

## RESEARCH PAPER

# Phenological match drives pollen-mediated gene flow in a temporally dimorphic tree

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## Keywords

*Acer opalus*; dichogamy; microsatellites; neighbourhood model; phenology; separate pollen dispersal kernels.

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## ABSTRACT

- Variation in flowering phenology is common in natural populations, and is expected to be, together with inter-mate distance, an important driver of effective pollen dispersal. In populations composed of plants with temporally separated sexual phases (*i.e.* dichogamous or heterodichogamous populations), pollen-mediated gene flow is assumed to reflect phenological overlap between complementary sexual phases. In this study, we conducted paternity analyses to test this hypothesis in the temporally dimorphic tree *Acer opalus*.
- We performed spatially explicit analyses based on categorical and fractional paternity assignment, and included tree size, pair-wise genetic relatedness and morph type as additional predictors. Because differences between morphs in flowering phenology may also influence pollination distances, we modelled separate pollen dispersal kernels for the two morphs.
- Extended phenological overlap between male and female phases (mainly associated with inter-morph crosses) resulted in higher siring success after accounting for the effects of genetic relatedness, morph type and tree size, while reduced phenological overlap (mainly associated with intra-morph crosses) resulted in longer pollination distances achieved. Siring success also increased in larger trees.
- Mating patterns could not be predicted by phenology alone. However, as heterogeneity in flowering phenology was the single morph-specific predictor of siring success, it is expected to be key in maintaining the temporal dimorphism in *A. opalus*, by promoting not only a prevalent pattern of inter-morph mating, but also long-distance pollination resulting from intra-morph mating events.

## INTRODUCTION

The evolution of the exceptional sexual diversity in flowering plants, as compared to most animal groups, has been mainly attributed to their sessile nature (Barrett 2010). Seeds and pollen grains are the main mobile stages in a plant's life cycle. Thus, gene spread in plants principally depends on the spatial position of pollen or seed producers. As seeds disperse locally in many species, gene flow relies primarily on effective pollen dispersal, which is therefore crucial to prevent genetic erosion and inbreeding, especially in small and genetically isolated populations (Kramer *et al.* 2008). In addition to spatial arrangement and plant density, asynchronous flowering phenology may also shape pollen movement (Burczyk & Prat 1997; Gérard *et al.* 2006; De Cauwer *et al.* 2012). Indeed, heterogeneity in flowering schedules has been shown to promote assortative mating between phenologically similar groups (Fox 2003; Weis & Kossler 2004; Gérard *et al.* 2006), leading eventually to allochronic reproductive isolation (Devaux & Lande 2008).

Differences in the time of pollen dispersal and stigma receptivity among individuals are common within plant populations (Kudo 2006; Elzinga *et al.* 2007; Munguía-Rosas *et al.* 2011), and are expected to be especially critical in regulating mating

in dichogamous species, where sexual phases are separated in time. Heterodichogamy is a particular case of dichogamy based on differences in phenological schedules between two genetically determined sexual morphs (Gleeson 1982; Lloyd & Webb 1986; Renner 2001), in which mating patterns are expected to be governed by phenological overlap between the complementary sexual phases of the two morphs. Although it has been reported to be an infrequent breeding system (though its frequency may be underestimated; Renner 2001), it is phylogenetically widespread (Renner 2014) and may have evolved as a way of maximising pollen transfer efficiency while reducing sex ratio selection expected in dichogamous populations (Sargent *et al.* 2006). Heterodichogamous populations usually comprise two temporally reciprocal dichogamous morphs: a protandrous morph expressing a male to female flowering sequence, and a protogynous morph expressing the opposite (female to male) sequence (Renner 2001). Although mating events within heterodichogamous populations are predominantly disassortative (*i.e.* occurring between temporally reciprocal morphs), mating between individuals of the same morph (*i.e.* assortative mating) and within individuals (*i.e.* selfing) may occasionally occur (Bai *et al.* 2007; Gleiser *et al.* 2008a; Shang *et al.* 2012) and lead to unbalanced sex ratios (Shang *et al.* 2012). Also,

gene exchange between the two morphs may be asymmetric due to sexual specialisation in pollen export and/or receipt (Dommée *et al.* 1990; Gleiser *et al.* 2008a), which may be reflected in biased sex allocation patterns.

Ecological and evolutionary studies using molecular-based paternity analyses to measure siring success in heterodichogamous populations confirmed the prevalence of disassortative mating (Bai *et al.* 2007; Gleiser *et al.* 2008a; Shang *et al.* 2012). Also, flowering phenology surveys have demonstrated greater phenological overlap between sexual phases of complementary morphs (e.g. Sato 2002; Bai *et al.* 2006; Kikuchi *et al.* 2009). However, the premise that effective pollen dispersal in heterodichogamous populations should be driven solely, or predominantly, by phenological schedules has seldom been tested explicitly (but see Kikuchi *et al.* 2009; although based on very few individuals). Furthermore, the spatial dependence of pollen-mediated gene flow has never been properly accounted for in paternity studies performed on heterodichogamous populations. Paternity models estimating pollen dispersal kernels together with mating parameters constitute a robust approach to account for spatial effects, and thus, to reduce biases in the estimation of siring success (Oddou-Muratorio *et al.* 2005; Chybicki & Burczyk 2013). As heterogeneity in flowering phenology may affect pollination distances (e.g. Oddou-Muratorio *et al.* 2006), the temporal dimorphism characterising heterodichogamous species may result in differences in the pollen dispersal patterns of the two sexual morphs. It would therefore be relevant to model the possibility of differential pollen dispersal in heterodichogamous species by fitting paternity models with separate dispersal kernels for the sexual morphs (Lagache *et al.* 2013).

In this study, we conducted molecular marker-based paternity analyses to test the assumption that phenological match governs effective pollen transfer between individuals of the temporally dimorphic species *Acer opalus*. This species has been proposed to be originally heterodichogamous (*i.e.* its populations being composed by protandrous and protogynous individuals in equal proportions; Gleiser *et al.* 2008b), but has gone through a process of sexual specialisation, probably triggered by a reduction in the quality of seeds produced towards the end of the flowering season (Pannell & Verdú 2006; Gleiser *et al.* 2008a). A previous study showed that mating in this species was predominantly disassortative, and that the sexual morphs differed in their phenological mating opportunities and siring success (Gleiser *et al.* 2008a). However, the link between siring success and flowering phenology was only inferred indirectly, and without considering the effect of other potentially confounding factors. Here, we specifically assessed the role of flowering phenology as a determinant of siring success in *A. opalus* by applying spatially explicit paternity models, and considering genetic relatedness between mating partners, morph type and plant size as other possible correlates of siring success. Paternity assignment was based in both categorical and fractional paternity. In the latter case, we developed an approach that allowed modelling separate pollen dispersal kernel parameters for each sexual morph.

## MATERIALS AND METHODS

### Study system

*Acer opalus* subsp. *granatense* (Boiss.) Font Quer & Rothm (*A. opalus* hereafter) is a small endemic tree distributed in SE

Spain, Mallorca Island and northern Morocco (López González 2004). Populations are sexually polymorphic, being composed of male (M hereafter), protandrous (PA hereafter) and protogynous (PG hereafter) individuals. Individuals of the PA morph are specialised in the male function and produce very few female flowers or frequently fail to produce them, effectively functioning as M individuals. Individuals of the PG morph always express both sexual functions and make most of the maternal contribution in the population, both in terms of seed quantity and quality (Gleiser *et al.* 2008a,b). Siring success of M and PA largely exceeds that of PG individuals (Gleiser *et al.* 2008a). Then, basically, populations are functionally dimorphic, being composed of M/PA and PG morphs in equal proportions, with the former functioning essentially as male, but producing a few late-flowering female flowers from time to time (Gleiser *et al.* 2008a,b). Flowering occurs in spring, and pollination is completely entomophilous (Gleiser *et al.* 2008b). Seeds are winged samaras and are dispersed by gravity; the mean dispersal distance of seeds was reported to be around 2–4 m, with a maximum observed distance of 12.5 m (Gómez-Aparicio *et al.* 2007).

The study was conducted in a typical population of *A. opalus* occurring on a north-facing slope in the Font Roja Natural Park, eastern Spain (38°40'N, 0°30'W). This site was also the focus of other reproductive biology studies characterising reproductive patterns in *A. opalus* (Verdú *et al.* 2004; Gleiser *et al.* 2008a,b).

### Sampling, genotyping and phenology records

A total of 129 trees (67 M/PA and 62 PG) were marked within an area of 1000 × 550 m and the spatial coordinates of each tree were recorded with a GPS. Tree size was estimated by measuring trunk diameter (see Gleiser *et al.* 2008a). Leaves from each tree were collected ( $N = 129$  adults), dried in silica gel and stored at  $-20\text{ }^{\circ}\text{C}$  until DNA extraction. Progeny arrays used for paternity analyses were obtained by collecting 24 seeds from each of 22 PG mothers ( $N = 526$  offspring, as two seeds had to be discarded due to amplification problems) in autumn 2004, just before the dispersal period.

The DNA extraction protocols for both leaf and seed material are given elsewhere (see Gleiser *et al.* 2008a). Candidate fathers, mothers and offspring (total  $N = 655$ ) were genotyped using seven microsatellite markers: Aop122, Aop132, Aop450, Aop820, Aop918, Aop943 (Segarra-Moragues *et al.* 2008) and Map9 (Pandey *et al.* 2004), which together provided a cumulative paternity exclusion probability of 0.999 (Segarra-Moragues *et al.* 2008), and unique multilocus genotypes for each candidate father. For a detailed description of PCR conditions, scoring protocols and levels of polymorphism obtained in the study population, see Pandey *et al.* (2004), Segarra-Moragues *et al.* (2008) and Gleiser *et al.* (2008a).

Phenological data were obtained during the flowering season of 2004, which was characterised by a high frequency and intensity of flowering. Phenology was recorded on 90 out of the 129 genotyped trees (41 PG and 49 M/PA) during the flowering season (beginning of April to end of May), by recording whether a tree was in female phase (carrying female flowers with receptive stigmas) or in male phase (carrying male flowers releasing pollen) every 2–9 days, depending on the rate of flowering. The extent of phenological overlap of a given tree

releasing pollen with sampled individuals carrying female flowers was calculated as:

$$PO_k = \left(\frac{1}{n}\right) \sum_{j=1}^n \text{ovl}_{kj}/d_j \quad (1)$$

where PO reflects the mean relative phenological overlap during the flowering season of any  $k$  individual releasing pollen (belonging to any of the two sexual morphs) with the sampled  $j$  individuals with receptive stigmas (*i.e.* including only PG trees from which progeny arrays were sampled; see above);  $\text{ovl}_{kj}$  is the number of days during which the  $k$ -th and  $j$ -th individuals were simultaneously releasing pollen and carrying receptive female flowers, respectively;  $n$  is the number of individuals carrying ovules that were sampled as mothers for paternity analyses; and  $d_j$  the total number of days during which the mother  $j$  carried receptive stigmas. This phenological overlap index ranged from zero, when a focal tree released pollen without matching any receptive ovules in the mother trees sampled for paternity analyses, to one, when it released pollen completely overlapping the period over which sampled mothers carried receptive stigmas.

#### Paternity analyses

We applied two different spatially explicit approaches to assess the role of phenological overlap between mating partners in driving effective pollen dispersal. First, the categorical assignments already reported in Gleiser *et al.* (2008a) were regressed against phenological overlap and other candidate correlates of siring success (see below). Then, we applied a full probability mating model based on fractional assignment, which allowed joint estimation of mating, flowering synchrony, fecundity and pollen dispersal kernel parameters. Categorical assignment is still the most popular approach and it is more biologically intuitive, as it assigns whole offspring genotypes to most likely fathers, while full probability paternity models usually produce less biased estimation of population-level parameters (Hadfield *et al.* 2006; Jones *et al.* 2010; Harrison *et al.* 2013). The latter approach requires a non-standard statistical treatment, however (*e.g.* Chybicki & Burczyk 2013; see below).

#### Categorical assignment

Categorical assignments were obtained using the approach implemented by the software FaMoz (Gerber *et al.* 2003; see Gleiser *et al.* 2008a). In this analysis, paternity of each offspring is assigned on the basis of LOD (log of the likelihood ratio; Meagher 1986) scores. LOD score thresholds for assigning paternity are established by simulations that build an empirical statistical test aiming to minimise both type I and II errors (Gerber *et al.* 2000). Simulations were run by considering a departure from Hardy-Weinberg equilibrium of 0.1 and a mistyping error of  $1 \times 10^{-4}$  (Gleiser *et al.* 2008a). Only those candidate fathers for which the LOD score fell above the threshold value and which did not have any genotypic mismatch with the offspring were considered as the true fathers. By running this analysis, we could assign paternity to 164 offspring (after excluding 30 selfing events), 77% being fathered by M/PA and 23% by PG trees (see Gleiser *et al.* 2008a). Based on this information, siring success (*i.e.* number of sired offspring) of each candidate father with each potential mother

(*i.e.* all possible candidate father–mother pairs were considered) was used as dependent variable in sequential regression models, all of which contained pair-wise spatial distances (*i.e.* the Euclidean distance between the candidate father and the mother) to account for spatial effects. Tested models included, besides the effect of phenological overlap between a given candidate father and mother (*i.e.* each individual term  $\text{ovl}_{kj}/d_j$  of the equation above), candidate father size (as evaluated by trunk diameter), sexual morph (coded as zero if the father was M/PA or as one if it was PG), and genetic relatedness between the candidate father and the mother. Pair-wise genetic relatedness between trees was estimated from genetic data using the software SPAGeDi (Hardy & Vekemans 2002). Two coefficients which make no assumptions about Hardy-Weinberg equilibrium proportions were computed: Loiselle's kinship coefficient (Loiselle *et al.* 1995) and Queller & Goodnight's (1989) relationship coefficient; however, given the high correlation obtained between these two measures (Mantel's  $r = 0.93$ ,  $P = 0.001$ ), only models including Loiselle's coefficient were kept. The relationship between siring success and the mentioned covariates was modelled through zero-inflated generalised mixed linear models with a negative binomial error structure, because our dependent variable contained many zeros (see Ison *et al.* 2014 for a similar approach). Both mothers and candidate fathers were considered as random effects. Multiple regressions were run using the *glmmadmb* function from the *glmmADMB* (Fournier *et al.* 2012; Skaug *et al.* 2014) R package (R Core Team 2016); predictor variables were  $z$ -transformed to obtain comparable slope estimates. Collinearity was inspected by estimating variance inflation factors (VIFs) in all multiple regression models with the random effects removed using the *vif* function from the *car* (Fox & Weisberg 2011) R package (R Core Team 2016); none of the models considered had significant collinearity problems, the highest VIF obtained being 1.77 (Table S1), much lower than the critical value of 10 (Quinn & Keough 2002, p. 128).

#### Mating model

The second approach consisted in a fractional paternity analysis based on the spatially explicit full probability mating model known as the *neighbourhood model* (see Burczyk *et al.* 2002; Oddou-Muratorio *et al.* 2005; Klein *et al.* 2008; Chybicki & Burczyk 2013). In this model, we assumed that the  $i$ -th seed collected from the  $j$ -th (mother) tree can result either from pollination with pollen coming from outside the study plot, self-fertilisation of the  $j$ -th tree or cross-fertilisation between the  $j$ -th tree and the  $k$ -th (father) tree ( $k = 1 \dots N$ , where  $N$  is the number of trees in the study plot). The probability that the  $i$ -th seed in the  $j$ -th family has the  $O_{i,j}$  genotype is:

$$\begin{aligned} \Pr(O_{i,j}) = & m \times \Pr(O_{i,j}|T_j, B) + s \times \Pr(O_{i,j}|T_j, T_j) \\ & + (1 - m - s) \times \sum_k^N \varphi_{jk} \times \Pr(O_{i,j}|T_j, T_k) \end{aligned} \quad (2)$$

where  $\Pr(O_{i,j}|T_j, B)$  is the Mendelian probability of  $O_{i,j}$  given the  $j$ -th maternal genotype  $T_j$  and the background allele frequencies ( $B$ ),  $\Pr(O_{i,j}|T_j, T_j)$  is the Mendelian probability of  $O_{i,j}$  given the  $j$ -th maternal genotype  $T_j$  after self-fertilisation,

$\Pr(O_{i,j}|T_j, T_k)$  is the Mendelian probability of  $O_{i,j}$  given the  $j$ -th maternal genotype  $T_j$  and paternal genotype  $T_k$ ,  $m$  is the probability of pollen immigration,  $s$  is the probability of self-fertilisation, and  $\varphi_{jk}$  is the probability that the  $k$ -th tree is a pollen donor for the  $j$ -th tree.  $\varphi_{jk}$  is often referred to as the relative mating success of the  $k$ -th father tree, because, for any  $j$ ,  $\sum_{k=1}^N \varphi_{jk} = 1$ . The likelihood of the model was computed, assuming that seeds were drawn independently, as the probability of observing the genotypes of all sampled progeny individuals, *i.e.*  $L = \prod_i \Pr(O_{i,j})$ .

In our model,  $\varphi_{jk}$  was treated as a function of three factors: pollen dispersal kernel, fecundity of the  $k$ -th father tree and flowering synchrony between the  $j$ -th mother and the  $k$ -th father trees. As in earlier implementations, we assumed that pollen dispersal follows the exponential-power dispersal kernel with scale parameter,  $a$ , and shape parameter,  $b$  (Oddou-Muratario *et al.* 2005; Chybicki & Burczyk 2013). To address the possibility of unequal pollen dispersal depending on sexual morph, pollen dispersal was modelled using two separate kernel functions. Thus, the probability of pollen dispersal at a distance equal to the Euclidean distance between the  $j$ -th and the  $k$ -th tree was:

$$\pi_{jk} = \begin{cases} c \times \exp\left(-\left(\frac{\sqrt{(x_j - x_k)^2 + (y_j - y_k)^2}}{a_1}\right)^{b_1}\right) & \text{if the } k\text{-th tree is the 1st morph type} \\ c \times \exp\left(-\left(\frac{\sqrt{(x_j - x_k)^2 + (y_j - y_k)^2}}{a_2}\right)^{b_2}\right) & \text{if the } k\text{-th tree is the 2nd morph type} \end{cases}$$

where  $x_j$  and  $y_j$  are Cartesian coordinates for the  $j$ -th tree (given in metres), and  $c$  is the normalising constant. Because we did not have data on individual pollen production, we used tree size as a proxy for male fecundity. The fecundity of the  $k$ -th tree was then proportional to:

$$f_k \propto \exp(\beta \times z_k)$$

where  $z_k$  is the standardised tree size for the  $k$ -th father tree and  $\beta$  is the effect of tree size on fecundity. Finally, flowering synchrony between the  $j$ -th and the  $k$ -th tree was modelled as:

$$g_{jk} = \exp(\gamma \times \text{ovl}_{jk})$$

where  $\text{ovl}_{jk}$  is the phenological overlap in flowering days between the  $j$ -th mother and the  $k$ -th father tree (as defined above), and  $\gamma$  is the effect of flowering synchrony on mating success. Taking all these factors into account, the relative mating success for the  $k$ -th tree was computed as:

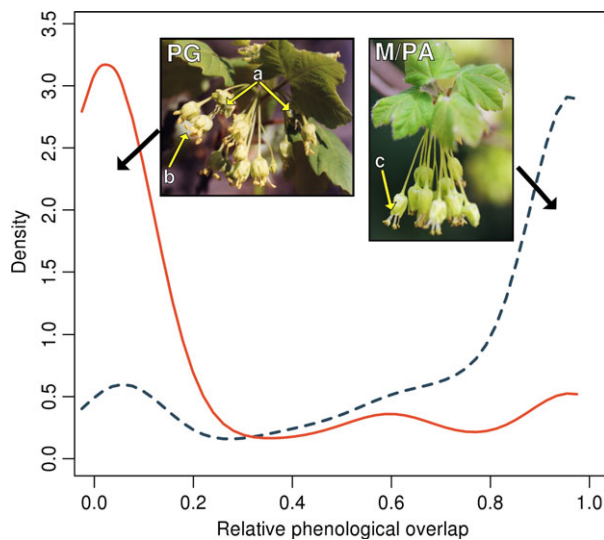
$$\varphi_{jk} = \frac{\pi_{jk} \times f_k \times g_{jk}}{\sum_{l=1}^N \pi_{jl} \times f_l \times g_{jl}}$$

To estimate the parameters of the model using the observed data, we used the Bayesian approach implemented in the NM2F software (Chybicki & Burczyk 2013), which was modified appropriately to account for separate dispersal

kernels and flowering overlap. Generally, the method is a class of Markov Chain Monte Carlo algorithms (MCMC) to generate a series of random samples from the posterior distribution of parameters. A single cycle of MCMC consisted of two steps. First, detailed genealogies of offspring were inferred (*i.e.* auxiliary variables, described in Chybicki & Burczyk 2013), given the current set of parameter values. Then, new parameter values were sampled based on those genealogies and assumed prior distributions. Unlike Chybicki & Burczyk (2013), we treated  $m$  and  $s$  to be common for all mother trees (families). In consequence, their values reflected average pollen immigration and self-fertilisation rates, respectively, across all families. We assumed that background allele frequencies ( $B$ ) were not differentiated among families. We also assumed that individual fecundities  $f_k$  were a function of tree size, and that they were not allowed to vary randomly as independent parameters. In this way, we avoided problems of over-parameterisation of the mating model, given our relatively small sample size. We ran 250,000 MCMC cycles; after 10,000 initial cycles, we recorded parameter values every 25 cycles. Consequently, we obtained 9600 random samples from the posterior distributions of parameters, for which a median and the 95% highest posterior density intervals (HPD) were computed. We compared the fit of eight alternative mating models, including different parameters (Table S2). To quantify the fit of a given model, we estimated the Deviance Information Criterion (DIC) as defined by Spiegelhalter *et al.* (2002). Models with DIC increased by 10 or more compared to the best model (the one with the lowest DIC) were considered as having essentially no empirical support, while those models with DIC only increased by less than 2 compared to the best model were considered as having substantial empirical support (Burnham & Anderson 2002, p. 70). Intermediate DIC differences provided considerably less support compared to the best model.

## RESULTS

We found strong evidence of complementarity between sexual morphs in flowering phenology (measured as  $PO$ ), as expected. Specifically, phenological overlap between the male phase of the M/PA trees and the female phase of the PG trees was greater than the overlap between the male and the female phases within the PG trees (mean overlap  $\pm$  standard error:  $0.79 \pm 0.01$  for M/PA *versus*  $0.22 \pm 0.01$  for PG; One-way ANOVA  $F_{1,21} = 78.6$ ,  $P < 0.001$ ; Fig. 1). This pattern was not related to differences in sexual morph size (mean size  $\pm$  SE:  $23.80 \pm 1.40$  cm for M/PA and  $23.85 \pm 1.58$  cm for PG; one-way ANOVA  $F_{1,88} = 0.001$ ,  $P = 0.98$ ). In addition, a Mantel test between the matrix of pair-wise Euclidean distances and a matrix depicting whether two trees belonged to the same (coded as one) or to different (coded as zero) sexual morphs allowed us to discard any significant spatial pattern of sexual morph aggregation (Mantel's  $r = 0.0009$ ,  $P = 0.63$ ). Similarly, genetic relatedness (measured with Loiselle's kinship coefficient) was not higher for alike sexual morphs (Mantel's  $r = 0.005$ ,  $P = 0.29$ ). However, the population was genetically structured, as evidenced by the significant decrease in pair-wise kinship (both sexual morphs considered together) with pair-wise spatial distance (slope =  $-0.014$ , 95% CI =  $-0.0020$ ,  $0.0016$ ; Figure S1).



**Fig. 1.** Density plots showing the distribution of relative phenological overlap (PO) estimates for M/PA individuals as sires (blue dashed line) and PG individuals as sires (red solid line). Curves were drawn using the *sm.density-compare* function from R package *sm* (Bowman & Azzalini 2014). Photo on the left: inflorescence from a PG tree with (a) developing winged fruits still retaining the stigmatic lobes, which lose turgidity once they become non-receptive, and (b) male flowers about to start pollen release. Photo on the right: inflorescence from a M/PA tree with (c) male flowers releasing pollen.

As expected, paternity success based on categorical assignment was highly dependent on the distance to the mother tree, with siring success decreasing with increasing distance between mates (Table 1). Model selection based on Akaike's information criterion (AIC) did not allow selection of a single model among all the categorical assignment models tested, with three models showing the best fit to data (Table 1; models G to I). Comparison of these models shows that both sexual morph and kinship between mates seemed to have poor explanatory power once the other variables were included in the models, as their inclusion did not significantly increase model fit to data (*i.e.* increasing the difference in AIC in more than two units). Therefore, from a conservative point of view, only spatial distance between mating partners, phenological overlap, and tree size predicted siring success (see Arnold 2010), with siring success increasing with decreasing distance between mates, increasing phenological overlap and when siring trees were larger.

A single best-fitting spatially explicit mating model was selected based on DIC (Table S2, Table 2). This model included positive effects on siring success of both phenological overlap and tree size, in agreement with regressions based on categorical paternity assignment. Selfing rate was low and similar in magnitude to that reported in a previous study (Gleiser *et al.* 2008a), confirming that the species is self-compatible, but that mating is predominantly allogamous. Pollen immigration from outside the study plot was considerable, with  $m = 0.47$ . Interestingly, the best fitting mating model also showed that pollen dispersal patterns differed between the sexual morphs, with the PG morph dispersing pollen at longer distances, as the posterior median of the dispersal distance (a less biased estimator of dispersal distance as

compared to the posterior mean; see Chybicki & Burczyk 2013) was over four-fold greater for PG than for M/PA. The estimation of the shape parameters for the two sexual morphs described fat-tailed kernels, indicating that, occasionally, both morphs disperse pollen at long distances.

## DISCUSSION

Variation in flowering phenology among individuals is common in natural populations, being, together with inter-mate distance, an important driver of effective pollen dispersal. In plant sexual polymorphisms based on flowering schedules, *i.e.* dichogamy and heterodichogamy, effective pollen dispersal is expected to reflect phenological overlap between complementary sexual phases; however, other factors may deviate effective mating from what is expected based on phenology alone. Using spatially explicit paternity analyses, we confirmed the significant role of phenological overlap in directing pollen movement in a temporally dimorphic *A. opalus* population. Flowering phenology was the single morph-related trait explaining siring success; however, it was not the only, nor the main determinant of paternity success. Not surprisingly, the main determinant of paternity success was plant proximity (see Table 1), with siring success decreasing with increasing distance between mates. Also, plant size (a proxy of flower production in a mast year, as that sampled) was as important as phenology as a determinant of siring success, with paternity probability increasing with plant size.

While the relevance of inter-mate distance in driving contemporary pollen flow patterns in plants is undeniable, the role of phenological match between mates has often been neglected (Ison *et al.* 2014), perhaps because scoring phenology requires greater sampling effort. Phenological match between mates has proved, however, to affect effective pollination distances (Kitamoto *et al.* 2006; Oddou-Muratorio *et al.* 2006), to maintain species hybrid zones (Gérard *et al.* 2006; Field *et al.* 2011) or to influence the amount of gene flow between crops and weedy relatives (Roumet *et al.* 2015). In the case of temporally polymorphic species, phenological match between complementary sexual phases is indeed thought to be the main driver of mating patterns. In spite of this, the role of phenology as an effective pollen transfer driver, or whether other traits may deviate mating from expectations based on phenology alone, have seldom been assessed (but see Kikuchi *et al.* 2009). Indeed, even if paternity studies performed in heterodichogamous *Juglans mandshurica* (Bai *et al.* 2007) and *A. opalus* (Gleiser *et al.* 2008a) confirmed the prevalence of disassortative over assortative mating, these studies did not assess explicitly the link between phenology and siring success, and whether other factors may affect this potential association. In fact, a phenological survey in *J. mandshurica* predicted higher levels of potential assortative mating as compared to estimates of realized paternity. In this species, intra-morph relatedness was higher than inter-morph one, and it was suggested that the reduced level of assortative mating observed could have resulted from a decrease in fertility of intra-morph crosses (Bai *et al.* 2007). In contrast, in our study in *A. opalus*, levels of kinship within and between morphs were similar, and, in fact, siring success was independent of the genetic relatedness between mates.

Full probability mating models of paternity represent probably the most robust approach for accounting for spatial effects

**Table 1.** Akaike's information criterion (AIC) and standardised partial regression coefficients (with SE between brackets) for the mixed-effects models linking siring success with selected explanatory variables (see Material and Methods). Variables were standardised to zero mean and unit variance prior to analyses, to make regression coefficients comparable in magnitude.  $\Delta$ AIC is the difference between the AIC of a given model and the lower AIC value obtained. Significant regression coefficients are highlighted in bold.

Model name	Null	A	B	C	D	E	F	G	H	I
AIC	866.4	693.4	671.6	676.8	690	695.1	670.9	666.9	665.9	667.2
$\Delta$ AIC	200.5	27.5	5.7	10.9	24.1	29.2	5	1	0	1.3
Spatial distance (log)		<b>-1.30 (0.10)</b>	<b>-1.28 (0.09)</b>	<b>-1.30 (0.10)</b>	<b>-1.33 (0.10)</b>	<b>-1.32 (0.11)</b>	<b>-1.28 (0.09)</b>	<b>-1.31 (0.09)</b>	<b>-1.31 (0.09)</b>	<b>-1.34 (0.09)</b>
Phenological overlap			<b>0.63 (0.13)</b>				<b>0.46 (0.16)</b>	<b>0.63 (0.12)</b>	<b>0.45 (0.16)</b>	<b>0.47 (0.16)</b>
Sexual morph				<b>-0.60 (0.14)</b>			<b>-0.28 (0.17)</b>		<b>-0.28 (0.16)</b>	<b>-0.26 (0.17)</b>
Size					<b>0.31 (0.13)</b>	<b>-0.05 (0.09)</b>		<b>0.31 (0.11)</b>	<b>0.32 (0.11)</b>	<b>0.33 (0.11)</b>
Kinship										<b>-0.07 (0.08)</b>

in the estimation of mating and male fecundity parameters (Buczyk *et al.* 2002; Oddou-Muratorio *et al.* 2005; Klein *et al.* 2008; Chybicki & Buczyk 2013). In these models, usually one single kernel curve is fitted to describe overall pollen dispersal spatial patterns in a population (Austerlitz *et al.* 2004). However, effective pollen dispersal is expected to differ among individuals (Robledo-Arnuncio *et al.* 2014), and, in populations in which there are discrete phenotypic classes that can be distinguished (such as in heterodichogamous ones), it may be relevant to account for a plausible heterogeneity in pollen dispersal kernels. Consequently, here we modified the traditional neighbourhood model as implemented in the NM2F software (Chybicki & Buczyk 2013) to allow for the possibility that the two morphs in the studied *A. opalus* population may disperse pollen differentially, and indeed, we found that the two best models assumed separate kernels. Judging by posterior estimates of pollination distances (see Table 2), PG trees dispersed pollen at longer distances as compared to M/PA trees. The difference in pollination distances between morphs may have resulted from the difference in phenological match with the female phase of the PG morph. Indeed, it is probable that the sampled protogynous mothers were surrounded by far more M/PA than PG trees releasing pollen, which may have resulted in longer pollen dispersal distances achieved by the PG morph. In fact, long-distance pollination associated with phenological mismatches has already been reported elsewhere (*e.g.* Oddou-Muratorio *et al.* 2006; Ison *et al.* 2014).

The fact that the two sexual morphs disperse pollen at different distances may have interesting evolutionary implications. Because the M/PA morph sires considerable more seeds than the PG morph, the evolutionary maintenance of the male phase in the PG morph may seem unlikely at first sight. However, a previous theoretical model using data on sex allocation and mating opportunities predicted the evolutionary maintenance of this monoecious morph (Gleiser *et al.* 2008a). In addition to this, the longer pollination distances achieved by PG sires may confer further male fitness advantages to this morph. In genetically structured populations suffering from inbreeding depression, the potential for long distance pollen dispersal can be advantageous as it may overcome the negative genetic effects of mating between spatially (and thus genetically) close individuals. In the studied *A. opalus* population, pair-wise kinship decreased significantly with the distance between trees (Figure S1). Although the distance at which two plants are expected to be genetically unrelated must be taken with care (*x*-axis intercept in Figure S1) as it depends on sampling design (Vekemans & Hardy 2004), we expect PG trees to mate, on average, with less related PG mothers, and thus, their progeny to be more prone to escape from the negative effects of biparental inbreeding. Furthermore, given that seed dispersal is locally restricted, offspring sired by PG trees will probably establish far away from their fathers and thus, siring competition between PG fathers and their offspring (once they reach reproductive maturity) is expected to be reduced. Therefore, the maintenance of the male phase in the PG morph may be fuelled by a fitness advantage arising from siring progeny at longer distances. Although appealing, this idea contrasts with previous evidence, which showed that progeny from controlled PG-PG crosses grew less than progeny from inter-morph crosses during a 3-year period under greenhouse conditions (Gleiser *et al.* 2008b). Evidently, only long-term fitness studies will help to assess

**Table 2.** Estimates of selfing rate ( $s$ ), pollen immigration ( $m$ ), mean pollen dispersal distance in metres ( $\delta$ ) and shape ( $b$ ) of the pollen dispersal kernel (modelled as an exponential-power function), and regression slopes resulting from the effects of phenological overlap ( $\beta_{\text{overlap}}$ ) and tree size ( $\beta_{\text{size}}$ ) on male fecundity. These estimates were obtained from the best fitting model according to DIC criteria (see Table S2 for model comparison parameters), which included separate pollen dispersal kernels for the M/PA and PG morphs. Explanatory variables were standardised to zero mean and unit variance prior to analyses.

	$s$	$m$	Pollen dispersal kernel				$\beta_{\text{overlap}}$	$\beta_{\text{size}}$
			M/PA		PG			
			$\delta$	$b$	$\delta$	$b$		
Posterior mean	0.12	0.47	203.20	0.22	2869.16	0.16	0.46	0.50
Posterior median	0.12	0.47	73.84	0.22	326.75	0.16	0.45	0.50
2.5% CI	0.09	0.42	32.69	0.08	37.37	0.07	0.12	0.27
97.5% CI	0.15	0.52	1,358.04	0.41	25,324.92	0.29	0.81	0.72

whether pollination at long distances significantly increases male fitness in this monoecious morph.

## CONCLUDING REMARKS

Phenological overlap was an important driver of effective pollen dispersal in the temporally dimorphic species *A. opalus*. Although siring success also depended on the spatial distance between mates and on tree size (a proxy of reproductive effort), flowering phenology was the single morph-specific predictor of siring success, suggesting, in a general sense, that selection for higher phenological match is expected to be key in maintaining the temporal sexual polymorphism in this species. Nonetheless, the reduced phenological overlap between pollen release and stigma receptivity within the PG morph may explain their longer pollination distances achieved, which may in turn be important for the evolutionary maintenance of the male function in this morph, provided the decrease in genetic load in their progeny results in a fitness increase. This hypothesis suggests a trade-off between producing more offspring (through a higher phenological overlap) and producing offspring of higher quality (by siring seeds at longer distances, as a product of an incomplete intra-morph phenological overlap), which would be interesting to explore in future studies.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Mean kinship coefficients against spatial distance in the *Acer opalus* study population.

**Table S1.** Variance inflation factors (VIFs) calculated for all categorical paternity-based multiple regression models (models B to I as defined in Table 1).

**Table S2.** Model comparison parameters, with models ranked from best to worst according to DIC criteria.

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