

Phenotypic selection on flowering phenology and size in two dioecious plant species with different pollen vectors

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Abstract

Dioecious plants may be pollinated biotically by animals or abiotically via wind or water currents. It has been hypothesized that these two types of pollen vectors might impose contrasting selective pressures on plant flowering phenology. In the present study we describe the flowering phenology of two sympatric dioecious species with contrasting pollination modes: *Mercurialis perennis* (wind-pollinated) and *Tamus communis* (insect-pollinated). We estimated selection differentials and gradients for flowering time and flowering synchrony. As flowering time might depend on the accumulation of enough internal resources, we also estimated direct and indirect selection on plant size. Both species have male-biased sexual ratios, and males are bigger and produce larger flower displays than females, but only in *T. communis* do males bloom earlier and for longer than females. Selection gradients suggest that selection tends to favor early-flowering females of *T. communis*. There is no evidence of direct current selection on the flowering phenology of *M. perennis*. Intersexual differences in phenology fit with sex allocation and sexual selection theories. As we hypothesized, phenology of the animal-pollinated species is under stronger selection than that of the wind-pollinated species and we discuss the potential role of pollen vectors in shaping the flowering phenologies of the study species.

Keywords: dioecy, flowering phenology, mutualism, phenotypic selection, wind pollination.

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Introduction

Dioecy is a plant sexual system where all genets in a population are either male or female (Richards 1986). This sexual system is relatively rare among angiosperms; it occurs in only approximately 6% of species (Renner & Ricklefs 1995) and evolved either directly from hermaphroditism or indirectly via gynodioecy, androdioecy or monoecy (Bawa 1980). Although it is widely accepted that the evolution of dioecy resulted from selective pressures promoting out-crossing, other ecological factors such as resource availability, sexual selection and pollination vectors are also important (Bawa 1980; Ashman 2006). These ecological factors can also interact and together result in the evolution of separate sexes; for example,

resource-mediated changes in plant phenotype can alter the degree of pollination and pollinator movements, and thus ultimately the sexual system (Ashman 2006). There exist some interesting correlations between dioecy and life-history traits. Dioecious plants are typically perennials, have fleshy fruit and small inconspicuous flowers, and are pollinated either by insects or wind (Bawa 1980; Renner & Ricklefs 1995; Vamosi *et al.* 2003). Dioecious plants are frequently highly generalist (e.g. Matsuyama *et al.* 2009), although they may be more specialized (with fewer insect pollinators) in the tropics (Renner & Feil 1993).

In flowering plants as a whole, wind pollination evolved from insect-pollinated plants, very probably as a reproductive assurance strategy (Culley *et al.* 2002), although it is still relatively rare compared with animal pollination (Ollerton *et al.* in press). Wind pollination has evolved more frequently in dioecious plants because

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reproductive assurance by selfing is not possible in unisexual plants (Friedman & Barrett 2009). Since Darwin (1876) some authors have noted the apparent disadvantage of wind pollination relative to animal pollination. It was believed that wind pollination was less efficient in terms of pollen deposition on conspecific stigmas compared with animal-pollinated plants. Contrary to this, recent evidence suggests that pollen limitation in wind-pollinated plants may not be as common as it is in animal-pollinated species and pollen transfer efficiency is not substantially lower than in animal-pollinated plants as is often assumed (Friedman & Barrett 2009; Barrett 2010). This is important because pollen limitation maximizes the opportunity for phenotypic selection (*sensu* Endler 1986) on floral traits, that is, variants that maximize pollen receipt will be favored (Harder & Aizen 2010). Flowering phenology is a trait that is frequently shaped by natural selection (reviewed by Kudo 2006, but see Ollerton & Lack 1992). Although there is some experimental evidence of pollinator-mediated selection on flowering time in some pollen-limited plant populations (e.g. Sandring & Ågren 2009), we do not know of any experimental study looking at selection on phenology in wind-pollinated plants. However, comparative studies of the flowering phenologies of wind and animal pollinated species have revealed some interesting patterns. Temporal niche partitioning is less evident for wind-pollinated species than for animal-pollinated ones (Bolmgren *et al.* 2003, but see Erenler & Gillman 2010). In contrast, wind-pollinated plants tend to flower more synchronously than animal-pollinated species (Rabinowitz *et al.* 1981; Bolmgren *et al.* 2003). These differences in phenological patterns between animal-pollinated and wind-pollinated species might suggest differences in selective pressures imposed by these pollen vectors; for example, we might predict that early flowering individuals of dioecious, wind-pollinated species would be at a selective disadvantage because of the lack of available mates. Although the same could be argued for biotically pollinated species, the targeted, constant nature of pollinator foraging behavior makes this less likely. Alternatively, wind-pollinated plants might be under stronger selection to flower synchronously than animal-pollinated plants because in wind-pollinated species pollen decay is very rapid, so it is crucial that potential mates are flowering synchronously and also because the anthers of wind-pollinated plants are typically only exposed for a few hours before they dry out and release all their pollen (e.g. Wei-Ning *et al.* 2006; Michalski & Durka 2007).

In the present study, we describe the flowering phenology and quantify phenotypic selection of two phenological traits: flowering time (day of the year a plant starts producing flowers) and flowering synchrony (the overlap of individual phenologies) of two sympatric dioecious

species with contrasting pollination modes. *Mercurialis perennis* L. (Euphorbiaceae) is a wind-pollinated plant and *Tamus communis* L. (Dioscoreaceae) is pollinated by insects. In the study area the wind velocity averages 7.4 knots year around and windless days are very rare. This means that wind is a highly reliable pollen vector of *M. perennis*. In contrast, insect visitation to flowers of *T. communis* in Britain is very infrequent (Burkill 1944). This suggests that blooming at the right time should be more critical in the case of *T. communis* and selection on flowering time might be stronger in this species, although stronger selection for synchronous flowering is expected in the wind-pollinated species *M. perennis*. We also examined the effect of individual plant size because the adaptive response of flowering phenology can be restricted by the need for sufficient time for vegetative growth and resource accumulation before flowering (Lacey 1986; Karlsson & Jacobson 2001). Our specific research questions were: (i) do the flowering phenologies of these sympatric species show similar patterns; (ii) is there evidence of phenotypic selection on phenology (flowering onset and synchrony) and size in these species; and (iii) does selection on flowering phenology and size differ between these two species?

Materials and methods

Study site and species

The study was carried out in patches of semi-natural vegetation in and around the University of Northampton, Northamptonshire, UK (57°17'N, 0°55'W), including the adjacent Scrub Field Local Nature Reserve (see Lamborn & Ollerton 2000 for a site description of the latter). Vegetation in this area consists of a matrix of grasslands, scrub, ancient hedgerows and small woodland fragments, some of which are dominated by the introduced Black Pine (*Pinus nigra*). *Tamus communis* is abundant only in the scrubbiest areas of the Scrub Field, whereas *Mercurialis perennis* grows across the area, but is particularly abundant in the *P. nigra* patches.

Mercurialis perennis is a rhizomatous perennial herb. Flowers in males and females are greenish, unisexual and do not produce floral nectar. Males have relatively long inflorescences and the stamens produce abundant pollen. Females have shorter inflorescences and have smaller flowers than males. Male and female flowers were not observed to be visited by any insects during the present study and all evidence suggests that the genus *Mercurialis* is wind pollinated (e.g. Jefferson 2008; Vandepitte *et al.* 2009). The fruits bear two ant-dispersed seeds (Jefferson 2008).

Tamus communis is a tuberous perennial with deciduous climbing foliage. The flowers are unisexual, yellowish

green, arranged in long inflorescences; both male and female flowers produce nectar. Female plants are shorter and have fewer flowers than male plants. The mature fruits are red berries typically containing six seeds, which are dispersed by birds (Burkill 1944). This plant is pollinated by several species of insects, mainly bees and flies, although all of them visit flowers very infrequently (Burkill 1944; Miguel A. Munguía-Rosas, pers. obs., 2009). There is no evidence of apomictic production of seeds in either *M. perennis* (Jefferson 2008) or *T. communis* (Burkill 1944).

Field methods

To record the wind-dispersed pollen density we hung 20 pollen traps (slides with a petroleum jelly coating on one side) along two linear transects in the population of *M. perennis*. The traps were placed 1.5 m from the forest floor and 4 m apart from each other. Although *M. perennis* only reach approximately 0.60 m tall we hung the traps 1.5 m from the forest floor anticipating that taller sympatric species may produce some interference later in the season. Owing to the position of the pollen traps our approach may underestimate absolute pollen density, but this gives us valuable data in relative terms. The traps were replaced every week and pollen of *M. perennis* on the traps was counted using a microscope. Pollen from *M. perennis* is very distinctive from other sympatric wind-pollinated species and easily recognizable under a microscope; to differentiate *M. perennis*' pollen from other species we made a collection of pollen from sympatric species. The probability of confounding *M. perennis* pollen with species not considered in the reference pollen collection cannot be ruled out completely because pollen of unknown species (and similar to *M. perennis* pollen) from distant places may have reached the pollen traps. However, the probability of finding such pollen is low in comparison with the abundance of pollen of *M. perennis*. In addition, daily records of wind velocity were obtained from the local weather station at Pitsford Hall, which records wind velocity once per day at 09.00 hours GMT.

Apparently sexually mature plants of the two species under study were tagged before floral buds appeared. When the plants started flowering, open flowers and fruits were counted once or twice per week; monitoring ended when the fruits were mature. At that time either whole tagged stems (*M. perennis*) or inflorescences (*T. communis*) were collected to calculate fruit set. Just before collection, plant size was measured as plant height in the case of *M. perennis* and maximum stem diameter for *T. communis*. Owing to differences in the growth habit and spatial distribution of the two species, we carried out the sampling with some technical variations. *Mercurialis perennis* grows in dense, discontinuous (possibly clonal)

patches in the study area and one or two stems per patch were randomly tagged. In total we tagged 99 stems in 64 patches. *Tamus communis* frequently have no accessible inflorescences because they often flower at the top of dense scrub; therefore, we tagged four inflorescences per plant in the more accessible plants. To ensure that the inflorescences belonged to the same individual, we tagged consecutive inflorescences along the same stem. In total 123 individuals were tagged initially. In both species some plants died, did not produce flowers or were removed by people during the study. These individual plants were excluded from some analyses. Fieldwork was conducted from February to August 2009.

Data analysis

Wind velocity and pollen density We examined temporal variation in wind velocity and the mean number of pollen grains of *M. perennis* on traps using generalized linear models with gaussian (wind velocity) or quasipoisson error distributions (pollen grains); the latter models were also used to assess the effect of wind velocity and the number of males in flower on the mean number of pollen grains of *M. perennis* on traps.

Intersexual differences in phenology We tested whether sex ratios deviated from 1:1 in both species using a binomial test. In the case of *T. communis*, plants were not randomly selected; therefore, in addition to tagged individuals we identified all clearly independent individuals in the study area in which the sex could be distinguished (231 plants in total). We obtained the following descriptors of individual flowering phenology: (i) the day of the year the plant started flowering, hereafter 'flowering onset'; (ii) the number of flowers produced; and (iii) the duration (in days) of individual flowering season. We compared between males and females the phenological descriptors (i–iii) and plant size using ANOVAS. Examination of the residuals (Crawley 2007) revealed a good fit of the data to a gaussian error distribution.

Selection differentials and gradients We calculated directional selection differentials and gradients following the approach of Lande & Arnold (1983). In these analyses, the independent variables (flowering onset, flowering synchrony and plant size) were standardized to have a mean of zero and a standard deviation of one and female fitness (fruit set) was relativized, that is, female fitness was defined as a ratio of the fitness value to the population mean. Selection differentials are derived from univariate regression analyses, where the trait is the independent variable and relative fitness is the dependent variable. The selection differential estimates total selection on a given trait and represents both direct selection and selection

resulting from correlations with other traits subject to selection. Selection gradients are measures of direct selection on a trait, independent of other correlated traits included in the model. To estimate linear selection gradients we carried out multiple regression analyses using relative female fitness as the response variable and standardized trait values as explanatory variables. To quantify non-linear selection (stabilizing or disruptive), a quadratic term was introduced into the regression model. To assess significance we built 95% confidence intervals using the bias corrected and accelerated method from 1000 bootstrap samples of the data (Dixon 2001). We checked for co-linearity between phenological traits using the variance inflation index VIF (Fox & Monette 1992) and examined any correlation between traits using Pearson correlation tests.

The phenological descriptors considered in the selection models (flowering onset and flowering synchrony) were estimated in an equivalent form in the two species. Flowering onset was considered to be the day of the year when we observed for the first time open flowers on the plant. Flowering synchrony refers to individual female synchrony with males, this calculus was based on a modification of Augspurger's (1983) index. The synchrony index of any i -th female to any males in the population (X_i) was defined as follows:

$$X_i = \left(\frac{1}{n}\right) \left(\frac{1}{f_i}\right) \sum_{j=1}^n e_{ij}$$

where e_{ij} is the number of census dates when the i -th female and j -th male are both in flower, f_i is the number of census dates when the female i -th is in flower and n is the number of males in the population. X_i varies from 0 (no overlap with males) to 1 (female flowering overlaps completely with all males). Male fitness was not estimated because it is technically very difficult in plants without pollen packaged in pollinia (but see Discussion). We carried out the analyses in R 2.10.1 (R Development Core Team 2009). Although flowering synchrony could be seen as a population level characteristic, it could arise or persist by natural selection because fitness may fluctuate as a function of the number of simultaneously flowering individuals (Parra-Tabla & Vargas 2007).

Results

Wind velocity and pollen density

During the flowering season of *M. perennis*, the wind velocity averaged 8.2 knots (standard error [SE] = 0.655). The relationship between the wind velocity and the day of the year did not show a slope different from zero (coefficient 0.001 ± 0.004 , $t_{57} = 0.286$, $P = 0.776$), indicating that

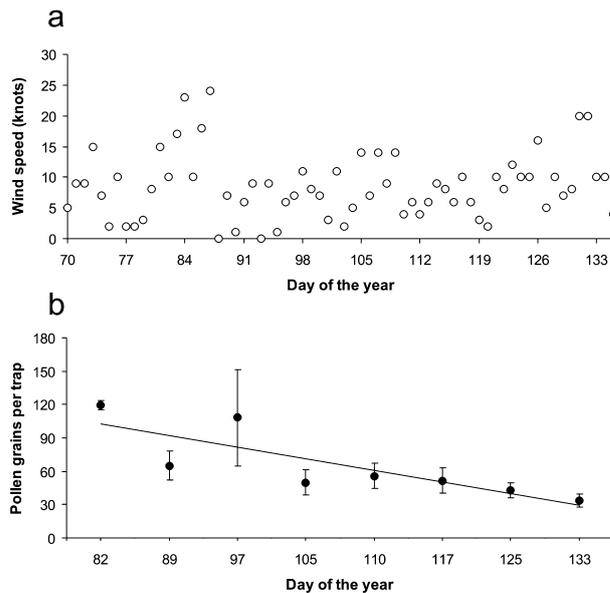


Fig. 1 (a) Daily records of the wind velocity in the study area and (b) the number of pollen grains of *Mercurialis perennis* found on the pollen traps. Data in (a) are from the closest meteorological station and data in (b) are mean ± 1 standard error. The regression line is also shown in (b).

the wind velocity during this period was relatively constant (Fig. 1a). Temporal variation in pollen grains on traps cannot be explained by wind velocity (coefficient 0.031 ± 0.035 , $F_{1,4} = 6.061$, $P = 0.069$) or number of males in flower (coefficient 0.002 ± 0.007 , $F_{1,4} = 0.076$, $P = 0.794$). However, the mean number of *M. perennis* pollen grains on traps decreased over the flowering season (coefficient 0.021 ± 0.006 , $F_{1,4} = 8.107$, $P = 0.046$; Fig. 1b).

Intersexual differences

Flowers of *M. perennis* were observed for the first time in March (70th day of the year) and flowers of *T. communis* in May (day 136). Flowering of *M. perennis* finished in May (day 133) and that of *T. communis* in July (day 185; Fig. 2). In the case of *T. communis*, males were larger, bloomed earlier and produced more flowers for a longer time than females (Table 1). Like *T. communis*, males of *M. perennis* were larger and produced more flowers than females (Table 1). However, unlike *T. communis*, males and females of *M. perennis* did not differ in flowering onset or in the duration of the reproductive event. The sex ratio was male skewed in both species (Table 1).

Selection differentials and gradients

Fruit set during the study was very high (mean = 0.852 per stem, minimum = 0.36, maximum = 1) and right

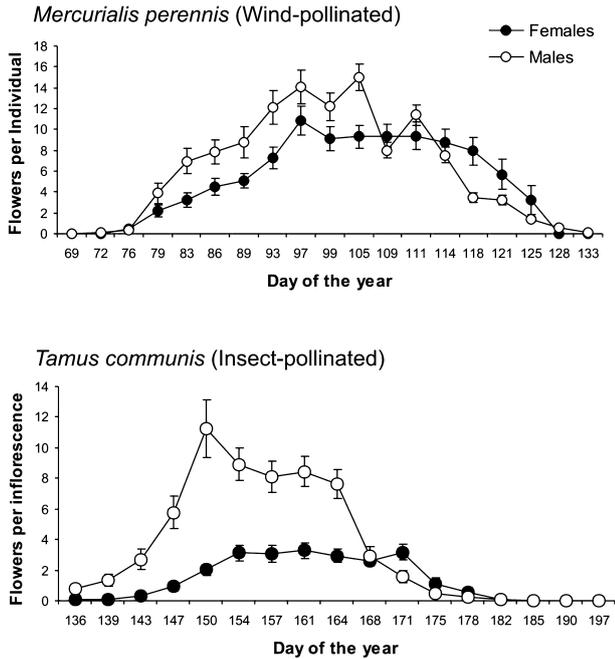


Fig. 2 Flowering phenology of two dioecious plant species with different pollen vectors: *Mercurialis perennis* is wind-pollinated and *Tamus communis* is insect-pollinated. The data shown (Y-axis) are mean production of flowers per plant (*M. perennis*) or inflorescence (*T. communis*) ± 1 standard error. The values on the X-axis are the days of the year, 1 = 1 January.

skewed in *M. perennis*; on the contrary, it was low (mean = 0.289 per inflorescence, minimum = 0, maximum = 0.86) and left skewed in *T. communis*. Only the linear selection differential for size (0.20) was significantly

different from zero in *M. perennis*. However, no selection gradient for any of the traits under study was statistically significant in this species (Table 2). In the case of *T. communis*, linear selection differentials for flowering onset (-0.35) and plant size (0.43) were statistically different from zero (Table 2). Among the selection gradients of the traits studied only linear (-0.58) and non-linear (-0.41) selection gradients for flowering onset were significant in this species. However, the linear selection gradient was greater and the confidence interval narrower than the non-linear selection gradient (Table 2).

Of the traits evaluated, we only identified significant correlations between flowering synchrony and flowering onset in both species: *M. perennis* ($r = 0.835$) and *T. communis* ($r = 0.580$). However, VIF values were slightly greater than three in *M. perennis* (flowering onset, 3.34; flowering synchrony, 3.44; size, 1.054) and lower than two in the case of *T. communis* (flowering onset, 1.449; flowering synchrony, 1.613; size, 1.1059). This suggests that there is no strong co-linearity between the traits examined.

Discussion

In the present study we found evidence that the reproductive behavior and flowering phenologies of the wind-pollinated species (*M. perennis*) and the insect-pollinated species (*T. communis*) have some similarities. In both species the sex ratio was male-skewed. In addition, males were larger and had a greater flower display than females. However, we also found some dissimilarities in terms of

Table 1 Summary of intersexual differences and associated statistics in two dioecious plant species with contrasting pollen vectors: *Tamus communis* (insect-pollinated) and *Mercurialis perennis* (wind-pollinated)

Species	Trait	Male	Female	Statistics
<i>M. perennis</i>	Sex ratio (plants)	50	21	0.70**
	Flowers (number)	20.27 \pm 1.588	11.65 \pm 1.42	$F_{1,69} = 10.25^{**}$
	Duration (days)	39.27 \pm 1.404	41.65 \pm 2.108	$F_{1,69} = 0.83$
	Onset (day)	82.96 \pm 1.07	81.30 \pm 1.59	$F_{1,69} = 0.70$
	Size (cm)	34.41 \pm 0.90	29.45 \pm 1.68	$F_{1,69} = 7.74^{**}$
<i>T. communis</i>	Sex ratio (plants)	131	100	0.57*
	Flowers (number)	15.91 \pm 1.73	5.81 \pm 0.46	$F_{1,83} = 32.52^{**}$
	Duration (days)	27.31 \pm 1.05	21.58 \pm 1.03	$F_{1,83} = 15.09^{**}$
	Onset (day)	144.45 \pm 0.76	151.12 \pm 0.96	$F_{1,83} = 29.15^{**}$
	Size (cm)	3.61 \pm 0.17	3.05 \pm 0.18	$F_{1,83} = 4.91^*$

The traits evaluated were sex ratio, flower production (Flowers) at inflorescence (*T. communis*) or stem level (*M. perennis*), duration of flowering period in days (Duration), the day of the year that the plants start to flowering (Onset) and the size of the plant (Size): stem diameter (*T. communis*) or plant height (*M. perennis*). The statistics shown are the F-values of one-way ANOVAS except for the sex ratio where the expected probability of success (i.e. males) relative to failure (i.e. females) in a Bernoulli experiment is shown. Sample size was 50 males and 21 females for *M. perennis* and 47 males and 43 females for *T. communis*; for the sex ratio analysis the sample size was different in the case of *T. communis*: 131 males and 100 females. * $P < 0.05$; ** $P < 0.01$.

Table 2 Linear selection differentials (S_i), linear selection gradients (β_i), non-linear selection differentials (C_i) and non-linear gradients (γ_{ii}) for flowering onset, flowering synchrony to male plants and plant size of two dioecious species with contrasting pollination mode: *Mercurialis perennis* (wind-pollinated) and *Tamus communis* (insect-pollinated)

Species	Trait	S_i	β_i	C_i	γ_{ii}
<i>M. perennis</i>	Onset	-0.01 (-0.41, 0.01)	-0.01 (-0.57, 0.30)	-0.01 (-0.61, 0.25)	0.19 (-0.85, 0.56)
	Synchrony	-0.09 (-0.30, 0.03)	0.07 (-0.21, 0.29)	-0.28 (-0.29, 0.03)	-0.11 (-0.53, 0.22)
	Size	0.20 (0.06, 0.42)	0.13 (-0.08, 0.35)	0.13 (-0.07, 0.35)	-0.08 (-0.36, 0.05)
<i>T. communis</i>	Onset	-0.35 (-0.67, -0.82)	-0.58 (-1.09, -0.19)	0.02 (-0.26, 0.30)	-0.41 (-0.97, -0.03)
	Synchrony	-0.07 (-0.49, 0.17)	-0.74 (-1.35, 0.04)	-0.18 (-0.42, 0.08)	0.01 (-0.24, 0.37)
	Size	0.43 (0.11, 0.69)	0.31 (-0.17, 0.67)	0.001 (-0.17, 0.30)	0.03 (-0.13, 0.45)

Confidence intervals are shown in parentheses below the gradients. Intervals excluding zero are statistically significant (in bold).

phenology between these two species. Males of *T. communis* bloomed earlier and for longer than females, whereas no differences in these phenological descriptors were found in the case of *M. perennis* (Fig. 2). The intersexual differences in phenology of males and females observed in *T. communis* agree with those predicted by sex allocation and sexual selection theories. In contrast, we found evidence of directional selection acting on flowering time in *T. communis* through female fitness (Table 2), which seems to fit to our expectation of stronger selection on flowering onset in the animal-pollinated species.

According to sex allocation theory, plants have limited resources for reproduction and both male and female fitness gain curves are limited by different factors (Charnov 1982; de Jong & Klinkhamer 2005). In dioecious plant species, the reproductive investment of males is restricted to flowering, whereas females must not only produce flowers, but also invest in developing embryos as well as the accessory structures that help in dispersal. Owing to these differences in resource investment gain of fitness is expected to be resource limited in females and mate limited in males (Charnov 1982; Anderson & Iwasa 1996). If investment in reproduction is at the expense of investment in other functions one would expect males of dioecious plants to be more abundant than females, develop larger flowering displays and bloom earlier than females (Stephenson & Bertin 1983) as suggested by sexual selection theory (Skogsmyr & Lankinen 2002). The predictions of these theories appear to be true for the two dioecious species we studied. Males are more abundant, larger and produce more flowers than females in both species (Table 1). However, males of *T. communis* also tend to flower earlier and for longer than females. We suggest that this might be the result of stronger male-male competition in *T. communis* than among males of *M. perennis*. This could be because of the paucity of pollinators in *T. communis*. According to our observations, male-male competition for pollinators should be stronger in *T. communis* owing to the scarcity of pollen visitors we saw in the field and reported in a previous study (Burkill 1944).

Selection gradient analyses suggest that selection on flowering onset of *T. communis* is stronger than on the onset of *M. perennis*. Selection on flowering onset is significant and negative in the case of *T. communis*; female fitness is greater in early flowering plants probably because early flowering females encounter more males with which to mate (Fig. 2b). In the long run, early flowering females will have a greater chance of being fertilized than plants flowering later in the season. Our results agree with a recent review on the evolution of flowering time, it appears that in flowering plants (mainly hermaphroditic species) selection tends to favor early flowering phenotypes (Elzinga *et al.* 2007; M. A. Munguía-Rosas *et al.* unpubl. data, 2010). Previous studies carried out in other animal-pollinated dioecious plants have also found evidence of selection on flowering onset (e.g. Biere & Honders 1996, but see Abe 2001). In contrast, we do not know of any study looking at selection on flowering time of a wind-pollinated dioecious species. Recent studies suggest that pervasive pollen limitation found in animal-pollinated relative to wind-pollinated plants may have favored the evolution of wind pollination from insect pollination in dioecious species (Friedman & Barrett 2009; Barrett 2010). It is expected that under pollen limitation, selection optimizes those traits that improve reproductive success (Harder & Aizen 2010). Female fitness in *T. communis* (29%) is far lower than female fitness in the wind-pollinated species, *M. perennis* (85%), which suggests that as expected the animal-pollinated species is more pollen limited than the wind-pollinated species. Although our results back up the prediction of stronger selection on the animal-pollinated species, we should recognize that there are some confounding factors, the most obvious being phylogenetic lineage and growth form. Although a comparison between related species may have reduced confounding factors drastically, such a comparison was not possible in the study area owing to a lack of suitable dioecious species. Dioecy is a rare sexual system confined to 6% of angiosperms (Renner & Ricklefs 1995), although it occurs in close to half of all families of flowering plants (Vamosi *et al.* 2003). In addition, in some floras many fami-

lies with dioecious species are either completely animal-pollinated or wind-pollinated (Kay & Stevens 1986). Therefore, to find a pair of dioecious species in the British flora for comparison without any confounding environmental factors (e.g. growth form, habitat) would be very difficult.

Although we expected stronger selection for synchronous flowering, particularly in the wind-pollinated species, phenotypic selection on synchrony was not significant in either species. A previous study suggested that flowering synchrony has no adaptive value in a hermaphroditic plant (Gómez 1993; but see Parra-Tabla & Vargas 2007 for an example in deceit-pollinated species) and this seems to be the case in the plants that we studied. Selection differentials for plant size were significant in both species, but the selection gradients for size were not significant. When selection differentials are significant, but selection gradients are not, indirect selection is invoked (Kingsolver *et al.* 2001). That means that size is usually correlated with female fitness, but does not have a selective value per se because selection is actually working on another size-correlated trait.

In the present study the phenotypic selection results we have shown are based only on female fitness and we did not evaluate male fitness. Evaluating male fitness in plant species with free pollen is technically very difficult. Although we did not evaluate selection on flowering onset through male fitness for the study species, we already know that in terms of selection on flowering onset, previous studies have shown that in hermaphrodites bearing pollinia, male and female fitness are highly concordant (O'Connell & Johnston 1998; Parra-Tabla & Vargas 2007; Sun *et al.* 2009).

Although insects are infrequent visitors to the flowers of *T. communis*, wind is clearly a reliable pollen vector of *M. perennis* pollen. Pollen on the traps shows a constant decay over the course of the flowering season of *M. perennis*, suggesting that pollen density is not affected by vector availability. Progressive decay of pollen on traps might be the result of the emergence of sympatric species or canopy closure reducing wind flow (and therefore pollen transport) late in the flowering season. Although pollen on the traps does not correlate with wind velocity, one should keep in mind that stigmas of wind pollinated species are far more efficient at capturing conspecific pollen than traps, owing to the anatomical and biochemical properties of their stigmas (Niklas 1985).

In conclusion, we found intersexual differences in the phenology of *M. perennis* and (particularly) *T. communis*. Only in the latter species did we find evidence of direct selection on flowering onset. Pollinator scarcity and competition for pollinators might have influenced this result. We recognize that owing to the contrasting phylogenetic

membership of the species we studied, we cannot suggest pollination mode as a major factor shaping the flowering phenology of dioecious plants in general. However, we hope that this study stimulates colleagues to carry out selection studies examining the phenology of dioecious species with different pollination modes in different places of the world and from diverse phylogenetic lineages so that general patterns can be analyzed in the future.

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