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Sexual interference revealed by joint study of male and female pollination success in chestnut

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Abstract

Most seed plants produce both pollen and ovules. In principle, pollen export could interfere with pollen import through self-pollination, resulting in ovule usurpation and reduced fruit set. Evidence for such interference exists under experimental settings but its importance under natural conditions is unknown. To test for sexual interference in nature, it is necessary to study together mating system, through paternity analyses, and fruit set, the proportion of flowers giving seeds or fruits. We developed a new model combining both processes, using chestnut (*Castanea*) as case study. We carried out a paternity analysis in an intensively studied plot of 273 trees belonging to three interfertile chestnut species and including a range of individuals with more or less functional stamens, resulting in a large data set of 1924 mating events. We then measured fruit set on 216 of these trees. Fruit set of male-fertile trees was much lower than that of male-sterile trees. Our process-based model shows that pollen is not limiting in the study site and hence cannot account for reduced fruit set. It also indicates that self-pollination is high (74%) but selfing rate is low (4%). Self-pollen is less competitive than cross-pollen, reducing sexual interference, but not sufficiently, as many ovules end up being self-fertilized, 95% of which abort before fruit formation, resulting in the loss of 46% of the fruit crop. These results suggest that the main cause of reduced reproductive potential in chestnut is sexual interference by self-pollen, raising questions on its evolutionary origins.

KEYWORDS
Castanea, fruit set, interspecific barriers, paternity analyses, self-incompatibility, spatially explicit mating model

1 | INTRODUCTION

Most seed plants are cosexual, producing both pollen and ovules (Lloyd, 1982). Hence, individual plants, if they manage to successfully export pollen from their anthers to a receptive compatible stigma and import pollen from a compatible pollen donor to their own receptive stigmas, will function as both paternal and maternal parents to the next generation. However, conflicts in their parental roles during pollination and mating could result in gamete wastage and loss of reproductive potential, called sexual interference or self-interference (Barrett, 2002; Lloyd & Webb, 1986). Is that mechanism truly significant in plant populations? The question remains largely open. For instance, Barrett (2002) provides numerous evidence in favour of the existence of sexual interference in plants but predicts

that it would be difficult to detect it in nature "because extant traits should have evolved to minimize its intensity in contemporary populations".

Cosexual plants, if they emit pollen when they are receptive, can deposit it on their own stigmas, resulting in self-pollination, a potentially important source of sexual interference. First, if self-fertilization takes place following self-pollination, and if there is inbreeding depression in the self-fertilized offspring, the corresponding ovules can be lost for reproduction (Charlesworth & Charlesworth, 1987). Inbreeding depression can result in the loss of the embryo early on, before a seed is produced (early inbreeding depression), or later on, for instance at the time of seed germination, with similar consequences for maternal fitness but not for fruit set (defined as the proportion of flowers giving mature fruits or seeds). Second, self-pollination can be detrimental to plant female fitness for reasons other than inbreeding depression. For instance, if too abundant on stigmas, self-pollen could restrict access of cross pollen, a phenomenon called pollen clogging (Aizen & Harder, 2007; Barrett, 2002). In plants that have a late-acting form of self-incompatibility (Seavey & Bawa, 1986), self-pollen tubes might reach the ovary but fail to penetrate the ovule; or they might penetrate the ovules but syngamy does not take place; or they might fertilize the ovule but the zygote is not recognized by ovarian tissues and degenerates (Gibbs, 2014). All these mechanisms result in ovule usurpation (loss of cross-fertilization opportunities when ovules are disabled by self-pollination) or embryo loss and hence reduced fruit set, even when there is an unlimited supply of pollen. In this context, it is not surprising that many plants equipped with physiological mechanisms of self-incompatibility nevertheless also possess floral traits that limit self-pollination (Bertin, 1993; Harder & Barrett, 1995; Lloyd & Webb, 1986; Webb & Lloyd, 1986).

Previous investigations of the costs of self-pollination in outcrossing species have employed experimental approaches using mixtures of self and cross pollen applied together or successively on stigmas. Most available studies report significant reductions in female fertility when self-pollen proportion increases (e.g., Bertin & Sullivan, 1988; Broyles & Wyatt, 1993; Byers, 1995; Galen et al., 1989; Husband & Schemske, 1996; Johnson et al., 2019; Waser & Price, 1991). However, such approaches, because of their manipulative nature, cannot estimate the amount of sexual interference actually taking place under natural conditions. To evaluate the consequences of sexual interference in nature, we need to test if increased pollen production and export is associated with less successful pollen import. Unfortunately, these two processes tend to be studied with different approaches, in line with the largely independent development of genetic and ecological perspectives in evolutionary biology (Harder & Barrett, 1996). Pollen export and corresponding male fitness are typically studied with genetic markers and paternity analyses whereas pollen import and female mating success are typically studied using fruit set measurements. An integrated model of pollen export and pollen import would represent a major achievement in pollination science, making it possible to study the interactions between the two processes.

To develop this approach, we can build on earlier mating system studies. Research during the last 30 years has led to the development of spatially explicit mating models informed by powerful DNA-based marker data. For hermaphrodite plants, researchers have relied on mixed-mating models, which consider the production of a mixture of self-fertilized (selfed) and outbred (outcrossed) seeds. They have been considerably refined since the first publications on the topic (Adams et al., 1992; Adams & Birkes, 1991; Schoen & Stewart, 1986). They can now be used to identify intrinsic and extrinsic factors influencing male fecundity (Burczyk et al., 1996; Chybicki et al., 2021; Klein et al., 2008; Lagache et al., 2014; Roeder, Devlin & Lindsay, 1989; Tani et al., 2009). They can clarify how far pollen travels, which travel path is used by pollinators, and which pairs of individuals can mate, depending on their flowering phenology and other factors (Burczyk & Prat, 1997; Klein et al., 2008; Lander et al., 2013; Oddou-Muratorio et al., 2005). The models have also been extended to accommodate hybridization (Klein et al., 2017), allowing to derive field-based estimates of interspecific mating barriers (Lagache et al., 2013). However, whereas intergenerational models exist that integrate pollen and seed dispersal to study male and female fecundity (Bontemps et al., 2013; Burczyk et al., 2006; Chybicki & Oleksa, 2018; Oddou-Muratorio et al., 2010; Oddou-Muratorio & Klein, 2008), female mating success is never considered. Indeed, all mixed-mating models are rooted in the genetic analysis of extant seeds or seedlings, ignoring failed female mating attempts.

Instead, the focus in most field studies of pollination ecology is on the proportion of flowers that do not develop into fruits because of pollen limitation in quantity or in quality (Burd, 1994; Larson & Barrett, 2000; Stephenson, 1981). In animal-pollinated plants, failures to set fruits can be caused by a lack of pollenizers (pollen donor plants), a lack of pollinators (animals that move pollen from the male anther of a flower to the female stigma of a flower), inefficient pollen transfer, low pollen-tube survival or zygote death (Harder & Aizen, 2010; Wilcock & Neiland, 2002). Just as for male fecundity, female mating success depends on a range of intrinsic and extrinsic factors, including the ability of individual plants to attract pollinators (Hegland & Totland, 2008; Mitchell et al., 2009; Totland & Sottocornola, 2001). At first sight, studying female mating success seems more straightforward and accessible than investigating male mating success, as the proportion of empty and filled seeds can be directly counted with no need for heritable markers (Bernasconi, 2003). However, the information acquired on female mating success by comparing fruit set measurements with the spatial distribution of potential pollen donors is very limited. This is due to the absence of a mechanism linking fruit set with the distribution of pollen donors and with pollen dispersal (Ahee et al., 2014; Knapp et al., 2001; Platt et al., 1974; Silander, 1978) and to the lack of consideration of self-pollination rates.

At first sight, measuring the effect of self-pollination on fruit set under natural conditions seems particularly difficult, as it requires that we distinguish between different mechanisms responsible for failures for flowers to set fruits, which can be caused

either by a lack of pollen or by poor quality pollen. In fact, fruit set measurements have long been used as indicators of pollen limitation, without distinguishing between pollen quantity and pollen quality effects (Aizen & Harder, 2007). For our study, we sought to model separately flowers that fail to set fruits because of self-pollination and flowers that fail to set fruits because of lack of pollen.

A promising method to investigate together male and female mating success would be to combine marker-based male fecundity and pollen dispersal studies with fruit set studies, thereby integrating genetic and ecological data. This approach would make it possible to test if plants that produce and export a lot of pollen have decreased fruit set due to sexual interference caused by self-pollination. To reconstruct the composition of the pollen pool at the time of pollination, the model needs to consider pollen movement among plants and the possible advantage of outcross-pollen over self-pollen (Christopher et al., 2020; Darwin, 1877). To model self-incompatibility, two types of processes need to distinguished, one acting early on after pollination and another one at the time of fertilization or slightly afterwards. First, we need to consider the difference in competitive ability of self- and outcross-pollen during pollen tube growth, as it could modify their proportion between pollination and fertilization. Second, we need to account for the differential mortality of selfed- versus crossed-fertilized ovules or embryos caused by late-acting self-incompatibility or early inbreeding depression, as it could reduce fruit set. By combining these two mechanisms, it should be possible to model a range of responses, from no effect on fruit-set when pollen is not limiting (simple competition between self- and cross-pollen) to abortion of all self-fertilized ovules through late-acting self-incompatibility or early inbreeding depression.

Here, we develop for the first time such a complete pollination model, by coupling a spatially explicit mating model based on paternity analyses with a fruit set model. Sexual interference caused by self-pollination is likely to be most severe in species with large floral displays, such as trees, because the transfer of pollen between flowers on the same plant (geitonogamy) inevitably increases with plant size (de Jong et al., 1993). Chestnut (genus *Castanea*, Fagaceae), an insect-pollinated tree cultivated for its fruits (Larue, Austruy, et al., 2021), was chosen as case study. Chestnuts are large insect-pollinated mass-flowering trees producing huge amounts of pollen, with record-high pollen: ovule ratios (Larue, Austruy, et al., 2021; Petit & Larue, 2022). The main chestnut pollinators (beetles and calyptrate flies) are not very mobile (Larue, 2021). In keeping with these observations, single pollen grain genotyping experiments have shown that most of the pollen deposited on stigmas is self-pollen (Hasegawa et al., 2009). Chestnut trees may also be characterized by prezygotic late-acting self-incompatibility and early-acting inbreeding depression (Seavey & Bawa, 1986; Xiong et al., 2019). Together, these biological features could lead to high rates of ovule discounting and reduced fruit set. Actually, emasculation experiments in chestnuts have shown that artificially reducing self-pollination greatly increases fruit set (Larue, 2021; Zhao & Liu, 2009), supporting the hypothesis that sexual interference is involved.

By focusing on an exhaustively sampled isolated chestnut population (Larue, Barreneche, & Petit, 2021a), we hope to source most fruits to their corresponding pollen donor using powerful genotyping techniques (Larue, Guichoux, et al., 2021). This should provide detailed description of the pollen pool sampled by each mother tree. In combination with fruit set measurements, this study design should eventually allow us to create a complete pollination model, by coupling male and female mating success models. Here, we derive model parameters and assess model performance. We then use the model to test the hypothesis that, under natural conditions, self-pollination does reduce fruit set in chestnut. This approach should help evaluate whether sexual interference, an outcome of sexual selection (Barrett, 2002), represents an important factor in chestnut reproduction and evolution.

2 | MATERIALS AND METHODS

2.1 | Study site

The 273 studied trees are located in Villenave d'Ornon, near Bordeaux, in south-western France (44.788319 N, −0.577062 E). The study site has been previously described (Larue, Barreneche, & Petit, 2021a). Most trees (242) belong to the INRAE chestnut germplasm collection hosted in two nearby orchards. The trees belong to three chestnut species and their hybrids. The three species are the European chestnut (*Castanea sativa*), the Japanese chestnut (*C. crenata*) and the Chinese chestnut (*C. mollissima*). The first orchard, planted in 1970, comprises only 29 large widely spaced trees on 2.3 ha. The second orchard, planted in 1990, comprises 213 trees on 3.5 ha. All 242 trees are grafted on two rootstocks: "Marsol" (CA07) and "Maraval" (CA74), two hybrids of *C. sativa* and *C. crenata*. We also identified 24 small but already fertile trees in the nursery and seven adult trees located within a distance of one kilometre from the studied orchards. All these trees were geolocated using a Garmin 64st gps and their coordinates are expressed in lambert 93 (Figure 1). We verified tree positions and corrected them using QGIS Software (Qgis Desktop 3.16.4) and satellite photos from IGN BdOrtho (https://geoservices.ign.fr/bdortho).

2.2 | Monitoring of flowering phenology

To study temporal compatibility between trees, we described in detail the flowering phenology of each individual tree using a scale specifically developed for chestnuts (Larue, Barreneche, & Petit, 2021b). We scored phenology during the late spring of 2018, twice a week during six weeks, between June and mid-July. During each monitoring session, we gave three scores to each tree: one for male flowers of unisexual male catkins (PhM), another for female inflorescences

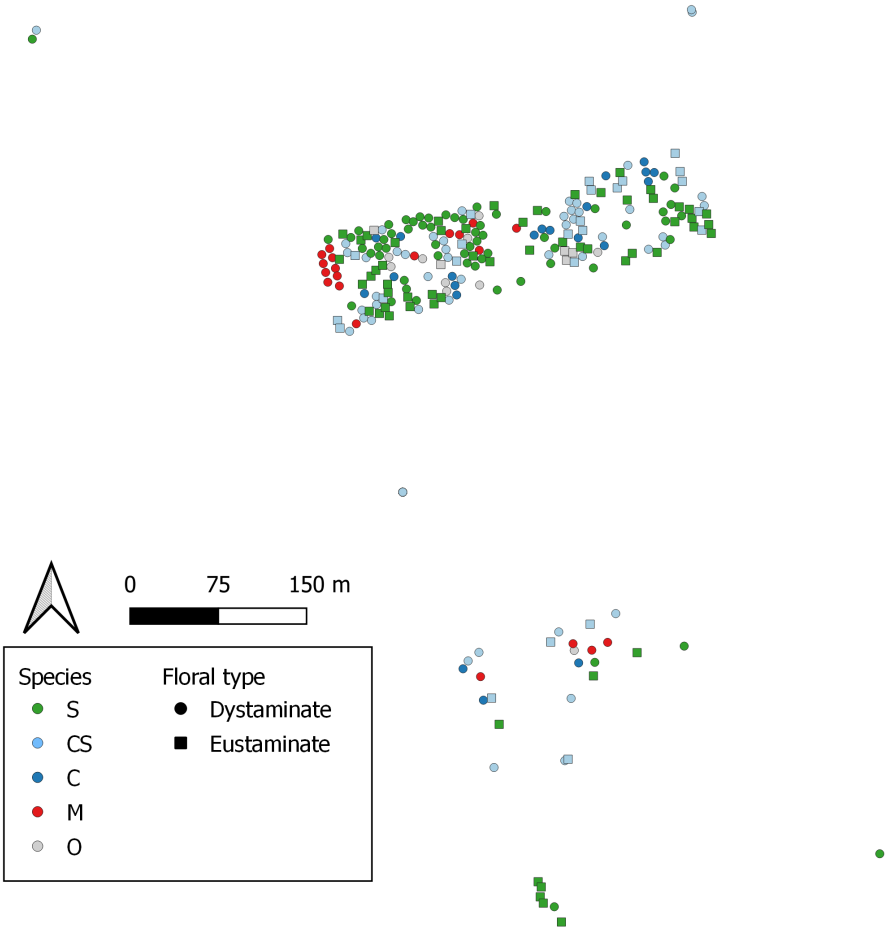


FIGURE 1 Map of all trees from the INRAE chestnut collection and from chestnut trees growing in the orchards vicinity used in this study. Shape of symbols indicates male flower type, whereas its colour indicates the species.

of bisexual catkins (PhF) and a final one for male flowers of bisexual catkins. For the model presented in this article, we used only the two first scores (i.e., PhM and PhF).

2.3 | Phenotype characterization

To obtain proxies of male fitness, we described the capacity of the trees to produce pollen in spring 2018 (Larue, Barreneche, & Petit, 2021a). After examining male flowers of each tree (i.e., number of stamens and length of stamen filaments), we assigned it to one of four flower type categories: astaminate, brachystaminate, mesostaminate and longistaminate (Solignat & Chapa, 1975). For subsequent analyses, we considered only two categories of flowers (FType covariate). The first, which we named "dystaminate", includes trees with male catkins made of male flowers with poorly developed stamens (i.e., astaminate, brachystaminate and mesostaminate trees). The second category, called "eustaminate", includes all the other trees with male flowers having fully developed stamens (i.e., longistaminate trees). For each tree, we also measured canopy average diameter (in metres) and calculated its canopy area (CA covariate, measured in square metres) by assuming that the canopy is half-spherical and visually estimating the proportion of missing canopy. Finally, we measured the average length of the male catkins of each tree (the FLength covariate, measured in centimetres). When phenotypic data were missing for a tree, we replaced these by the mean value of the corresponding variable.

2.4 | Fruit set estimation

In chestnuts, female inflorescences are composed of three female flowers located side by side, the central one flowering slightly before the two lateral ones (Solignat & Chapa, 1975). Each flower, if pollinated, produces an indehiscent fruit protected by a pericarp of maternal origin. A fruit typically contains a single seed, seed set being thus equivalent to fruit set. If pollination of a flower fails, the pericarp is still present, but remains empty. After flowering, female inflorescences turn into spiny infrutescences called burrs, in which three (full or empty) fruits are enclosed. If all three fruits are empty, the burr can fail to develop and fall early in the season. Sampling a large number of burrs is straightforward and fruit set is calculated by counting the number of developed or empty fruits present in each burr (Larue, Austruy, et al., 2021). During fall 2018, we collected at least 20 burrs, corresponding to 60 fruits, in the canopy of each tree or on the ground underneath the canopy. In fall 2019, we targeted at least 30 burrs per tree (90 fruits) to increase resolution. We estimated fruit set by counting the number of developed and aborted fruits per burr, excluding burrs with only aborted fruits.

2.5 | Chestnut germplasm collection

We previously genetically characterized all trees from the collection with SNP markers (Larue, Barreneche, & Petit, 2021a). Briefly, in

May 2018, we collected one or two young leaves from all trees. We isolated DNA from 50 mg of leaves using a custom-made CTAB protocol with 1.4 M NaCl lysis buffer (Larue, Guichoux, et al., 2021). We then assigned each tree to a species (Larue, Guichoux, et al., 2021). We identified 121 *C. sativa*, 94 *C. sativa* × *C. crenata* hybrids, 21 *C. crenata*, 20 *C. mollissima*, and 17 other interspecific crosses subsequently pooled in the analyses (2 *C. crenata* × *C. mollissima* hybrids, 10 *C. sativa* × *C. mollissima* hybrids and 5 three-ways hybrids). Among these trees, there were 187 eustaminate trees (68%) and 86 dystaminate trees (32%) (Table 1).

2.6 | Fruit sampling and DNA isolation

To perform paternity analysis, we collected at least 40 burrs and up to 95 on 43 mother trees in September 2018 (Table 2). We chose genets with multiple ramets randomly distributed to vary the pollen environment. These 43 mother trees belong to the three chestnut species and one category of hybrids (*C. sativa* × *C. crenata*). For each burr, we isolated DNA either from one randomly selected fruit or from all three fruits. All samples were stored at −20°C until DNA isolation. We isolated DNA from 50 mg of chestnut tissue using CTAB custom DNA isolation protocol for 96 well plate format with 2.4 M NaCl lysis buffer (Larue, Guichoux, et al., 2021). We checked the quality of isolated DNA with a spectrophotometer Thermo Scientific NanoDrop 8000.

2.7 | SNP genotyping

We characterized all individuals at 79 SNP using Agena MassARRAY Platform (Larue, Guichoux, et al., 2021) and checked the raw data using MassARRAY Typer Analyser 4.0.26.75 (Agena Biosciences). We excluded loci with weak or ambiguous signal (i.e., unclear cluster delimitation) and retained 68 SNP to perform the subsequent analyses.

2.8 | Categorical paternity analysis

A categorical paternity analysis was performed with the software CERVUS (Kalinowski et al., 2007). We calculated allelic frequencies using the 113 unique genotypes of all adult trees constituting the INRAE chestnut germplasm collection. We list all parameters used for the simulation of paternity analysis in Supporting Information 1; self-fertilization was enabled. We calculated confidence intervals using LOD score and delta LOD with a relaxed level of 95% and a strict level of 99%.

2.9 | Pollen limitation and sexual interference

When a plant receives high quality pollen mixture, in which all pollen grains are viable and compatible, only the amount of pollen received limits pollination success (Figure 2, green curve). A plateau is reached rapidly after which nearly all female flowers produce a fruit. Before that, a slight pollen increase will have a strong positive effect on pollination success (limitation by quantity). However, the mixture of pollen received by a plant is rarely entirely compatible. If a fraction of the pollen grains cause ovule abortion for different reasons, such as late-acting self-incompatibility or early inbreeding depression in the case of self-pollen (Figure 2, red curve), this reduces fruit set. After some threshold, an additional amount of pollen no longer increases pollination success (limitation by quality).

Usually, to estimate pollen limitation, researchers manually add pollen to female flowers in so-called pollen supplementation experiments. Aizen and Harder (2007) have criticized this method, arguing that if the pollen mixture received by control plants differs from that used in supplementation experiments, the results will be difficult to interpret. The precise composition of the pollen mixture naturally received by a mother plant is generally unknown. Hence, these supplementation experiments cannot differentiate the effects of pollen quantity and quality on pollination success. We designed the model presented below to fill that gap.

TABLE 1 Composition of the studied plot: Chestnut tree sample sizes by species and flower type, expressed in number of clones (grafted varieties) and ramets (trees)

Species	Sativa		Hybrids		Crenata		Mollissima		Others		Total		All
Flower type	E[a]	D[b]	E	D	E	D	E	D	E	D	E	D	
No. clones	26	19	27	8	7	0	17	0	6	3	83	30	113
No. ramets	64	57	70	24	21	0	20	0	12	5	187	86	273
% clones	0.23	0.17	0.24	0.07	0.06	0.00	0.15	0.00	0.05	0.03	0.73	0.27	1.00
% ramets	0.23	0.21	0.26	0.09	0.08	0.00	0.07	0.00	0.04	0.02	0.68	0.32	1.00

[a]E, eustaminate trees.
[b]D, dystaminate trees.

TABLE 2 List of mother trees sampled and results of the paternity analysis for all genotyped seeds

					Fathers					
		Ext[a]	Self[b]		*C. sativa*[c]	Hybrid	*C. crenata*	*C. mollissima*	Others	Total
	Father relative abundance	–	–		0.37	0.39	0.10	0.09	0.06	1
Mothers	*C. sativa*	# Fruits sired	6	27	318	244	52	18	51	716
	Hybrid		5	38	117	417	338	45	48	1008
	C. crenata		0	0	5	58	6	7	4	80
	C. mollissima		0	10	2	4	4	66	34	120
	C. sativa	Frequencies	0.01	0.04	0.44	0.34	0.07	0.03	0.07	1.00
	Hybrid		0.00	0.04	0.12	0.41	0.34	0.04	0.05	1.00
	C. crenata		0.00	0.00	0.06	0.73	0.08	0.09	0.05	1.00
	C. mollissima		0.00	0.08	0.02	0.03	0.03	0.55	0.28	1.00

[a]Ext, number/frequency of fruits sired by fathers located outside of the study site.
[b]Self, number/frequency of selfed fruits.
[c]Number/frequency of fruits sired by fathers from each species.

2.10 | Modelling and parameter estimation

2.10.1 | Spatially-explicit mating model

We used a spatially explicit individual-based mating model to investigate intra- and interspecific mating events with pollen from inside and outside the studied stand. This approach allows a simultaneous estimation of all the parameters influencing male fecundity and pollen dispersal using a Bayesian approach for random effect mating model (Chybicki et al., 2019; Klein et al., 2008; Klein & Oddou-Muratorio, 2011). We added several components to adjust to the present data set and to the questions of interest. Specifically, (i) we coupled the standard likelihood of pollen pool composition with the likelihood of fruit set; (ii) we considered several species with fertilization barriers between each species pair; (iii) we modelled the effect of male and female flowering dates on mating probabilities (for the male flowering date, only the first peak of pollen emission was considered); (iv) we included a clone-ramet information with some degree of self-sterility among ramets of the same clone; and (iv) we used two models for the fruit set likelihood, one with self-sterility only and the other with self-sterility together with pollen limitation. In both models, we investigated the effects of covariates on fruit set. We also accounted for mistyping at a maximum of two loci per genotype (using a mistyping rate of 0.001 at all loci) and for the presence of null alleles at 12 of the 68 loci. We identified these null alleles by checking for Mendelian segregation in mother trees using MismatchFinder, a custom made software (Larue, Guichoux, et al., 2021).

2.10.2 | Likelihoods

More specifically, the full likelihood of the data writes:

$$L(\text{data}|\ \text{parameters}) = L_{\text{genet}} \times L_{\text{fructi}} \quad (1)$$

$$L_{genet}(data|\ parameters) = \prod_{o:\text{genotyped seeds}} P\left(g_o \mid g_{j_o}\right)$$

where g_o is the genotype of a seed and g_{j_o} is the genotype of its mother-tree j_o.

$$L_{fructi}(data|\ parameters) = \prod_{b:burrs} P\left(n_b \mid j_b\right)$$

where n_b is the number of formed fruits in the burr b sampled on the mother-tree j_b. For each mother tree, we used only the burrs collected in 2018, as we have flowering data only for that year. When fruit set data from 2018 were not available (23% of the cases), we used burrs collected in 2019 instead to estimate fruit set, rather than using mean fruit set values. Our rationale is that fruit sets were positively correlated across years ($r = 0.62$, $n = 160$).

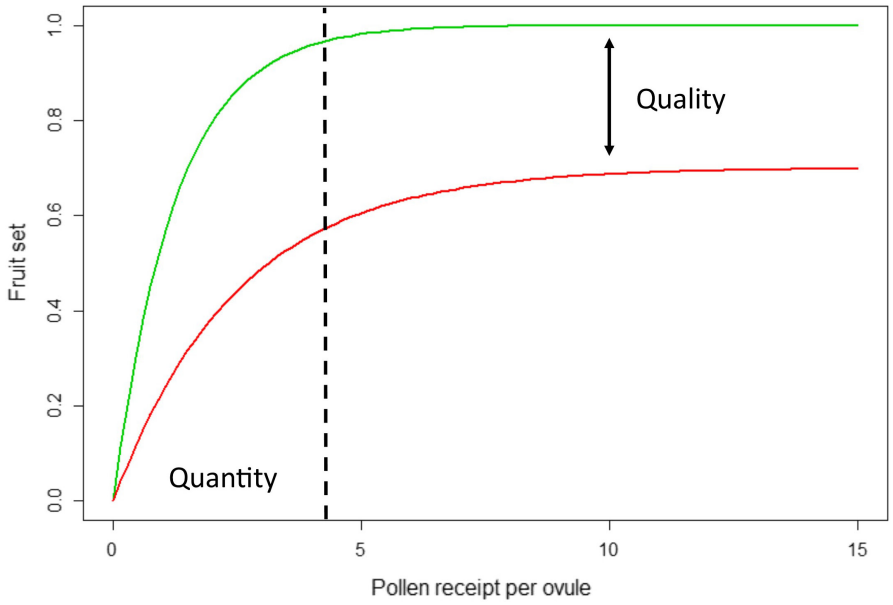


FIGURE 2 Pollination success (fruit set) as a function of pollen receipt per ovule. Green curve: Quantity limitation only. Red curve: Limitation by the quality of pollen received (e.g., a late-acting self-incompatibility barrier or early inbreeding depression), in addition to quantity limitation. Pollen quantity effects are only visible when the quantity of pollen received by the ovules is low, whereas pollen quality effects occur regardless of pollen quantity but are most visible when the quantity of pollen received by ovules is high. Adapted from Aizen and Harder (2007).

For the first component of Equation (1), the probability that a seed o from mother g_{j_o} has genotype g_o is:

$$P(g_o|g_{j_o}) = mig \times T(g_o|g_{j_o}, AF) + (1-mig) \times \sum_{k:candidates} \pi_{j_o k} \times T(g_o|g_{j_o}, g_k) \quad (2)$$

where $T(g_o|\, g_{j_o}, g_k)$ are the Mendelian probabilities of generating the offspring's genotype g_o from the known genotypes of the two parents; AF are the SNP allelic frequencies in the external population, mig corresponds to the pollen migration rate from outside the site (to be estimated); and π_{jk} is the relative contribution of the candidate father k to the pollen pool of mother j (detailed below).

For the second component of Equation (1), the probability that a burr b from a mother j_b has n_b formed fruits follows a zero-deflated binomial distribution.

$$P(n_b = 1|j_b) = \frac{3\alpha_{j_b}(1-\alpha_{j_b})^2}{1-(1-\alpha_{j_b})^3} \quad (3)$$

$$P(n_b = 2|j_b) = \frac{3\alpha_{j_b}^2(1-\alpha_{j_b})}{1-(1-\alpha_{j_b})^3} \quad (4)$$

$$P(n_b = 3|j_b) = \frac{3\alpha_{j_b}^3}{1-(1-\alpha_{j_b})^3} \quad (5)$$

where α_{j_b} is the probability for a single fruit to be formed, that is, fertilization occurs and no abortion occurs (see below). We obtained these equations by assuming that the different fruits in the same burr are independent of each other (given the amount and composition of pollen received) and that burrs with zero fruit were not sampled. Different parameterized models for α_{j_b} were built to account for different processes (see below). Figure 3 shows how observations, latent variables and parameters interact in the Directed Acyclic Graph of this hierarchical model.

2.10.3 | Modelling the relative contributions of candidate fathers to pollen pools (π_{jk})

Central in Equation (2), the relative contribution π_{jk} of the candidate father k to the pollen pool of mother j results from the competition with pollen from all other known candidate fathers. Following Smouse and Sork (2004), we considered three kinds of factors determining the pollen pool available to each mother-tree j: factors affecting the male fecundity of each father tree k of the stand (F_k); factors affecting the dispersal from location of father k to the location of mother j ($DISP_{jk}$); and factors affecting the compatibility between mother j and father k ($COMPAT_{jk}$) (including phenology, species barriers, reduced competitive ability of pollen coming from ramets of the same clone):

$$\pi_{jk} = \frac{F_k \times DISP_{jk} \times COMPAT_{jk}}{\sum_l F_l \times DISP_{jl} \times COMPAT_{jl}} \quad (6)$$

$$F_k = exp(v_1 FType_k + v_2 CA_k + v_3 FLength_k + E_k) \quad (7)$$

where v_1, v_2 and v_3 are the fixed effects of the three covariates of interest (flower-type (Dys = 0; Eu = 1), canopy area, catkin length) and E_k is an individual random effect of variance V_E.

$$DISP_{jk} = \frac{s}{2\pi a^2 \Gamma(2/s)} exp\left(-\frac{d_{jk}^s}{a^s}\right) \quad (8)$$

where s is the shape parameter and a is a scale parameter related to the mean dispersal distance δ following $\delta = a\frac{\Gamma(3/s)}{\Gamma(2/s)}$. The parameters s and δ were estimated. Note that for selfing, i.e., for $j = k$ and $d_{jj} = 0$, we used

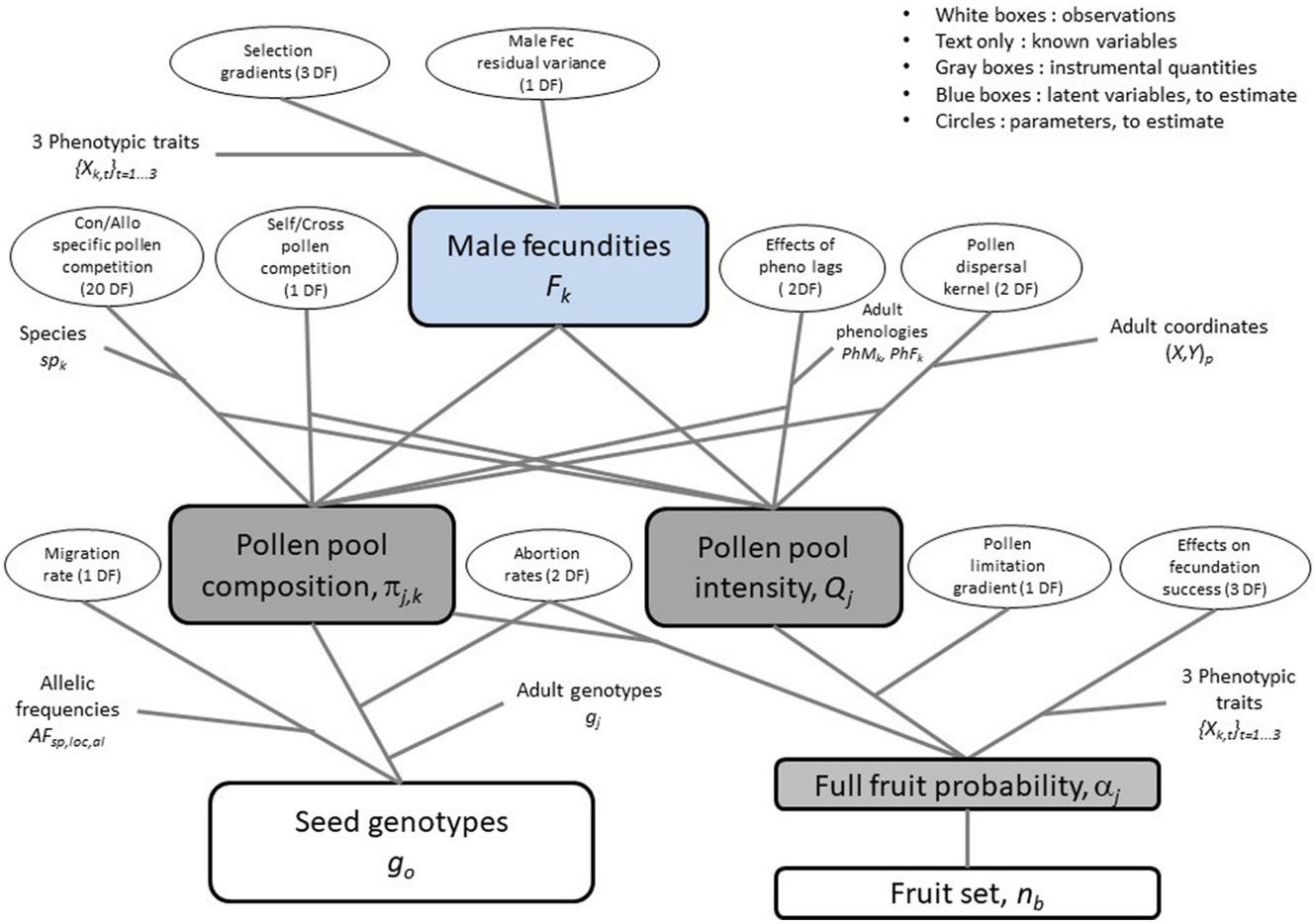


FIGURE 3 Directed acyclic graph illustrating the model used. « pollen pool » here is considered at the time of fecundation, i.e., after pollen competition and before abortion of ovules or embryos. It combines a pollen pool model for the relative contributions of the fathers to the pollen pool (πjk) and a fruit set model, based on probability to have a filled fruit, αj. The pollen pool composition (πjk) determines the genotypic data of seeds and depends on male fecundities (controlled by three phenotypic traits), geographical coordinates and genotypes of adult trees, and mating compatibilities between them. These compatibilities between trees depend in turn on barriers against self-pollen and against heterospecific pollen and on the temporal compatibility between flowering trees. The probability to have a filled fruit, αj, determines fruit set. It is modelled as a function of phenotypic traits, of the barriers against selfed ovules or embryos and of pollen pool intensity Qj (i.e., limitation by pollen quantity, model 2 only, see below), which is controlled by the same parameters as pollen pool composition πjk.

the integral of the exponential power kernel over a disk of radius 3 m mimicking the canopy of an average tree:

$$DISP_{jj} = \int_{r=0}^{r=3} \frac{sr}{a^2\Gamma(2/s)} exp\left(-\frac{r^s}{a^s}\right) dr.$$

$$COMPAT_{jk} = SI_{jk} \times h_{sp_j,sp_k} \times e^{-\frac{\left(PhM_k - PhF_j - \Delta_{opt1}\right)^2}{\sigma_1^2}} \quad (9)$$

where $SI_{jk} = b_{SI}$ if j and k belong to the same clone of the same species and $SI_{jk} = 1$ if j and k belong to different species or different clones of the same species. In this equation SI_{jk} represents the relative competitive ability of the pollen: i.e., a pollen grain has a chance to win the race for fertilization weighted by a factor SI_{jk} (b_{SI} is expected <1). b_{SI} is thus the parameter for early-acting self-incompatibility, and Equation 6 models pollen pools prior to the fecundation stage. Below (in Equations 10 and 11), we will also consider the effect of late-acting self-incompatibility, acting at fertilization stage, or through postzygotic barriers at the survival stage (embryo abortion or early inbreeding depression).

In Equation (9) h_{sp_j,sp_k} represents the relative fertilization success on a mother j of species sp_j of each pollen grain from a father k of species sp_k, relatively to a compatible pollen of species sp_j (thus any $h_{sp,sp} = 1$). Finally, PhM_k and PhF_j are the phenological indices for male blooming and female receptivity; the parameter Δ_{opt1} determines the optimal phenological lag that provide the best chance of pollination success and parameter σ_1 determines the sensitivity to phenological lag (smaller σ_1 means a quicker loss of pollination success when departing from the optimal lag).

Parameters b_{SI}, $h's$, Δ_{opt1}, σ_1 are to estimate.

2.10.4 | Modelling the probability of a filled fruit (α_j)

Central in Equations 3–5, the probabilities of a filled fruit α_j, for all mothers j, were modelled in two different ways described below. In both models, the probability to abort due to late-acting self-incompatibility or early inbreeding depression is the principal determinant of α_j. In model 1 below, we consider a barrier, where selfed ovules or embryos have different abortion rates than outcrossed ones. Model 2 is identical to model 1 except for the addition of a mechanism for pollen limitation as a determinant of fruit set.

Model 1

Fruit set determined by differential abortion rates. The survival rate of an embryo of mother j is written.

$$\alpha_j = \frac{exp(i + w_2 CA + w_3 FLength_j)}{1 + exp(i + w_2 CA_j + w_3 FLength_j)} \sum_k \pi_{jk}\alpha_{jk} \quad (10)$$

where π_{jk} is written as above and corresponds to the probability of fertilization by father k, and α_{jk} is the survival rate at fruit stage of an ovule fertilized by father k. Here self-incompatibility is the only determinant of abortion with $\alpha_{jk} = a_{SI}$ (expected <1) if j and k belong to the same clone and $\alpha_{jk} = 1$ if j and k belong to different clones. Parameters w_2 and w_3 are the effects of covariates on fertilization success and i is the intercept. The left component of α_j is a logit link that expresses the probability of fertilization success (between 0 and 1) as a function of the covariates and the right component expresses the probability that the embryo survives.

In that model, due to differential survival rates, the proportion of father k in nonaborted fruits becomes

$$\pi'_{jk} = \frac{\pi_{jk} \times \alpha_{jk}}{\sum_l \pi_{jl} \times \alpha_{jl}}$$

(i.e., π_{jk} is thus replaced by π'_{jk} in Equation (2) of L_{genet}).

Model 2

Fruit set determined by differential abortion rates and pollen limitation:

$$\alpha_j = \frac{exp(i + w_2 CA_j + w_3 FLength_j + w_{PL} Q_j)}{1 + exp(i + w_2 CA_j + w_3 FLength_j + w_{PL} Q_j)} \sum_k \pi_{jk}\alpha_{jk} \quad (11)$$

where π_{jk}, α_{jk}, w_2, w_3 and i are defined as above and where

$$Q_j = \sum_{l:father\ trees} F_l \times DISP_{jl} \times COMPAT_{jl}$$

is the total amount of effective pollen received by the mother-tree j. Here "effective" means "after competition, accounting for early-acting penalty of self-pollen grains". The parameter w_{PL} of Equation 11 thus measures the importance of pollen limitation as a determinant of fruit set.

Here again, the proportion of father k in the nonaborted fruits, used in Equation (2), is replaced by

$$\pi'_{jk} = \frac{\pi_{jk} \times \alpha_{jk}}{\sum_l \pi_{jl} \times \alpha_{jl}}$$

2.10.5 | Estimation procedure

We used a Bayesian framework and a Markov chain Monte-Carlo (MCMC) with a Metropolis-Hasting algorithm to estimate jointly all latent variables, that is, the E_k's random components of fecundity, and all parameters, that is, mig, δ, s, v_1, v_2, v_3, h's, Δ_{opt1}, σ_1, b_{SI}, a_{SI}, w_2, w_3, w_{PL}.

Each MCMC was run for 1,000,000 iterations and the first 250,000 were discarded. We visually checked the good convergence of the chains towards a stationary state and ran several independent chains to check that all reached the same stationary state. We kept one iteration every 50th iteration to compute posterior distributions and posterior means for the parameters, the individual fecundities, and some additional variables enabling to evaluate the goodness-of-fit of the models.

2.11 | Statistical analysis

We tested the significance of the relationships between predicted and observed variables with a linear model and the differences in male fertility between flower types and among species with one-way ANOVAs. We drew interspecific barriers plots with basic functions implemented in R (version 3.6.0; R Core Team, 2013), and scatter plots, boxplots and histograms with ggplot2 (version 3.6.3; Wickham, 2016), ggthemes (version 4.2.4; Arnold, 2016) and cowplot (version 1.1.1; Wilke, 2020) packages.

3 | RESULTS

3.1 | Flowering and fruiting

We have obtained phenological, phenotypic and fruit set data for most trees in the study site (Larue, Barreneche, & Petit, 2021a). The canopy area (CA) and catkin length ($FLength$) of the trees were highly variable: $CA = 20.9\,m^2$ (0.04–224) and $FLength = 18.7\,cm$ (9.4–35.2). There is a large overlap of flowering phenology among species, with however a tendency for *C. mollissima* to bloom first, followed by *C. crenata*, *C. sativa* × *C. crenata* hybrids, and finally *C. sativa*. Fruit set, assessed on 216 trees, is much higher for dystaminate trees (0.85, ranging from 0.64 to 0.99) than for eustaminate trees (0.45, ranging from 0.14 to 0.63).

3.2 | Paternity analysis with CERVUS

We performed paternity analysis on 1939 seeds (Table S1, Figure 3e). From this, 15 seeds were excluded because their genotypes had more than 50% of missing data, 11 seeds were sired by an unknown father, presumably from outside the studied area, and 1913 seeds (i.e., 98.7% of the total) were assigned to a single father, including 1825 assigned at the strict threshold of 99%. Among the 1924 seeds correctly genotyped (Table 2), 75 are self-pollinated (overall selfing rate of 3.9%). Selfing rate of mother trees varied across individuals (0%–36%, Table S1). Conspecific trees sired a large but variable proportion of the seeds of each mother tree. For *C. sativa* mother trees, *C. sativa* sired 48% of the seeds; for *C. sativa* × *C. crenata* hybrid mother trees, *C. sativa* × *C. crenata* hybrids sired 45% of the seeds; and for *C. mollissima* mother trees, *C. mollissima* sired 63% of the seeds. However, for *C. crenata*, represented by only seven clones in the study site, conspecific *C. crenata* trees sired only 8% of the seeds whereas *C. sativa* × *C. crenata* hybrids sired as many as 73% of the seeds (Table 2).

Dystaminate trees, which represent 32% of the adult population studied, sired only 145 of the 1913 seeds with an identified father (8% of the total). In contrast, eustaminate trees, which represent 68% of the adult population studied, sired 1768 seeds (92% of the total). Hence, eustaminate trees sired on average 5.6 times more seeds than dystaminate trees.

3.3 | Model comparison

Our attempts with model 2, in which fruit set is determined not only by differential abortion rates of self-fertilized versus outcrossed ovules and embryos but also by pollen limitation, did not result in an improved fit compared to model 1 in which fruit set is determined only by differential abortion rates. We therefore focused on model 1, which combines a spatially explicit mixed-mating model and a fruit set model. We compared it with the standard MEMM version, which does not include fruit set (Table 3). The likelihood of the standard version of MEMM is (as expected) lower than the likelihood of the corresponding genetic part of the new model: 62,584 versus 62,872, illustrating a better fit to that part of the data.

With the new model, male fecundity (Fk) is on average five times larger for eustaminate trees than for dystaminate ones (parameter v1 in Table 3 and Figure 4, left; ANOVA, $p < 10^{-15}$), which is close to the value found for siring success (5.6-fold difference, see above). In contrast, the standard MEMM model yields a much larger difference (Table 3). There is also evidence for male fecundity Fk differences across species (ANOVA, $p < .004$), but the only significant pairwise comparison is that between *C. sativa* and *C. sativa* × *C. crenata* hybrids, the two most abundant and best sampled taxa, with hybrids having a slightly higher male fecundity (Tukey's test, $p < .003$).

With the standard MEMM model, only the value of a prezygotic barrier against self-pollen can be estimated ($bSI = 0.01$), suggesting that there is a 100-fold advantage for cross-pollen over self-pollen. With the new model, two parameters are estimated instead: an early barrier against self-pollen caused the competitive advantage of cross-pollen over self-pollen ($bSI = 0.20$), and a delayed barrier against self-pollen caused by a mechanism of late-acting self-incompatibility or early inbreeding depression resulting in a competitive advantage through ovule or embryo abortion (overall abortion rate $\alpha_{SI} = 95\%$, Table 3). Once combined, these two mechanisms result in an overall selfing rate of about 4% (see Figure 5).

The migration rate of pollen from outside the site is very similar in both models (about 4.5% in the new model, see Table 3). This finding differs from that based on paternity analyses, as we identified only 11 seeds (0.6%) with unknown parents.

3.4 | Model predictions and model performance

By disentangling the different processes accounted for in the model, we could predict the proportions of self-pollen received by each mother tree at different stages. The first stage corresponds to the pollen pool received on stigmas at the time of pollination (Figure 5a). Self-pollen received by mother trees represents on average 59% of the pollen pool deposited on stigmas, with large variation among trees (from 5% to 98%). For instance, in eustaminate mother trees, the average proportion of self-pollen reaches 74%. In the second stage, the five-fold difference in competitive ability between self- and cross-pollen further shapes the proportion of self-pollen making it to the ovules (Figure 5b). This results in a large decrease in the proportion of self-pollen, down to 35% (48% in eustaminate trees). The third stage corresponds to the effect of late-acting self-incompatibility and early inbreeding depression (Figure 5c). The proportion of self-pollinated fruits is reduced to 4% (0–19), due to the abortion of almost all ovules fertilized by self-pollen ($a_{si} = 0.95$). For eustaminate trees, this implies that 46% the ovules abort (95% of 48%). The last picture (Figure 5d) illustrates the selfing rate assessed on fruits of each mother-tree, estimated with the paternity analysis. This proportion is low, around 4% (0–36), matching well with the estimate of the fitted model. The overall relationship between observed and predicted self-pollination is marginally not significant (F test, $p < .06$, $r^2 = 0.06$), with one tree (E_39_A) having an unusually high selfing rate.

Observed fruit set matched very well with predicted fruit set (F test, $p < 10^{-16}$, $r^2 = 0.9$) (Figure 6), suggesting that our model for fruit set satisfactorily catches the underlying processes. The predictions are good for all investigated species (Figure 6a) regardless of flower types (Figure 6b). Data from both dystaminate (black dots, larger fruit sets) and eustaminate mother plants (red dots, widely variable fruit sets, including weak values) closely correspond to predictions in the full range of observed values (from 0.14 up to 0.99). Abortion rate of selfed ovules or embryos have a strong effect on fruit set, whereas other variables, such as tree phenotypes and phenology, have only a minor impact. Catkin length had no effect on male fecundities or on fruit set, whereas canopy area had a slightly positive effect on male fecundities as

TABLE 3 Comparison of parameters estimated values of the new model and the standard MEMM model

		New model			Standard MEMM model		
Abbreviation	**Description**	**Mean**	**CI[a] min**	**CI max**	**Mean**	**CI[a] min**	**CI max**
Likelihoods							
-log(L(data))	Total likelihood	72,740					
-log(L_{genet})	Likelihood of seed genetic model	62,872			62,584		
-log(L_{fructi})	Likelihood of fruit set model	9869					
Parameters							
V_E	Variance of the random component of ♂ fecundity per ramet	0.9	0.7	1.1	1.2	0.8	1.6
mig	Pollen migration rate from outside the site	0.045	0.035	0.056	0.046	0.036	0.056
δ	Pollen mean dispersal distance	5319	1265	9732	2725	324	8923
s	Shape parameter of dispersal function	0.07	0.06	0.10	0.09	0.06	0.15
b_{SI}	Prezygotic barrier against selfing	0.20	0.14	0.27	0.01	0.00	0.02
a_{SI}	Survival rate of selfed embryos (1 – postzygotic barrier)	0.05	0.03	0.06	/	/	/
Δ_{opt1} (days)	First ♂ peak - ♀ receptivity	−0.8	−3.5	1.8	−5.4	−9.5	−1.0
σ_1 (days)	Sensibility of male pollination success to departure to optimal phenological lag between mother and father	13.5	12.1	15.3	14.8	12.3	18.5
Interspecific barriers (h's)							
S × CS	*Sativa* × *hybrid*	0.54	0.37	0.77	0.43	0.26	0.68
S × C	*Sativa* × *crenata*	0.45	0.25	0.75	0.46	0.23	0.85
S × M	*Sativa* × *mollissima*	0.13	0.06	0.23	0.17	0.07	0.33
CS × S	*Hybrid* × *sativa*	0.46	0.30	0.67	0.66	0.38	1.06
CS × C	*Hybrid* × *crenata*	4.18	2.54	6.68	4.61	2.52	7.94
CS × M	*Hybrid* × *mollissima*	0.63	0.35	1.06	0.89	0.41	1.73
C × S	*Crenata* × *sativa*	0.08	0.02	0.20	0.33	0.04	1.19
C × CS	*Crenata* × *hybrid*	0.79	0.45	1.27	0.65	0.17	1.92
C × M	*Crenata* × *mollissima*	0.90	0.32	1.88	1.35	0.24	4.59
M × S	*Mollissima* × *sativa*	0.01	0.00	0.04	0.01	0.00	0.03
M × CS	*Mollissima* × *hybrid*	0.04	0.01	0.09	0.01	0.00	0.02
M × C	*Mollissima* × *crenata*	0.39	0.15	0.84	0.07	0.01	0.18
Effect on male fecundity							
v_1[b]	Male fecundity of eustaminate vs. dystaminate trees	5.0	3.5	7.1	40.5	16.5	99.5
v_2	Effect of canopy area on ♂ fecundity	0.15	0.01	0.32	0.42	0.24	0.62
v_3	Effect of catkin length on ♂ fecundity	0.10	−0.06	0.27	0.29	0.09	0.48
Effect on fruit set							
I[c]	Intercept of the logit model for fruit set	3.0	2.7	3.5	/	/	/
w_2	Effect of canopy area on fruit set	0.8	0.3	1.4	/	/	/
w_3	Effect of catkin length on fruit set	−0.2	−0.4	0.1	/	/	/

[a]Credibility interval.

[b]Logit value of the intercept.

[c]Exponential value of the effect.

well as on fruit set (Table 3). Regarding flowering phenology, we found that mating between trees is maximum when full flowering of unisexual male catkins happens close in time to the full receptivity of female flowers (Table 3, Δ_{opt1} within one day). However, the window of compatibility is relatively wide since a flowering gap of +/− 14 days (σ_1) only reduced the chances of pollination by a factor 10. We provide parameters for interspecific barriers and pollen dispersal in Supporting Information 2 and 3.

4 | DISCUSSION

To investigate sexual interference in chestnuts under natural conditions, we successfully developed a spatially explicit individual-based model coupling the standard likelihood of pollen pool composition with the fruit set likelihood. Hence, we could estimate female mating success and male fecundity in the same modelling framework. In theory, the pollen pool (pollen composition/quality and pollen amount/quantity) should influence female mating success, as predicted by Levin (1988). This fully justifies coupling the two processes for more realistic predictions of fruit set. Here, for the first time, we used information on the pollen cloud composition at the time of pollination, obtained thanks to genetic markers, including the proportion of self-pollen, to predict fruit set.

Our biological system proved very effective for these new developments. Nearly all chestnut trees could be geolocated, sampled, genotyped and phenotyped. We evaluated their fruit set using a simple but effective procedure that closely reflects pollination success (Larue, 2021). We found that most mating events take place within the study plot: gene flow from outside the study site (i.e., from distances >1 km, given our nearly exhaustive sampling strategy) involved as little as 0.6% of the fruits, as inferred using paternity exclusion. This makes sense given that the surrounding landscape is mostly urban with no chestnut forests or woods. Moreover, chestnut is pollinated by small beetles and flies (Larue, Austruy, et al., 2021), which are probably less effective for long-distance pollen dispersal than either wind or large bees (Hasegawa et al., 2015; Wessinger, 2021). In most cases, we could identify the father with great confidence, providing a powerful platform for clarifying complex mating processes. Overall, the model accurately predicted pollen-pool composition and fruit set. Trees' flower types (dystaminate or eustaminate trees) strongly determined male fecundity (i.e., eustaminate >> dystaminate), confirming the existence of different genders in European chestnut and in its hybrids, including completely or partly male-sterile chestnut trees.

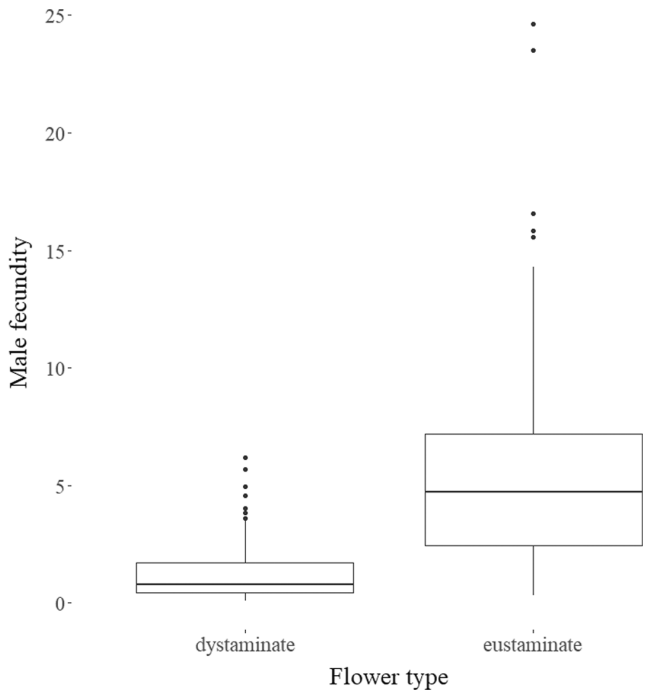


FIGURE 4 Individual male fecundities predicted according to trees' flower types.

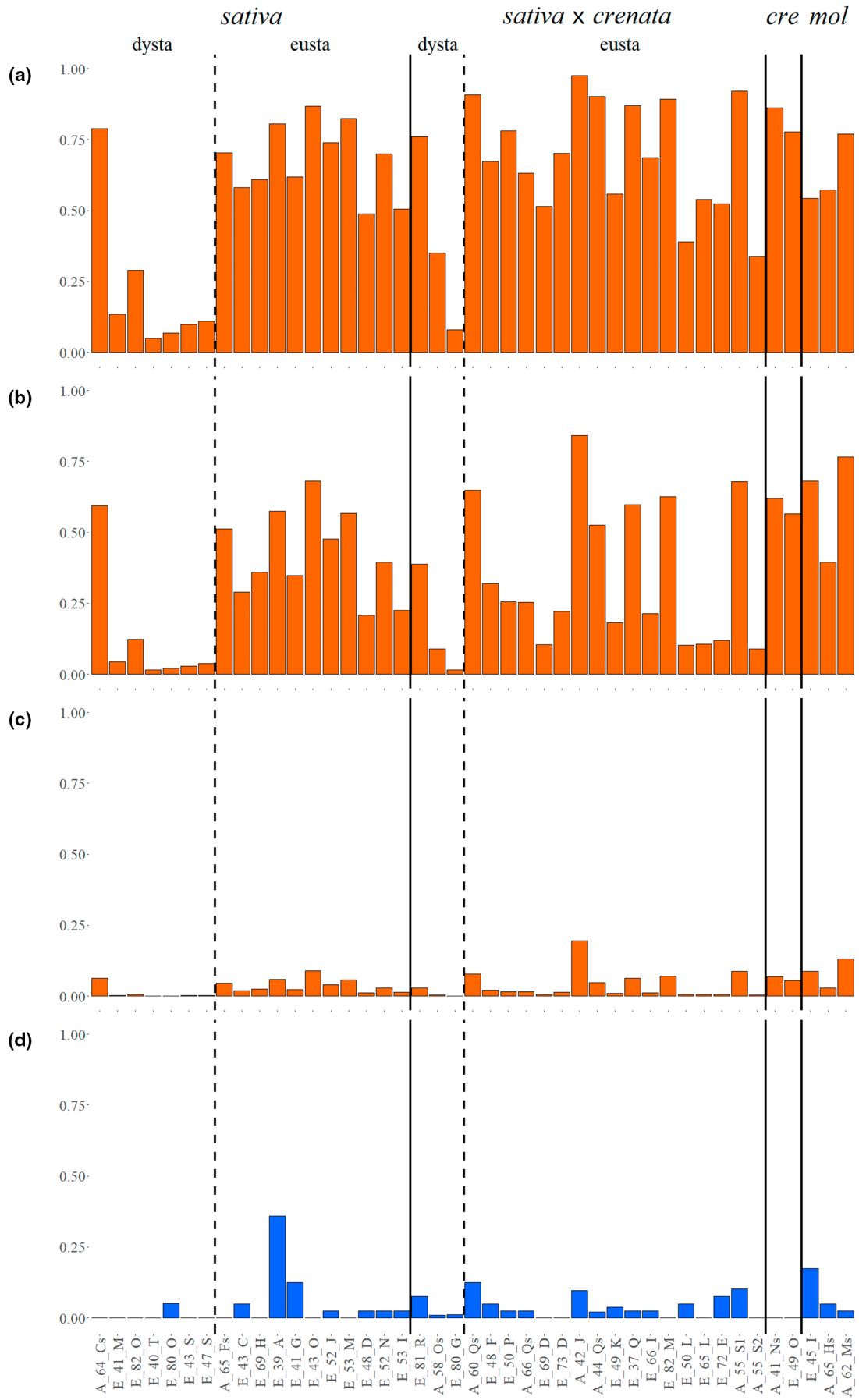
We also found a positive effect of canopy area on both male fecundity and fruit set. Previous studies have also reported a positive relationship between male fecundity and tree size, including in insect-pollinated species, as expected given that pollen production increases with tree size (Burczyk et al., 2002; Latouche-Hallé et al., 2004; Oddou-Muratorio et al., 2005). In contrast, the positive relationship between fruit set and tree size was unexpected as large trees are expected to experience increased self-pollination and to have decreased fruit set compared to male-fertile trees. More work is needed to confirm this trend and clarify its cause.

We obtained accurate predictions of fruit set only when considering differential abortion rates of selfed- and outcrossed ovules and embryos. In contrast, there was no need to invoke pollen limitation to predict fruit set. The high fruit set observed in male-sterile trees (over 95% in some trees) supports this conclusion. We hypothesise that in this site, characterized by a large diversity of potential pollen donor trees located at reduced distance from each other and by abundant insect visitors (Larue, Austruy, et al., 2021), cross pollen is not limiting. Hence, we conclude that sexual interference is largely responsible for the reduced fruit set observed in male-fertile trees.

Unlike the standard MEMM model that uses a single parameter to estimate the barrier against self-pollen, our new model uses two parameters: the relative competitive ability of self-pollen compared to cross-pollen and the viability of selfed ovules or embryos caused by late-acting self-incompatibility or early inbreeding depression. We found that cross-pollen is five times more competitive than self-pollen and that roughly 19 selfed ovules out of 20 give rise to an empty fruit. Using these two barriers in combination, we could accurately predict the observed selfing rate and fruit set. Fruit mortality thus depends on the proportion of self-pollen remaining after pollen competition. We argue that this proves the importance of sexual interference and our ability to reproduce this mechanism in equations.

Several related findings confirm that sexual interference is involved. First, as already pointed out, eustaminate (male-fertile) trees, which produce large amounts of pollen, have a greatly reduced fruit set compared to dystaminate (mostly male-sterile) trees. Second, for both eustaminate and dystaminate trees, increased male fecundity is associated with decreased fruit set, as expected if self-pollination usurps ovules. These findings are supported by

FIGURE 5 Proportion of self-pollen predicted and observed on each mother tree. (a) Expected proportion of self-pollen arriving on the stigmas. (b) Expected proportion of self-pollen after the operation of pollen competition. (c) Expected proportion of self-pollen after abortion of self-pollinated ovules or selfed embryos. (d) Proportion of fruits of each mother tree actually resulting from a selfing event.



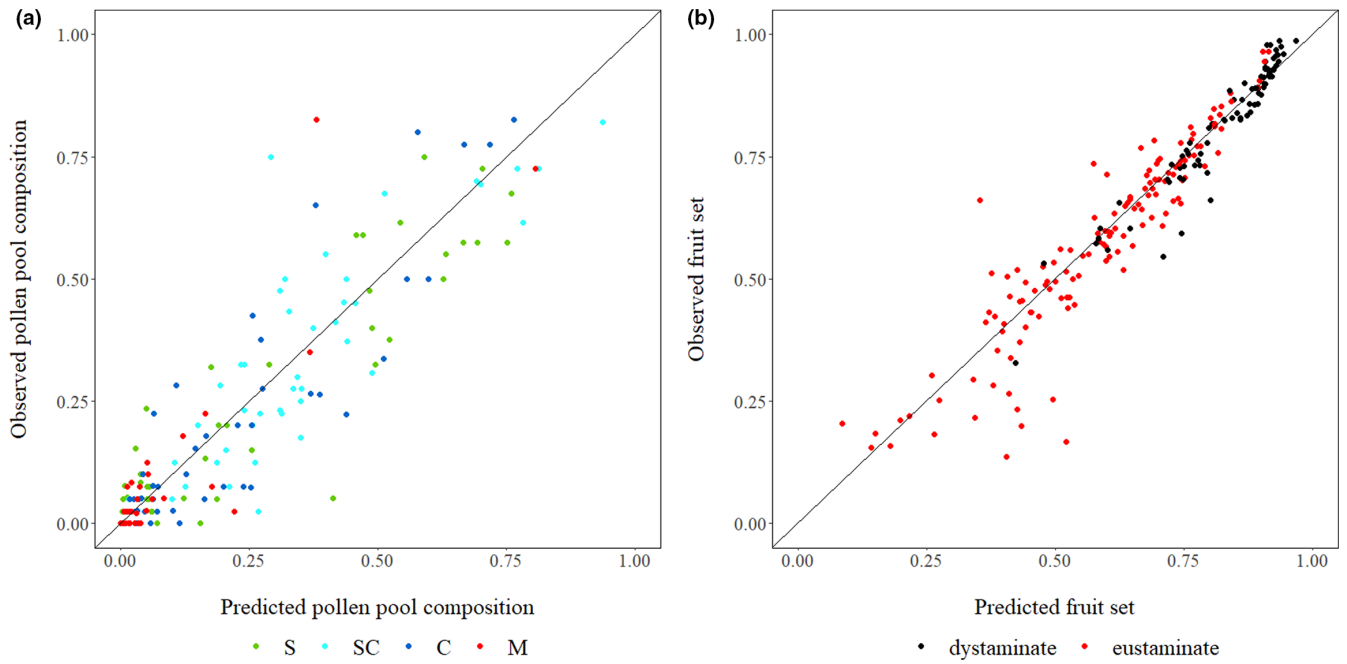


FIGURE 6 Relationship between predicted and observed pollen pool composition. (a) for each mother tree, we compared the observed fraction of the pollen pool donated by each of the four paternal taxa with model predictions. (b) Relationship between observed and expected fruit set for each tree (ramet). Eustaminate trees are in red and dystaminate ones are in black.

emasculation experiments (removal of male catkins) of whole trees or branches, where we observed increased fruit set in emasculated male-fertile but not in "emasculated" male-sterile varieties of *C. sativa* × *C. crenata* hybrids (Larue, 2021; Larue & Petit 2022). Similarly, Zhao and Liu (2009) observed increased fruit yield using chemical emasculation of male-fertile *C. mollissima*.

Since Darwin (1877), researchers have interpreted floral traits of outcrossing plant species as the result of natural selection against self-fertilization. Under this framework, the high rate of ovule loss caused by self-pollination observed in chestnut, a largely self-incompatible tree, is surprising, if we consider along with Barrett (2002) that selection should have minimized the consequences of sexual interference. One possibility to explain this counter-intuitive finding is that natural selection is not the only mechanism at work. As increasingly acknowledged in the literature, sexual selection to increase male mating success applies not only to animals but also to plants, including cosexual ones (Moore & Pannell, 2011; Willson, 1979). We have previously shown that chestnuts produce huge amounts of tiny pollen grains and make large efforts to attract pollinators (Larue, Austruy, et al., 2021). Hence, both sexual and natural selection must be considered, which could result in evolutionary trade-offs including sexual interference (Moore & Pannell, 2011). The large pollen production in chestnut is probably driven not only by low pollinator reliability but also by competition among males for access to ovules (Willson, 1979). Because of incomplete dichogamy (Hasegawa et al., 2017) as well as limited movement of some of the pollinators on trees canopies, abundant geitonogamous pollination takes place, leading to the coating of stigmas by self-pollen, as shown in Japan by Hasegawa et al. (2009). Our work confirmed this finding: we found that on average 74% (and up to 98%) of pollen received by male-fertile trees is self-pollen, whereas the selfing rate is only 4%. Despite the greater competitive advantage of cross-pollen over self-pollen, almost half of the ovules abort in male-fertile trees, a massive rate, illustrating the importance of sexual interference in the field. By construction, male-sterile individuals do not experience sexual interference. Their presence in natural populations of European chestnut (Larue, 2021; Bodénès, Larue and Petit, personal observation) suggests that this advantage is large enough to compensate for their reduced male fitness, as discussed elsewhere (Larue & Petit 2022).

Our model was able to distinguish between two types of barriers against self-fertilization that occur successively, both contributing to decrease the proportion of selfed seeds in the offspring. Alone, the lower competitive ability of self-pollen compared to cross-pollen should have no effect on fruit set when pollen is nonlimiting. However, this mechanism interacts with the subsequent barrier (abortion of selfed ovules or abortion of embryos). This interaction helps reduce sexual interference compared to a situation where only late-acting self-incompatibility or early-inbreeding depression would take place. At first sight, the earlier barrier appears much more effective than the later one, because it does not impact fruit set. This finding raises the question of why this first barrier did not evolve to become even more effective, thereby further reducing fruit mortality. In their seminal paper, Seavey and Bawa (1986) mention two possible advantages of late-acting self-incompatibility barriers that could explain their persistence in nature. They propose that such barriers would result in "an extended period of time over which pollen genotypes

may be evaluated by the maternal parent and greater flexibility in the choice of male parents". Ghazoul and Satake (2009) cite another possible advantage, which they call the "sacrificial sibling" hypothesis. High fruit abortion rate, combined with difficulties for seed predators to distinguish between aborted and viable fruits, could dilute predation pressure, ultimately benefitting to mother plant fitness. In chestnuts, the spiny burrs are a vivid testimony of the important pressure exerted by seed predators such as chestnut weevils during fruit growth. These interesting but largely neglected questions could only emerge once we had demonstrated that self-pollination reduces fruit set in nature, illustrating the importance of acknowledging the existence of sexual interference and evaluating its demographic effects in plant populations.

4.1 | Future challenges and opportunities

We found no evidence for pollen limitation in our study. It would be useful to explore situations in which there is pollen limitation, including under natural conditions in chestnut forests, to explore how this process interacts with sexual interference. In contrast, we found massive rates of hybridization in this mixed-species stand. In our model, we considered that interspecific barriers are early acting, with no incidence on fruit set. In principle, along with self-pollen, allospecific pollen could also represent a source of low-quality pollen interfering with fruit set, a topic that deserves further study. We found that canopy size influenced male and female pollination success. It should exert contrasting pressures during pollination, as pollinating insects may preferentially visit large trees, whereas the negative effect of self-pollination should increase with plant size (de Jong et al., 1993). To go a step further, it would be interesting to consider plant size effects separately for male-sterile and for male-fertile trees.

Despite some remaining uncertainties, the model developed is remarkably informative and allowed the largely neglected but massive effect of self-pollen interference on fruit set in chestnut to be explored. Because our model is process-based, it enables predictions to be achieved in various configurations and scenarios. All these advances have potential practical consequences for orchards conception and management as well as for conservation. They are also relevant for fundamental studies aiming at comprehensively characterizing male and female mating success and their interactions.

AUTHOR CONTRIBUTIONS

Data curation was done by Clément Larue. Conceptualization of the model was done by Clément Larue, Rémy J. Petit and Etienne K. Klein. This model was coded by Etienne K. Klein. Validations were performed by Clément Larue, Rémy J. Petit and Etienne K. Klein. Statistical analyses and illustration were performed by Clément Larue with advice from Rémy J. Petit and Etienne K. Klein. The first draft of this article was jointly written by Clément Larue and Rémy J. Petit.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.57745/B9KJNH.

DATA AVAILABILITY STATEMENT

All data used have been described in a data paper (Larue, Barreneche, & Petit, 2021a) and have been made available at: https://data.inrae.fr/dataset.xhtml?persistentId=doi:10.15454/GSJSWW. Associated metadata have been made available at: https://metadata-afs.nancy.inra.fr/geonetwork/srv/fre/catalog.search#/metadata/02c5ca07-1536-4f89-9a0c-9e8d44a91287. The C/C++ code used to model male and female mating success is available upon request to one of the coauthors, Etienne Klein (etienne.klein@inrae.fr).

BENEFIT-SHARING STATEMENT

Benefits generated: A research collaboration was developed, all collaborators are included as coauthors, and the results of research have been shared with the provider communities and the broader scientific community. The research addresses a priority concern, pollination and fruit set, which are relevant to human alimentation and to plant conservation.

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REFERENCES

Adams, W. T., & Birkes, D. S. (1991). Estimating mating patterns in forest tree populations. In S. Fineschi, M. E. Malvotti, F. Cannata, & H. H. Hattemer (Eds.), *Biochemical markers in the population genetics of Forest trees*, *Netherland* (pp. 157–172). SPB Academic Publishing.

Adams, W. T., Birkes, D. S., & Erickson, V. J. (1992). Using genetic markers to measure gene flow and pollen dispersal in forest tree seed orchards. In R. Wyatt (Ed.), *Ecology and evolution of plant reproduction* (pp. 37–61). Chapman and Hall.

Ahee, J., Drunen, W., & Dorken, M. (2014). Analysis of pollination neighbourhood size using spatial analysis of pollen and seed production in broadleaf cattail (*Typha latifolia*). *Botany*, *93*, 1–10. https://doi.org/10.1139/cjb-2014-0169

Aizen, M. A., & Harder, L. D. (2007). Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology*, *88*, 271–281. https://doi.org/10.1890/06-1017

Arnold, J. B. (2016). *ggthemes: Extra themes, scales and geoms for "ggplot2"*. R package version 4.2.4. https://cran.r-project.org/web/packages/ggthemes/

Barrett, S. (2002). Sexual interference of the floral kind. *Heredity*, *88*, 154–159. https://doi.org/10.1038/sj.hdy.6800020

Bernasconi, G. (2003). Seed paternity in flowering plants: An evolutionary perspective. *Perspectives in Plant Ecology, Evolution and Systematics*, *6*, 149–158. https://doi.org/10.1078/1433-8319-00075

Bertin, R. I. (1993). Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. *American Journal of Botany*, *80*, 557–560. https://doi.org/10.2307/2445372

Bertin, R. I., & Sullivan, M. (1988). Pollen interference and cryptic self-fertility in *Campsis radicans*. *American Journal of Botany*, *75*, 1140–1147. https://doi.org/10.2307/2444097

Bontemps, A., Klein, E. K., & Oddou-Muratorio, S. (2013). Shift of spatial patterns during early recruitment in *Fagus sylvatica*: Evidence from seed dispersal estimates based on genotypic data. *Forest Ecology and Management*, *305*, 67–76. https://doi.org/10.1016/j.foreco.2013.05.033

Broyles, S. B., & Wyatt, R. (1993). The consequences of self-pollination in *Asclepias exaltata*, a self-incompatible milkweed. *American Journal of Botany*, *80*, 41–44. https://doi.org/10.2307/2445118

Burczyk, J., Adams, W. T., Birkes, D. S., & Chybicki, I. J. (2006). Using genetic markers to directly estimate gene flow and reproductive success parameters in plants on the basis of naturally regenerated seedlings. *Genetics*, *173*, 363–372. https://doi.org/10.1534/genetics.105.046805

Burczyk, J., Adams, W. T., Moran, G. F., & Griffin, A. R. (2002). Complex patterns of mating revealed in a *Eucalyptus regnans* seed orchard using allozyme markers and the neighbourhood model. *Molecular Ecology*, *11*, 2379–2391. https://doi.org/10.1046/j.1365-294X.2002.01603.x

Burczyk, J., Adams, W. T., & Shimizu, J. Y. (1996). Mating patterns and pollen dispersal in a natural knobcone pine (*Pinus attenuata* Lemmon.) stand. *Heredity*, *77*, 251–260. https://doi.org/10.1038/hdy.1996.139

Burczyk, J., & Prat, D. (1997). Male reproductive success in *Pseudotsuga menziesii* (Mirb.) Franco: The effects of spatial structure and flowering characteristics. *Heredity*, *79*, 638–647. https://doi.org/10.1038/hdy.1997.210

Burd, M. (1994). Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. *The Botanical Review*, *60*, 83–139. https://doi.org/10.1007/BF02856594

Byers, D. L. (1995). Pollen quantity and quality as explanations for low seed set in small populations exemplified by *eupatorium* (Asteraceae). *American Journal of Botany*, *82*, 1000–1006. https://doi.org/10.2307/2446229

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, *18*, 237–268. https://doi.org/10.1146/annurev.es.18.110187.001321

Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in flowering plants. *Annals of Botany*, *125*, 1–9. https://doi.org/10.1093/aob/mcz159

Chybicki, I. J., Iszkuło, G., & Suszka, J. (2019). Bayesian quantification of ecological determinants of outcrossing in natural plant populations: Computer simulations and the case study of biparental inbreeding in English yew. *Molecular Ecology*, *28*, 4077–4096. https://doi.org/10.1111/mec.15195

Chybicki, I. J., & Oleksa, A. (2018). Seed and pollen gene dispersal in *Taxus baccata*, a dioecious conifer in the face of strong population fragmentation. *Annals of Botany*, *122*, 409–421. https://doi.org/10.1093/aob/mcy081

Chybicki, I. J., Oleksa, A., & Dering, M. (2021). Identification of determinants of pollen donor fecundity using the hierarchical neighborhood model. *Molecular Ecology Resources*, *21*, 781–800. https://doi.org/10.1111/1755-0998.13307

Darwin, C. (1877). *The effects of cross and self fertilisation in the vegetable kingdom*. John Murray.

de Jong, T. J., Waser, N. M., & Klinkhamer, P. G. (1993). Geitonogamy: The neglected side of selfing. *Trends in Ecology & Evolution*, *8*, 321–325. https://doi.org/10.1016/0169-5347(93)90239-L

Galen, C., Gregory, T., & Galloway, L. F. (1989). Costs of self-pollination in a self-incompatible plant, *Polemonium viscosum*. *American Journal of Botany*, *76*, 1675–1680. https://doi.org/10.1002/j.1537-2197.1989.tb15152.x

Ghazoul, J., & Satake, A. (2009). Nonviable seed set enhances plant fitness: The sacrificial sibling hypothesis. *Ecology*, *90*, 369–377.

Gibbs, P. E. (2014). Late-acting self-incompatibility – The pariah breeding system in flowering plants. *New Phytologist*, *203*, 717–734. https://doi.org/10.1111/nph.12874

Harder, L., & Aizen, M. (2010). Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society B*, *365*, 529–543. https://doi.org/10.1098/rstb.2009.0226

Harder, L., & Barrett, S. (1996). Pollen dispersal and mating patterns in animal-pollinated plants. In D. G. Lloyd & S. C. H. Barrett (Eds.), *Floral biology: Studies on floral evolution in animal-pollinated plants* (pp. 140–190). Springer.

Harder, L. D., & Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite plants. *Nature*, *373*, 512–515. https://doi.org/10.1038/373512a0

Hasegawa, Y., Suyama, Y., & Seiwa, K. (2009). Pollen donor composition during the early phases of reproduction revealed by DNA genotyping of pollen grains and seeds of *Castanea crenata*. *The New Phytologist*, *182*, 994–1002. https://doi.org/10.1111/j.1469-8137.2009.02806.x

Hasegawa, Y., Suyama, Y., & Seiwa, K. (2015). Variation in pollen-donor composition among pollinators in an entomophilous tree species, *Castanea crenata*, revealed by single-pollen genotyping. *PLoS One*, *10*, e0120393. https://doi.org/10.1371/journal.pone.0120393

Hasegawa, Y., Suyama, Y., & Seiwa, K. (2017). Flowering phenology of a duodichogamous self-incompatible tree species, *Castanea crenata*. *Japanese Journal of Ecology*, *67*, 31–39. https://doi.org/10.18960/seitai.67.1_31

Hegland, S., & Totland, O. (2008). Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos*, *117*, 883–891. https://doi.org/10.1111/j.0030-1299.2008.16561.x

Husband, B. C., & Schemske, D. W. (1996). Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, *50*, 54–70. https://doi.org/10.1111/j.1558-5646.1996.tb04472.x

Johnson, S. D., Butler, H. C., & Robertson, A. W. (2019). Breeding systems in *Cyrtanthus* (Amaryllidaceae): Variation in self-sterility and potential for ovule discounting. *Plant Biology*, *21*, 1008–1015. https://doi.org/10.1111/plb.13025

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Klein, E., Desassis, N., & Oddou-Muratorio, S. (2008). Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. IV. Whole inter-individual variance of male fecundity estimated jointly with the dispersal kernel. *Molecular Ecology*, *17*, 3323–3336. https://doi.org/10.1111/j.1365-294X.2008.03809.x

Klein, E., Lagache, L., & Petit, R. (2017). Demographic and spatial determinants of hybridization rate. *Journal of Ecology*, *105*, 29–38. https://doi.org/10.1111/1365-2745.12674

Klein, E., & Oddou-Muratorio, S. (2011). Pollen and seed dispersal inferred from seedling genotypes: The Bayesian revolution has passed here too. *Molecular Ecology*, *20*, 1077–1079. https://doi.org/10.1111/j.1365-294X.2011.05020.x

Knapp, E. E., Goedde, M. A., & Rice, K. J. (2001). Pollen-limited reproduction in blue oak: Implications for wind pollination in fragmented populations. *Oecologia*, *128*, 48–55. https://doi.org/10.1007/s004420000623

Lagache, L., Klein, E. K., Ducousso, A., & Petit, R. J. (2014). Distinct male reproductive strategies in two closely related oak species. *Molecular Ecology*, *23*, 4331–4343. https://doi.org/10.1111/mec.12766

Lagache, L., Klein, E. K., Guichoux, E., & Petit, R. J. (2013). Fine-scale environmental control of hybridization in oaks. *Molecular Ecology*, *22*, 423–436. https://doi.org/10.1111/mec.12121

Lander, T. A., Klein, E. K., Stoeckel, S., Mariette, S., Musch, B., & Oddou-Muratorio, S. (2013). Interpreting realized pollen flow in terms of pollinator travel paths and land-use resistance in heterogeneous landscapes. *Landscape Ecology*, *28*, 1769–1783. https://doi.org/10.1007/s10980-013-9920-y

Larson, B., & Barrett, S. C. H. (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, *69*, 503–520. https://doi.org/10.1006/bijl.1999.0372

Larue, C. (2021). *De la pollinisation à la formation des graines: le cas du châtaignier* [PhD Thesis]. Université de Bordeaux.

Larue, C., Austruy, E., Basset, G., & Petit, R. J. (2021). Revisiting pollination mode in chestnut (*Castanea* spp.): An integrated approach. *Botany Letters*, *168*, 348–372. https://doi.org/10.1080/23818107.2021.1872041

Larue, C., Barreneche, T., & Petit, R. J. (2021a). An intensive study plot to investigate chestnut tree reproduction. *Annals of Forest Science*, *78*, 90. https://doi.org/10.1007/s13595-021-01104-w

Larue, C., Barreneche, T., & Petit, R. J. (2021b). Efficient monitoring of phenology in chestnuts. *Scientia Horticulturae*, *281*, 109958. https://doi.org/10.1016/j.scienta.2021.109958

Larue, C., Guichoux, E., Laurent, B., Barreneche, T., Robin, C., Massot, M., Delcamp, A., & Petit, R. J. (2021). Development of highly validated SNP markers for genetic analyses of chestnut species. *Conservation Genetics Resources*, *13*, 383–388. https://doi.org/10.1007/s12686-021-01220-9

Latouche-Hallé, C., Ramboer, A., Bandou, E., Caron, H., & Kremer, A. (2004). Long-distance pollen flow and tolerance to selfing in a neotropical tree species. *Molecular Ecology*, *13*, 1055–1064. https://doi.org/10.1111/j.1365-294X.2004.02127.x

Larue, C., & Petit, R. J. (2022). Self-interference and female advantage in chestnut. *bioRxiv*. https://doi.org/10.1101/2022.08.01.502348

Levin, D. A. (1988). The paternity pools of plants. *The American Naturalist*, *132*, 309–317. https://doi.org/10.1086/284854

Lloyd, D. G. (1982). Selection of combined versus separate sexes in seed plants. *The American Naturalist*, *120*, 571–585. https://doi.org/10.1086/284014

Lloyd, D. G., & Webb, C. J. (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. *New Zealand Journal of Botany*, *24*, 135–162. https://doi.org/10.1080/0028825X.1986.10409725

Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M., & Karron, J. D. (2009). New frontiers in competition for pollination. *Annals of Botany*, *103*, 1403–1413. https://doi.org/10.1093/aob/mcp062

Moore, J. C., & Pannell, J. R. (2011). Sexual selection in plants. *Current Biology*, *21*, R176–R182. https://doi.org/10.1016/j.cub.2010.12.035

Oddou-Muratorio, S., Bontemps, A., Klein, E., Chybicki, I., Vendramin, G. G., & Suyama, Y. (2010). Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in *Fagus sylvatica* and *Fagus crenata*. *Forest Ecology and Management*, *259*, 2151–2159.

Oddou-Muratorio, S., & Klein, E. (2008). Comparing direct vs. indirect estimates of gene flow within a population of a scattered tree species. *Molecular Ecology*, *17*, 2743–2754. https://doi.org/10.1111/j.1365-294X.2008.03783.x

Oddou-Muratorio, S., Klein, E., & Austerlitz, F. (2005). Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success inferred from parent–offspring analysis. *Molecular Ecology*, *14*, 4441–4452. https://doi.org/10.1111/j.1365-294X.2005.02720.x

Petit, R. J., & Larue, C. (2022). Confirmation that chestnuts are insect-pollinated. *Botany Letters*, *0*, 1–5. https://doi.org/10.1080/23818107.2022.2088612

Platt, W. J., Hill, G. R., & Clark, S. (1974). Seed production in a prairie legume (*Astragalus canadensis* L.). *Oecologia*, *17*, 55–63. https://doi.org/10.1007/BF00345095

R Core Team. (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Roeder, K., Devlin, B., & Lindsay, B. G. (1989). Application of maximum likelihood methods to population genetic data for the estimation of individual fertilities. *Biometrics*, *45*, 363–379. https://doi.org/10.2307/2531483

Schoen, D. J., & Stewart, S. C. (1986). Variation in male reproductive investment and male reproductive success in white spruce. *Evolution*, *40*, 1109–1120. https://doi.org/10.1111/j.1558-5646.1986.tb05737.x

Seavey, S. R., & Bawa, K. S. (1986). Late-acting self-incompatibility in angiosperms. *The Botanical Review*, *52*, 195–219. https://doi.org/10.1007/BF02861001

Silander, J. A. (1978). Density-dependent control of reproductive success in *Cassia biflora*. *Biotropica*, *10*, 292–296. https://doi.org/10.2307/2387681

Smouse, P. E., & Sork, V. L. (2004). Measuring pollen flow in forest trees: An exposition of alternative approaches. *Forest Ecology and Management*, *197*, 21–38. https://doi.org/10.1016/j.foreco.2004.05.049

Solignat, G., & Chapa, J. (1975). *La Biologie florale du Châtaignier*. Institut National de Vulgarisation pour les Fruits Légumes et Champignons.

Stephenson, A. G. (1981). Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology, Evolution, and Systematics*, *12*, 253–279.

Tani, N., Tsumura, Y., Kado, T., Taguchi, Y., Lee, S. L., Muhammad, N., Ng, K. K. S., Numata, S., Nishimura, S., Konuma, A., & Okuda, T. (2009). Paternity analysis-based inference of pollen dispersal patterns, male fecundity variation, and influence of flowering tree density and general flowering magnitude in two dipterocarp species. *Annals of Botany*, *104*, 1421–1434. https://doi.org/10.1093/aob/mcp252

Totland, O., & Sottocornola, M. (2001). Pollen limitation of reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. *American Journal of Botany*, *88*, 1011–1015. https://doi.org/10.2307/2657082

Waser, N. M., & Price, M. V. (1991). Reproductive costs of self-pollination in *Ipomopsis aggregata* (polemoniaceae): Are ovules usurped? *American Journal of Botany*, *78*, 1036–1043. https://doi.org/10.1002/j.1537-2197.1991.tb14511.x

Webb, C. J., & Lloyd, D. G. (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *New Zealand Journal of Botany*, *24*, 163–178. https://doi.org/10.1080/0028825X.1986.10409726

Wessinger, C. A. (2021). From pollen dispersal to plant diversification: Genetic consequences of pollination mode. *New Phytologist*, *229*, 3125–3132. https://doi.org/10.1111/nph.17073

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.

Wilcock, C. C., & Neiland, M. R. M. (2002). Pollination failure in plants: Why it happens and when it matters. *Trends in Plant Science*, *7*, 270–277. https://doi.org/10.1016/S1360-1385(02)02258-6

Wilke, C. O. (2020). *Cowplot: Streamlined plot theme and plot annotations for "ggplot2"*. R package version 1.1.1. https://cran.r-project.org/web/packages/cowplot/

Willson, M. F. (1979). Sexual selection in plants. *The American Naturalist*, *113*, 777–790.

Xiong, H., Zou, F., Guo, S., Yuan, D., & Niu, G. (2019). Self-sterility may be due to prezygotic late-acting self-incompatibility and early-acting inbreeding depression in Chinese chestnut. *Journal of the American Society for Horticultural Science*, *144*, 172–181. https://doi.org/10.21273/JASHS04634-18

Zhao, Z., & Liu, K. (2009). Effect of chemical thinning catkins on Chinese chestnut yield and quality. *Acta Horticulturae*, *844*, 457–460. https://doi.org/10.17660/ActaHortic.2009.844.64

SUPPORTING INFORMATION

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