Mating systems and avoidance of inbreeding depression as evolutionary drivers of pollen limitation in animal-pollinated self-compatible plants

Celine Devaux, Emmanuelle Porcher, Russell Lande

Type of article: Original article

Title: Mating systems and avoidance of inbreeding depression as evolutionary drivers of pollen limitation in animal pollinated self-compatible plants

Céline Devaux[1*], Emmanuelle Porcher[2] and Russell Lande[3]

[1]Institut des Sciences de l'Evolution de Montpellier, UMR 5554, 34095 Montpellier, France

[2]Centre d'Ecologie et des Sciences de la Conservation, UMR 7204, Muséum national d'Histoire naturelle, Centre National de la Recherche Scientifique, Sorbonne Université, 75005 Paris, France

[3]Center for Biodiversity Dynamics, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

Running title: Mating systems drive evolution of pollen limitation

[*]For correspondence. Email: celine.devaux@umontpellier.fr

ABSTRACT

Background and Aims Most theory addressing the evolution of pollen limitation in flowering plants focuses on stochasticity in relative abundance of plant and pollinator populations affecting trade-offs in resource allocation to ovule production or pollinator attraction vs. seed maturation. Mating system evolution is an underappreciated but potentially widespread additional mechanism for the evolutionary emergence of pollen limitation in animal pollinated self-compatible plants.

Methods We model individual plant flowering phenologies influencing both pollinator attraction and geitonogamous self-fertilization caused by pollinator movements among flowers within plants, incorporating demographic but not environmental stochasticity. Plant

phenology and the resulting pollen limitation are analyzed at evolutionarily stable equilibria (ESS). Pollen limitation is measured by two quantities: the proportion of unpollinated flowers and the reduction in maternal fitness caused by inbreeding depression in selfed seeds.

Key results When pollinators visit multiple flowers per plant, pollen limitation is never minimized at an ESS and results from the evolution of flowering phenologies balancing the amount and genetic composition (outbred versus inbred) of pollen receipt.

Conclusions Results are consistent with previous theory demonstrating that pollen limitation can be an evolved property, and not only a constraint; they complement existing models by showing that plant avoidance of inbreeding depression constitutes a genetic mechanism contributing to evolution of pollen limitation, in addition to ecological mechanisms previously studied.

KEYWORDS

embryo quality; evolutionarily stable equilibria; inbreeding depression; individual flowering phenologies; geitonogamy; pollen quantity; pollen limitation; pollinator limitation.

INTRODUCTION

Limitation of seed or fruit production is widespread in animal pollinated plants and has three non-exclusive proximate causes related to pollinators, embryo quality and resource availability (Bierzychudek 1981; Ashman *et al.* 2004; Knight *et al.* 2005). Limited reproductive success of a plant can be caused by insufficient pollen receipt: some flowers or some of their ovules remain unfertilized because pollinators are scarce, little attracted to the plant, or visit only a fraction of the flowers open (Geber 1985; Charlesworth 1989; Burd 1995; Snow *et al.* 1996; Moeller *et al.* 2012; Schreiber *et al.* 2015), or because pollinators carry insufficient pollen loads (e.g. generalist pollinators depositing heterospecific rather than conspecific pollen, Waser 1978). Even with sufficient conspecific pollen receipt, some seeds on a plant may not mature because embryos are of low quality due to inbreeding (Husband and Schemske 1996; Angeloni *et al.* 2011) or outbreeding depression (Whitlock *et al.* 2013), or because female resources are limited and induce high rates of abortion in otherwise viable seeds or fruits (Willson and Rathcke 1974).

Theoretical work has explored ultimate causes of pollen limitation and showed that it can evolve as a result of trade-offs among three costly reproductive functions for plants: ovule production, seed provisioning, and pollinator attraction. Studies of these functions and their trade-offs revealed that within or among plant variation in pollination rates can generate the frequently observed pollen limitation (Bierzychudek 1981; Haig and Westoby 1988; Burd 1995, 2008; Schreiber *et al.* 2015), although this is sometimes debated (Rosenheim *et al.* 2014, 2016; Burd 2016). Much of this theory was devoted to the role of environmental stochasticity, i.e. random spatial variation or random temporal variation in pollen deposition rate affecting all individual plants in a population simultaneously, as a primary determinant of reproductive trade-offs and consequent pollen limitation (Burd 1995, 2008; Richards *et al.* 2009; Schreiber *et al.* 2015). In doing so, most models took an ecological rather than a

population genetics point of view, emphasizing maternal fitness only (e.g. Haig and Westoby 1988; Burd 1994, 1995; but see Bell 1985; Charlesworth 1989; Burd and Callahan 2000; Harder and Aizen 2010; Thomson 2001). They generally gave little consideration to seed quality and mating system, which are however major evolutionary drivers of plant reproductive strategies (Ashman *et al.* 2004; Devaux *et al.* 2014a) and have proved to correlate with pollen limitation (e.g. self-compatibility for animal-pollinated plant species, Larson and Barrett 2000; Knight *et al.* 2005; Alonso *et al.* 2010).

Here we complement previous evolutionary studies of pollen limitation by examining how selection on the mating system can also cause limitation of seed production and fitness in self-compatible animal-pollinated plants. Our aim is not to question the well-established role of environmental stochasticity in the evolution of pollen limitation (see reviews cited above), but instead to demonstrate that additional mechanisms are likely to be at play. We focus on floral display size, the number of flowers simultaneously open on a plant, a key trait that influences pollen limitation (Dudash 1991, 1993) via its role in pollinator attraction (e.g. Willson and Schemske 1980; Bauer *et al.* 2017) and between-flower self-pollination (geitonogamous selfing as in Lloyd 1992; Lau *et al.* 2008; Karron and Mitchell 2012), hence post-zygotic inbreeding depression. Evolution of floral display size is analyzed by modelling how individual plants allocate a constant total number of flowers through the season (i.e. the individual flowering phenology) under the constraints of pollinator foraging behavior among and within plants. Therefore, we analyze how ecological and genetic mechanisms (insufficient ovule fertilization via pollinator attraction and insufficient embryo quality via inbreeding depression, respectively) jointly constrain evolution of a trait influencing pollen limitation. Unlike previous models, we intentionally do not address the role of seed provisioning or environmental stochasticity but instead focus on the role of mating system on the emergence of pollen limitation. Yet, we incorporate the minimal amount of demographic stochasticity to

portray the basic elements of pollination ecology, i.e. random variation in daily floral display, pollinator visitation and pollinator behavior, and include them in a mechanistic model of pollinator foraging behavior. Our model distinguishes two ultimate causes of pollen limitation, i.e. whether plants at evolutionarily stable equilibria (Devaux *et al.* 2014a) are pollen limited because they produce too few flowers in a day to attract pollinators or because they produce too many flowers to avoid geitonogamous selfing and inbreeding depression.

METHOD

We examine pollen limitation at an evolutionarily stable equilibrium (ESS) predicted by the model of Devaux et al. (2014a). The total number of flowers produced by a given plant throughout the flowering period is Poisson distributed with mean $\bar{N}$. These flowers are open sequentially according to a normal distribution with standard deviation σ (see e.g. Fig. 1). Standard deviation in individual flowering phenology, σ, is the trait under selection: it describes how individual plants distribute their flowers among days within a season, and relates directly to floral display and duration of flowering time: small values of σ correspond to large floral displays over short periods (e.g. mass blooming), whereas large values of σ produce a small floral display over long periods. The standard deviation in flowering phenology therefore modifies all components of plant fitness through floral display size: male and female outcrossed fitness via pollinator attraction, and geitonogamous (between-flower) self-pollination rates via the foraging behavior of pollinators among flowers within plants.

Pollinator behavior and the severity of inbreeding depression of selfed seeds following geitonogamous self-pollination impose trade-offs between maternal and paternal components of plant fitness that govern the ESS. We investigate the ESS by examining the fate of an initially rare modifier of flowering time (σ^*) in a resident plant population at equilibrium, assuming infinite population size and a uniform distribution of the average flowering time, i.e.

aseasonal reproduction. The ESS can be expressed in terms of standard deviations in individual flowering phenology, σ, derived from the maximal expected relative fitness of the rare modifier (σ^*) in the resident plant population ($\partial w^*/\partial \sigma^* = 0$ at $\sigma^* = \sigma$):

$$w^* = G(\sigma^*)\bar{w}_{self}T(\sigma^*) + \frac{1}{2}[1 - G(\sigma^*)]\bar{w}_{out}T(\sigma^*) + \frac{1}{2}[1 - G(\sigma)]\bar{w}_{out}\frac{P(\sigma^*)}{P(\sigma)}T(\sigma) \quad (1)$$

with $G(\sigma)$, $T(\sigma)$ and $P(\sigma)$ respectively the fraction of selfed seeds produced by geitonogamous selfing (i.e. geitonogamous pollination rate), number of flowers visited (~ ovules fertilized) and pollen export of genotypes with standard deviation in flowering phenology σ, and $\bar{w}_{self}$ and $\bar{w}_{out}$ the mean fitnesses of selfed and outcrossed individuals controlling inbreeding depression ($\delta = 1 - \bar{w}_{self}/\bar{w}_{out}$). The three terms of equation (1) correspond respectively to seed production via selfing, seed production via outcrossing and cross-fertilization of ovules on other plants.

We choose a mechanistic model of pollination, instead of phenomenological functions generally used to describe the relationships between $G(\sigma)$, $T(\sigma)$ and $P(\sigma)$. With this model (described below), $G(\sigma)$, $T(\sigma)$ and $P(\sigma)$ depend on individual flowering phenology, pollinator abundance and pollinator behavior. Trade-offs between fitness components, such as pollen discounting, are emerging properties instead of being hypothesized. We make several simplifying but realistic assumptions to keep the mechanistic model of pollination general (Devaux *et al.* 2014a) and introduce a minimal amount of demographic stochasticity. To derive equation (1) we assume that:

(i) individual pollinators are generalist, such that their density does not depend on the floral density of the focal plant species but on the density of all plant species in a community; this assumption ensures that there is no environmental stochasticity,

(ii) pollinators are constant, i.e. faithful to the focal plant species within a foraging bout (Chittka *et al.* 1999). Relaxing this assumption would likely lower the number of pollinator visits and the amount of conspecific pollen deposited on stigmas and exported to conspecifics, with the same predicted effects as a variation in pollen abundance M or pollen loads A on pollinators (see below),

(iii) the number of daily pollinator visits per plant is Poisson distributed, with a mean determined by both pollinator abundance (M) and the function of pollinator attraction to plants with a given floral display (e.g. Klinkhamer *et al.* 1989; Klinkhamer and de Jong 1990); this assumption introduces a minimal amount of demographic stochasticity, which, unlike environmental stochasticity, operates independently among individual plants in the population. It also depicts the observed correlation between pollinator visitation rates and inflorescence size (e.g. Willson and Schemske 1980; Bauer *et al.* 2017),

(iv) pollinators have the same pollen carryover $1-\rho$, where ρ is the rate of pollen uptake and deposition by pollinator; this assumption ensures the observed decay of pollen deposition from a single flower to subsequent flowers (Price and Waser 1982),

(v) pollinators have a constant probability τ to leave a plant after each flower visited, such that they visit more flowers, but a smaller proportion of flowers, on plants with larger vs. smaller floral displays (Snow *et al.* 1996; Chittka *et al.* 1999; Ohashi and Yahara 2001; Harder *et al.* 2004; Ishii and Harder 2006),

(vi) the sequence of flower visitation by pollinators on a plant is either random (hereafter "random-rank") or constant (e.g. always visiting flowers from bottom to top, hereafter "constant rank"). These two extreme behaviors are likely to encompass the variability of visiting patterns across pollinators (with e.g. a higher tendency for constant sequences in bumblebees; Best and Bierzychudek 1982; Harder and Barrett 1995; Harder *et*

al. 2000) and floral architectures (e.g. more constant sequences in racemes vs. umbels; Jordan and Harder 2006),

(vii) a single pollinator visit is sufficient to fertilize all ovules on a flower, which requires few ovules per flower, or large and constant pollen loads of *A* pollen grains for pollinators that groom little among visited flowers (pollen saturation as in de Jong *et al.* 1993 with *A* pollen grains on the pollinator body). This assumption, although not always verified (Harder and Thomson 1989), best models nectarivorous pollinators (Castellanos *et al.* 2003),

(vii) individual pollinators visit a given flower only once, which is observed frequently (Best and Bierzychudek 1982; Goulson *et al.* 1998; Ohashi and Yahara 1999; Stout and Goulson 2001),

(viii) all plants have the same ovule number per flower,

(ix) because of infinite population size, reproduction is never limited by mate availability, regardless of plant phenology.

With these assumptions, $T(\sigma)$ and $w(\sigma)$ can be expressed either in numbers of flowers visited or numbers of seeds produced, as they are proportional. Although all these assumptions may not always be observed in natural populations (see Devaux *et al.* 2014a for more details), changing them should not affect our main conclusions that pollen limitation evolves as a byproduct of selection for increased pollinator attraction but also for avoidance of inbreeding depression. The few assumptions that can change qualitatively (and not just quantitatively) the results of the model are either explored by changing parameter values (see below), or thoroughly discussed.

Devaux *et al.* (2014a) showed that this model predicts two types of equilibria: (1) evolutionarily stable equilibria (ESS) determined by a trade-off between pollinator attraction to large floral displays and avoidance of inbreeding depression due to selfing, with intermediate geitonogamous selfing rates and (2) ecologically stable equilibria, corresponding

to extremely long or short flowering phenologies constrained by pollinator behavior only, which yield minimal or maximal selfing rates. The latter equilibria are rarely observed in natural populations, in which they are constrained by mechanisms not included in the present model. Therefore we ignore them for our study of mechanisms leading to pollen limitation.

We explore the causes of pollen limitation by inspecting three fitness components at the evolutionarily stable standard deviation in flowering time: (1) the total number of flowers pollinated T, i.e. not including inbreeding depression and thus reflecting only a limitation in the number of pollinator visits, (2) viable seed production (first two terms in equation 1, hereafter "maternal fitness"), including pollinator shortage and inbreeding depression but not pollen export, and, (3) plant total fitness (expressed in number of flowers, as it is proportional to seed production), thus including limitation in the number of seeds produced and the amount of pollen exported accounting for pollinator shortage and inbreeding depression. Note that even if the paternal outcross component of fitness is not included in the first two components, it does constrain the existence and position of the ESS. We quantify pollen limitation at the ESS with two measures, within which most empirical estimates fall. We exclude pre-zygotic effects of pollen quality on pollen limitation (e.g., slow pollen tube growth) by assuming that all conspecific pollen fertilizes equally ovules and that inbreeding depression acts only on post-zygotic components of fitness (i.e. seed viability). We also exclude components of pollen limitation due to costs of producing ovules or maturating seeds (i.e. plants have enough resources to mature all viable seeds), and thus focus on the joint effects of the number of pollinator visits and inbreeding depression on pollen limitation. Pollen limitation is first measured at an ESS as the fraction of unfertilized ovules or equivalently in our model the fraction of unpollinated flowers, $(\overline{N} - T)/\overline{N}$. This measure of pollen limitation describes the potential shortage in pollinator visits. Second, we incorporate embryo quality by measuring pollen limitation as the reduction in plant maternal fitness w_m at the ESS (viable seeds) due to

inbreeding depression: $(w_{m,\max} - w_m)/w_{m,\max}$ with $w_{m,\max} = \bar{N}(1+G)/2$ when $\bar{w}_{self} = \bar{w}_{out} = 1$. This second measure is used to portray situations for which all flowers receive sufficient pollen to fertilize all ovules (no pollinator shortage), yet seed production is still increased by manual supplementation with outcross pollen (Eckert *et al.* 2010). In other words, this second measure is positive only if inbreeding depression, and not pollinator availability, is responsible for fitness loss. We further assess whether an ESS occurs at the strategy σ' that both maximizes total plant fitness w' and minimizes pollen limitation $(\bar{N} - w')/\bar{N}$, given the constraints generated by pollinators. If $w \leq w'$, we determine whether the evolutionary equilibrium (or equilibria when several exist) corresponds to a larger daily floral display (a shorter plant flowering period $\sigma < \sigma'$) or a smaller display (a longer plant flowering period $\sigma > \sigma'$) than the phenology that minimizes pollen limitation at σ'.

We focus on a reference case chosen to match typical observations (see Devaux *et al.* 2014a for details and Table S1 **[Supplementary Information]**) and then vary some parameters that most strongly influence pollinator behavior and mating system evolution. In the reference *base* case, plants produce $\bar{N} = 100$ flowers, pollen carry-over of pollinators is $1 - \rho = 0.75$. Limitation occurs in pollinator abundance with $M = 100$ pollinators per day, $A = 100$ pollen grains on pollinators body and a probability that a pollinator departs a plant after visiting a flower of $\tau = 0.33$. Limitation also occurs in pollinator attraction via a positive relationship between the number of visits $v(F)$ and daily floral display F $v(F) = F/\left[(F+1)\left(1 + a\, e^{-bF}\right)\right]$, with $a = 50$, $b = 0.1$ (Fig. S1 **[Supplementary Information])**. Inbreeding depression either evolves with the selfing rate (i.e. its purging is possible via lower survival of individuals carrying more deleterious mutations; genomic rate to nearly recessive lethal alleles $U = 0.02$, dominance coefficient $h = 0.02$) or is constant (background inbreeding depression, $d = 0.25$). This parameterization is chosen to match values observed in natural populations, in which not all flowers are expected to be pollinated because plants

receive a finite number of pollinator visits and pollinators leave a plant before visiting all of its open flowers (Ohashi and Yahara 1999). For example with these parameter values ca. 3 flowers among 10 displayed are visited in a single bout for the random-rank model of pollinator foraging behaviour. We investigate the relative contribution of mechanisms driving pollen limitation over a wide range of parameter values that govern pollinator abundance limitation, pollinator attraction limitation, pollinator movements among flowers of the same plant, severity of inbreeding depression and total number of flowers produced per plant (Table S1 **[Supplementary Information]**).

RESULTS

None of the evolutionarily stable equilibria maximizes mean total fitness ($w < w'$) for any set of pollinator and genetic constraints we model (Figs. 1-3 and Figs. S2-S3 for the constant-rank model **[Supplementary Information]**). This occurs because of frequency-dependent selection on the individual flowering phenology and selfing rate: Fisher's automatic genetic advantage of a rare, completely selfing mutant is 50% in a strictly outcrossing population and decreases to 0 when the selfing genotype has completely invaded the population. The ESS also do not correspond to flowering phenologies that maximize the fraction of flowers that can be pollinated by either self or outcross pollen. Nevertheless some ESS are close to the strategy that minimizes pollen limitation by mass blooming on a single day (with standard deviation in flowering time much lower than 1), thus maximizing pollinator attraction (Fig. 1A). As explained above, these rarely observed equilibria are not discussed further.

When multiple ESS exist, the most realistic ones (with $\sigma > 1$ day, see above) always consist of extended flowering phenologies with a small fraction of total flowers open per day. These equilibria involve a trade-off between pollinator attraction to daily floral display and seed quality determined by inbreeding depression and geitonogamous selfing, and they

depend on the foraging behavior of pollinators. For the *base* case consisting of plants with $\bar{N} = 100$ flowers, with limitation of both pollinator abundance and pollinator attraction and with substantial inbreeding depression, a random-rank visitation sequence of flowers on a plant by individual pollinators generates high (maternal and/or paternal) outcross reproductive success independent of floral display, whereas a constant-rank visitation sequence produces higher outcross reproductive success only if fewer flowers are open per day (Fig. 1). This pattern is created because different pollinators deposit outcross pollen on different flowers under random movement, but under the constant movement different flowers can be outcrossed only if flowers are open on different days. For the same reasons and all else being equal, pollen limitation is higher at an ESS under constant-rank rather than random-rank visitation sequences of flowers (Table 1; Figs. 1-3 and Figs. S2-S3 **[Supplementary Information]**).

The strong effect of the genetic composition of pollen receipt on pollen limitation is demonstrated by analyzing flowering phenologies that evolve under reduced inbreeding depression of selfed seeds (Figs. 2A and S2A **[Supplementary Information]** for the random- vs. constant-rank pollinator visitation sequence of flowers on a plant). With lower inbreeding depression, flowering phenologies at evolutionary equilibria are shorter, plants display more flowers per day, a higher proportion of them is pollinated because plants receive more pollinator visits, and thus pollen limitation is diminished. The interaction between genetic and ecological constraints is again exemplified by the great difference in pollen limitation at equilibrium under random- versus constant-rank visitation sequence of flowers (9% vs 35%; Table 1). In the latter case, more flowers per plant are pollinated only if they are open on different days. Therefore equilibrium individual flowering phenologies are longer under the constant- than the random-rank visitation sequence model ($\sigma \sim 10$ in Fig. S2A vs. $\sigma < 1$ in Fig. 2A).

Two modifications of the base conditions alter the intensity of pollinator abundance limitation: a change in the mean pollinator abundance on a given day (M) and a change in the expected number of open flowers that a pollinator visits on plants (via the leaving probability τ). Decreasing pollinator abundance increases pollen limitation at an ESS despite the shorter plant flowering phenologies that evolve to maintain a substantial visitation rate from pollinators. With low pollinator abundance the difference in individual flowering phenologies, and resulting pollen limitation, between the two models of pollinator visitation are small. This pattern is expected because the few pollinator visits lead to similar numbers of cumulative flowers visited for the two visitation models (Table 1; Figs. 2B and S2B **[Supplementary Information]** for the random- vs constant-rank visitation sequence of flowers). Similarly, greatly increasing the number of open flowers pollinators visit shortens the plant flowering phenology and shrinks the difference between the visitation patterns of pollinators. Pollen limitation at these equilibria is mostly due to large inbreeding depression of geitonogamous seeds and not to pollinator limitation (Figs. 2C and S2C **[Supplementary Information]** panels for the random- vs constant-rank visitation sequence of flowers).

The ESS depend critically on the intensity of pollinator attraction limitation (Devaux *et al.* 2014a), which can be altered in two ways: by changing either the pollinator attraction function (Fig. S1 for its shape and intensity **[Supplementary Information]**) or the expected total number of flowers per plant (Figs. 3 and S3; number of flowers decreased or increased by an order of magnitude **[Supplementary Information]**). For the same pollinator attraction function, species that produce fewer flowers per plant are predicted to have shorter flowering phenologies to sustain pollinator visitation, with strong pollen limitation (Figs. 3A and S3A **[Supplementary Information]** for the random- vs constant-rank visitation sequence; Table 1). Differences between pollinator visitation patterns of flowers on a plant are intensified with increased flower production per plant. A random-rank visitation sequence generates multiple

stable equilibria: flowering phenologies of a few weeks characterized by pollen limitation mostly due to the low quality of selfed seeds rather than a shortage of pollinator visits, and much longer flowering phenologies with high pollen limitation due mainly to low pollinator attraction, rather than pollen genetic composition. In contrast, a constant-rank visitation sequence generates only extended flowering phenologies favoring outcross pollination; these phenologies are strongly pollen limited because of low pollinator attraction (Figs. 3B and S3B **[Supplementary Information]** for the random- vs constant-rank visitation sequence; Table 1).

DISCUSSION

This study complements earlier theoretical work on pollen limitation by highlighting a potential additional mechanism driving evolution of limited seed production in natural populations of animal-pollinated plants. We show that both the quantity and genetic composition of pollen receipt of self-compatible animal-pollinated plant species control the evolutionary equilibrium flowering phenologies and consequent pollen limitation. Mean fitness is not maximized, and pollen limitation is never minimized at equilibrium. Non-maximization of mean fitness is explained by frequency-dependent selection, which violates the assumption of constant genotypic fitnesses required for Wright's (1931, 1969) principle of evolutionary maximization of mean fitness. In this model, as in earlier ones (e.g. Bierzychudek 1981; Haig and Westoby 1988; Burd 1995, 2008; Harder and Aizen 2010; Schreiber *et al.* 2015) pollen limitation is an evolved emergent property; however, here pollen limitation is constrained by a trade-off between the maternal self and paternal outcross components of fitness that involves both genetic and ecological constraints (Devaux *et al.* 2014a), whereas most previous models only considered ecological constraints. The ecological constraints include pollinator abundance, pollinator attraction to large floral displays,

expected pollinator bout length and pollinator visitation patterns of flowers on a plant; the genetic constraints include inbreeding depression on plant viability and Fisher's automatic advantage of selfing.

Our theoretical approach is mechanistic, which allows an analysis of the causes of pollen limitation among several parameters describing pollinator behavior, floral traits and inbreeding depression. However, as all models, it relies on several necessary simplifying assumptions and omits some potentially important ecological mechanisms that can also influence the evolution of pollen limitation. In the following, we first discuss some implications of our results and identify predictions that could be tested in natural populations. We then outline the main limitations of our approach and some useful perspectives to broaden our evolutionary understanding of pollen limitation.

Relevance of our model to study pollen limitation in natural populations

The predicted flowering phenologies depend on the pollinator foraging behavior among flowers on a plant. These phenologies are expected to be longer when different pollinators visit flowers of a plant in the same order, and generate higher selfing rates, as experimentally found for bees (Jordan and Harder 2006), and consequently higher pollen limitation than when pollinators visit flowers on a plant in random order. The differences generated by pollinator movements on a plant are reduced if pollinators with constant visitation sequence among flowers also carry more pollen and/or visit more flowers per plant. Higher pollen limitation under the constant vs. random rank visitation pattern is caused both by a lower number of flowers visited by pollinators and by inbreeding depression in selfed seeds. Pollen limitation is thus predicted to critically depend on pollinator species, inflorescence size and architecture, all of which are known to impact the foraging path among flowers on a plant. Although our model was not designed to examine the effect of inflorescence architecture on the evolution of pollen limitation, it could be used to test the following prediction: plant

species with racemes have been demonstrated to elicit more constant pollinator pathways among flowers than plant species with umbels (Jordan and Harder 2006), such that plant species with racemes are expected to suffer higher pollen limitation. However, an accurate test of this prediction should be based on a model that incorporates explicitly inflorescence architecture.

Regardless of pollinator behavior, geitonogamous (between-flower within plant) selfing imposed by pollinators and its associated inbreeding depression often cause evolution of long flowering phenologies in which plants produce few flowers per day and thus avoid inbreeding depression at the cost of reduced pollinator attraction. Our results therefore predict that plant species with lower inbreeding depression would evolve shorter flowering phenologies with larger daily floral displays, which would enhance pollinator attraction and thus reduce pollen limitation. This could be tested by examining the relationship between pollen limitation and inbreeding depression in natural populations. Note that there are potential caveats (see below), the main one being that the expected positive relationship between pollen limitation and inbreeding depression may also be caused by environmental stochasticity: the intensity of temporal fluctuations in pollinator abundance within season correlates positively with the duration of flowering phenologies (Devaux and Lande 2010), and is also expected with pollen limitation, as observed in temporal cohorts within a season (Thomson 2010). The predicted extended flowering phenologies under higher inbreeding depression suffer a high risk of daily pollination failure due to temporal fluctuations in pollinator abundance or activity among days within seasons (Devaux and Lande 2010), and depend crucially on floral constancy of generalist pollinators among plant species, which is nonetheless frequently observed in plant communities (Chittka *et al.* 1999).

Pollen limitation measured over the entire individual flowering phenology, as we do here, may differ from that measured over parts of the phenology (Knight *et al.* 2006) or that

measured as the difference in seed production under natural and artificially supplemented pollination (Knight *et al.* 2005). The first type of discrepancy highlights the role of resource allocation in pollen limitation. The second discrepancy can be generated by abortion of inbred embryos; therefore pollen limitation may be frequently overestimated for partially selfing species because it is usually measured by supplementing large amounts of outcross pollen (Aizen and Harder 2007). In other terms, comparing the number of viable seeds under supplemental outcrossed and selfed pollen can help distinguish the cause of pollen limitation, between a shortage of pollinator visits (increased seed production with outcrossed or selfed pollen) and inbreeding depression (smaller increase in seed production with self vs. outcross pollen). Estimating pollen limitation in species whose selfing rates are constrained by pollinators (Devaux *et al.* 2014b; a) while accounting for inbreeding depression of selfed seeds is a difficult but necessary task if the causes of pollen limitation are to be determined, as already mentioned by Eckert *et al*. (2010), and several experimental methods that also account for plant resources are available (Calvo and Horvitz 1990; Aizen and Harder 2007; Wesselingh 2007; Alonso *et al.* 2012; Arceo-Gomez and Ashman 2014).

Limitations and perspectives to model the evolution of pollen limitation

Pollen limitation evolves in this study by mechanisms different from those analyzed in previous theory. First and most importantly, we deliberately excluded environmental stochasticity for the sake of simplicity : it proved to be the main driver of within and among plant variation in pollination and the evolution of pollen limitation in previous models (Burd 2008; Richards *et al.* 2009; Rosenheim *et al.* 2014; Schreiber *et al.* 2015) and is undoubtedly responsible for some pollen limitation in natural populations. Instead we include demographic stochasticity (operating independently among individuals, unlike environmental stochasticity) to produce variation in the number of open flowers a given day, variation in the number of pollinator visits to a plant, and variation in the number of flowers visited per plant per

pollinator visit. Because our model involves an infinitely large population, such demographic stochasticity has little impact on evolutionary equilibria in comparison to the temporal environmental stochasticity that is synchronized among all individuals in other models. Pollen limitation in our model evolves because of a genetic trade-off between pollinator attraction (as well as other aspects of pollinator behavior) and inbreeding depression after zygote formation. Predicting how these mechanisms may interact with environmental stochasticity is not straightforward. In self-compatible insect-pollinated plants, both inbreeding depression with selfing and temporal fluctuations in pollinator availability (i.e. pollinator visits per plant, which could be due to fluctuations in pollinators and/or plant population density, Thomson 2010) should contribute to the evolution of pollen limitation. Obviously, in a highly stochastic environment, highly variable pollinator availability is much more likely important than is inbreeding depression with selfing. In a more constant environment with stable pollinator availability, the contribution of inbreeding depression to pollen limitation depends on both the selfing rate and how much inbreeding depression can be purged.

Evolution of individual flowering phenologies, and consequent pollen limitation, may also be driven by genetic and ecological factors not considered here, acting at both the individual and community levels: our mechanistic model of pollinator behavior is simplified, to address ubiquitous genetic and ecological mechanisms responsible for the emergence of pollen limitation, and cannot portray the immense variation among pollinator species. First, plant resources are limited in our model as all plants display the same expected number of flowers, but we neglect allocation to seed provisioning considered by previous authors (Bierzychudek 1981; Haig and Westoby 1988; Ashman *et al.* 2004). Resource allocation may be particularly crucial to understand pollen limitation in iteroparous species (Crone *et al.* 2009), which are not considered in our model. Instead we model allocation to flowers among days in the flowering phenology of individual plants and allow plants to mature all seeds

without reproductive compensation. Second, autonomous selfing has been proposed many times as a reproductive assurance strategy under pollinator limitation (Fishman and Willis 2008; Marten-Rodriguez and Fenster 2010; Thomann *et al.* 2013); its evolution towards increased selfing was found in natural populations (Moeller 2006) and in experimental populations experiencing pollinator abundance limitation (Bodbyl Roels and Kelly 2011). Allowing autonomous selfing and its evolution can have complex effects on the evolution of flowering phenologies (Devaux *et al.* 2014a) and pollen limitation (Morgan and Wilson 2005; Harder *et al.* 2008). We also do not account for facilitated selfing (c.f. Lloyd and Schoen 1992) as little empirical information exists on this intra-flower component of selfing except for specific flower morphologies (Johnson *et al.* 2005; Owen *et al.* 2007; Vaughton *et al.* 2008). Evolution of sterile flowers can reduce pollen limitation by increasing attraction of pollinators at low energetic and genetic costs (Morales *et al.* 2013). We further do not address pollen competition between self and outcross pollen, or among multiple sires (Lankinen and Armbruster 2007; Richards *et al.* 2009) possibly complicated by pollen precedence (Waser and Fugate 1986), the evolution of aggregated pollen that occurs in orchids (Harder and Johnson 2008), or mechanisms such as dichogamy or herkogamy that can prevent geitonogamous selfing. Our model also omits several factors operating at the community level among plant species that can affect both the amount and genetic composition of pollen receipt: e.g. facilitation and competition among species (Moeller 2004; Vamosi *et al.* 2006; Hegland and Totland 2008; Devaux and Lande 2009; Sargent *et al.* 2011; Lazaro *et al.* 2014), which partly depends on the constancy of pollinators to a plant species and the transfer of heterospecific pollen, and can potentially affect the evolution of autonomous selfing.

CONCLUSION

Ecological constraints alone predict that many flowers remain unpollinated because pollen or plant resources for fruit production and seed maturation are limited. Beyond the role of trade-

offs among costly reproductive functions, our results show that pollen limitation is an evolved property that depends also on genetic mechanisms and pollinator behaviors that constrain mating systems and the evolution of plant flowering phenologies. Our model suggests that, despite strong pollinator attraction limitation, plants do not evolve short phenologies with an excess of flowers to attract pollinators, but instead evolve long flowering phenologies with relatively few flowers open per day to favor outcross pollination, resulting in pollen limitation due to reduced pollinator attraction to daily floral displays. Future research on pollen limitation should examine how genetic processes interact with more commonly studied ecological processes (resource limitation and environmental stochasticity) to drive evolution of pollen limitation.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: Ecological and genetic parameters, along with the values investigated and their units. Figure S1: Pollinator attraction function as a function of (log) daily floral display. Figure S2: Fitness components and floral display under a constant-rank visitation sequence of flowers on a plant and under decreased inbreeding depression, decreased number of pollinators available, and increased fraction of open flowers visited by pollinators. Figure S3: Fitness components and floral display under a constant-rank visitation sequence of flowers on a plant and under decreased and increased flower production per plant.

FUNDING

This work was supported by the French CNRS program PICS grant #5273 to E.P., and a grant from the Balzan Foundation and a Royal Society Research Professorship to R.L.

LITERATURE CITED

Aizen MA, Harder LD. **2007**. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* **88**: 271–281.

Alonso C, Herrera CM, Ashman T-L. **2012**. A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. *New Phytologist* **193**: 532–542.

Alonso C, Vamosi JC, Knight TM, Steets JA, Ashman T-L. **2010**. Is reproduction of endemic plant species particularly pollen limited in biodiversity hotspots? *Oikos* **119**: 1192–1200.

Angeloni F, Ouborg NJ, Leimu R. **2011**. Meta-analysis on the association of population size and life history with inbreeding depression in plants. *Biological Conservation* **144**: 35–43.

Arceo-Gomez G, Ashman T-L. **2014**. Patterns of pollen quantity and quality limitation of pre-zygotic reproduction in *Mimulus guttatus* vary with co-flowering community context. *Oikos* **123**: 1261–1269.

Ashman TL, Knight TM, Steets JA, *et al.* 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**: 2408–2421.

Bauer AA, Clayton MK, Brunet J. **2017**. Floral traits influencing plant attractiveness to three bee species: Consequences for plant reproductive success. *American Journal of Botany* **104**: 772–781.

Bell G. **1985**. On the function of flowers. *Proceedings of the Royal Society B-Biological Sciences* **224**: 223–265.

Best LS, Bierzychudek P. **1982**. Pollinator foraging on foxglove (*Digitalis purpurea*): a test of a new model. *Evolution* **36**: 70–79.

Bierzychudek P. **1981**. Pollinator limitation of plant reproductive effort. *American Naturalist* **117**: 838–840.

Bodbyl Roels SA, Kelly JK. **2011**. Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution* **65**: 2541–2552.

Burd M. **1994**. Bateman principle and plant reproduction - The role of pollen limitation. *Botanical Review* **60**: 83–139.

Burd M. **1995**. Ovule packaging in stochastic pollination and fertilization environments. *Evolution* **49**: 100–109.

Burd M. **2008**. The Haig-Westoby model revisited. *American Naturalist* **171**: 400–404.

Burd M. **2016**. Pollen limitation is common-Should it be? *American Naturalist* **187**: 388–396.

Burd M, Callahan HS. **2000**. What does the male function hypothesis claim? *Journal of Evolutionary Biology* **13**: 735–742.

Calvo R, Horvitz C. **1990**. Pollinator limitation, cost of reproduction, and fitness in plants - a transition-matrix demographic approach. *American Naturalist* **136**: 499–516.

Castellanos MC, Wilson P, Thomson JD. **2003**. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**: 2742–2752.

Charlesworth D. **1989**. Evolution of low female fertility in plants - Pollen limitation, resource-allocation and genetic load. *Trends in Ecology & Evolution* **4**: 289–292.

Chittka L, Thomson JD, Waser NM. **1999**. Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* **86**: 361–377.

Crone EE, Miller E, Sala A. **2009**. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. *Ecology Letters* **12**: 1119–1126.

Devaux C, Lande R. **2009**. Displacement of flowering phenologies among plant species by competition for generalist pollinators. *Journal of Evolutionary Biology* **22**: 1460–1470.

Devaux C, Lande R. **2010**. Selection on variance in flowering time within and among individuals. *Evolution* **64**: 1311–1320.

Devaux C, Lande R, Porcher E. **2014a**. Pollination ecology and inbreeding depression control individual flowering phenologies and mixed mating. *Evolution* **68**: 3051–3065.

Devaux C, Lepers C, Porcher E. **2014b**. Constraints imposed by pollinator behaviour on the ecology and evolution of plant mating systems. *Journal of Evolutionary Biology* **27**: 1413–1430.

Dudash M. **1991**. Plant size effects on female and male function in hermaphroditic *Sabatia angularis* (Gentianaceae). *Ecology* **72**: 1004–1012.

Dudash M. **1993**. Variation in pollen limitation among individuals of *Sabatia angularis* (Gentianaceae). *Ecology* **74**: 959–962.

Eckert CG, Kalisz S, Geber MA, *et al.* 2010. Plant mating systems in a changing world. *Trends in Ecology & Evolution* **25**: 35–43.

Fishman L, Willis JH. **2008**. Pollen limitation and natural selection on floral characters in the yellow monkeyflower, *Mimulus guttatus*. *New Phytologist* **177**: 802–810.

Geber MA. **1985**. The relationship of plant size to self-pollination in *Mertensia ciliata*. *Ecology* **66**: 762–772.

Goulson D, Hawson SA, Stout JC. **1998**. Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Animal Behaviour* **55**: 199–206.

Haig D, Westoby M. **1988**. On limits to seed production. *American Naturalist* **131**: 757–759.

Harder LD, Aizen MA. **2010**. Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 529–543.

Harder LD, Barrett SCH. **1995**. Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**: 512–515.

Harder LD, Barrett SCH, Cole WW. **2000**. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **267**: 315–320.

Harder LD, Johnson SD. **2008**. Function and evolution of aggregated pollen in angiosperms. *International Journal of Plant Sciences* **169**: 59–78.

Harder LD, Jordan CY, Gross WE, Routley MB. **2004**. Beyond floricentrism: The pollination function of inflorescences. *Plant Species Biology* **19**: 137–148.

Harder LD, Richards SA, Routley MB. **2008**. Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution* **62**: 157–172.

Harder L, Thomson J. **1989**. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* **133**: 323–344.

Hegland SJ, 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.

Husband BC, Schemske DW. **1996**. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

Ishii HS, Harder LD. **2006**. The size of individual *Delphinium* flowers and the opportunity for geitonogamous pollination. *Functional Ecology* **20**: 1115–1123.

Johnson SD, Neal PR, Harder LD. **2005**. Pollen fates and the limits on male reproductive success in an orchid population. *Biological Journal of the Linnean Society* **86**: 175–190.

de Jong TJ, Waser N, Klinkhamer P. **1993**. Geitonogamy: the neglected side of selfing. *Trends in Ecology & Evolution* **8**: 321–325.

Jordan CY, Harder LD. **2006**. Manipulation of bee behavior by inflorescence architecture and its consequences for plant mating. *American Naturalist* **167**: 496–509.

Karron JD, Mitchell RJ. **2012**. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Annals of Botany* **109**: 563–570.

Knight TM, Steets JA, Ashman TL. **2006**. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany* **93**: 271–277.

Knight TM, Steets JA, Vamosi JC, *et al.* 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology Evolution and Systematics* **36**: 467–497.

Lankinen A, Armbruster WS. **2007**. Pollen competition reduces inbreeding depression in *Collinsia heterophylla* (Plantaginaceae). *Journal of Evolutionary Biology* **20**: 737–749.

Larson BMH, Barrett SCH. **2000**. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* **69**: 503–520.

Lau JA, Miller RE, Rausher MD. **2008**. Selection through male function favors smaller floral display size in the common morning glory *Ipomoea purpurea* (Convolvulaceae). *American Naturalist* **172**: 63–74.

Lazaro A, Lundgren R, Totland O. **2014**. Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. *Oikos* **123**: 1037–1048.

Lloyd DG. **1992**. Self-fertilization and cross-fertilization in plants. 2. The selection of self-fertilization. *International Journal of Plant Sciences* **153**: 370–380.

Lloyd DG, Schoen DJ. **1992**. Self-fertilization and cross-fertilization in plants. 1. Functional dimensions. *International Journal of Plant Sciences* **153**: 358–369.

Marten-Rodriguez S, Fenster CB. **2010**. Pollen limitation and reproductive assurance in Antillean Gesnerieae: a specialists vs. generalist comparison. *Ecology* **91**: 155–165.

Moeller DA. **2004**. Facilitative interactions among plants via shared pollinators. *Ecology* **85**: 3289–3301.

Moeller DA. **2006**. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. *Ecology* **87**: 1510–1522.

Moeller DA, Geber MA, Eckhart VM, Tiffin P. **2012**. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. *Ecology* **93**: 1036–1048.

Morales CL, Traveset A, Harder LD. **2013**. Sterile flowers increase pollinator attraction and promote female success in the Mediterranean herb *Leopoldia comosa*. *Annals of Botany* **111**: 103–111.

Morgan MT, Wilson WG. **2005**. Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* **59**: 1143–1148.

Ohashi K, Yahara T. **1999**. How long to stay on, and how often to visit a flowering plant? A model for foraging strategy when floral displays vary in size. *Oikos* **86**: 386–392.

Ohashi K, Yahara T. **2001**. Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits (L Chittka and JD Thomson, Eds.). *Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution*: 274–296.

Owen K, Vaughton G, Ramsey M. **2007**. Facilitated autogamy and costs of selfing in the perennial herb *Bulbine bulbosa* (Asphodelaceae). *International Journal of Plant Sciences* **168**: 579–585.

Price M, Waser N. **1982**. Experimental studies of pollen carryover - Hummingbirds and *Ipomopsis aggregata*. *Oecologia* **54**: 353–358.

Richards SA, Williams NM, Harder LD. **2009**. Variation in pollination: causes and consequences for plant reproduction. *American Naturalist* **174**: 382–398.

Rosenheim JA, Williams NM, Schreiber SJ. **2014**. Parental optimism versus parental pessimism in plants: how common should we expect pollen limitation to be? *American Naturalist* **184**: 75–90.

Rosenheim JA, Williams NM, Schreiber SJ, Rapp JM. **2016**. Modest pollen limitation of lifetime seed production is in good agreement with modest uncertainty in whole-plant pollen receipt (A reply to Burd). *American Naturalist* **187**: 397–404.

Sargent RD, Kembel SW, Emery NC, Forrestel EJ, Ackerly DD. **2011**. Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. *American Journal of Botany* **98**: 283–289.

Schreiber SJ, Rosenheim JA, Williams NW, Harder LD. **2015**. Evolutionary and ecological consequences of multiscale variation in pollen receipt for seed production. *American Naturalist* **185**: E14–E29.

Snow AA, Spira TP, Simpson R, Klips RA. **1996**. The ecology of geitonogamous pollination In: *Floral Biology. Studies on floral evolution in animal-pollinated plants*. New York: Chapman and Hall, 191–216.

Stout JC, Goulson D. **2001**. The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behaviour* **62**: 183–189.

Thomann M, Imbert E, Devaux C, Cheptou P-O. **2013**. Flowering plants under global pollinator decline. *Trends in Plant Science* **18**: 353–359.

Thomson JD. **2001**. Using pollination deficits to infer pollinator declines: Can theory guide us? *Conservation Ecology* **5**: 6.

Thomson JD. **2010**. Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 3187–3199.

Vamosi JC, Knight TM, Steets JA, Mazer SJ, Burd M, Ashman TL. **2006**. Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 956–961.

Vaughton G, Ramsey M, Simpson I. **2008**. Does selfing provide reproductive assurance in the perennial herb *Bulbine vagans* (Asphodelaceae)? *Oikos* **117**: 390–398.

Waser N. **1978**. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* **59**: 934–944.

Waser N, Fugate M. **1986**. Pollen precedence and stigma closure - A mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* **70**: 573–577.

Wesselingh RA. **2007**. Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist* **174**: 26–37.

Whitlock R, Stewart GB, Goodman SJ, *et al.* 2013. A systematic review of phenotypic responses to between-population outbreeding. *Environmental Evidence* **2**: 13.

Willson M, Rathcke B. **1974**. Adaptive design of floral display in *Asclepias syriaca L.* *American Midland Naturalist* **92**: 47–57.

Willson M, Schemske D. **1980**. Pollinator limitation, fruit production, and floral display in pawpaw (*Asimina triloba*). *Bulletin of the Torrey Botanical Club* **107**: 401–408.

Wright S. **1931**. Evolution in Mendelian populations. *Genetics* **16**: 97–159.

Wright S. **1969**. *Evolution and the genetics of populations. Vol. 2. Theory of gene frequencies*. Chicago, USA: University of Chicago Press.

TABLES

Table 1. Minimal vs. realized pollen limitation, expressed either as percent of unpollinated flowers $(PL_{unpoll} = 100 \times (\bar{N} - T)/\bar{N})$ or percent of deviation from theoretical maximal maternal fitness $(PL_{devmax} = 100 \times (w_{m,max} - w_m/w_{m,max})$, at evolutionarily stable equilibria described by the individual flowering phenologies σ^* in the *base* case and for several deviations from the *base* case. For a given combination of parameter values, the minimal pollen limitation appears on the first line ("Min").

Parameter values	Random-rank visitation sequence			Constant-rank visitation sequence		
	σ^*	PL_{unpoll}	PL_{devmax}	σ^*	PL_{unpoll}	PL_{devmax}
Base case[1]	Min	5.2	30.7	Min	29.8	50.6
	0.6	7.1	32.1	-	-	-
	34	29.6	57.8	53	31.4	58.6
Lower inbreeding depression (U = 0.02, d = 0)	Min	5.2	6.7	Min	29.8	34.3
	0.59	7.1	8.7	10	30.9	35.2
Reduced pollinator abundance (M = 10)	Min	72	79.5	Min	85.8	89.7
	0.59	72	79.5	0.8	86	89.8
	20	88	92.6	22	88.1	93.3
Reduced pollinator leaving rate (τ = 0.01)	Min	9×10^{-6}	32	Min	9×10^{-6}	32.1
	0.5	0.1	34.2	0.5	0.15	34.5
Reduced attraction (a = 20, b = 0.01)	-	-	-	-	-	-
Smaller floral display ($\bar{N} = 10$)	Min	23.7	44.0	Min	29.8	50.1
	3.35	29.5	57.3	5.25	31.1	56.4
Larger floral display ($\bar{N} = 1000$)	Min	4.6	30.2	Min	29.9	50.6
	5.65	4.8	30.3	-	-	-
	350	29.7	57.7	550	31.4	56.9

[1]Note: *Base* case: $M = 100$ pollinators are available, they carry $A = 100$ pollen grains, their probability of leaving a plant after visiting a flower is $\tau = 0.33$, their pollen carry-over is $1 - \rho = 0.75$, and their visitation rate is defined by $a = 50$ and $b = 0.1$ (Fig. S1 **[Supplementary Information]**); inbreeding depression is due to deleterious mutations that

can or cannot be purged ($U = 0.2$, $h = 0.02$, and $d = 0.25$), plants produce $\bar{N} = 100$ flowers. Evolutionary equilibria are ranked according to increased σ (flowering period of plants).

FIGURE LEGENDS

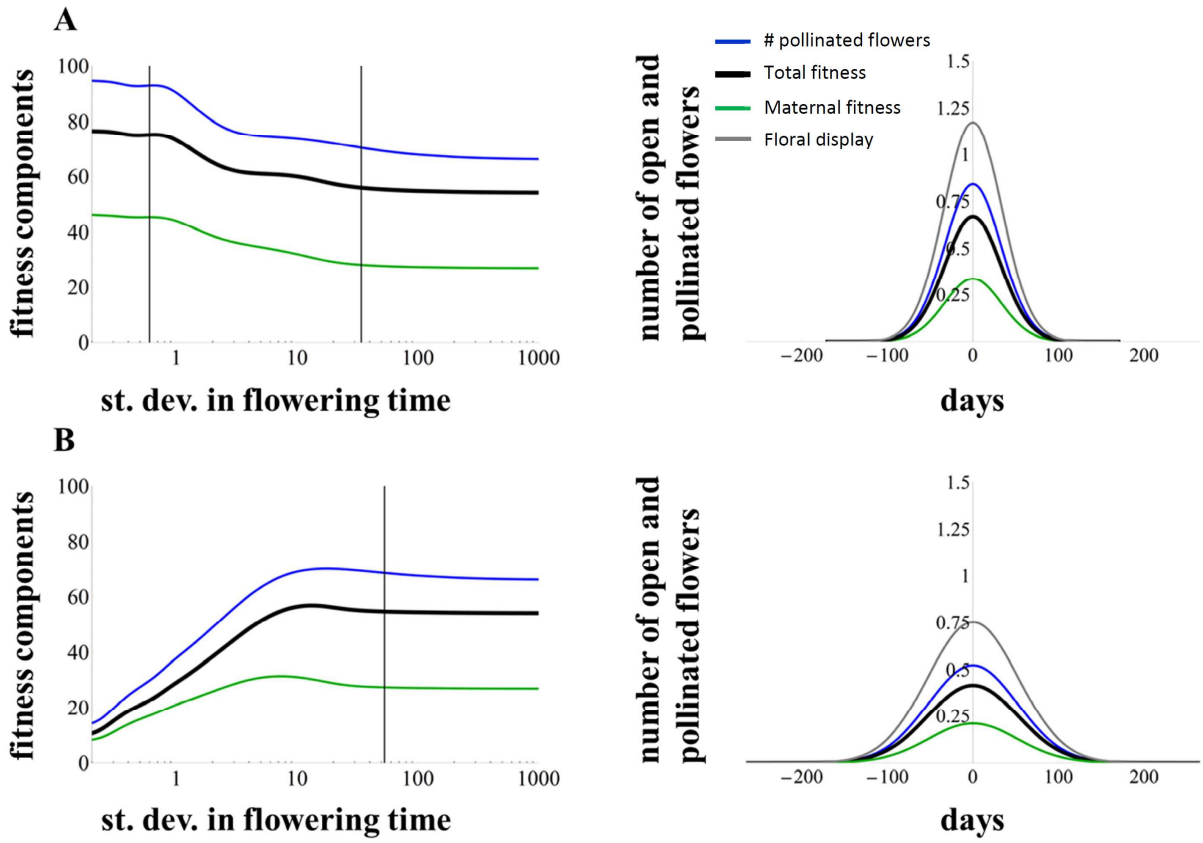

Fig. 1: Fitness components and floral display under two pollinator visitation sequences of flowers on a plant (random-rank for panels A vs. constant-rank visitation sequence for panels B). Left panels: total fitness (w, thick black line), maternal fitness (w_m, green line) and number of pollinated flowers (T, blue line) as a function of (log) standard deviation in flowering time. The solid vertical lines indicate the evolutionarily stable standard deviation(s) in flowering time. Right panels: floral display (gray line), number of pollinated flowers (blue line), maternal fitness (w_m, green line), and total fitness (thick black line) as a function of days at the ESS with the highest standard deviation in flowering time. Pollen limitation can be visualized by comparing floral display (number of open flowers) vs. number of pollinated flowers, number of pollinated flowers vs. total fitness, or number of pollinated flowers vs. maternal fitness (See Table 1 for quantitative measures of pollen limitation). Pollinator attraction limitation defined by $a = 50$ and $b = 0.1$, pollinator abundance limitation by $M =$

100 pollinators, $\tau = 0.33$ and $A = 100$, pollen deposition rate $\rho = 0.25$, $\bar{N} = 100$ flowers per plant, $U = 0.2$, $h = 0.02$ and $d = 0.25$ for inbreeding depression.

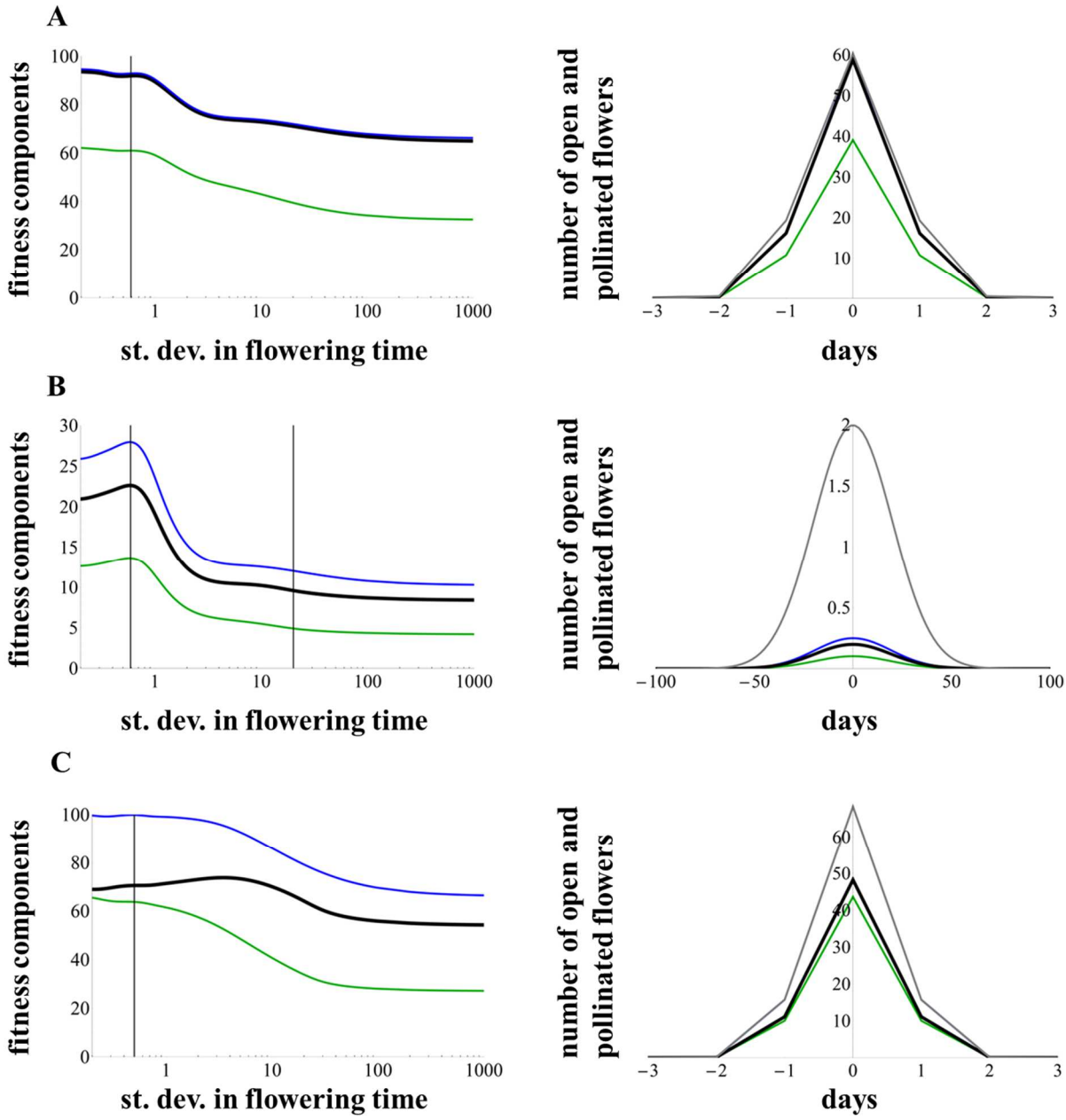

Fig. 2: Fitness components and floral display under decreased inbreeding depression ($U = 0.02$ and $d = 0$, panels A), decreased number of pollinators available ($M = 10$, panels B), and increased fraction of open flowers visited by pollinators ($\tau = 0.01$, panels C). Random-rank visitation sequence of flowers on a plant; other parameters and symbols as in Fig. 1.

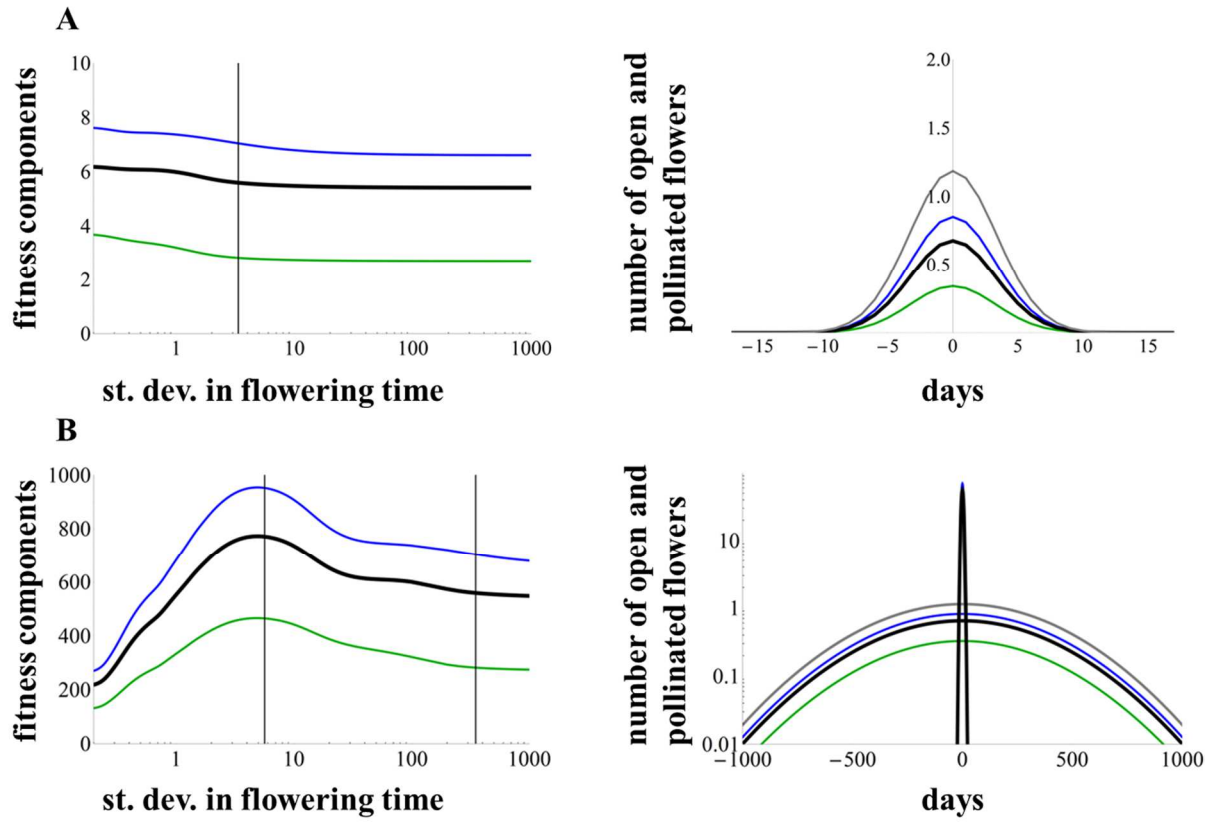

Fig. 3: Fitness components and floral display under decreased ($\bar{N} = 10$, panels A) and increased ($\bar{N} = 1000$, panels B) flower production per plant. Random-rank visitation sequence of flowers on a plant; other parameters and symbols as in Fig. 1. The stable equilibria for both small and large standard deviations in flowering time for plants producing $\bar{N} = 1000$ are shown on a log-scale.