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1 **Type of article:** Original article
2 **Title: Mating systems and avoidance of inbreeding depression as evolutionary drivers of**
3 **pollen limitation in animal pollinated self-compatible plants**

4

5 Céline Devaux^{1*}, Emmanuelle Porcher² and Russell Lande³

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

7 ²Centre d'Ecologie et des Sciences de la Conservation, UMR 7204, Muséum national
8 d'Histoire naturelle, Centre National de la Recherche Scientifique, Sorbonne Université,
9 75005 Paris, France

10 ³Center for Biodiversity Dynamics, Norwegian University of Science and Technology, N-
11 7491 Trondheim, Norway

12

13 **Running title:** Mating systems drive evolution of pollen limitation

14 *For correspondence. Email: celine.devaux@umontpellier.fr

15

16 **ABSTRACT**

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

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

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

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

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

12

13 **KEYWORDS**

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

16

17

1 **INTRODUCTION**

2 Limitation of seed or fruit production is widespread in animal pollinated plants and has three
3 non-exclusive proximate causes related to pollinators, embryo quality and resource
4 availability (Bierzychudek 1981; Ashman *et al.* 2004; Knight *et al.* 2005). Limited
5 reproductive success of a plant can be caused by insufficient pollen receipt: some flowers or
6 some of their ovules remain unfertilized because pollinators are scarce, little attracted to the
7 plant, or visit only a fraction of the flowers open (Geber 1985; Charlesworth 1989; Burd
8 1995; Snow *et al.* 1996; Moeller *et al.* 2012; Schreiber *et al.* 2015), or because pollinators
9 carry insufficient pollen loads (e.g. generalist pollinators depositing heterospecific rather than
10 conspecific pollen, Waser 1978). Even with sufficient conspecific pollen receipt, some seeds
11 on a plant may not mature because embryos are of low quality due to inbreeding (Husband
12 and Schemske 1996; Angeloni *et al.* 2011) or outbreeding depression (Whitlock *et al.* 2013),
13 or because female resources are limited and induce high rates of abortion in otherwise viable
14 seeds or fruits (Willson and Rathcke 1974).

15 Theoretical work has explored ultimate causes of pollen limitation and showed that it
16 can evolve as a result of trade-offs among three costly reproductive functions for plants: ovule
17 production, seed provisioning, and pollinator attraction. Studies of these functions and their
18 trade-offs revealed that within or among plant variation in pollination rates can generate the
19 frequently observed pollen limitation (Bierzychudek 1981; Haig and Westoby 1988; Burd
20 1995, 2008; Schreiber *et al.* 2015), although this is sometimes debated (Rosenheim *et al.*
21 2014, 2016; Burd 2016). Much of this theory was devoted to the role of environmental
22 stochasticity, i.e. random spatial variation or random temporal variation in pollen deposition
23 rate affecting all individual plants in a population simultaneously, as a primary determinant of
24 reproductive trade-offs and consequent pollen limitation (Burd 1995, 2008; Richards *et al.*
25 2009; Schreiber *et al.* 2015). In doing so, most models took an ecological rather than a

1 population genetics point of view, emphasizing maternal fitness only (e.g. Haig and Westoby
2 1988; Burd 1994, 1995; but see Bell 1985; Charlesworth 1989; Burd and Callahan 2000;
3 Harder and Aizen 2010; Thomson 2001). They generally gave little consideration to seed
4 quality and mating system, which are however major evolutionary drivers of plant
5 reproductive strategies (Ashman *et al.* 2004; Devaux *et al.* 2014a) and have proved to
6 correlate with pollen limitation (e.g. self-compatibility for animal-pollinated plant species,
7 Larson and Barrett 2000; Knight *et al.* 2005; Alonso *et al.* 2010).

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

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

7

8 **METHOD**

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

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

1 aseasonal reproduction. The ESS can be expressed in terms of standard deviations in
2 individual flowering phenology, σ , derived from the maximal expected relative fitness of the
3 rare modifier (σ^*) in the resident plant population ($\partial w^*/\partial \sigma^* = 0$ at $\sigma^* = \sigma$):
4

5 $w^* = G(\sigma^*)\bar{w}_{self}T(\sigma^*) + \frac{1}{2}[1 - G(\sigma^*)]\bar{w}_{out}T(\sigma^*) + \frac{1}{2}[1 - G(\sigma)]\bar{w}_{out}\frac{P(\sigma^*)}{P(\sigma)}T(\sigma)$ (1)

6
7 with $G(\sigma)$, $T(\sigma)$ and $P(\sigma)$ respectively the fraction of selfed seeds produced by
8 geitonogamous selfing (i.e. geitonogamous pollination rate), number of flowers visited (~
9 ovules fertilized) and pollen export of genotypes with standard deviation in flowering
10 phenology σ , and \bar{w}_{self} and \bar{w}_{out} the mean fitnesses of selfed and outcrossed individuals
11 controlling inbreeding depression ($\delta = 1 - \bar{w}_{self}/\bar{w}_{out}$). The three terms of equation (1)
12 correspond respectively to seed production via selfing, seed production via outcrossing and
13 cross-fertilization of ovules on other plants.

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

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

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

6 (iii) the number of daily pollinator visits per plant is Poisson distributed, with a mean
7 determined by both pollinator abundance (M) and the function of pollinator attraction to
8 plants with a given floral display (e.g. Klinkhamer *et al.* 1989; Klinkhamer and de Jong
9 1990); this assumption introduces a minimal amount of demographic stochasticity, which,
10 unlike environmental stochasticity, operates independently among individual plants in the
11 population. It also depicts the observed correlation between pollinator visitation rates and
12 inflorescence size (e.g. Willson and Schemske 1980; Bauer *et al.* 2017),

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

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

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

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

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

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

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

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

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

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

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

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

1 inbreeding depression: $(w_{m,\max} - w_m)/w_{m,\max}$ with $w_{m,\max} = \bar{N}(1 + G)/2$ when $\bar{w}_{self} = \bar{w}_{out}$
2 = 1. This second measure is used to portray situations for which all flowers receive sufficient
3 pollen to fertilize all ovules (no pollinator shortage), yet seed production is still increased by
4 manual supplementation with outcross pollen (Eckert *et al.* 2010). In other words, this second
5 measure is positive only if inbreeding depression, and not pollinator availability, is
6 responsible for fitness loss. We further assess whether an ESS occurs at the strategy σ' that
7 both maximizes total plant fitness w' and minimizes pollen limitation $(\bar{N} - w')/\bar{N}$, given the
8 constraints generated by pollinators. If $w \leq w'$, we determine whether the evolutionary
9 equilibrium (or equilibria when several exist) corresponds to a larger daily floral display (a
10 shorter plant flowering period $\sigma < \sigma'$) or a smaller display (a longer plant flowering period
11 $\sigma > \sigma'$) than the phenology that minimizes pollen limitation at σ' .

12 We focus on a reference case chosen to match typical observations (see Devaux *et al.*
13 2014a for details and Table S1 [**Supplementary Information**]) and then vary some
14 parameters that most strongly influence pollinator behavior and mating system evolution. In
15 the reference *base* case, plants produce $\bar{N} = 100$ flowers, pollen carry-over of pollinators is
16 $1 - \rho = 0.75$. Limitation occurs in pollinator abundance with $M = 100$ pollinators per day,
17 $A = 100$ pollen grains on pollinators body and a probability that a pollinator departs a plant
18 after visiting a flower of $\tau = 0.33$. Limitation also occurs in pollinator attraction via a
19 positive relationship between the number of visits $v(F)$ and daily floral display F $v(F) =$
20 $F/[(F + 1)(1 + a e^{-bF})]$, with $a = 50$, $b = 0.1$ (Fig. S1 [**Supplementary Information**]).
21 Inbreeding depression either evolves with the selfing rate (i.e. its purging is possible via lower
22 survival of individuals carrying more deleterious mutations; genomic rate to nearly recessive
23 lethal alleles $U = 0.02$, dominance coefficient $h = 0.02$) or is constant (background
24 inbreeding depression, $d = 0.25$). This parameterization is chosen to match values observed
25 in natural populations, in which not all flowers are expected to be pollinated because plants

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

9

10 RESULTS

11 None of the evolutionarily stable equilibria maximizes mean total fitness ($w < w'$) for any set
12 of pollinator and genetic constraints we model (Figs. 1-3 and Figs. S2-S3 for the constant-
13 rank model [Supplementary Information]). This occurs because of frequency-dependent
14 selection on the individual flowering phenology and selfing rate: Fisher's automatic genetic
15 advantage of a rare, completely selfing mutant is 50% in a strictly outcrossing population and
16 decreases to 0 when the selfing genotype has completely invaded the population. The ESS
17 also do not correspond to flowering phenologies that maximize the fraction of flowers that
18 can be pollinated by either self or outcross pollen. Nevertheless some ESS are close to the
19 strategy that minimizes pollen limitation by mass blooming on a single day (with standard
20 deviation in flowering time much lower than 1), thus maximizing pollinator attraction (Fig.
21 1A). As explained above, these rarely observed equilibria are not discussed further.

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

1 depend on the foraging behavior of pollinators. For the *base* case consisting of plants with
2 $\bar{N} = 100$ flowers, with limitation of both pollinator abundance and pollinator attraction and
3 with substantial inbreeding depression, a random-rank visitation sequence of flowers on a
4 plant by individual pollinators generates high (maternal and/or paternal) outcross reproductive
5 success independent of floral display, whereas a constant-rank visitation sequence produces
6 higher outcross reproductive success only if fewer flowers are open per day (Fig. 1). This
7 pattern is created because different pollinators deposit outcross pollen on different flowers
8 under random movement, but under the constant movement different flowers can be
9 outcrossed only if flowers are open on different days. For the same reasons and all else being
10 equal, pollen limitation is higher at an ESS under constant-rank rather than random-rank
11 visitation sequences of flowers (Table 1; Figs. 1-3 and Figs. S2-S3 [**Supplementary**
12 **Information**]).

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

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

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

1 stable equilibria: flowering phenologies of a few weeks characterized by pollen limitation
2 mostly due to the low quality of selfed seeds rather than a shortage of pollinator visits, and
3 much longer flowering phenologies with high pollen limitation due mainly to low pollinator
4 attraction, rather than pollen genetic composition. In contrast, a constant-rank visitation
5 sequence generates only extended flowering phenologies favoring outcross pollination; these
6 phenologies are strongly pollen limited because of low pollinator attraction (Figs. 3B and S3B
7 [Supplementary Information] for the random- vs constant-rank visitation sequence; Table
8 1).

9

10 DISCUSSION

11 This study complements earlier theoretical work on pollen limitation by highlighting a
12 potential additional mechanism driving evolution of limited seed production in natural
13 populations of animal-pollinated plants. We show that both the quantity and genetic
14 composition of pollen receipt of self-compatible animal-pollinated plant species control the
15 evolutionary equilibrium flowering phenologies and consequent pollen limitation. Mean
16 fitness is not maximized, and pollen limitation is never minimized at equilibrium. Non-
17 maximization of mean fitness is explained by frequency-dependent selection, which violates
18 the assumption of constant genotypic fitnesses required for Wright's (1931, 1969) principle of
19 evolutionary maximization of mean fitness. In this model, as in earlier ones (e.g.
20 Bierzychudek 1981; Haig and Westoby 1988; Burd 1995, 2008; Harder and Aizen 2010;
21 Schreiber *et al.* 2015) pollen limitation is an evolved emergent property; however, here pollen
22 limitation is constrained by a trade-off between the maternal self and paternal outcross
23 components of fitness that involves both genetic and ecological constraints (Devaux *et al.*
24 2014a), whereas most previous models only considered ecological constraints. The ecological
25 constraints include pollinator abundance, pollinator attraction to large floral displays,

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

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

12 **Relevance of our model to study pollen limitation in natural populations**

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

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

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

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

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

16 **Limitations and perspectives to model the evolution of pollen limitation**

17 Pollen limitation evolves in this study by mechanisms different from those analyzed in
18 previous theory. First and most importantly, we deliberately excluded environmental
19 stochasticity for the sake of simplicity : it proved to be the main driver of within and among
20 plant variation in pollination and the evolution of pollen limitation in previous models (Burd
21 2008; Richards *et al.* 2009; Rosenheim *et al.* 2014; Schreiber *et al.* 2015) and is undoubtedly
22 responsible for some pollen limitation in natural populations. Instead we include demographic
23 stochasticity (operating independently among individuals, unlike environmental stochasticity)
24 to produce variation in the number of open flowers a given day, variation in the number of
25 pollinator visits to a plant, and variation in the number of flowers visited per plant per

1 pollinator visit. Because our model involves an infinitely large population, such demographic
2 stochasticity has little impact on evolutionary equilibria in comparison to the temporal
3 environmental stochasticity that is synchronized among all individuals in other models. Pollen
4 limitation in our model evolves because of a genetic trade-off between pollinator attraction (as
5 well as other aspects of pollinator behavior) and inbreeding depression after zygote formation.
6 Predicting how these mechanisms may interact with environmental stochasticity is not
7 straightforward. In self-compatible insect-pollinated plants, both inbreeding depression with
8 selfing and temporal fluctuations in pollinator availability (i.e. pollinator visits per plant,
9 which could be due to fluctuations in pollinators and/or plant population density, Thomson
10 2010) should contribute to the evolution of pollen limitation. Obviously, in a highly stochastic
11 environment, highly variable pollinator availability is much more likely important than is
12 inbreeding depression with selfing. In a more constant environment with stable pollinator
13 availability, the contribution of inbreeding depression to pollen limitation depends on both the
14 selfing rate and how much inbreeding depression can be purged.

15 Evolution of individual flowering phenologies, and consequent pollen limitation, may
16 also be driven by genetic and ecological factors not considered here, acting at both the
17 individual and community levels: our mechanistic model of pollinator behavior is simplified,
18 to address ubiquitous genetic and ecological mechanisms responsible for the emergence of
19 pollen limitation, and cannot portray the immense variation among pollinator species. First,
20 plant resources are limited in our model as all plants display the same expected number of
21 flowers, but we neglect allocation to seed provisioning considered by previous authors
22 (Bierzychudek 1981; Haig and Westoby 1988; Ashman *et al.* 2004). Resource allocation may
23 be particularly crucial to understand pollen limitation in iteroparous species (Crone *et al.*
24 2009), which are not considered in our model. Instead we model allocation to flowers among
25 days in the flowering phenology of individual plants and allow plants to mature all seeds

1 without reproductive compensation. Second, autonomous selfing has been proposed many
2 times as a reproductive assurance strategy under pollinator limitation (Fishman and Willis
3 2008; Marten-Rodriguez and Fenster 2010; Thomann *et al.* 2013); its evolution towards
4 increased selfing was found in natural populations (Moeller 2006) and in experimental
5 populations experiencing pollinator abundance limitation (Bodbyl Roels and Kelly 2011).
6 Allowing autonomous selfing and its evolution can have complex effects on the evolution of
7 flowering phenologies (Devaux *et al.* 2014a) and pollen limitation (Morgan and Wilson 2005;
8 Harder *et al.* 2008). We also do not account for facilitated selfing (c.f. Lloyd and Schoen
9 1992) as little empirical information exists on this intra-flower component of selfing except
10 for specific flower morphologies (Johnson *et al.* 2005; Owen *et al.* 2007; Vaughton *et al.*
11 2008). Evolution of sterile flowers can reduce pollen limitation by increasing attraction of
12 pollinators at low energetic and genetic costs (Morales *et al.* 2013). We further do not address
13 pollen competition between self and outcross pollen, or among multiple sires (Lankinen and
14 Armbruster 2007; Richards *et al.* 2009) possibly complicated by pollen precedence (Waser
15 and Fugate 1986), the evolution of aggregated pollen that occurs in orchids (Harder and
16 Johnson 2008), or mechanisms such as dichogamy or herkogamy that can prevent
17 geitonogamous selfing. Our model also omits several factors operating at the community level
18 among plant species that can affect both the amount and genetic composition of pollen
19 receipt: e.g. facilitation and competition among species (Moeller 2004; Vamosi *et al.* 2006;
20 Hegland and Totland 2008; Devaux and Lande 2009; Sargent *et al.* 2011; Lazaro *et al.* 2014),
21 which partly depends on the constancy of pollinators to a plant species and the transfer of
22 heterospecific pollen, and can potentially affect the evolution of autonomous selfing.

23 CONCLUSION

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

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

11

12 **SUPPLEMENTARY DATA**

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

21

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25

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1 **TABLES**

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

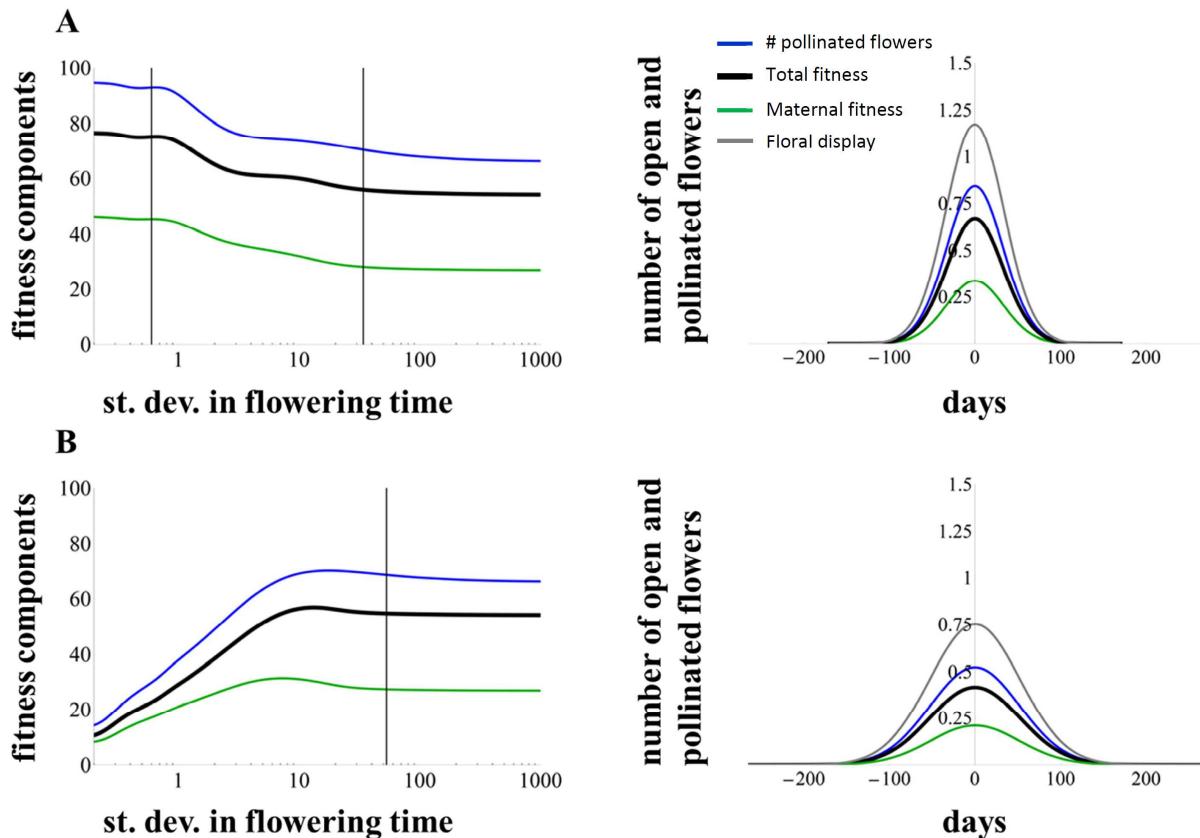
Parameter values	Random-rank visitation sequence			Constant-rank visitation sequence		
	σ^*	PL_{unpoll}	PL_{devmax}	σ^*	PL_{unpoll}	PL_{devmax}
<i>Base case</i> ¹	Min	5.2	30.7	Min	29.8	50.6
	0.6	7.1	32.1	-	-	-
	34	29.6	57.8	53	31.4	58.6
Lower inbreeding depression ($U = 0.02, d = 0$)	Min	5.2	6.7	Min	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 ($\tau = 0.01$)	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

8 ¹Note: *Base case*: $M = 100$ pollinators are available, they carry $A = 100$ pollen grains, their

9 probability of leaving a plant after visiting a flower is $\tau = 0.33$, their pollen carry-over is $1 -$
 10 $\rho = 0.75$, and their visitation rate is defined by $a = 50$ and $b = 0.1$ (Fig. S1
 11 [Supplementary Information]); inbreeding depression is due to deleterious mutations that

- 1 can or cannot be purged ($U = 0.2$, $h = 0.02$, and $d = 0.25$), plants produce $\bar{N} = 100$ flowers.
- 2 Evolutionary equilibria are ranked according to increased σ (flowering period of plants).
- 3
- 4

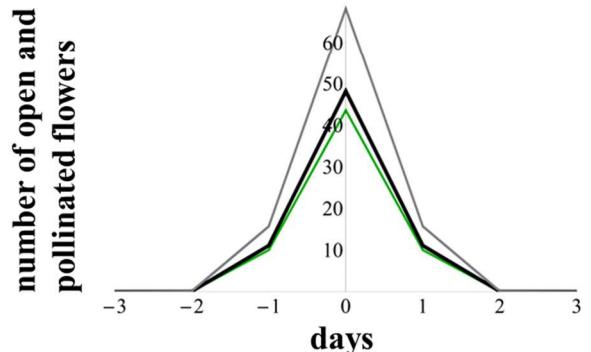
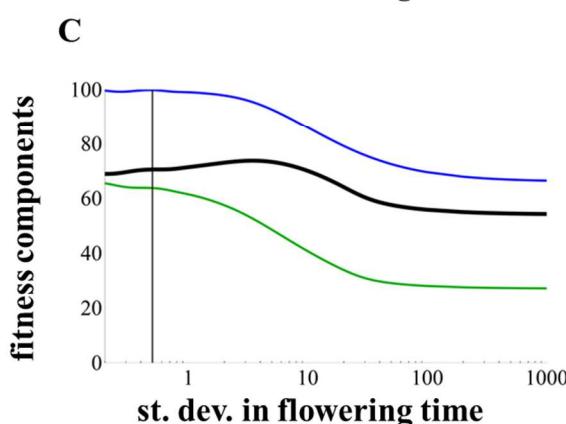
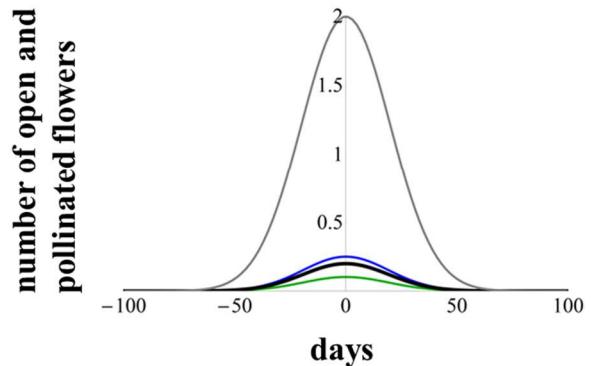
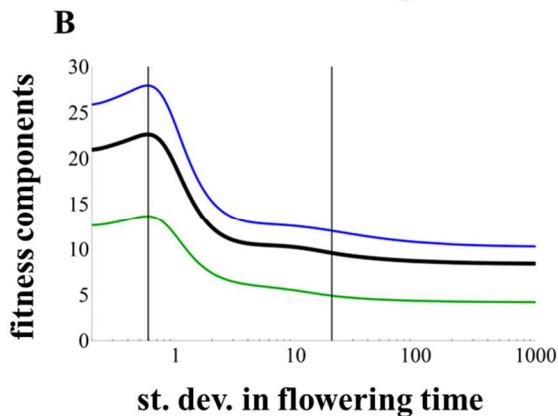
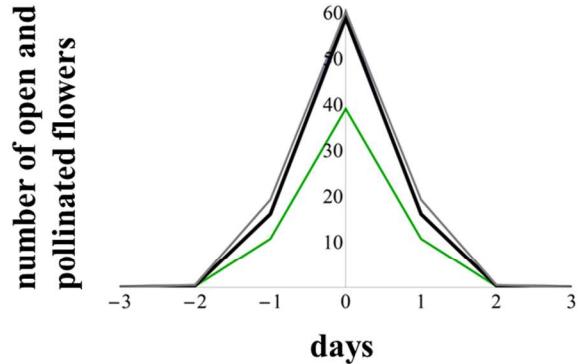
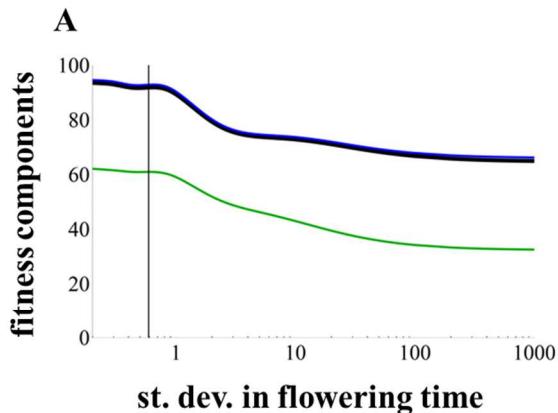
1 **FIGURE LEGENDS**



2
3 **Fig. 1:** Fitness components and floral display under two pollinator visitation sequences of
4 flowers on a plant (random-rank for panels A vs. constant-rank visitation sequence for panels
5 B). Left panels: total fitness (w , thick black line), maternal fitness (w_m , green line) and
6 number of pollinated flowers (T , blue line) as a function of (log) standard deviation in
7 flowering time. The solid vertical lines indicate the evolutionarily stable standard deviation(s)
8 in flowering time. Right panels: floral display (gray line), number of pollinated flowers (blue
9 line), maternal fitness (w_m , green line), and total fitness (thick black line) as a function of
10 days at the ESS with the highest standard deviation in flowering time. Pollen limitation can be
11 visualized by comparing floral display (number of open flowers) vs. number of pollinated
12 flowers, number of pollinated flowers vs. total fitness, or number of pollinated flowers vs.
13 maternal fitness (See Table 1 for quantitative measures of pollen limitation). Pollinator
14 attraction limitation defined by $a = 50$ and $b = 0.1$, pollinator abundance limitation by $M =$

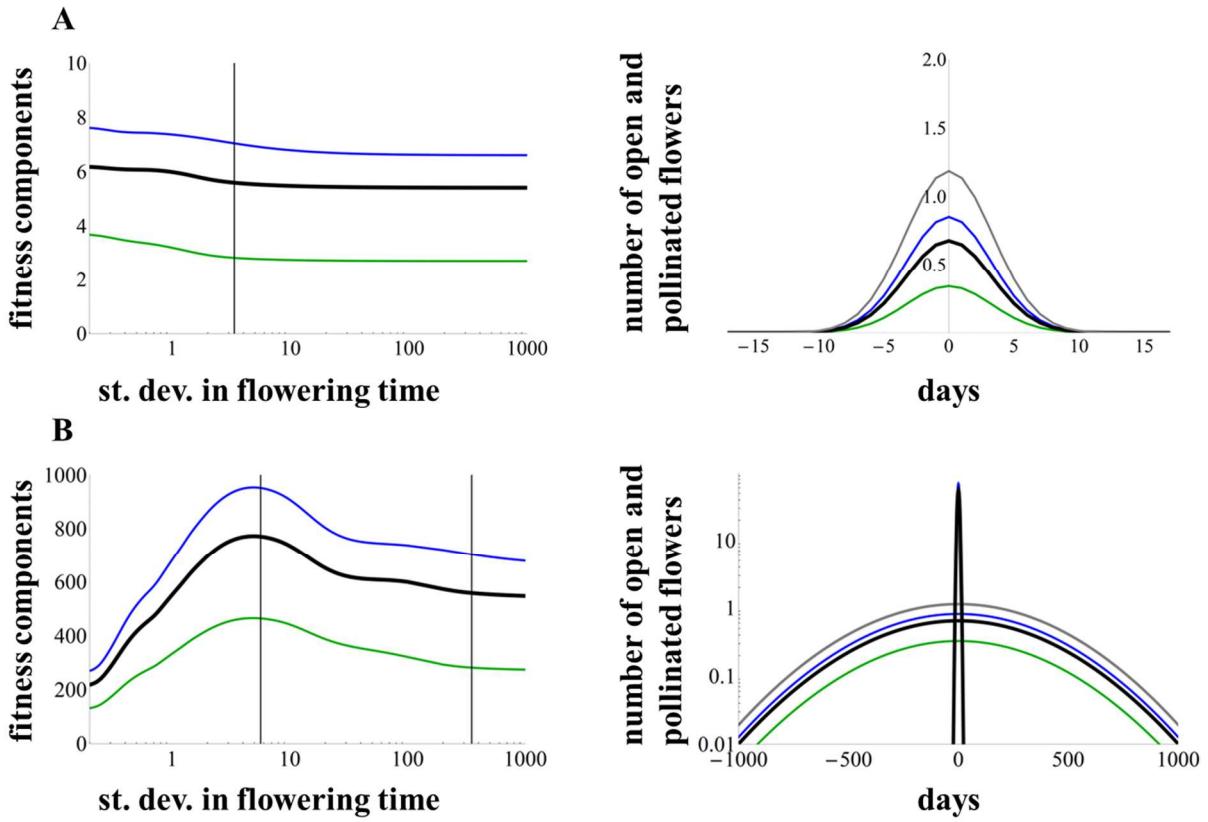
- 1 100 pollinators, $\tau = 0.33$ and $A = 100$, pollen deposition rate $\rho = 0.25$, $\bar{N} = 100$ flowers
- 2 per plant, $U = 0.2$, $h = 0.02$ and $d = 0.25$ for inbreeding depression.
- 3

1
2



3

4 **Fig. 2:** Fitness components and floral display under decreased inbreeding depression ($U =$
5 0.02 and $d = 0$, panels A), decreased number of pollinators available ($M = 10$, panels B),
6 and increased fraction of open flowers visited by pollinators ($\tau = 0.01$, panels C). Random-
7 rank visitation sequence of flowers on a plant; other parameters and symbols as in Fig. 1.
8



1
2 **Fig. 3:** Fitness components and floral display under decreased ($\bar{N} = 10$, panels A) and
3 increased ($\bar{N} = 1000$, panels B) flower production per plant. Random-rank visitation
4 sequence of flowers on a plant; other parameters and symbols as in Fig. 1. The stable
5 equilibria for both small and large standard deviations in flowering time for plants producing
6 $\bar{N} = 1000$ are shown on a log-scale.