, we used dry mass, and N and P as allocation currencies. The number of male and female flowers was also used in the comparison of male *versus* female structures. We utilised type II regression or RMA (Reduced Major Axis) on log₁₀-transformed variables (LaBarbera 1989) and tested whether the slope of the regression significantly departed from 1 using a *t*-test (Zar 1999). Slopes of non-significant regressions ($P > 0.05$) were not analysed.

Effects of inflorescence traits on female reproductive success

To study how the variation in inflorescence traits influenced female reproductive success, we marked 40 inflorescences in each population from 3 February to 17 March 2004. Inflorescence traits considered were number of male and female flowers, head diameter (to the nearest 0.1 mm), scape length (to the nearest 0.1 mm), ray length (estimated as mean value of ten rays per head to the nearest 0.1 mm), number of scapes, and femaleness index G. After flowering, heads were bagged to prevent seed dispersal. We collected the ripe heads to count the number of seeds and weigh the fruits. We compared inflorescence traits between populations by means of a *t*-test, except for scape length, head diameter and ray length,

in which the Mann–Whitney *U*-test was utilised due to a lack of normality.

Female reproductive success was estimated in three ways: as the probability of an inflorescence producing fruits, as seed set, and as number of seeds. Seed set was calculated as the number of seeds divided by the number of female florets. We studied the extent to which each inflorescence trait independently explained each of these three variables using two approaches: (i) correlation analyses and (ii) hierarchical partitioning of regression analysis (Chevan & Sutherland 1991; MacNally 2000) for each population separately.

First, we evaluated the potential effect of inflorescence traits on female reproductive success by means of correlation analyses. Logistic regression was utilised to test the correlations between inflorescence traits and the probability of fruit production. Pearson correlation was utilised for the other two variables.

Second, independent variables, which were all inflorescence components, were highly correlated and thus produced multicollinearity. Severe collinearity can have important, and detrimental, effects on the estimated regression parameters (MacNally 2000, 2002; Quinn & Keough 2002). Moreover, seeking a single model may not be the most effective way of identifying variables most likely to influence variation in the response variable (MacNally 2000). A possible solution is hierarchical partitioning, which allows identification of those predictor variables whose independent correlation (*i.e.*, independent of other predictor variables) with a dependent variable may be important, as distinct from predictor variables that have little independent effect on the dependent variable (Chevan & Sutherland 1991; MacNally 2000). The latter may still have a high, or even the highest, pair-wise correlation with the dependent variable, but this is due to joint action with other independent variables. For example, a predictor variable may have a high correlation with the response variable but much of this may be ‘shared’ with other variables that are correlated in a similar way, suggesting that this is not an important causal relationship. In contrast, if much of the variance is ‘independent’, then it suggests that an important causal relationship may be evident. For each female reproductive success measurement (probability of seed production, seed set and number of seeds), MacNally’s (2002) randomisation method was used, with 500 simulations, to assess the statistical significance of the independent contribution of each inflorescence trait. All analyses were conducted using the hier.part package (MacNally & Walsh 2004) of the R statistical software (R Development Core Team. 2008).

RESULTS

Variation in inflorescence traits

Inflorescences in both populations showed a broad range of variation in number of male and female flowers (Table 1). One of the populations (Noreña) even included inflorescences with no or few male flowers. In general, inflorescences had 10-times more female than male flowers (Table 1). The two populations differed significantly in the number of female flowers and in scape length (Table 1). In addition, infructescence production was significantly higher in Noreña (60%) than in Saús (37.5%) ($\chi^2_1 = 4.05, P = 0.044$).

Table 1. Inflorescence traits of *Tussilago farfara* in two populations.

inflorescence traits	Noreña			Saús			test	P
	mean \pm SD	range	n	mean \pm SD	range	n		
number of male flowers	28 \pm 12	0–60	80	30 \pm 7	13–50	80	$t_{158} = -1.117$	0.2657
number of female flowers	267 \pm 72	92–455	80	300 \pm 65	143–508	79	$t_{157} = -3.052$	0.0027
number of pollen grains per flower	3846 \pm 1849	1058–9090	20	4070 \pm 1942	855–8438	20	$t_{38} = -0.374$	0.7105
scape length (mm)	69.5 \pm 30.0	17.3–154.2	80	104.5 \pm 37.5	33.4–228.4	80	$U = 1462$	0.0000
head diameter (mm)	24.4 \pm 4.1	13.2–32.7	40	26.5 \pm 5.06	16.5–36.2	39	$U = 609.5$	0.0955
ray length (mm)	10.8 \pm 0.4	10.4–12.8	40	10.9 \pm 2.0	6.1–14.2	40	$U = 766$	0.7472

Differences between populations were analysed using *t*-tests except for scape length, head diameter and ray length, which were tested with the Mann-Whitney *U* test. Bold type for significant differences ($P < 0.05$).

Variation in sexual expression and its relationship with resource availability

There was a broad variation in phenotypic gender within populations (Fig. 1). Nevertheless, the femaleness index *G* was not significantly correlated with scape dry mass (Noreña: $n = 40$, $r = -0.099$, $P = 0.544$; Saús: $n = 39$, $r = 0.268$, $P = 0.099$) or with TNC in rhizomes (Noreña: $n = 7$, $r = 0.111$, $P = 0.813$; Saús: $n = 21$, $r = 0.084$, $P = 0.718$).

Allometric variation in allocation to primary sexual and attraction structures

The allometric variation in sex expression differed between populations. In Noreña, male flower number per head increased with increasing ovary number per head, with a slope significantly >1 (Table 2). In Saús, however, male flower dry mass per head increased with increasing ovary dry mass per head, with a slope significantly lower than 1 (Table 2).

Regarding primary sexual *versus* attraction structure allocation, in Noreña, ray allocation increased with increasing primary sexual allocation, with a slope not significantly different than one (Table 2). In Saús, an increase in dry mass, *N* and

P allocation to primary sexual structures entailed a disproportionate increase in allocation to attraction structures (Table 2).

Effects of inflorescence traits on female reproductive success

In Noreña, the number of male flowers was positively related to the probability of seed production (Table 3). In addition, seed set was positively related to scape length, head diameter and number of male flowers, and negatively related to the femaleness index (Table 3). The number of seeds was positively related to head diameter and number of male flowers, and negatively related to the femaleness index (Table 3). In Saús, inflorescence traits were not significantly related to female reproductive success (Table 3).

In Noreña, the hierarchical partitioning analysis showed that only head diameter and the femaleness index had independent explanatory power on the probability of seed production variance (Fig. 2A). Although scape number explained the greatest proportion of variance, it was not significantly higher than that expected by chance (Fig. 2A). Four variables had independent explanatory power on seed set in Noreña (Fig. 2B); femaleness index (11%) and head diameter (10%) had the highest independent contribution. The number of male flowers and femaleness index had independent explanatory power on the number of seeds (Fig. 2C). In Saús, hierarchical partitioning analysis showed that inflorescence traits had no independent power to explain seed production or seed set (Fig. 2A and B). Only scape length had independent explanatory power for seed number (Fig. 2C).

DISCUSSION

Inflorescence variation in phenotypic gender and its relationship with resource availability

This study showed intraspecific variation for phenotypic gender in *Tussilago farfara* inflorescences. Intraspecific variation in phenotypic gender has also been reported in other monoecious Asteraceae such as *Leptinella* (Lloyd 1972) and *Ambrosia artemisiifolia* (McKone & Tonkyn 1986). In the wind-pollinated *A. artemisiifolia*, larger plants were male-biased (Ackerly & Jasiński 1990), as expected according to sex allocation theory (Bickel & Freeman 1993). In the entomophilous *T. farfara*, we did not find a significant correlation between the femaleness index (*G*) and measures of overall resource availability.

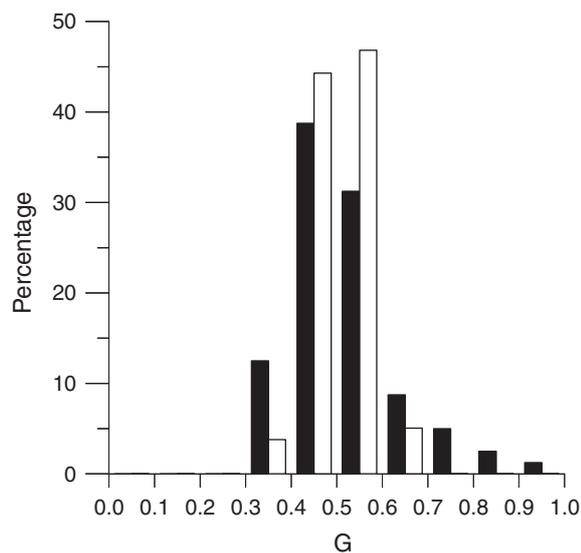


Fig. 1. Femaleness index (*G*) in two populations of *Tussilago farfara*. Black bars: Noreña; white bars: Saús. $n = 80$ inflorescences per site.

Table 2. Allocation to male flowers versus ovaries or to attractive versus primary sexual structures in inflorescences of *Tussilago farfara*.

currency	structure (y versus x)	n	r	b _{RMA} ± SE	df	t	P
Noreña							
number	male Flowers versus Ovaries	40	0.513	1.860 ± 0.261	38	3.299	**
dry mass	male Flowers versus Ovaries	29	0.330 ns	–	–	–	–
dry mass	attractive versus Primary sexual	29	0.784	1.146 ± 0.137	27	1.062	ns
Saús							
number	male Flowers versus Ovaries	40	0.286 ns	–	–	–	–
dry mass	male Flowers versus Ovaries	39	0.405	0.760 ± 0.114	37	2.1	*
N	male Flowers versus Ovaries	24	0.569	1.034 ± 0.181	22	0.186	ns
P	male Flowers versus Ovaries	23	0.086 ns	–	–	–	–
dry mass	attractive versus Primary sexual	39	0.779	1.765 ± 0.190	37	4.034	***
N	attractive versus Primary sexual	22	0.571	2.135 ± 0.392	20	2.896	**
P	attractive versus Primary sexual	22	0.411	1.807 ± 0.368	20	2.191	*

Reduced Major Axis (RMA) regression was utilised to obtain the slope (b_{RMA}) and significant departure from b = 1 was tested by a t-test. This test is not reported when correlation was not significant. All variables were log₁₀ transformed. r = Pearson correlation coefficient ; SE = standard error. *P < 0.05; **P < 0.01; ***P < 0.001.

Table 3. Bivariate correlations between inflorescence traits and female reproductive success.

inflorescence traits	n	probability of fruit production coefficient	n	seed set coefficient	n	no. of seeds coefficient
Noreña						
no. of scapes	31	0.108 (P = 0.070)	21	0.170 (P = 0.462)	21	0.138 (P = 0.550)
scape length	40	0.004 (P = 0.347)	24	0.462 (P = 0.023)	24	0.383 (P = 0.065)
head diameter	40	0.020 (P = 0.136)	24	0.421 (P = 0.040)	24	0.495 (P = 0.014)
ray length	40	0.037 (P = 0.212)	24	–0.284 (P = 0.179)	24	–0.184 (P = 0.390)
femaleness index	40	0.395 (P = 0.499)	24	–0.599 (P = 0.002)	24	–0.524 (P = 0.009)
no. of female flowers	40	0.002 (P = 0.102)	24	–0.050 (P = 0.816)	24	0.264 (P = 0.213)
no. of male flowers	40	0.021 (P = 0.047)	24	0.493 (P = 0.014)	24	0.656 (P = 0.000)
Saus						
no. of scapes	40	–0.092 (P = 0.192)	15	0.071 (P = 0.801)	15	0.053 (P = 0.851)
scape length	40	–0.004 (P = 0.133)	15	–0.293 (P = 0.290)	15	–0.207 (P = 0.458)
head diameter	39	–0.016 (P = 0.181)	15	–0.093 (P = 0.741)	15	0.013 (P = 0.964)
ray length	40	–0.043 (P = 0.148)	15	0.066 (P = 0.815)	15	0.125 (P = 0.657)
femaleness index	40	–1.042 (P = 0.109)	15	–0.235 (P = 0.399)	15	–0.128 (P = 0.649)
no. of female flowers	40	–0.001 (P = 0.182)	15	–0.220 (P = 0.430)	15	–0.012 (P = 0.966)
no. of male flowers	40	–0.013 (P = 0.221)	15	0.015 (P = 0.958)	15	0.087 (P = 0.759)

For the probability of fruit production, the coefficient of the logistic regression is shown; for the other two variables, the value of Pearson's r is shown. Bold type for significant correlations (P < 0.05).

Allometric variation in allocation to primary sexual and attraction structures

Previous studies have documented allometric variation in allocation to floral or inflorescence structures, either disproportionate increases in female allocation (Koelewijn & Hunscheid 2000; Méndez & Traveset 2003; Hiraga & Sakai 2007), male allocation (Méndez 2001; Ishii 2004) or in allocation to attraction structures (Méndez 2001). However, the patterns in the present study were more intricate. Namely, we obtained contrasting allometric relationships in different populations. Based on a tentative pilot study showing partial self-compatibility in our study sites (results not shown), we expected that larger inflorescences would show a bias towards female allocation because partially or completely selfing plants are expected to allocate less to attraction and male function (Charlesworth & Charlesworth 1981; Charlesworth & Morgan 1991). Increases in inflorescence size entailed disproportionate increases in male allocation at one site, but disproportionate increases in both female and attraction allo-

cation at the other one. Although it is rather likely that allometric variation in sexual allocation is population-specific rather than species-specific (e.g., Delesalle & Mazer 2009), we are only aware of one other species in which the allometry of sexual allocation differs between sites. In *Cardocrinum cordatum*, one population growing under a sparse canopy increased maleness with increasing plant size, while another population under a closed canopy increased femaleness with increasing plant size (Cao & Kudo 2008). Cao & Kudo (2008) suggest that contrasting photosynthetic gain in each environment could be responsible for their results. *T. farfara* lacks photosynthetic tissue at flowering time, but differences between populations in photosynthetic gain later in the season, or in storage, could mediate this response because TNC concentration was higher in the open than in the shaded site (results not shown). This hypothesis needs further examination under experimental conditions.

Two other alternative explanations deserve further experimental attention. The first is that contrasting light environments differentially affected visibility of the flowers to

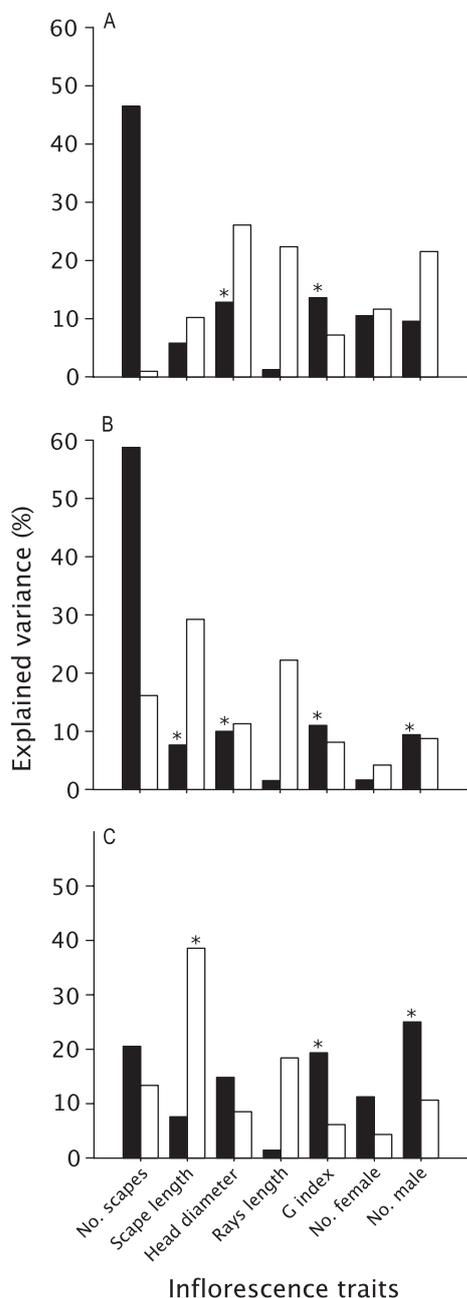


Fig. 2. Independent contributions of each inflorescence trait estimated from hierarchical partitioning for female reproductive success. The bars indicate percentage of explained variance in female reproductive success, estimated as A: the probability of seed production, B: seed set, and C: number of seeds, explained for each of the seven inflorescence traits by hierarchical partitioning in Noreña (black bars) and Saús (white bars). Variables marked with an asterisk independently explained a greater proportion of variance that would be expected by chance (with $P < 0.05$). No. scapes = number of scapes; Sc. length = scape length; Head diam. = head diameter; No. female = number of female flowers; No. male = number of male flowers.

pollinators, thus influencing the potential gain through male and female functions. Pollen limitation is very likely in our sites because we only detected three syrphids visiting *T. farfara* in 200 min of systematic observations. The second is that the extent of selfing could also differ among sites.

Unexpectedly, we found an increase in both female and attraction structures at one of our study sites. Although attraction structures are expected to increase male function (Bell 1985), we suggest that the increase in allocation to these structures cannot be separated from that to female structures in Asteraceae, because it is not possible to produce a ray without producing a female flower and *vice versa*. Under this scenario, it is not possible to disentangle whether it was female or attraction allocation that was actually favoured in Saús. This means that the unequivocal relationship between structure and function often assumed in the study of reproductive and sexual allocation (Bazzaz *et al.* 2000) is misleading in this case.

Effects of inflorescence traits on female reproductive success

In the open site, we found higher female reproductive success in larger, showier inflorescences, *i.e.*, those with longer scape and head diameter. This is consistent with previous findings in other Asteraceae (Andersson 1991; Nielsen *et al.* 2002; Kirchner *et al.* 2005). Again, our results revealed outcomes not expected from our initial portrayal of the influence of sex expression on female reproductive success. In monoecious species, fertility is positively correlated with femaleness (*e.g.*, Lázaro & Méndez 2007). We found exactly the opposite, *i.e.*, a positive influence of male flowers and a negative influence of femaleness.

A similar violation of the univocal relationship between structure and function in the capitulum of Asteraceae could explain the positive influence of male flowers on female reproductive success. We suggest that such influence could be due to a role of male flowers in pollinator attraction. Male flowers produce pollen, which is a reward for floral visitors. Furthermore, odour emission has been reported at the petal tips, anthers and pollen grains of male flowers (Wild *et al.* 2003). Additional evidence comes from our pilot mating system experiment, because emasculated exogamous inflorescences produced significantly lower seed number and seedset than unmanipulated control inflorescences (result not shown). This result was also found by Wild *et al.* (2003). Furthermore, given the observed capacity of geitonogamous selfing in this species, self-pollination would be increased by increasing the number or proportion of male florets.

Allocation currency

In general, patterns of allometric variation in sexual allocation were consistent across allocation currencies. For allocation to male *versus* female structures, only the number of flowers in Noreña and dry mass in Saús showed an allometric variation in allocation, although the trends were opposite in these two populations. Regarding allocation to attraction *versus* primary sexual structures, patterns of allometric variation were consistent across allocation currencies. Our findings differ from those of Méndez & Traveset (2003), where only number of structures but none of the other currencies (dry mass, N and P) showed an allometric variation in allocation.

In conclusion, there was allometric variation in allocation to inflorescence structures in *T. farfara*. Unexpectedly, the two studied populations showed contrasting patterns of allocation to inflorescence structures with an increase of inflores-

cence size. These allometric patterns were not easily interpretable when naïvely assuming an unequivocal relationship between structure and function for the inflorescence structures. First, it was not possible to disentangle whether attraction or female function drove the allometric pattern found in one of the sites because it is not possible to produce rays without producing female flowers and *vice versa*. Second, male flowers could have a relevant role in attraction in the other site. Thus, experimental manipulation may be the only way to disentangle the causes of the allometric patterns in some species that have inflorescences as pollination units.

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REFERENCES

- Ackerly D.D., Jasiński M. (1990) Size-dependent variation of gender in high density stands of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae). *Oecologia*, **82**, 474–477.
- Andersson S. (1991) Floral display and pollination success in *Achillea ptarmica* (Asteraceae). *Holarctic Ecology*, **14**, 186–191.
- Andersson S. (1996) Floral display and pollination success in *Senecio jacobaea* (Asteraceae): interactive effects of head and corymb size. *American Journal of Botany*, **83**, 71–75.
- Arroyo M.T.K., Till-Bottraud I., Torres C., Henríquez C.A., Martínez J. (2007) Display size preferences and foraging habits of high Andean butterflies pollinating *Chaetanthera lycopodioides* (Asteraceae) in the subnival of the central Chilean Andes. *Arctic, Antarctic and Alpine Research*, **39**, 347–352.
- Ashman T.-L., Baker I. (1992) Variation in floral sex allocation with time of season and currency. *Ecology*, **73**, 1237–1243.
- Bazzaz F.A., Ackerly D.D., Reekie E.G. (2000) Reproductive allocation in plants. In: Fenner M. (Ed.), *Seeds: the ecology of regeneration in plant communities*. CAB International, Wallingford, UK: pp. 1–29.
- Bell G. (1985) On the function of flowers. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **223**, 224–265.
- Bickel A.M., Freeman D.C. (1993) Effects of pollen vector and plant geometry on floral sex ratio in monoecious plants. *American Midland Naturalist*, **130**, 239–247.
- Bostock S.J., Benton R.A. (1979) The reproductive strategies of five perennial compositae. *Journal of Ecology*, **67**, 91–108.
- Brunet J. (1992) Sex allocation in hermaphroditic plants. *Trends in Ecology and Evolution*, **7**, 79–84.
- Burt B.L. (1961) Compositae and the study of functional evolution. *Transactions of the Botanical Society of Edinburgh*, **39**, 216–232.
- Cao G.-X., Kudo G. (2008) Size-dependent sex allocation in a monocarpic perennial herb, *Cardiocrinum cordatum* (Liliaceae). *Plant Ecology*, **194**, 99–107.
- Carroll S.B., Delph L.F. (1996) The effects of gender and plant architecture on allocation to flowers in dioecious *Silene latifolia* (Caryophyllaceae). *International Journal of Plant Sciences*, **157**, 493–500.
- Charlesworth D., Charlesworth B. (1981) Allocation of resources to male and female functions in hermaphrodites. *Biological Journal of Linnean Society*, **15**, 57–74.
- Charlesworth D., Morgan M.T. (1991) Allocation of resources to sex functions in flowering plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **332**, 91–102.
- Charnov E.L. (1982) *The theory of sex allocation*. Princeton University Press, Princeton, NJ, USA.
- Chevan A., Sutherland M. (1991) Hierarchical partitioning. *The American Statistician*, **45**, 90–96.
- Conner J.K., Rush S. (1996) Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia*, **105**, 509–516.
- Cruden R.W. (1977) Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution*, **31**, 32–46.
- Cruzan M.B., Neal P.R., Willson M.F. (1988) Floral display in *Phyla incisa*: consequences for male and female reproductive success. *Evolution*, **42**, 505–515.
- Delesalle V.A., Mazer S.J. (2009) Size-dependent pollen:ovule ratios and the allometry of floral sex allocation in *Clarkia* (Onagraceae) taxa with contrasting mating systems. *American Journal of Botany*, **96**, 968–978.
- Fox J.F. (1993) Size and sex allocation in monoecious woody plants. *Oecologia*, **94**, 110–113.
- Freeman D.C., McArthur E.D., Harper K., Blauer A.C. (1981) Influence of environment on the floral sex ratio of monoecious plants. *Evolution*, **35**, 194–197.
- Fryxell P.A. (1957) Mode of reproduction of higher plants. *The Botanical Review*, **23**, 135–233.
- Goldman D.A., Willson M.F. (1986) Sex allocation in functionally hermaphroditic plants: a review and critique. *The Botanical Review*, **52**, 157–194.
- Gómez J.M., Bosch J., Perfectti F., Abdelaziz M., Fernandez J.D., Camacho J.P.M. (2008) Association between floral traits and reward in *Erysimum mediohispanicum* (Brassicaceae). *Annals of Botany*, **101**, 1413–1420.
- Harder L.D., Barrett S.C.H. (1995) Mating cost of large floral displays in hermaphrodite plants. *Nature*, **373**, 512–515.
- Hiraga T., Sakai S. (2007) The effects of inflorescence size and flower position on biomass and temporal sex allocation in *Lobelia sessiflora*. *Plant Ecology*, **188**, 205–214.
- Ishii H.S. (2004) Increase of male reproductive components with size in an animal-pollinated hermaphrodite, *Nartheicum asiaticum* (Liliaceae). *Functional Ecology*, **18**, 130–137.
- Ishii H.S., Sakai S. (2002) Temporal variation in floral display size and individual floral sex allocation in racemes of *Nartheicum asiaticum* (Liliaceae). *American Journal of Botany*, **89**, 441–446.
- de Jong T.J. (2000) From pollen dynamics to adaptive dynamics. *Plant Species Biology*, **15**, 31–41.
- Kawarasaki S., Hori H. (1999) Effect of flower number on the pollinator attractiveness and the threshold plant size for flowering in *Pertya triloba* (Asteraceae). *Plant Species Biology*, **14**, 69–74.

- Kirchner F., Luijten S.H., Imbert E., Riba M., Mayol M., González-Martínez S.C., Mignot A., Colas B. (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos*, **111**, 130–142.
- Klinkhamer P.G.L., de Jong T.J., Metz H. (1997) Sex and size in cosexual plants. *Trends in Ecology and Evolution*, **12**, 260–265.
- Koelewijn H.P., Hunscheid M.P.H. (2000) Intraspecific variation in sex allocation in hermaphroditic *Plantago coronopus* (L.). *Journal of Evolutionary Biology*, **13**, 302–315.
- LaBarbera M. (1989) Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics*, **20**, 97–117.
- Lázaro A., Méndez M. (2007) Variation in sexual expression in the monoecious shrub *Buxus balearica* at different scales. *Plant Biology*, **9**, 1–9.
- Lloyd D.G. (1972) Breeding systems in *Cotula* L. (Compositae, Anthemideae). II Monoecious populations. *New Phytologist*, **71**, 1195–1202.
- Lloyd D.G. (1980) Sexual strategies in plants. III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany*, **18**, 103–108.
- Lloyd D.G. (1987) Allocations to pollen, seeds and pollination mechanisms in self-fertilizing plants. *Functional Ecology*, **1**, 83–89.
- Lloyd D.G., Bawa K.S. (1984) Modification of the gender of seed plants in varying conditions. *Evolutionary Biology*, **17**, 255–338.
- Lovett Doust J., Harper J.L. (1980) The resource costs of gender and maternal support in an andromonoecious umbellifer, *Smyrniolobos olusatrum* L. *New Phytologist*, **85**, 251–264.
- MacNally R. (2000) Regression and model-building in conservation biology, biogeography and ecology: the distinction between – and reconciliation of – ‘predictive’ and ‘explanatory’ models. *Biodiversity and Conservation*, **9**, 655–671.
- MacNally R. (2002) Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. *Biodiversity and Conservation*, **11**, 1397–1401.
- MacNally R., Walsh C.J. (2004) Hierarchical partitioning public-domain software. *Biodiversity and Conservation*, **13**, 659–660.
- McKone M.J., Tonkyn D.W. (1986) Intrapopulation gender variation in common ragweed (Asteraceae: *Ambrosia artemisiifolia* L.), a monoecious, annual herb. *Oecologia*, **70**, 63–67.
- Méndez M. (1998) Modification of phenotypic and functional gender in *Arum italicum* (Araceae). *American Journal of Botany*, **85**, 225–234.
- Méndez M. (2001) Sexual mass allocation in species with inflorescences as pollination units: a comparison between *Arum italicum* and *Arisaema* (Araceae). *American Journal of Botany*, **88**, 1781–1785.
- Méndez M., Traveset A. (2003) Sexual allocation in single-flowered hermaphroditic individuals in relation to plant and flower size. *Oecologia*, **137**, 69–75.
- Morris D. (1948) Quantitative determination of carbohydrates with Dreywood’s anthrone reagent. *Science*, **107**, 254–255.
- Nielsen L.R., Philipp M., Siegmund H.R. (2002) Selective advantage of ray florets in *Scalesia affinis* and *S. pedunculata* (Asteraceae), two endemic species from the Galápagos. *Evolutionary Ecology*, **16**, 139–153.
- Nordenstam B. (2007). Tribe senecioneae cass. In: Kadereit J. W., Jeffrey C. (Eds), *The families and genera of flowering plants*. Vol. 8. Springer, New York, NY, USA: pp. 208–241.
- Ogden J. (1974) The reproductive strategies of higher plants II. The reproductive strategies of *Tussilago farfara* L. *The Journal of Ecology*, **62**, 291–324.
- Ohara M., Higashi S. (1994) Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). *Oecologia*, **98**, 25–30.
- Olano J.M., Menges E.S., Martinez E. (2006) Carbohydrate storage in five resprouting Florida scrub plant across a fire chronosequence. *New Phytologist*, **170**, 99–106.
- Pfeiffer T., Günzel C., Frey W. (2008) Clonal reproduction, vegetative multiplication and habitat colonisation in *Tussilago farfara* (Asteraceae): a combined morpho-ecological and molecular study. *Flora*, **203**, 281–291.
- Quinn G.P., Keough M.J. (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge.
- R Development Core Team. (2008) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rodríguez-Robles J.A., Melendez E.J., Ackerman J.D. (1992) Effects of display size, flowering phenology, and nectar availability on effective visitation frequency in *Compartmentia falcata* (Orchidaceae). *American Journal of Botany*, **79**, 1009–1017.
- Salsman K.J., Jordan D.N., Smith S.D., Neuman D.S. (1999) Effect of Atmospheric CO₂ enrichment on root growth and carbohydrate allocation of *Phaseolus* spp. *International Journal of Plant Sciences*, **160**, 1075–1081.
- Sarkissian T.S., Barrett S.C.H., Harder L.D. (2001) Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology*, **82**, 360–373.
- Schmid-Hempel P., Speiser B. (1988) Effects of inflorescence size on pollination in *Epilobium angustifolium*. *Oikos*, **53**, 98–104.
- Stanton M.L., Galloway L.F. (1990) Natural selection and allocation to reproduction in flowering plants. In: Mangel M. (Ed.), *Some mathematical questions in biology – sex allocation and sex change: experiments and models*. American Mathematical Society, Providence, RI, USA: pp. 1–50.
- Stanton M.L., Snow A.A., Handel S.N. (1986) Floral evolution: attractiveness to pollinator increases male fitness. *Science*, **232**, 1625–1627.
- Sutherland S., Delph L.F. (1984) On the importance of male fitness in plants: patterns of fruit-set. *Ecology*, **65**, 1093–1104.
- Thomson J.D. (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of trapping pollinators of *Aralia hispida*. *Evolutionary Ecology*, **2**, 65–76.
- Trapnell D.W., Hamrick J.L. (2006) Floral display and mating patterns within populations of the neotropical epiphytic orchid, *Laelia rubescens* (Orchidaceae). *American Journal of Botany*, **93**, 1010–1018.
- Wild J.-D., Mayer E., Gottsberger G. (2003) Pollination and reproduction of *Tussilago farfara* (Asteraceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, **124**, 273–285.
- Zar J.H. (1999) *Biostatistical analysis*, 4th edn. Prentice Hall, Upper Saddle River, NJ, USA.

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