

Temporal dynamics of sexual dimorphism in a dioecious species

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• **Background and Aims** Sexual dimorphism for floral traits is common in dioecious plant species. Beyond its significance for understanding how selection acts on plant traits through male vs. female reproductive function, sexual dimorphism has also been proposed as a possible risky characteristic for insect-pollinated plants, as it could drive pollinators to forage mostly on male plants. However, even though most flowering plant species spread their flowering across several weeks or months, the temporal variation of floral phenotypes and sexual dimorphism have rarely been investigated.

• **Methods** We performed a survey of male and female plants from the dioecious generalist-pollinated *Silene dioica* (Caryophyllaceae) in a common garden experiment, over two consecutive flowering seasons. Flower number and floral size were measured each week, as well as pollen quantity and viability in male plants.

• **Key Results** Sexual dimorphism was found for all investigated floral traits, with males showing an overall higher investment in flower production and flower size. Males and females showed a similar temporal decline in flower size. The temporal dynamics of daily flower number differed between sexes, with males showing a peak in the middle of their flowering season, whereas flower production by females was quite stable over time. At the scale of the experimental population, both individual and floral sex ratios appeared to vary across the flowering season. Moreover, because the onset of flowering varied among plants, the magnitude of sexual dimorphism in floral size also fluctuated strongly through time.

• **Conclusions** Capturing male/female differences with only one temporal measurement per population may not be informative. This opens stimulating questions about how pollinator behaviour and resulting pollination efficiency may vary across the flowering season.

Key words: *Silene dioica*, red campion, sexual dimorphism, floral traits, temporal variation, flower size, floral display, pollen.

INTRODUCTION

Unlike in animals, the occurrence of separate sexes (i.e. dioecy) is a rare reproductive strategy in flowering plants (6 % of species only; Renner, 2014). One common observation in dioecious plant species is the occurrence of sexual dimorphism for one or several traits, such as flower number, flower size, flowering duration, quantity and composition of floral scents, quantity and composition of rewards for pollinators, plant longevity or plant architecture (Delph *et al.*, 1996; Eckhart, 1999; Ashman, 2009; Barrett and Hough, 2013). This can be explained by sex-specific differences in selective pressures acting on traits that affect either fertility or survival (natural selection) or access to mating partners (sexual selection; Geber *et al.*, 1999). In particular, the fact that male plants of insect-pollinated species apparently invest more than females in pollinator attraction (larger floral display, larger flower size, higher quantities of floral scents) is usually interpreted as a possible result of sexual selection, with male reproductive success being more sensitive to their ability to attract pollinators (reviewed by Delph *et al.*, 1996; Ashman, 2009; Barrett and Hough, 2013). Beyond

its significance for understanding how selection acts on plants traits through male vs. female reproductive function, sexual dimorphism has also been proposed as a possible risky character for insect-pollinated plants, as it could drive pollinators to forage mostly on the most attractive sex (Vamosi and Otto, 2002; Vamosi *et al.*, 2006; Glaetli and Barrett, 2008). Such a mechanism has been invoked to explain the overall rarity of dioecy within flowering plants (Heilbuth, 2000; Vamosi and Otto, 2002), in addition to other possible risks incurred by dioecious species, such as the lack of reproductive assurance.

Studies investigating sexual dimorphism usually report one single value of male/female difference for a given floral trait (based either on one measurement per plant or on several measurements averaged over several dates), thus ignoring possible within-individual trait variation throughout the flowering season (Williams and Conner, 2001). Indeed, temporal variation of floral phenotypes across the flowering season (e.g. biomass of reproductive parts, flower size) is a common phenomenon, possibly resulting from a decline in nutrients and available resources over time and/or from variation in developmental constraints among early and late

flowers (Wolfe, 1992; Diggle, 1997). Although this has not been investigated to our knowledge, temporal variation in floral traits is likely to differ between males and females in dioecious species. Indeed, temporal variation in biomass/nutrient supply to floral parts has been found to differ among floral tissues of hermaphroditic flowers, in particular between male and female tissues (Ashman and Baker, 1992; Zhao et al., 2010). One reason for this is that the nature and the quantity of nutrients required for the production of ovules/seeds vs. pollen can differ strongly, leading to different dynamics of resource limitation (e.g. McDowell et al., 2000; Obeso, 2002; Harris and Pannell, 2008; Van Drunen and Dorken, 2012). Moreover, the temporal variation in opportunities to mate through male vs. female function should select for changes in sex allocation throughout the flowering season (Brunet and Charlesworth, 1995). This type of temporal variation in floral traits implies variation in attractive signals and rewards for pollinators across the flowering season, and such changes could thus be different between males and females in dioecious species. To date, integration of temporal dynamics in the study of sexual dimorphism has been done in studies that investigated sexual differences in plant size and in allocation to reproduction vs. survival or growth (Sánchez Vilas and Pannell, 2011; Cipollini et al., 2013; Teitel et al., 2016), but not for floral traits involved in pollinator attraction. Integrating temporal dynamics of floral phenotypes and potential sex-related variation should help to capture the variation perceived by foraging pollinators and its possible consequences for pollination efficiency.

This study aims to measure the variation in several floral traits in *Silene dioica* (Caryophyllaceae), both within individuals (across time) and among individuals, in particular between males and females. Sexual dimorphism for floral traits has been reported in many dioecious and gynodioecious species from the family Caryophyllaceae and the genetic variation behind these sexual differences has been investigated in several cases (e.g. Weller et al., 2007; Delph et al., 2010; Campbell et al., 2011). In *S. dioica*, previous studies have shown that male plants produce more and larger flowers (Kay et al., 1984; Hemborg, 1998). Male flowers have also been shown to be more attractive to pollinators than female flowers (Carlsson-Granér et al., 1998). Because this species is known to exhibit a large inter-individual variation in floral traits such as flower number and size (Kay et al., 1984), we chose to investigate trait variation in a common garden experiment, to minimize environmental and age-related variations. We measured flower numbers and size to compare these traits and their possible temporal variation between males and females and we examined how this variation translated in terms of sexual dimorphism at the population scale. We also measured pollen quantity and viability for two reasons. First, we aimed to explore the possible trade-offs between fecundity and other investigated floral traits. Second, because pollen can play a role in pollinator attraction, the quantity of available pollen and its variation through time could also impact pollinator behaviour. Because the temporal dynamics of flowering and floral phenotypes have the potential to be triggered by environmental cues that can vary from year to year, we repeated the study across two consecutive flowering seasons.

MATERIAL AND METHODS

Plant species and plant collection

The red campion, *Silene dioica* (L.) Clairv. (Caryophyllaceae), is a perennial, dioecious herb with a generalist pollination system. The main pollinators are Hymenoptera (long-tongued *Bombus* species) and Lepidoptera (*Pieris* spp.). While it has been clearly established that pollinators, in particular bumblebees, display a marked preference for male plants (Kay et al., 1984; Carlsson-Granér et al., 1998), it is not known which trait combination is responsible for pollinator attraction. Because bumblebees collect both pollen and nectar (L.M., pers. obs.), they should be able to find resources on both sexes, but to date it is not clear whether the preference towards males is driven by the absence of pollen on females, by sexual dimorphism for one/several other traits or by a combination of these characteristics. This species grows in disturbed habitats, roadsides, woodlands and wet meadows throughout most of northern and central Europe (Rameau et al., 1989). It is one of the most common insect-pollinated dioecious plant species in north-western Europe (Kay et al., 1984). *Silene dioica* flowers from April to June. Male flowers usually remain open for 2–5 d, whereas female flowers can be receptive for a longer period of time, depending on the pollination context (M.D., pers. obs.). Progeny sex ratios are typically female-biased in this species (Van Nigtevecht, 1966; Taylor, 1994).

Experimental plants were derived from seeds collected in July 2015 (cohort 1) and July 2016 (cohort 2) from eight populations in northern France (see Supplementary Data, Table S1), three of which were sampled in both years (MO1, MO2 and MA: 13, 22 and 30 maternal plants respectively). Of the remaining five populations, three were sampled only in 2015 (MO3, MO4 and SA: five, 16 and 21 maternal plants) and two were sampled only in 2016 (MO5 and MO6: 23 and 15 maternal plants). Several natural populations were sampled to maximize genetic variation in the experimental population. All source populations were located along forest paths. Ten seeds per mother plant were sown in October 2015 and October 2016 for cohort 1 and cohort 2 respectively and three randomly selected seedlings were kept for phenotype measurements. Two months after germination, plants were vernalized for 10 weeks at 6 °C to induce flowering. All the steps from sowing to vernalizing were conducted in Lille University's glasshouse. In mid-March 2016, plants were placed in a randomized block design in an experimental garden, accessible to pollinators. From 4 April to 1 July, 2016, 228 individuals (140 females and 88 males) from 89 mother plants (cohort 1) were surveyed at Lille University's experimental garden (50°36'31.4"N, 3°08'39.3"E). A second survey was performed from 3 April to 2 June, 2017 on plants from cohort 2 (75 females and 104 males, from 90 mother plants) at the University of Louvain's experimental garden (105 km from Lille University, 50°39'56.3"N, 4°37'10.2"E). Floral display and floral size were measured in both years using the same methodology (see below), providing a detailed picture of flowering strategy for the two cohorts during their first year of flowering. In addition, pollen production was measured on male plants during the 2016 flowering season ($n = 84$ males). Finally, plants from cohort 1 that flowered in both years were used to test for correlations in floral

phenotypes across years. All phenotypic measurements are described in the section below.

Survey of floral traits

Floral size and floral display. At the onset of flowering, we recorded the date and the sex of individuals. Then, each week, we counted the number of open flowers and we measured flower and petal size on one randomly selected flower per individual. Corolla diameter and calyx height were measured directly on the plants, using a digital caliper precise to 0.01 mm. The length from paracorolla to the apical part of petals and the largest width were measured on one petal of the same flower, after taping it to a sheet of paper.

Pollen production and viability. In 2016, one nearly opened bud was collected each week on the main floral stem of surveyed male plants, when available (one to nine buds collected per male plant over the flowering season). Each bud was dissected to keep four anthers. The proportion of viable pollen grains was estimated using a staining method developed by Peterson (2010) on a mix of pollen from two of the four collected anthers. Pollen was placed on a glass slide with one drop of staining solution. These samples were observed under a light microscope at 100× magnification. We estimated the proportion of viable pollen grains (stained in purple/red) on a sample of 200 pollen grains. The two remaining anthers were stored together in 95 % ethanol until pollen counts. We used a particle counter (CASY, model TT, Innovatis, Bielefeld, Germany) to estimate the number of pollen grains in these samples, following the protocol described by Dufay et al. (2008, 2010). We estimated the number of viable pollen grains by multiplying the total number of pollen grains per anther by the proportion of viable pollen grains determined from the pollen staining tests.

Statistical analyses

Statistical analyses were performed to investigate: (1) phenotypic correlations among the different traits; (2) inter-individual, temporal and sexual variation in floral traits; (3) the stability of flowering phenotypes across two consecutive years; (4) inter-individual and temporal variation in pollen quantity and viability in male plants; and (5) the degree and the direction of population-level sexual dimorphism in floral traits.

Phenotypic correlations among floral traits. Inter-correlations among the different floral traits were explored with Spearman rank correlations and the Holm correction for multiple comparisons, separately analysing male and female plants. Phenotypic correlations were tested for each cohort separately, on data averaged for each plant across the flowering season when several values were measured per individual plant.

Within- and between-sex variation of floral traits. We assessed male/female differences for daily flower number and the four measurements of flower size (corolla diameter, calyx height, petal length and petal width). We analysed two additional composite variables: (1) the daily floral display (flower number multiplied

by corolla diameter, both measured on the same day) as an estimator of the intensity of the visual signal and a possible proxy for pollinator attraction, and (2) an estimate of the total number of flowers (sum of daily flower numbers across the flowering season for each plant), a proxy of overall flower production. It must keep in mind that this sum is likely to be an under-estimation of the total number of flowers, because individual flowers generally remain open for less than a week in both sexes (Kay et al., 1984). Moreover, because the lifespan of male flowers was shorter than females (M.D., pers. obs.) this under-estimation could be stronger in male plants. Variables that were measured every week across the flowering season were analysed with generalized linear mixed-effects models with a negative binomial distribution for daily flower number and with linear mixed-effects models with Gaussian distributions for flower size traits and for daily flower display. We tested for an effect of sex and population of origin (treated as fixed factors), while controlling for the effect of two co-variables, the date of flowering onset and the flowering week (number of weeks that have elapsed since the beginning of flowering). Mother plant identity was included as a random factor, as well as plant identity. For the variable for which only one measure per individual was available (the estimate of overall flower production), a linear mixed-effects model was used to test for the occurrence of sexual dimorphism. Explanatory variables were sex, population of origin and the date of flowering onset. Mother plant identity was again included as a random effect.

All models were fitted in Rgui (version 3.2.2; R Development Core Team, 2008) using the lme4 package (Bates et al., 2015). We plotted residuals of each model against fitted values as well as a QQ-plot of the residuals to graphically validate our models. For all models, except for daily flower number, residuals exhibited homogeneity and normality. To summarize results, we calculated type-II ANOVA tables (Anova function in R).

Inter-annual variation. We tested whether individual floral traits were stable across years of flowering. Only individuals from cohort 1 that flowered both in 2016 and in 2017 were included in this analysis ($n = 85$, including 50 females and 35 males). For each individual, we kept the same number of observations for the two years and calculated mean and maximum values for flower size descriptors (corolla diameter, calyx height, petal length and petal width) and daily flower number. We used linear regressions to test whether measures for 2017 were dependent on measures for 2016, analysing males and females separately.

Pollen production and viability. The total quantity of pollen grains per anther, as well as the proportion and the quantity of viable pollen grains per anther were analysed using generalized linear mixed-effects models. Models included population of origin (fixed factor), as well as three co-variables: week of flowering, daily flower number (allowing us to explore potential trade-offs between flowering intensity and gamete production) and corolla diameter (allowing us to investigate a possible allometric relationship between flower size and gamete production). Because daily flower number was not statistically independent from the date of flowering onset (see Results), the latter was not included in the models. Mother plant identity and individual identity were included as random factors.

Variation of population sex ratio and sexual dimorphism over the flowering season. In the analyses presented above, time was treated as a relative variable (time that has elapsed since the onset of flowering). We also graphically investigated how population attributes varied at the population level across the flowering season, treating flowering time as an absolute variable (calendar week). We investigated the temporal dynamics of (1) operational sex ratio (i.e. the proportion of males among flowering individuals, at each date), (2) floral sex ratio (i.e. the proportion of male flowers at each date), (3) sexual dimorphism in flower width and (4) sexual dimorphism in floral display.

Data are presented as means $\pm$ s.d. throughout the paper.

RESULTS

Phenotypic correlations among floral traits

Most of the correlations were similar between the two study years and among populations (see Table 1 and Supplementary Data Table S2 for correlations across populations in cohort 1 and cohort 2 respectively, as well as Tables S3 and S4 for within-population, within-cohort correlations). In both males and females, we observed positive and significant correlations between the four measurements of flower size (corolla diameter, calyx height, petal length and petal width). The total number of flowers and the average daily flower number were also strongly positively correlated within both sexes, and were often negatively correlated with the date of flowering onset, indicating that plants that started to flower early produced more flowers. In addition, in 2017, males that started to flower later produced flowers with significantly wider petals (see Table S2). Phenotypic trade-offs (negative correlations) between floral traits were found in a very limited number of cases (e.g. proportion of viable pollen and petal width, proportion of viable pollen and pollen quantity in males from the MO2 population and the first cohort, see Table S3; calyx height and total number of flowers in females from the MA population and the first cohort, see Table S3) and were not significant when tested on the complete dataset (Table 1 and Table S2). Overall females even showed positive correlations between three of the four measurements of flower size and the average number of open flowers, but only in 2016, although this

was driven by one population only (MO2). Both pollen quantity per anther and proportion of viable pollen grains were positively correlated with the estimated number of viable pollen grains per anther. The average quantity of pollen per anther was positively correlated with average petal width.

Within- and between-sex variation in floral traits

Regarding floral number and size, both cohorts (2016 and 2017) provided similar results. For the sake of clarity, result descriptions and figures focus on data collected on cohort 1 (2016). The statistics for both cohorts can be found in Table 2.

Number of flowers (Figs 1 and 2A, Table 2). Daily flower production was significantly higher in male plants than in female plants and was significantly influenced by the date of flowering onset, as mentioned above. The temporal dynamics of flower production differed between males and females, as confirmed by the significant interaction between sex and flowering week: whereas females always carry a limited number of open flowers, male plants show a clear peak in the middle of their flowering season, with over 20 flowers on average. The total number of flowers observed per individual for cohort 1 (i.e. sum of daily flower numbers across the flowering season) was thus much higher for males (148.41 ± 81.54 flowers in 2016) than females (11.68 ± 16.25 flowers in 2016, $\chi^2_1 = 746.97$, $P < 2 \times 10^{-16}$) as visualized in Fig. 1, with a large among-males variation found for all populations of origin (ranging from three to 377 flowers). This variable was also significantly influenced by the date of onset of flowering ($\chi^2_1 = 123.5$, $P < 2 \times 10^{-16}$) and marginally influenced by population of origin ($\chi^2_5 = 10.12$, $P = 0.07$).

Floral size (Fig. 2B–E, Table 2). Significant sexual dimorphism was detected for all measurements of floral size: male flowers had significantly larger corolla diameters and calyx heights, as well as wider and longer petals than female flowers. Overall flower size decreased with flowering week in both sexes. Petal size was larger for plants that started to flower earlier. Beyond sexual dimorphism, when focusing on

TABLE 1. Pearson's correlation coefficients among floral traits in male (upper right half of the matrix) and female individuals (bottom left half of the matrix) based on traits measured in spring 2016 on cohort 1. Bold characters indicate significant correlations, based on P-values after Holm correction. Apart from flowering onset and total number of flowers, all traits were averaged for each individual over the flowering season. Onset flow. = date of onset of flowering, Cor. diam. = corolla diameter. Pollen viability (viab.) was analysed in terms of both quantity of viable pollen grains (quant.) and proportion of viable pollen grains (prop.).

	Onset flow.	Calyx. height	Cor. diam.	Petal height	Petal width	Daily no. of flowers	Total no. of flowers	Pollen quant.	Pollen viab. (quant.)	Pollen viab. (prop.)
Onset flow.		−0.02	0.11	0.14	0.02	−0.34**	−0.7***	0.02	0.05	−0.09
Calyx height	0.014		0.49***	0.43***	0.22*	−0.09	−0.01	0.05	0.07	0.06
Cor. diam.	−0.03	0.23**		0.93***	0.53***	−0.09	−0.11	0.14	0.15	0.12
Petal height	−0.04	0.32***	0.74***		0.56***	−0.13	−0.16	0.18	0.13	0.07
Petal width	−0.09	0.21*	0.57***	0.66***		−0.11	−0.07	0.24*	0.02	−0.14
Daily no. of flowers	−0.29***	−0.12	0.23**	0.22**	0.19*		0.89***	−0.02	0.02	0.06
Total no. of flowers	−0.63***	−0.1	0.04	0	0.04	0.74***		−0.05	−0.01	0.13
Pollen quant.	—	—	—	—	—	—	—		0.64***	−0.1
Pollen viab. (quant.)	—	—	—	—	—	—	—	—		0.41***

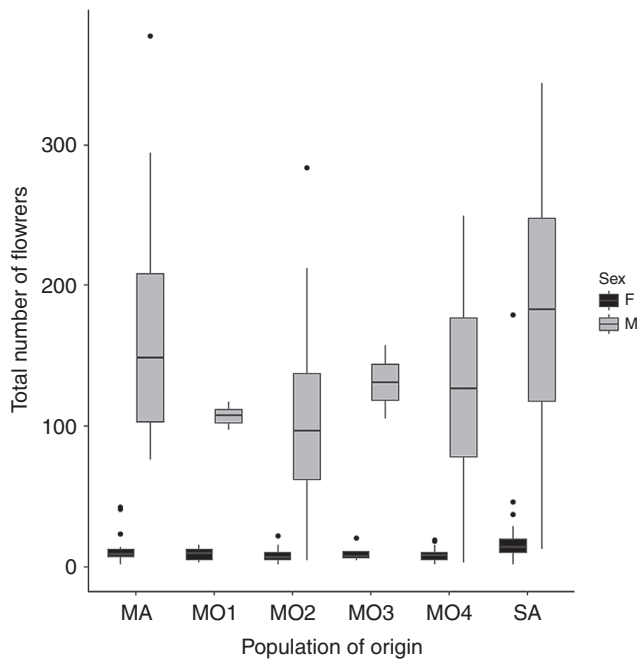


FIG. 1. Total number of flowers observed per individual for cohort 1 (i.e. sum of daily flower numbers across the flowering season), according to plant sex and population of origin. The horizontal line within the box indicates the median. The boxes contain the observed values from the lower quartile (25 %) to the upper quartile (75 %) of the distribution and include 50 % of the observed values. The box whiskers encompass 98 % of the observed values (1–99 % of the distribution) and the black circles represent outliers.

the maximum values of floral size for each individual (generally, the first value collected on the plant), we observed high variability within both males and females (Supplementary Data Fig. S1). For instance, in 2016, maximum individual corolla diameter ranged from 11.97 to 29.79 mm in females and from 16.82 to 33.85 mm in males. Similar levels of within-sex variation were recorded for all traits linked to floral size (Fig. S1).

Floral display (Fig. 2F, Table 2). Daily floral display (number of open flowers multiplied by corolla diameter) was significantly larger for males and extremely variable within both males and females. Temporal dynamics differed between sexes, with males exhibiting a clear peak in flowering effort during their fifth flowering week, on average, while females showed a steadier effort across the flowering season.

Inter-annual variation

Mean flower numbers observed in 2016 were positively correlated with the same data observed in 2017 for males (t value = 7.369, $P < 0.001$, coefficient of correlation $R = 0.376$) but not for female individuals (t value = -1.015 , $P = 0.313$, Fig. 3). Corolla diameters observed in 2016 were positively correlated with the same data in 2017 (t value = 2.901, $P = 0.005$, $R = 0.326$; t value = 4.530, $P < 0.001$, $R = 0.395$ for females and males respectively, Fig. 3). Date of flowering onset

was marginally repeatable for females only (t value = 2.02, $P = 0.04$).

Pollen production and viability

Males produced on average 2622 ± 785 pollen grains per anther. This value was positively correlated with corolla diameter, but did not depend on the number of open flowers (Table 3). The number of pollen grains per anther was negatively affected by the week of flowering, indicating a decrease of pollen production per flower throughout the flowering period. On average, individuals produced 2855 ± 811 pollen grains per anther during the first week of flowering and 2239 ± 578 pollen grains per anther if they reached the ninth week of flowering. The proportion of viable pollen grains remained stable across the flowering season and did not depend on the number of flowers or on flower size. As a result, the number of viable pollen grains per flower was negatively affected by the week of flowering and positively correlated with corolla diameter.

Variation of population sex ratio and sexual dimorphism over the flowering season

Even during the 2016 survey that included more females in the collection, the operational sex ratio (in terms of flowering individuals) was at first balanced from April to the beginning of June, and afterwards strongly male-biased, due to the longer duration of flowering in males (Fig. 4A). Floral sex ratio was always strongly male-biased (reflecting sexual dimorphism in terms of flower number), this bias globally increasing across the flowering season (Fig. 4B). Finally, sexual dimorphism in terms of both flower size and floral display was always in favour of males, the male floral display being on average three to nine times larger than the female floral display, depending on the calendar date. This ratio fluctuated across the flowering season, but with no particular temporal trend (Fig. 4C, D).

DISCUSSION

The aim was to investigate the variation in several floral traits within individuals through time, among populations of origin and between sexes. We found some variation at all investigated levels. In particular, we found that all floral traits varied through time, and that male and female plants do not always show the same patterns of temporal variation. The number of open flowers in male plants showed a clear peak in the middle of the flowering season, whereas it was quite stable in females. Therefore, and also because the operational sex ratio became strongly male-biased at the end of the survey, the ratio of open male/female flowers increased strongly at the end of the flowering season. Besides, the average sexual dimorphism in flower size also showed some striking variation through time. Before discussing the possible causes of such sex-specific

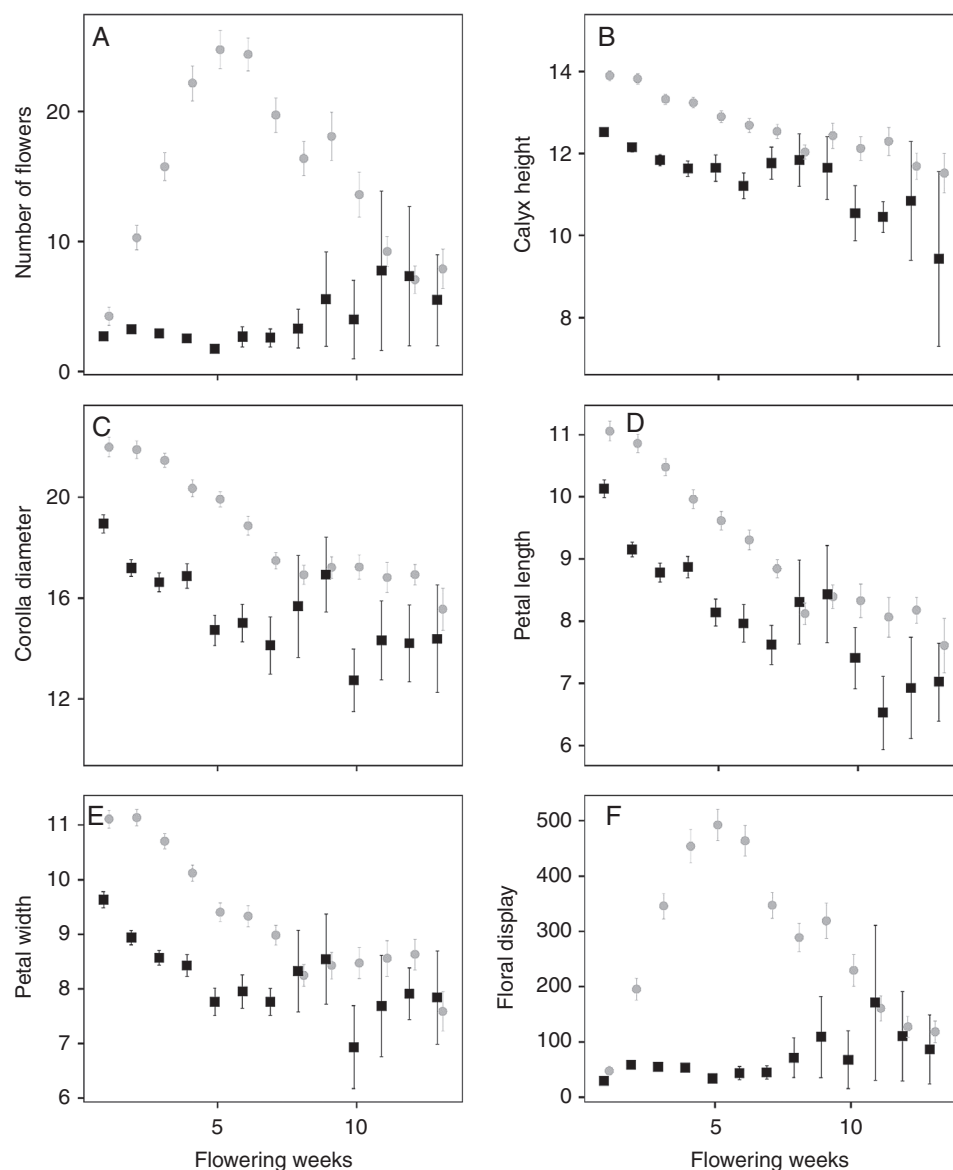


FIG. 2. Variation of floral traits in females (black) and males (grey) as a function of time (number of weeks that have elapsed since the beginning of flowering of each individual) for cohort 1 (2016 survey). (A) Mean daily number of flowers; mean flower size expressed as (B) calyx height (mm) and (C) corolla diameter (mm); mean petal size with (D) petal length (mm) and (E) petal width (mm); (F) average floral display (number of flowers multiplied by corolla diameter).

temporal variation in floral traits and its possible consequences on both pollinator behaviour and pollination efficiency, we will briefly discuss the correlations that we found among floral traits, as well as the overall difference between males and females reported in this study.

Correlation among traits

We found significant phenotypic correlations between several pairs of floral traits. The four measures of flower size (calyx height, corolla diameter, petal length and petal width) were all strongly and positively correlated, probably a result of genetic correlations and/or selection acting on several traits simultaneously (Conner and Via, 1993; Ishii and Morinaga, 2005;

Conner *et al.*, 2014). Of note, such correlations could also result from variation among individuals in plant size, which was not directly assessed in the current study. This result corroborated what has been seen in a previous study of *S. dioica*, which also documented significant phenotypic correlations between petal width, petal length and calyx height (Giles *et al.*, 2006).

In males, pollen quantity was found to correlate positively with flower size and to be independent of flower number, both when considering the average phenotype over the flowering season and when working on repeated measurements through time. As the proportion of viable pollen grains was independent of these variables, males seem to incur no trade-off between floral display and fertility. The total number of pollen grains thus appears to be a linear function of total flower number, with no cost on other measured floral traits. Because of the high

TABLE 2. Analysis of deviance table for the generalized linear models testing for the effect of sex, flowering week, population of origin, date of flowering onset and the flowering week by sex interaction on different estimators of flower size (calyx height, corolla diameter, petal length and petal width), on daily flower number and on daily floral display (daily flower number multiplied by corolla diameter). Plant identity and mother plant were included as random factors.

	2016			2017		
	χ^2	d.f.	P	χ^2	d.f.	P
Daily flower number						
Sex	477.01	1	$<2 \times 10^{-16}$	403.47	1	$<2 \times 10^{-16}$
Week of flowering	1.31	1	0.252	2138.08	1	$<2 \times 10^{-16}$
Population	5.51	5	0.356	36.27	4	<0.001
Onset of flowering	20.30	1	<0.001	128.75	1	$<2 \times 10^{-16}$
Week of flowering $\times$ Sex	6.30	1	0.012	124.28	1	$<2 \times 10^{-16}$
Calyx height						
Sex	91.53	1	$<2 \times 10^{-16}$	30.57	1	<0.001
Week of flowering	384.54	1	$<2 \times 10^{-16}$	6.82	1	0.009
Population	8.79	5	0.117	5.79	4	0.215
Onset of flowering	2.93	1	0.087	0.24	1	0.626
Week of flowering $\times$ Sex	0.93	1	0.334	0.03	1	0.867
Corolla diameter						
Sex	129.99	1	$<2 \times 10^{-16}$	76.63	1	$<2 \times 10^{-16}$
Week of flowering	500.18	1	$<2 \times 10^{-16}$	14.75	1	<0.001
Population	24.04	5	<0.001	6.41	4	0.170
Onset of flowering	42.44	1	0.15	11.36	1	0.001
Week of flowering $\times$ Sex	0.01	1	0.938	0.01	1	0.951
Petal length						
Sex	61.56	1	<0.001	43.35	1	<0.001
Week of flowering	847.41	1	$<2 \times 10^{-16}$	86.53	1	$<2 \times 10^{-16}$
Population	28.81	5	<0.001	3.81	4	0.432
Onset of flowering	58.63	1	<0.001	40.96	1	<0.001
Week of flowering $\times$ Sex	0.19	1	0.665	0.00	1	0.994
Petal width						
Sex	109.03	1	$<2 \times 10^{-16}$	127.30	1	$<2 \times 10^{-16}$
Week of flowering	834.52	1	$<2 \times 10^{-16}$	5.46	1	0.019
Population	7.51	5	0.185	0.19	4	0.996
Onset of flowering	38.06	1	<0.001	4.19	1	0.041
Week of flowering $\times$ Sex	0.88	1	0.348	0.56	1	0.455
Daily floral display						
Sex	522.92	1	$<2 \times 10^{-16}$	150.43	1	$<2 \times 10^{-16}$
Week of flowering	0.12	1	0.74	358.03	1	$<2 \times 10^{-16}$
Population	9.53	5	0.089	6.56	5	0.16
Onset of flowering	30.39	1	3.5×10^{-8}	88.39	1	$<2 \times 10^{-16}$
Week of flowering $\times$ Sex	7.38	1	0.006	4.85	1	0.029

variance in the number of flowers among male plants, we can expect a very high variance in total pollen production among males. In particular, although this should be confirmed by further studies, this implies that the most attractive males are potentially the most fertile and that natural and sexual selection should not act in opposite directions, at least regarding floral display and gamete production.

Sexual dimorphism

Our results confirm the patterns already documented in *S. dioica* by Kay (1984). Sexual dimorphism was found for all investigated floral traits. Male plants produce more and larger flowers than females. The most dimorphic trait is flower number, including both the average daily number of open flowers, which is expected to be one of the signals perceived by foraging pollinators, and the overall number of flowers produced at the end of the flowering season, which reflects the total investment in flower production. In our dataset, the sum of daily

flower number across the flowering season was 13 times higher in males than in females. Because flower lifespan is on average shorter in males compared to females (Kay *et al.*, 1984), this result is likely to be an under-estimation of the male/female difference in investment in flower production. Sexual dimorphism in flower number and/or size is common in dioecious species (Delph *et al.*, 1996; Barrett and Hough, 2013). Because no trade-off was found between these two traits, *S. dioica* provides a simple pattern of sexual dimorphism, with males exhibiting unequivocally higher visual attractiveness to pollinators. This is consistent with the expected effects of sexual selection in insect-pollinated flowering plants, which should lead to stronger selection to attract pollinators in male individuals, with female reproductive success being more limited by resources allocated to seed production (Bateman, 1948; Delph *et al.*, 1996; Moore and Pannell, 2011; Barrett and Hough, 2013). Petal size has been found to be heritable in *S. dioica* (Giles *et al.*, 2006) and is thus likely to respond to both natural and sexual selection. Nothing is known for now about the heritability of flower number, but the fact that average daily flower number correlates

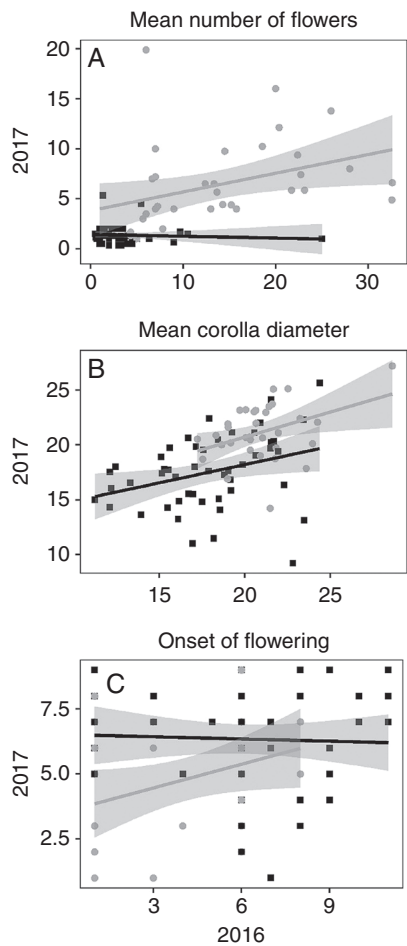


FIG. 3. Between-year correlations for floral traits measured on the same individuals during the 2016 and 2017 flowering seasons (cohort 1) for male (grey) and female (black) plants.

within individuals across years suggests that this trait might also have some genetic basis. However, because our study only investigated inter-annual phenotypic correlations, this result can also be explained by an inter-individual variance in the capacity of acquiring resources and/or different levels of inbreeding.

Temporal dynamics

We studied temporal dynamics at two scales: at the individual level, in order to capture within-individual variation of phenotypes across their first flowering season, and at the experimental population level, in order to estimate whether foraging visitors potentially experience different situations in terms of floral trait variation within a given plant population. Both males and females exhibited a similar decrease in floral size with time, and males also showed a decrease in pollen quantity. A decrease in biomass, nutrient supply or size of floral tissues has been observed in several hermaphroditic species and has been traditionally interpreted as the result of a shortage of resources, due to fruit and seed development from the first flowers (Wolfe, 1992; Brunet and Charlesworth, 1995; Diggle, 1997; Williams and Conner, 2001). However, as underlined by several authors,

TABLE 3. Analysis of deviance table for models testing for the effect of week of flowering, population of origin, flower number and corolla diameter on total pollen quantity per anther, proportion of viable pollen grains per anther and quantity of viable pollen per anther. Plant identity and mother plant were included as random factors.

	χ^2	d.f.	P
Pollen quantity per anther			
Week of flowering	90.72	1	2×10^{-16}
Population	13.48	5	0.02
Flower number	0.775	1	0.38
Corolla diameter	5.82	1	0.02
Proportion of viable pollen			
Week of flowering	2.39	1	0.12
Population	5.48	5	0.36
Flower number	0.35	1	0.55
Corolla diameter	2.72	1	0.09
Quantity of viable pollen			
Week of flowering	38.25	1	6.2×10^{-10}
Population	8.29	5	0.14
Flower number	0.12	1	0.73
Corolla diameter	6.66	1	0.009

resource decline across time can be confounded with ontogenetic mechanisms, such as a reduction in the quantity of vascular tissues resulting from temporal changes in the plant architecture (Wolfe, 1992; Diggle, 1997). The fact that male and female plants of *S. dioica* present the same temporal decline in flower size suggests that the cost of fruit development is not the only process affecting floral changes across the flowering season. Both a shortage of resources unrelated to fruit and seed production and ontogenetic changes may lead to the decline of both flower size and pollen production experienced by male plants in our study.

As a result, because the proportion of male and female plants at the beginning vs. end of their flowering season was extremely variable among dates, we found that the magnitude of sexual dimorphism in floral size fluctuated strongly across the flowering season. If floral size impacts the probability of attracting pollinators, as has been found in many different species (e.g. Vaughton and Ramsey, 1998; Martin, 2004), our results suggest that the degree of preference that pollinators exhibit for male flowers in this species (Kay et al., 1984; Carlsson-Granér et al., 1998) may not be stable across time.

In terms of daily flower number, the temporal dynamics was very different between males and females, leading to an extremely variable floral sex ratio at the population level over the flowering season. Such a sex difference could have two opposite consequences for pollination efficiency. First, if floral aggregation plays a role in pollinator attraction (as suggested by Kay et al., 1984; Thomson, 1988; Campbell, 1989; Ishii, 2006; Brunet et al., 2015), the preference of pollinators for male plants could increase over time, possibly increasing pollen limitation in females. Increased pollen limitation could even be reinforced in our case by the decline in pollen quantity produced per anther. On the reverse, male-biased floral sex ratio should overall increase the average quantity of pollen deposited on stigmas (Carlsson-Granér et al., 1998), decreasing pollen limitation and possibly increasing pollen competition.

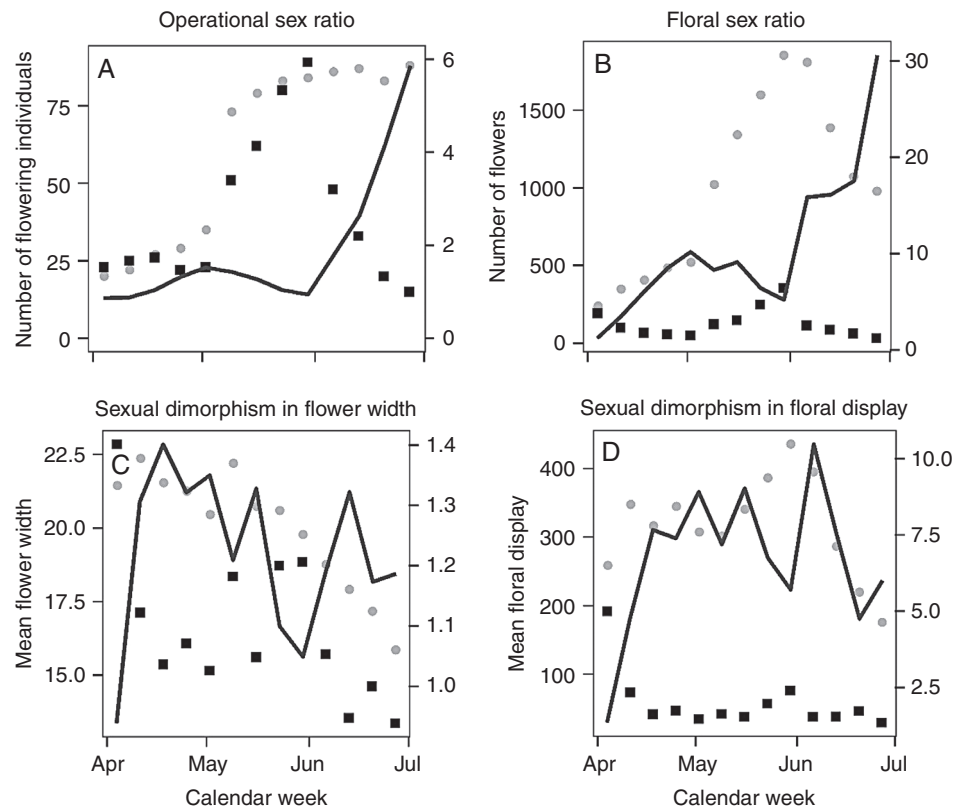


FIG. 4. Sex ratios and sexual dimorphism at the population level as a function of time across the flowering season in 2016. Black and grey dots show the average values for females and males, respectively. The ratios, visualized with the solid line (right y-axis), are all calculated as males/females, a value above 1 thus corresponding to a male bias. (A) Operational sex ratio taking into account the number of flowering individuals. (B) Floral sex ratio based on the number of open flowers of each sex per date. Sexual dimorphism expressed as the ratio between (C) the average floral size (corolla diameter) of males and average floral size in females and (D) the average floral display (number of flowers multiplied by corolla diameter) in males and in females.

Although the consequences in terms of pollinator behaviour and pollen dispersal now need to be dissected, our results underline the interest of investigating temporal variation in floral traits, as it probably affects pollination patterns across time.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Fig. S1: Individual maximum values of flower size traits according to population of origin and sex. Table S1: List of the eight populations of origin of the plants surveyed in the study. Table S2: Pearson's correlation coefficients among floral traits in male and female individuals based on traits measured in spring 2017 on cohort 2. Table S3: Pearson's correlation coefficients among floral traits in male and female individuals based on traits measured in spring 2016 on cohort 1, per study population. Table S4: Pearson's correlation coefficients among floral traits in male and female individuals based on traits measured in spring 2017 on cohort 2, per study population.

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